Vulvar Pathology

Mai P. Hoang
Maria Angelica Selim
Editors

Springer

Vulvar Pathology

Mai P. Hoang • Maria Angelica Selim
Editors

Vulvar Pathology

Springer

Editors
Mai P. Hoang
Harvard Medical School
Massachusetts General Hospital
Department of Pathology
Boston, MA, USA

Maria Angelica Selim, MD
Duke University Medical Center
Department of Pathology
Durham, NC, USA

ISBN 978-1-4939-1806-5 ISBN 978-1-4939-1807-2 (eBook)
DOI 10.1007/978-1-4939-1807-2
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014955477

To Carol and Hulse Wagner

—Mai P. Hoang, MD

To my family for always being there with unending support and wisdom; to my mentors who are a source of professional encouragement and inspiration; and to my colleagues and patients from whom I continue to learn. Finally, I would like to dedicate this book to you, the reader, committed to the care of women.

—Maria Angelica Selim, MD

Foreword

The time is now. Of all major body areas that the medical literature covers, the vulva is the one most neglected. Despite a long history of fascination since the beginning of humanity and its prominent display in the artwork of our early ancestors, the medical writings are less than might be anticipated by the seriousness and frequency of disease and the conditions that arise. To a large part, the cause may be that the diseases encountered fall outside the exclusive domain of any specific medical specialty. For example, the general pathologist and even the specialist in gynecologic pathology rarely see the dermatoses and other infectious disease processes afflicting the vulva. In turn, the general dermatopathologist typically sees the melanocytic lesions, but often not the other neoplasms, especially sarcomas, and other soft tissue conditions typically seen there. The average gynecologist and sometimes the dermatologist may initially be the first physicians the patient encounters with any condition involving the vulva, but to whom does the biopsy specimen go: To the general pathologist, to the gynecologic pathologist, or to the dermatopathologist?

Consequently and all too often, vulvar disease is not diagnosed or treated with the care it deserves. One manifestation of this contention is that few books have been written about the vulva – far fewer than textbooks on gynecologic pathology or dermatopathology – and few presented comprehensively. Within the last 3 years, only one other book listed in the Library of Congress on-line Catalogue has been written exclusively about vulvar pathology. It is therefore refreshing to see a new textbook written and edited by two experienced dermatopathologists who have spent much of their professional lives dealing with lesions found in the vulva. Their team includes dermatopathologists, gynecologic pathologists, dermatologists, and soft tissue experts, all of whom specialize in this area and have pooled their collective experience and wisdom.

Readers will enjoy that this book encompasses in-depth normal anatomy and embryology, inflammatory dermatoses, melanocytic and squamous proliferative lesions, squamous preneoplasia and cancer, cysts and glandular

lesions, and, finally, mesenchymal proliferations. Further, these major headings subdivide into 15 separate chapters presenting what are common to the not-so-common diagnostic challenges. The book is highly illustrated and easy to read, and should form a solid basis for someone involved in understanding vulvar disease.

Stanley J. Robboy, MD, FCAP, FFPath, RCPI, FRCPath, UK
Department of Pathology
Duke University Medical Center
Durham, NC, USA

Introduction

The word "vulva" originates from the Middle Latin word "*volva*" referring to the *"womb"* or *"female sexual organ."* This organ has been featured in rituals, Paleolithic art and mentioned in ancient texts like the Egyptian papyri from the second millennium BC, the Talmud, and the Bible. The first detailed medical description of vulvar diseases was rendered by Avicenna (Ibn Sina), illustrious philosopher-scientist of the pre-modern era. Through time, no single medical specialty has claimed vulvar diseases as its unique area of expertise. The most frequent diseases affecting the vulva are dermatologic, but most of such patients are cared for by family practitioners and gynecologists lacking sophisticated diagnostic and therapeutic skills in skin disorders. Conversely, dermatologists may have interest and expertise in the vulva, but only in a subset of its diseases. The challenge of diagnosing vulvar disorders is highlighted by considering three intersecting elements involved by a health care encounter with a patient with vulvar complaints:

- The *patient*'s social and cultural background colors how she perceives the problem. Reticence commonly leads to delay and/or attempted self-treatment, either of which may change or exacerbate the clinical appearance of the disease, adding to the diagnostic difficulty. This problem is of particular concern in sexually transmitted diseases, where the patient's partner(s) is/are at risk.
- The *vulva* is susceptible to the effects of friction, trauma, and maceration, all frequently encountered in this anatomic location. Itching is a common symptom in numerous disorders of the vulva; scratching in turn can produce secondary changes that obscure the original disease for the clinician and pathologist. In addition, the native vulvar environment, as well as secondary alterations, facilitates growth of microorganisms, making a large proportion of vulvar diseases multifactorial.
- The *physician* is typically not fully trained to care for the broad spectrum of vulvar diseases. Furthermore, skin diseases appear different when they occur in skin folds and on genital skin. It comes as no surprise, then, that vulvar diseases are frequently underreported, underinvestigated, or misdiagnosed. Delay in diagnosis and therapy is frustrating for both the patient and her physician. A multidisciplinary approach involving more than one specialist can minimize these problems and enable the patient to be seen, studied, and managed in one visit.

In response to the complexity of vulvar disorders, the *International Society for the Study of Vulvovaginal Disease* (ISSVD, http://www.issvd.org) was

founded with the objective of standardizing terminology and promulgating classifications that facilitate clear communication among different physicians caring for patients who suffer from non-neoplastic and neoplastic vulvar disease. A classification of inflammatory disorders based on histologic patterns (Table 1) was published on 2006 [1]. In 2011, a new classification incorporating the lesion type was presented in Paris (Table 2) [2] to assist in clinical diagnosis. Currently, the ISSVD considers the histologic and clinical classifications as complementary.

Table 1 ISSVD classification of vulvar dermatoses: pathologic subsets and their clinical correlates

Spongiotic pattern
Atopic dermatitis
Contact dermatitis
Allergic
Irritant
Acanthotic pattern
Lichen simplex chronicus
Primary (idiopathic)
Secondary (superimposed on other vulvar disease)
Psoriasis
Reiter's syndrome
Lichenoid pattern
Lichen sclerosus
Lichen planus
Dermal homogenization/sclerosis pattern
Lichen sclerosus
Vesiculobullous pattern
Bullous pemphigoid
Mucous membrane pemphigoid
Linear IgA disease
Pemphigus vulgaris
Pemphigus vegetans
Acantholytic pattern
Hailey-Hailey disease
Darier disease
Papular genitocrural acantholysis
Acantholytic acanthoma
Granulomatous pattern
Crohn disease
Melkersson-Rosenthal syndrome
Sarcoidosis
Vasculopathic pattern
Aphthous ulcers
Behcet disease
Plasma cell vulvitis

Used with permission from Lynch et al. [1] and from http://issvd.org/wordpress/wp-content/uploads/2014/02/2014-BIBLIOGRAPHY-CURRENT-ISSVD-TERMINOLOGYrev.pdf

Table 2 The 2011 ISSVD clinical classification of vulvar dermatological disorders

(A) Skin colored lesions
Papules and nodules
1. Papillomatosis of the vestibule and medial labia minora
2. Molluscum contagiosum
3. Warts (HPV infection)
4. Scar
5. Vulvar intraepithelial neoplasia
6. Skin tag
7. Nevus, intradermal type
8. Mucinous cysts of the vestibule and medial labia minora
9. Epidermal cyst
10. Anogenital mammary-like gland tumor (hidradenoma papilliferum)
11. Bartholin gland cyst and tumor
12. Syringoma
13. Basal cell carcinoma
Plaques
1. Lichen simplex chronicus (LSC) and other lichenified disease
2. Vulvar intraepithelial neoplasia
(B) Red lesions
Patches and plaques
Eczematous and lichenified diseases
1. Contact dermatitis, allergic and irritant
2. Atopic dermatitis (rarely seen as a vulvar presentation)
3. Eczematous changes superimposed on other vulvar disorders
4. Diseases clinically mimicking eczematous disease (e.g. candidiasis, Hailey-Hailey disease and extramammary Paget's disease)
5. Lichen simplex chronicus (lichenification with no preceding skin lesions)
6. Lichenification superimposed on an underlying preceding pruritic disease
Red patches and plaques with no epithelial disruption
1. Candidiasis
2. Psoriasis
3. Vulvar intraepithelial neoplasia
4. Lichen planus
5. Plasma cell (Zoon's) vulvitis
6. Bacterial soft-tissue infection (cellulitis and early necrotizing fasciitis)
7. Extramammary Paget's disease
Papules and nodules
Red papules
1. Folliculitis
2. Wart (HPV infection)
3. Angiokeratoma
4. Molluscum contagiosum (inflamed)
5. Hidradenitis suppurativa (early lesions)
6. Hailey-Hailey disease
Red nodules
1. Furuncles ("boils")
2. Wart (HPV infection)
3. Prurigo nodularis
4. Vulvar intraepithelial neoplasia

(continued)

Table 2 (continued)

5. Molluscum contagiosum (inflamed)
6. Urethral caruncle and prolapse
7. Hidradenitis suppurativa
8. Anogenital mammary-like gland adenoma (hidradenoma papilliferum)
9. Inflamed epidermal cyst
10. Bartholin duct abscess
11. Squamous cell carcinoma
12. Melanoma (amelanotic type)
(C) White lesions
Patches and plaques
1. Vitiligo
2. Lichen sclerosus
3. Post-inflammatory hypopigmentation
4. Lichenified diseases (when the surface is moist)
5. Lichen planus
6. Vulvar intraepithelial neoplasia
7. Squamous cell carcinoma
Papule and nodules
1. Fordyce spots
2. Molluscum contagiosum
3. Wart
4. Scar
5. Vulvar intraepithelial neoplasia
6. Squamous cell carcinoma
7. Milium (pl. milia)
8. Epidermal cyst
9. Hailey-Hailey disease
(D) Dark colored (brown, blue, gray or black) lesions
Patches
1. Melanocytic nevus
2. Vulvar melanosis (vulvar lentiginosis)
3. Post-inflammatory hyperpigmentation
4. Lichen planus
5. Acanthosis nigricans
6. Melanoma-in-situ
Papules and nodules
1. Melanocytic nevus (includes those with clinical and/or histologic atypia)
2. Warts (HPV infection)
3. Vulvar intraepithelial neoplasia
4. Seborrheic keratosis
5. Angiokeratoma (capillary angioma, cherry angioma)
6. Anogenital mammary-like gland adenoma (hidradenoma papilliferum)
7. Melanoma
(E) Blisters
Vesicles and bullae
1. Herpesvirus infections (herpes simplex, herpes zoster)
2. Acute eczema (see definitions in Part IV above)
3. Bullous lichen sclerosus
4. Lymphangioma circumscriptum (lymphangiectasia)

Table 2 (continued)

5. Immune blistering disorders (e.g. cicatricial pemphigoid, fixed drug eruption, Steven-Johnson syndrome, pemphigus)
Pustules
1. Candidiasis
2. Folliculitis
(F) Erosions and ulcers
Erosions
1. Excoriations
2. Erosive lichen planus
3. Fissures arising on normal tissue (idiopathic, intercourse related)
4. Fissures arising on abnormal tissue (candidiasis, lichen simplex chronicus, psoriasis, Crohn's disease, etc.)
5. Vulvar intraepithelial neoplasia, eroded variant
6. Ruptured vesicles, bullae and pustules
7. Extramammary Paget's disease
Ulcers
1. Excoriations (related to eczema, lichen simplex chronicus)
2. Aphthous ulcers (syn. aphthous minor, aphthous major, Lipschütz ulcer, occurring either as an idiopathic process or secondary to other diseases such as Crohn's, Behçet's, various viral infections)
3. Crohn's disease
4. Herpesvirus infection
5. Ulcerated squamous cell carcinoma
6. Primary syphilis (chancre)
(G) Edema
Skin-colored
1. Crohn's disease
2. Idiopathic lymphatic abnormality (congenital Milroy's disease)
3. Post-radiation and post-surgical lymphatic obstruction
4. Post-infectious edema (esp. staphylococcal and streptococcal cellulitis)
5. Post-inflammatory edema (esp. hidradenitis suppurativa)
Pink or red
Venous obstruction (e.g. pregnancy, parturition)
2. Cellulitis (primary or superimposed on already existing edema)
3. Inflamed Bartholin duct cyst/abscess
4. Crohn's disease
5. Mild vulvar edema may occur with any inflammatory vulvar disease

Used with permission from Lynch et al. [2] and from http://issvd.org/wordpress/wp-content/uploads/2014/02/2014-BIBLIOGRAPHY-CURRENT-ISSVD-TERMINOLOGYrev.pdf

The female genital tract is a complex system with multiple organs. Most textbooks of pathology, dermatopathology, and gynecologic pathology dedicate restricted space to vulvar diseases, with an understandable emphasis on neoplastic disorders due to their life-threatening nature. Yet, the demand for specialists in inflammatory and infectious vulvar disorders is increasing. This book focuses on setting forth our current knowledge of the full spectrum of vulvar pathology, utilizing a multidisciplinary approach. Our introductory chapter lays the groundwork for an understanding of vulvar diseases by

describing its embryologic development, normal anatomy, and histology. Inflammatory diseases are presented, organized by structures affected and histopathologic patterns. Mesenchymal processes are categorized based on cell of origin. Melanocytic and glandular lesions are reviewed in detail, so as to assist in diagnosis and classification. The changing and contending categorizations of squamous lesions are discussed, and the arguments for each are set out to assist the reader in understanding the logic behind each of the proposed classifications. The clinical presentation of each entity is described with emphasis of the peculiarities of presentation of each disease in the vulva. Extragenital manifestations of each disorder are enumerated, because not uncommonly they are the key to a correct diagnosis. Recent advances in understanding of the physiopathology, genetic, and molecular basis of vulvar diseases are thoroughly discussed. The histopathology of vulvar disease is presented in detail, with an emphasis on pathologic differential diagnosis and histopathologic mimickers.

To further enhance this book, we have included "take-away essentials," a section to which the reader can turn for a summary of practical points. Case vignettes at the end of each chapter provide an opportunity to observe how knowledge can be applied in actual cases. Each of these additions should be useful tools for professional development.

We hope the reader will find this book a useful, practical reference for the daily practice of vulvar disease diagnosis, an area of rapidly expanding knowledge and clinical need.

Duke University Medical Center
Department of Pathology
Durham, NC, USA

Maria Angelica Selim, MD

References

1. Lynch PJ, Moyal-Barracco M, Bogliatto F, Micheletti L, Scurry J 2006 ISSVD classification of vulvar dermatoses: pathologic subsets and their clinical correlates. J Reprod Med. 2007;52(1):3–9.
2. Lynch PJ, Moyal-Barracco M, Scurry J, Stockdale C 2011 ISSVD terminology and classification of vulvar dermatological disorders: an approach to clinical diagnosis. J Low Genit Tract Dis. 2012;16(4):339–44.

Acknowledgments

Special thanks to all authors for their exceptional efforts in putting together this book.

Many thanks to the Springer team – senior editor Richard Hruska, this book's developmental editor Elizabeth Corra, and Silembarasanh Panneerselvam and Arokianathan Vinita at SPi Global, for their patience and invaluable help.

We sincerely hope that this book will serve as a useful resource for the readers.

Contents

Contributors

Russell A. Ball Ball Dermpath, Greensboro, NC, USA

Sarah M. Bean Department of Pathology, Duke University Health System, Durham, NC, USA

Rex C. Bentley Department of Pathology, Duke University Health System, Durham, NC, USA

J. Andrew Carlson Department of Pathology, Albany Medical College, Albany, NY, USA

Libby Edwards Department of Internal Medicine, Carolinas Medical Center, Charlotte, NC, USA

Maria Teresa Fernández-Figueras Department of Anatomic Pathology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

Allison H.S. Hall Department of Pathology, Duke University Medical Center, Durham, NC, USA

Mai P. Hoang Department of Pathology, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA

Doina Ivan Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Dmitry V. Kazakov Department of Pathology, Charles University Medical Faculty Hospital, Pilsen, Czech Republic

Konstantinos Linos Department of Pathology, Dartmouth-Hitchcock Medical Center, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Cesar A. Llanos Department of Pathology, Miller School of Medicine, University of Miami, Miami, FL, USA

Martin C. Mihm Jr. Department of Dermatology, Brigham and Women's Hospital, Boston, MA, USA

Kristen M. Paral Department of Pathology, The University of Chicago Medical Center, Chicago, IL, USA

Viviana Parra Facultad de Ciencias Medicas Uncuyo, Hospital Luis Lagomaggioer, Mendoza, Argentina

Victor G. Prieto Department of Pathology and Dermatology, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Jason C. Reutter Department of Dermatopathology, Piedmont Pathology Associates, Hickory, NC, USA

Luis Requena Fundacion Jimenez Diaz, Madrid, Spain

Stanley J. Robboy Department of Pathology, Duke University Medical Center, Durham, NC, USA

Andrew E. Rosenberg Department of Pathology, University of Miami Hospital, Miami, FL, USA

Demaretta S. Rush Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL, USA

Martin A. Sangueza Department of Pathology, Caja Nacional de Salud, La Paz, Bolivia

Omar P. Sangueza Departments of Pathology and Dermatology, Wake Forest Baptist Medical Center, Wake Forest University School of Medicine, Winston Salem, NC, USA

Maria Angelica Selim Department of Pathology, Duke University Medical Center, Durham, NC, USA

Christopher R. Shea Department of Medicine, The University of Chicago Medicine, Chicago, IL, USA

Bruce Smoller Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Tien Anh Nguyen Tran Department of Pathology, Florida Hospital Orlando, Orlando, FL, USA

J. Matthew Velkey Department of Cell Biology, Duke University School of Medicine, Durham, NC, USA

Kelly L. West Ball Dermpath, Greensboro, NC, USA

Edward J. Wilkinson Department of Pathology, Immunology, and Laboratory Medicine, Shands Hospital, University of Florida College of Medicine, Gainesville, FL, USA

Part I

The Normal Vulva

Normal Vulva: Embryology, Anatomy, and Histology

1

J. Matthew Velkey, Allison H.S. Hall, and Stanley J. Robboy

Overview

The vulva consists of the female genital structures external to the vaginal opening—the introitus. Anatomically, the vulva lies within the perineum, which is a diamond-shaped region bounded anteriorly by the pubic symphysis, laterally by the left and right ischial tuberosities of the pelvic bones, and posteriorly by the coccyx (Figs. 1.1 and 1.2) [1]. The perineum further subdivides into an anterior urogenital triangle with the vulva and a posterior anal triangle with the anus and its external sphincter. The vulva comprises the mons pubis, the labia majora, the labia minora, the clitoris, and the vestibule. The vestibule itself is the specific region demarcated anteriorly by the clitoral prepuce, laterally by the labia minora, and posteriorly by the fourchette, which is a fold of skin where the labia minora join. Within this region are the clitoris, the vestibulovaginal bulbs and associated vestibular (Bartholin's) glands, the urethral meatus and associated periurethral (Skene's) glands, and the vaginal introitus. Before considering the anatomy and histology of these structures in more detail, however, it will be beneficial to first consider the development of the female reproductive tract.

J.M. Velkey (✉)
Department of Cell Biology, Duke University School of Medicine, Durham, NC, USA
e-mail: Matt.velkey@duke.edu

Embryology of the Female Reproductive Tract and External Genitalia

The urinary and reproductive systems in both males and females are embryologically and anatomically interrelated in that both develop from a urogenital ridge of intermediate mesoderm located along the posterior body wall in the developing abdominal cavity and both open into an endoderm-lined cloaca at the caudal end of the embryo (Table 1.1, Fig. 1.3). During the 4th week of development, excretory tubules of the mesonephros arise within the lateral, or mesonephric, portion of the urogenital ridge along the body axis extending from the thoracic to upper lumbar body segments [2]. The excretory tubules elongate into S-shaped loops encapsulating a rudimentary glomerular capillary tuft located along their medial portion. The lateral end of each excretory tubule attaches to a collecting duct running longitudinally known as the mesonephric or Wolffian duct, which opens into a portion of the cloaca that will invaginate to form the urogenital sinus. As the name implies, the urogenital sinus contributes to the lower urinary tract, namely, the bladder and urethra, as well as a portion of the reproductive tracts in both the male (prostate and prostatic urethra) and female (vagina and vestibule). Initially, the segmental nephrons of the mesonephros provide functional urine output but then regress as the definitive,

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_1,

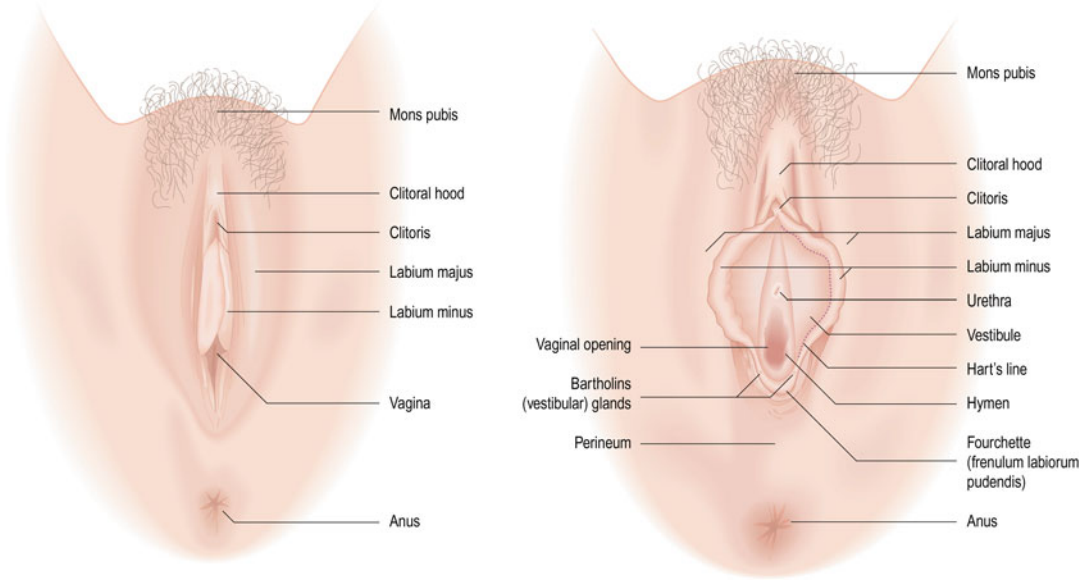


Fig. 1.1 External genitalia of the vulva (Used with permission from Robboy et al. [22]

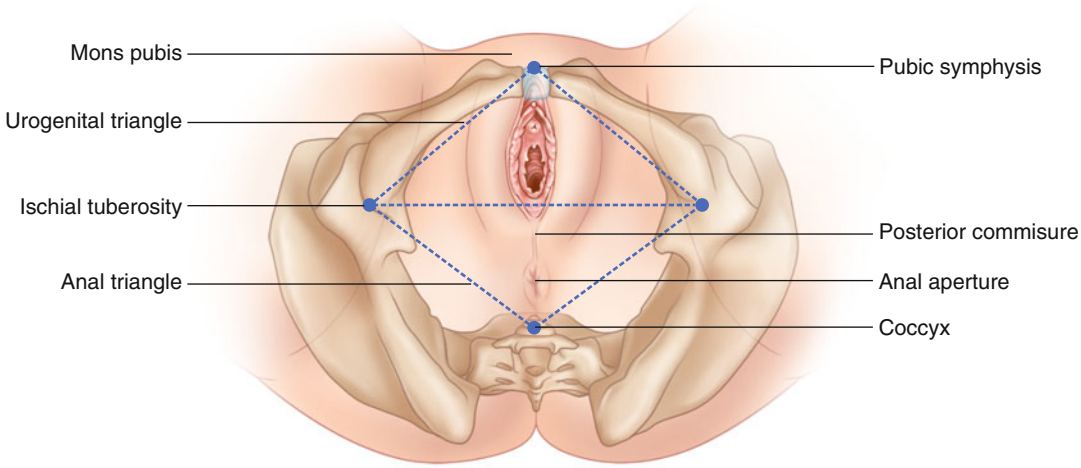


Fig. 1.2 Perineal surface anatomy

metanephric kidneys arise from the caudal intermediate mesoderm. The longitudinal mesonephric duct on each side persists and becomes part of a paired set of genital ducts that contribute significantly to the male reproductive tract. As described below, the mesonephric ducts largely regress in the female and normally only contribute to rudimentary structures.

During the 6th week, primordial germ cells appear within the medial, or genital, portion of the urogenital ridge. These germ cells initially arise in the embryo's epiblast, migrate through

Table 1.1 Homologues and origins of the human reproductive system

Indifferent	Germ layer	Female	Male
Gonad	Mesoderm	Ovary	Testis
Paramesonephric (Müllerian) duct	Mesoderm	Fallopian tubes	Appendix testis
Paramesonephric duct	Mesoderm	Uterus, vagina	Prostatic utricle
Mesonephric (Wolffian) duct	Mesoderm	Rete ovarii	Rete testis
Urogenital sinus	Endoderm	Skene's glands	Prostate
Urogenital sinus	Endoderm	Bladder, urethra	Bladder, urethra
Urogenital sinus	Endoderm	Bartholin's gland	Bulbourethral gland
Labioscrotal folds	Ectoderm	Labia majora	Scrotum
Urogenital folds	Mesoderm	Labia minora	Spongy urethra
Genital tubercle	Mixed	Vestibular bulbs	Bulb of the penis
Genital tubercle	Mixed	Clitoral glans	Glans penis
Genital tubercle	Mixed	Clitoral crura	Crus of the penis
Prepuce	Mixed	Clitoral hood	Foreskin
Gubernaculum	Mesoderm	Round ligament of the uterus	Gubernaculum testis

Modified from Ref. [21]

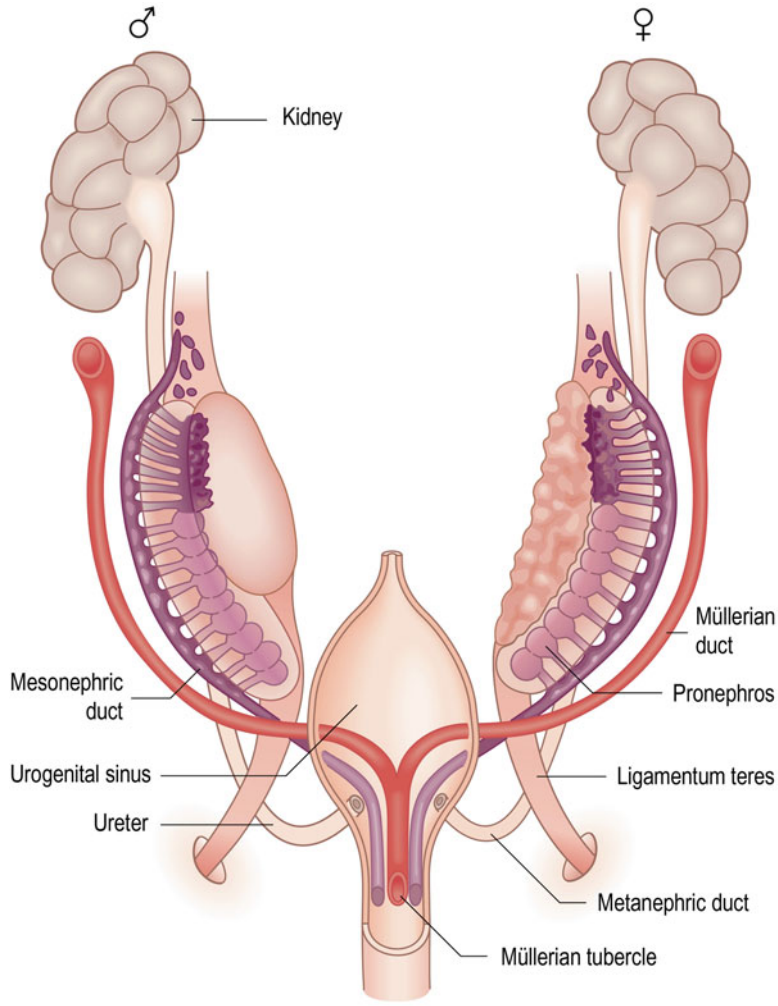


Fig. 1.3 Anlage of the genital organs in the indifferent, bisexual stage (Used with permission from Jaubert et al. [3]

the primitive streak during gastrulation in the 3rd week, and come to rest in the wall of the yolk sac near the forming allantois. Soon after gastrulation, the germ cells migrate back into the embryo along the dorsal mesentery of the hindgut and invade the medial edge of the urogenital ridge. In response to the germ cells, the overlying coelomic epithelium of the genital ridge proliferates and invades the mesenchymal tissue to form a series of irregularly shaped, primitive sex cords that remain connected to the surface epithelium and become closely associated with the germ cells. The surrounding mesenchymal cells, in turn, will develop into sex-specific interstitial cells of the gonads that contribute to the differentiation of the male or female phenotype.

During this same period, a second set of genital ducts, the paramesonephric (Müllerian) ducts, arise as the epithelium along the lateral edge of the genital ridge adjacent to the mesonephric ducts invaginates to form a longitudinal tube. At their cranial end, the paramesonephric ducts are lateral to the mesonephric ducts and end in a funnel-shaped opening into the abdominal cavity at about the same level as the superior aspect of the indifferent gonad. Caudally, the paramesonephric ducts continue lateral to the mesonephric ducts and then cross under (ventral to) the mesonephric ducts to course medially and partially fuse in the midline to form the uterine canal. The uterine canal projects caudally until it meets the wall of the urogenital sinus, where it causes a small swelling known as the paramesonephric, or Müllerian, tubercle to form [3].

At this point, the gonads of males and females are indistinguishable. The ductal systems are distinguishable by location only, but not histologically. However, from the 7th week on, the male and female systems diverge greatly as the process of sexual differentiation ensues. In the male, the sex-determining region of the Y-chromosome gene (*SRY*) acts in conjunction with *SOX9* to upregulate the expression of steroidogenic factor 1 (*SF1*) [4]. SF1 stimulates the differentiation of the epithelial sex cord cells into Sertoli cells, which then secrete anti-Müllerian hormone (AMH, also known as Müllerian inhibiting substance, or MIS) [5]. AMH is a transforming growth factor-beta (TGF-β) family member which induces apoptosis and regression of the paramesonephric ducts. The Sertoli cells also express CYP26, which degrades local retinoic acid, thus inducing meiotic arrest in the germ cells. In this milieu, the germ cells are directed to become spermatogonia [6]. SF1 also directs the differentiation of the interstitial cells of the male gonad into Leydig cells that secrete testosterone, which promotes growth and differentiation of the mesonephric ducts into the efferent ductules, epididymis, vas deferens, and seminal vesicles [7]. Testosterone is further converted by 5-α reductase into dihydrotestosterone, which stimulates growth and differentiation of the prostate and external genitalia.

In the female, *SRY* expression is absent, and instead the dominant genetic program is directed by *WNT4*, which initiates differentiation of the female phenotype [8]. Under this program, the surface epithelium of the female gonad proliferates rapidly to give rise to a secondary generation of sex cords known as cortical cords which will then become follicular cells rather than Sertoli cells. As there are no Sertoli cells, AMH is absent and the paramesonephric ducts persist. Moreover, retinoic acid signaling within the gonad induces progression of the germ cells into oogonia that proliferate and enter into the first meiotic division to form primary oocytes. The oocytes, in turn, direct the differentiation of the epithelial cord cells into follicular cells and cells of the surrounding mesenchyme into theca cells. Without Leydig cells, there is not a significant source of testosterone (and therefore DHT) to support the growth of the mesonephric ducts or male external genitalia. Instead, the theca and follicular cells secrete estrogens which promote the growth and development of the paramesonephric ducts and female external genitalia.

The uterine (Fallopian) tubes, uterine corpus, and uterine cervix develop exclusively from the paramesonephric ducts, whereas the vagina has contributions also from the urogenital sinus. The cranial portion of the paramesonephric ducts develop into the uterine (Fallopian) tubes, retaining their funnel-shaped openings into the coelomic cavity, which develop into the infundibulum and fimbriae, while the caudal portion of the paramesonephric ducts, when fused, gives rise to the uterus (Fig. 1.3). At this period, the entire presumptive uterus is a simple canal, without any of the later structural changes found in the adult

uterine body or cervix. The uterine canal grows toward and contacts the urogenital sinus at about the 7th week and induces the formation of the paramesonephric tubercle. Concurrent with this process, the endodermal epithelium of the urogenital sinus at the point of contact forms a pair of swellings (sinovaginal bulbs) that fuse into a solid vaginal plate that proliferates and extends cranially, thus pushing the paramesonephric tubercle and forming uterus away from the urogenital sinus. The growth of the vaginal plate and paramesonephric tubercle continues during the 3rd and 4th months followed by a process of canalization that is completed by the 5th month such that the mesenchymal walls of the vaginal fornices are derived from the paramesonephric tubercle and the squamous epithelial surfaces and possibly some of the lowest vagina are of urogenital sinus origin [3]. The initial site where growth of the vaginal plate from the wall of the urogenital sinus commenced usually does not fully canalize, thus leaving a membranous hymen that separates the vaginal canal from the urogenital sinus. While the mesonephric ducts mostly regress in the female, some remnants may remain as rudimentary tubules or cysts found adjacent to the ovary (epoophoron), uterine tube (paroophoron), or along the lateral wall of the uterus and/or vagina (Gartner's ducts/cysts) (Fig. 1.4).

The development of the external genitalia is apparent by the 4th week of development as the tissue along the rim of the cloacal opening thickens into cloacal folds [9]. Division of the cloaca into urogenital and anal portions occurs during the 5th and 6th weeks by the growth of the urorectal septum, which is a block of mesoderm-derived tissue that grows in between the alimentary and urogenital tracts and eventually fuses with the cloacal folds at the site of the perineal body. Anteriorly, the tissues of the cloacal folds fuse to form the genital tubercle. Posterior to the genital tubercle, the tissue surrounding the opening into

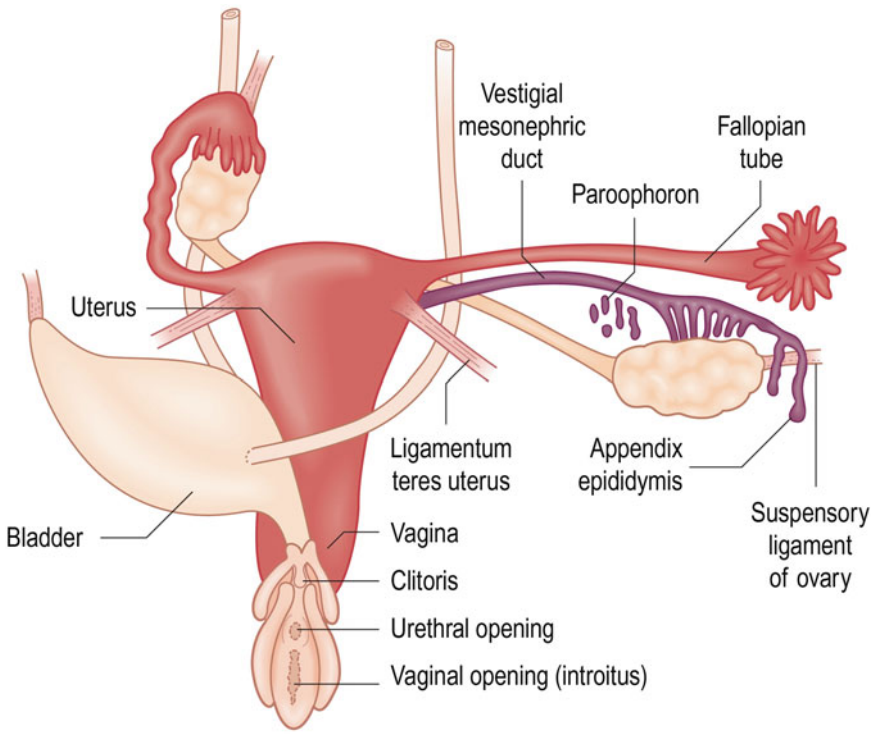


Fig. 1.4 Female differentiation of the genital organs (Used with permission from Jaubert et al. [3]

the forming urogenital sinus develops into urogenital folds. The posterior-most cloacal folds develop into anal folds that surround the forming anus. Lateral to the urogenital folds, a second set of swellings, the labioscrotal folds, appear. In the male (under androgenic influence, in particular dihydrotestosterone), the genital tubercle develops into the penile glans; the urogenital folds fuse and elongate to form the penile shaft; and the labioscrotal folds fuse to become the scrotum [8]. In the female (under the influence of estrogens rather than androgens), the genital tubercle develops into the clitoral glans, whereas the urogenital and labioscrotal folds give rise to the labia minora and majora, respectively [9] (Fig. 1.5). Anteriorly, the labia majora merge to form the mons pubis, and posteriorly, the labia majora fuse with the perineal body anterior to the anus. The remaining opening of the urogenital sinus exterior to the vaginal introitus (usually completely or partially covered by the hymen) and bounded by the labia minora expands to form the vestibule, the lining of which is thus endoderm derived. This is in contrast to the other structures of the vulva, which are of ectoderm and mesoderm origin and therefore respond differently to hormones or other stimuli evoked in either the normal or diseased state.

Overview of Vulvar Anatomy

As described above, the vulva consists of those structures within the urogenital triangle and external to the vaginal introitus, or hymen. Anteriorly, the most superficial vulvar structure is the mons pubis, which blends laterally with the labia majora found on either side of the vestibular opening. Posteriorly, the labia majora merge with the perineal body, which lies between the vaginal fourchette and the anus. The mons pubis and labia majora are of ectodermal origin and therefore are covered by hairy skin. Medial to the labia majora are the interlabial sulci, which separate the labia majora from the labia minora and also mark a transition point where the skin becomes hairless. The labia minora bound the opening into the vestibule. Anteriorly, each labium minus bifurcates into a medial and lateral fold. The medial folds from each labium minus unite posterior to the clitoris to form the frenulum of the clitoris, whereas the lateral folds unite anterior to the clitoris to form the hood or prepuce of the clitoris. Posteriorly, the labia minora merge to form the frenulum of the labia minora (or vaginal fourchette). Elements of the labia minora constitute an imaginary vestibular line (of Hart) that demarcates the vestibule, which is of endodermal origin, from the exterior elements of the vulva, which are of ectodermal origin. Hart's line, thus, is defined anteriorly by the prepuce of the clitoris, laterally by the labia minora, and posteriorly by the vaginal fourchette. Within the vestibule itself are the vaginal introitus, bounded by the hymen, the paired erectile vestibulovaginal bulbs and associated vestibular glands, and the urethral meatus and associated paraurethral glands.

Somatic innervation of the vulva is largely via the iliohypogastric, ilioinguinal, and genitofemoral nerves, which serve the mons pubis and anterolateral labia majora, and the pudendal nerve, which originates from the sacral plexus and carries fibers derived from sacral spinal cord segments S2 to S4 as well as sympathetic fibers derived from gray rami communicantes from the pelvic sympathetic chain and is the major nerve of the perineum [1]. The pudendal nerve enters the perineum by coursing along the lateral wall of the ischioanal fossa within the pudendal canal formed by the fascia associated with the obturator internus muscle. The pudendal nerve gives rise to three major branches:

1. The inferior rectal nerve, which serves the rectum and anus
2. The perineal nerve, which provides motor innervation to the muscles of the perineum, sensory innervation via the posterior labial nerve, and sympathetic innervation to blood vessels and sweat glands
3. The dorsal nerve of the clitoris, which passes through the perineal membrane just inferior to the pubic symphysis and courses along the dorsal body and glans of the clitoris to provide sensory innervation

Parasympathetic innervation of erectile tissue within the vestibular bulb and the clitoris occurs via pelvic splanchnic nerves that originate from the anterior rami of sacral spinal cord segments S2 to S4 and course into the inferior hypogastric plexus before then ramifying into fine branches that pierce the perineal membrane to enter into

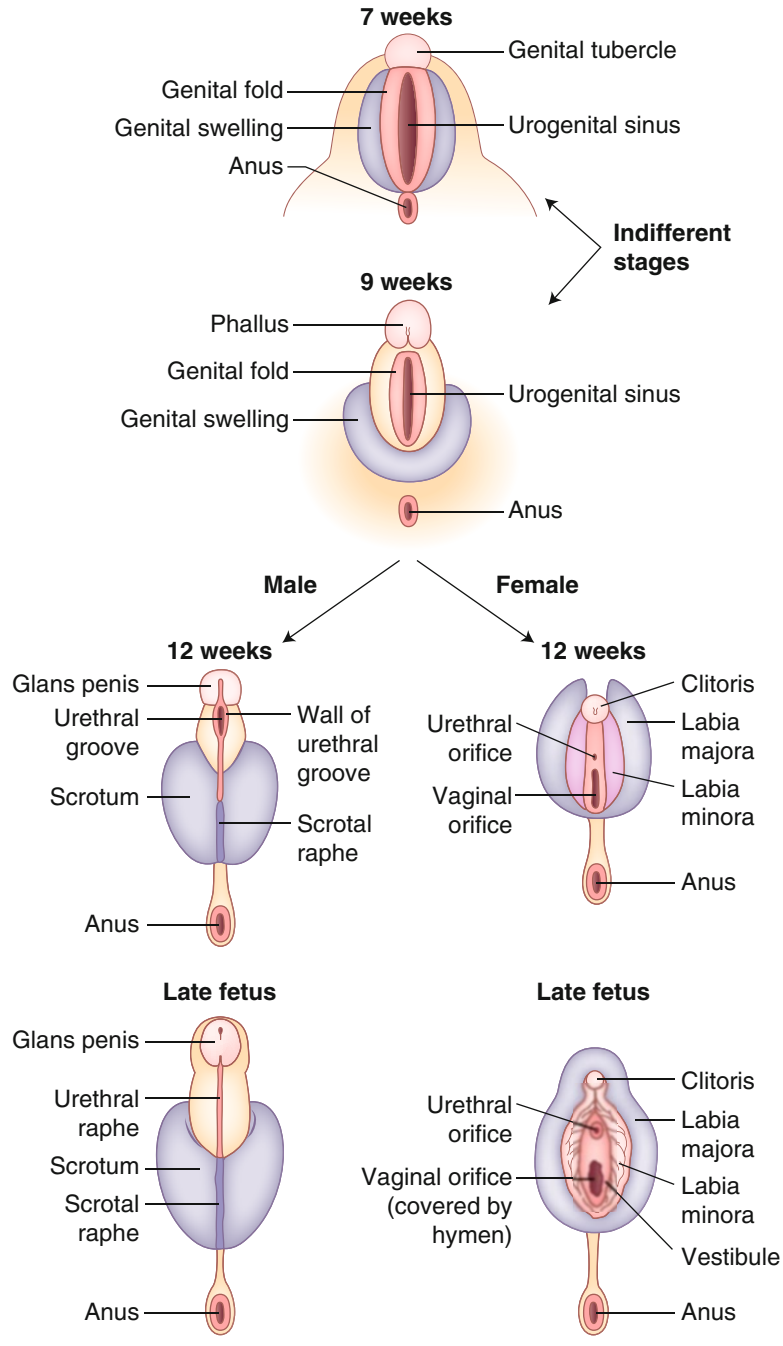


Fig. 1.5 Embryological development of the female and male external genitalia

the crura and glans of the clitoris and the bulbs of the vestibule [1].

Blood supply to the vulva is via the femoral artery, which sends both superficial and deep external branches that supply the mons pubis and anterolateral labia majora, respectively, and the internal iliac artery from which the internal pudendal artery derives [1]. The internal pudendal artery, in turn, branches in a similar pattern to the pudendal nerve to supply the rectum, anus, and perineal structures (via branches of the perineal artery such as the posterior and anterior labial

arteries that serve the labia minora and majora). The terminal internal pudendal artery gives rise to the arteries that serve the vestibular bulb as well as to the dorsal and deep arteries of the clitoris that supply erectile tissues within these organs. Venous drainage follows a similar pattern.

Lymphatic drainage from the perineal deep structures follows the internal pudendal artery into the internal iliac nodes in the pelvis [1]. Lymphatic drainage from the superficial tissues of the labia majora and mons pubis goes to the superficial inguinal nodes located superficial to the fascia lata near the emergence of the saphenous vein from the femoral vein. Lymphatic channels from the labia minora, clitoris, vestibule, and caudal-most vagina drain into deep inguinal nodes located deep to the fascia lata near the saphenous vein and external iliac nodes located along the external iliac artery. Lateral vulvar structures generally drain to the respective ipsilateral side, whereas medial structures drain centrally.

Regional Anatomy and Histology: Mons Pubis

The mons pubis overlies the pubic symphysis at the apex of the urogenital triangle. Being of ectodermal origin, it is covered by skin with a stratified squamous keratinized epithelium, hair follicles, sebaceous glands that empty into the hair follicles, eccrine sweat glands that empty onto the surface, and sensory receptors similar to other locations on the body. Touch receptors associated with the epithelium include Meissner's corpuscles and Merkel's tactile disks for vibratory sensation and free nerve endings for fine touch, pain, and temperature [10]. Vibratory sensation is further augmented by free nerve endings associated with hair follicles. Pacinian and Ruffini corpuscles for pressure sensation are present throughout the subcutaneous tissue, largely at the dermis-hypodermis interface, as are Dogiel-Krause receptors [10].

Substantial changes occur at puberty in the skin of the mons pubis. For one, the subcutaneous tissue becomes more prominent as the hypodermis progressively accumulates more adipose tissue. The hair follicles elongate and the hairs become coarser as puberty progresses up until about age 17 years when the final adult pubic hair pattern is set [11]. Racial and genetic factors affect the quality of pubic hair in terms of pigmentation, amount, and consistency.

Regional Anatomy and Histology: Labia Majora

The labia majora arise from the labioscrotal folds of the embryo, which, unlike those of the male, do not fuse but instead remain open as a pair of folds bounding the labia minora and the opening of the vulvar vestibule. The labia majora lie laterally to the labia minora, separated by the interlabial sulcus. Anteriorly, the labia majora merge with the mons pubis, whereas posteriorly, they join the perineal body located between the vaginal fourchette and the anus. Laterally, the labia majora merge with the inguinal-gluteal folds.

Like the mons pubis, the labia majora are of ectodermal origin and are therefore covered by skin with many of same features as those seen in the mons pubis (Fig. 1.6), although in addition to

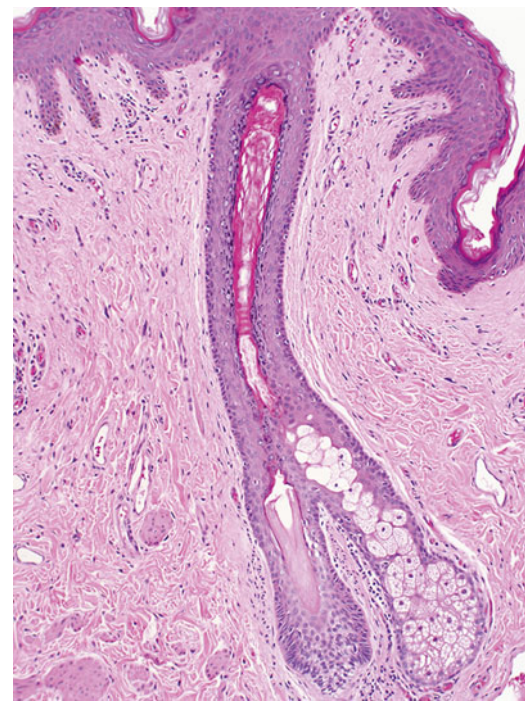


Fig. 1.6 The skin of the labium majus with hair follicle and sebaceous gland

the eccrine sweat glands, there are also numerous apocrine sweat glands associated with scent production (Fig. 1.7a, b).

Occasionally, cell clusters with eccrine and apocrine features may resemble a heterotopic mammary tissue, for which reason in the past this has been considered as heterotopic breast (Fig. 1.8a, b). Some studies report that in human embryos the mammary gland primordia do not extend beyond the axillary-pectoral area, arguing instead that the tissue and lesions arising from it originate from the native apocrine and eccrine glands normally found there in the form, embryonically, as the "Toker" cell, which is a cell, sometimes gland-like, where the cytoplasm is clear and CK7 reactive [12].

The epidermis of the labia may also disclose basal melanocytes (Fig. 1.9a, b), which are especially prominent in black women, and Langerhans cells (Fig. 1.10), which are involved in antigen acquisition, immune surveillance, and immune effector mechanisms, with dendrites covering expanses of cells. Quantitatively, Langerhans cells are twice as common in the vulva compared to the vagina [13].

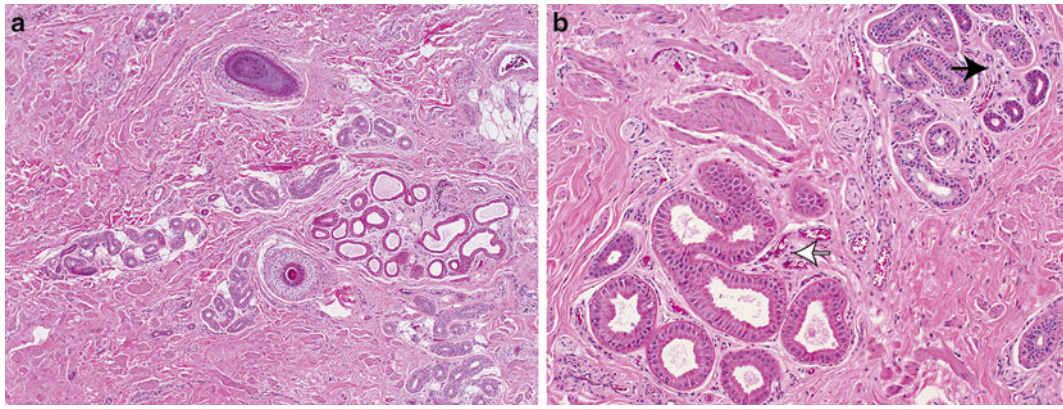


Fig. 1.7 (**a**) The labium majus with the apocrine and eccrine sweat glands. (**b**) In high power the apocrine glands show oxiphilic cytoplasm and holocrine secretion (*white arrow head*), not seen in eccrine glands (*black arrow head*)

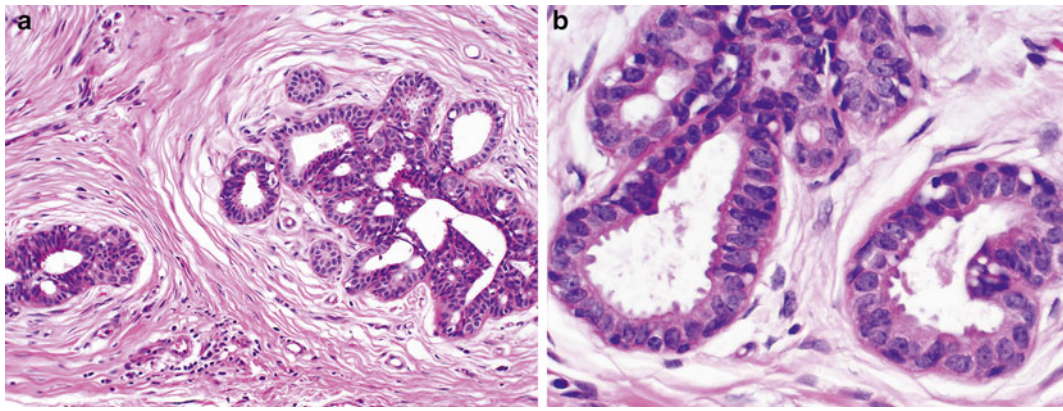


Fig. 1.8 (**a**, **b**) The mammary-like anogenital glands in the vulva with eccrine and apocrine features resembling those from the breast

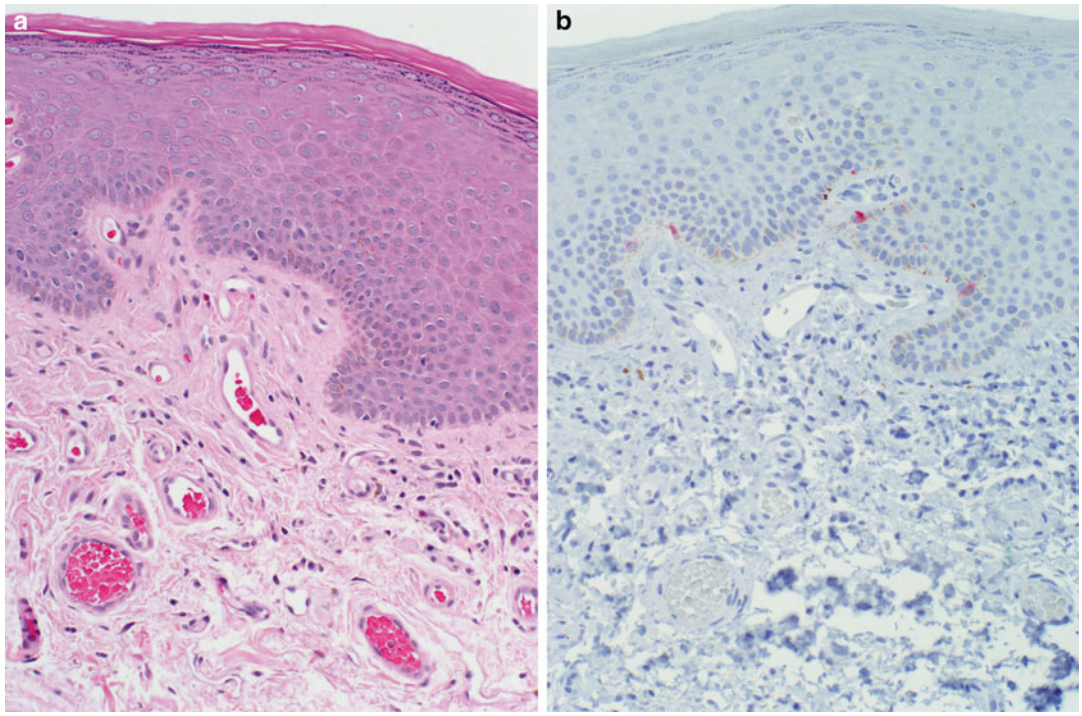


Fig. 1.9 (**a**, **b**) Melanocytes highlighted by MART-1 immunostain are seen at the basal aspect of the epidermis

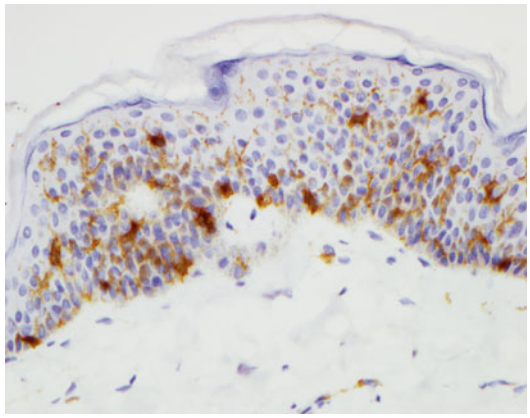


Fig. 1.10 CD1a immunostain highlights prominent Langerhans cells with dendrites within the epidermis

The medial side of the labia majora is thinly keratinized and lacks hair follicles and sweat glands, which is helpful in distinguishing medial from lateral based on the slide alone (Fig. 1.6, Fig. 1.11b). Despite the absence of hair follicles, the medial aspect of the labia majora contains abundant sebaceous glands visible to the naked eye as soft, tiny (Fordyce) spots that open directly onto the skin surface (Fig. 1.12).

The labia majora also change with aging and during pregnancy. At puberty, the labia majora increase in size, primarily the result of fat accumulation within the subcutaneous tissue, and there are changes in hair growth similar to that which occurs in the mons pubis. During pregnancy, the labia majora may distend from vasodilated subcutaneous vessels influenced by gestational hormones, sometimes resulting in venous varicosities that fail to later resolve [14]. After menopause, the labia majora typically atrophy, showing progressive loss of hair growth and overall reduced size due to the loss of subcutaneous fat [15].

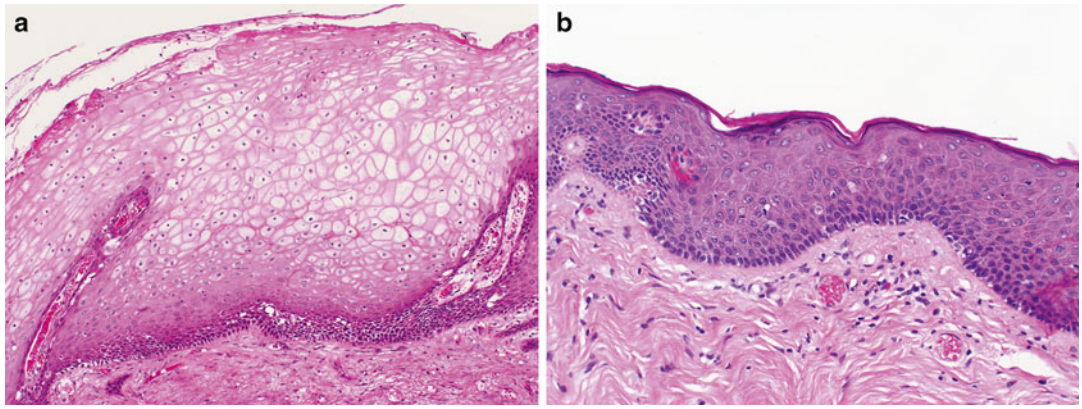


Fig. 1.11 (**a**) Highly glycogenated squamous epithelium lining the vulvar vestibule. (**b**) Squamous epithelium with a thin keratin layer, typical of the medial labia majora and lateral labia minora

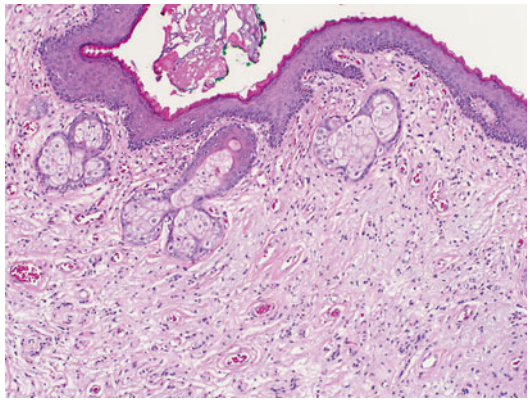


Fig. 1.12 Grossly visible sebaceous gland (Fordyce spot)

As the labia majora are developmentally homologous to the male scrotum, there are structures similarly associated with the inguinal canal related to the descent of the gonads. The round ligament of the uterus, a remnant of the gubernaculum, courses from the uterus to the internal inguinal ring, through the inguinal canal, out the external inguinal ring and finally ends within the dermis of the anterior labia majora, where it joins with a longitudinal bundle of smooth, or cremaster, muscle of the labia majora. Within the dermis of the labia majora, a delicate muscle layer derived from the transversus abdominis muscle (tunica dartos labialis) can be observed that is homologous to the dartos fascia of the scrotum. As in the male, these structures, related to the descent of the gonads, can help entrap the peritoneum within the anterior labia majora, which can lead to cysts. Similarly, indirect (inguinal) herniation of abdominal contents, also involving the anterior aspect of the labia majora, can occur, although this rarely happens in females [16].

Regional Anatomy and Histology: Labia Minora

Medial to the labia majora and the interlabial sulcus are the labia minora, which bound the opening into the vulvar vestibule. The labia minora arise from the left and right urogenital folds that arise at the lip of the urogenital sinus. In the male, these folds fuse to form the corpus spongiosum of the penis, whereas in the female the folds remain separated by the opening into the vulvar vestibule and vaginal introitus. Anteriorly, each labium minus divides into medial and lateral folds: the medial folds from each side fuse posterior to the clitoris to form the frenulum of the clitoris, whereas the lateral folds fuse anteriorly to form the prepuce. Posteriorly, the labia minora fuse to form the vaginal fourchette, which is anterior to the perineal body. The skin of the labia minora generally lacks skin appendages and is sparsely pigmented, although some sweat and sebaceous glands along with some degree of pigmentation may be found at the lateral and posterior borders (i.e., at the interlabial sulcus and perineal area) in some individuals [17]. As described above, Hart's line, which demarks the

endodermally derived epithelium of the urogenital sinus from the ectodermal epithelium of the urogenital folds, runs along structures associated with the labia minora. Lateral to this line, the epithelium is keratinized stratified squamous; medial to Hart's line, the lining is nonkeratinized, generally highly glycogenated stratified squamous epithelium (Fig. 1.11). The sensory receptors are like those for the labia majora, although peritrichial nerve endings are absent as the labia minora lack hair. Reflecting the developmental homology to the penile corpus spongiosum, the subcutaneous tissue of the labia majora is highly vascularized and erectile, supported by a loose connective tissue with elastic fibers. Deep to the labia minora, within the vestibule, are vestibular bulbs, which consist of erectile tissue invested by elastic fibers and skeletal muscle fibers of the bulbospongiosus muscles.

Regional Anatomy and Histology: Clitoris

The clitoris derives from the genital tubercle and is thus developmentally analogous to the corpora cavernosa of the male penis. It is anterior to the frenulum formed by the fused medial folds of the labia minora and covered anteriorly by the prepuce derived from the fused lateral folds of the labia minora. Like the penis, the clitoris has an attached root and a free body. However, the clitoris differs from the penis in that it lacks a bulbospongiosus component and is instead composed only of two crura and a single glans clitoris (although the bulbs of the vestibule are attached to the glans clitoris by thin bands of erectile tissue, they are considered to be vestibular rather than clitoral). The clitoral crura consist of erectile tissue (Fig. 1.13) similar to the corpus cavernosum of the penis, both entailing cavernous venous sinuses fed by centrally located muscular arteries and enveloped by a dense fibroelastic tunica albuginea and slips of skeletal ischiocavernosus muscle. The central muscular arteries within the erectile tissue receive parasympathetic innervation from segments S2 to S4 of the sacral spinal cord [1]. Nitric oxide-mediated relaxation of the arterial tunica media in response to parasympathetic stimulation leads to the engorgement of blood within the venous sinuses and tumescence of the erectile tissues [18]. As a derivative of the genital tubercle, the skin of the clitoris is of ectodermal origin and thus has a keratinized stratified squamous epithelium, but it lacks hair and sebaceous glands. As with the skin found elsewhere on the body, there are free nerve endings and sensory receptors associated with the skin of the clitoris, although peritrichial receptors are absent as there is no hair and the density of Meissner's corpuscles and Merkel's disks is somewhat less than what is found on the labia majora [10]. Pacinian corpuscles reside in very high numbers (Fig. 1.14).

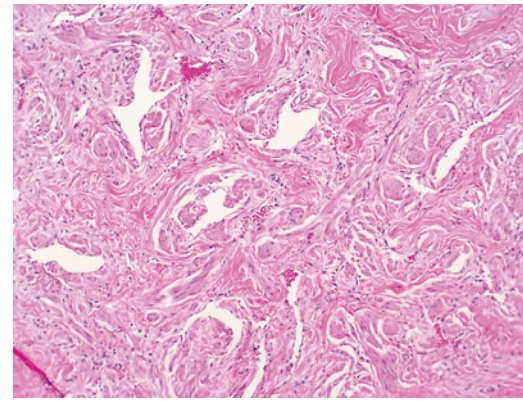


Fig. 1.13 Erectile tissue of the clitoris

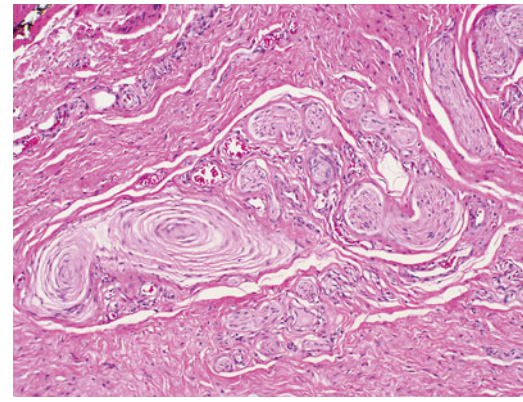


Fig. 1.14 Pacinian corpuscles (located *left*) and nerve bundles (located *right*) in the clitoris

Regional Anatomy and Histology: Vulvar Vestibule

The vulvar vestibule lies exterior to the hymen and interior to Hart's line. It is thus bounded anteriorly by the frenulum of the clitoris, laterally by the medial edge of the labia minora, and posteriorly by the vaginal fourchette. The posterior region between the hymen and fourchette known as the vestibular fossa is more concave compared to the remainder of the vestibule, which is typically flattened when the labia minora are spread apart. The vestibular lining is of endodermal origin (from the urogenital sinus) and is therefore predominantly nonkeratinized stratified squamous epithelium (Fig. 1.11a). The epithelium is also typically enriched in glycogen similar to that of the vagina and external os of the cervix. The vaginal introitus bounded by the hymenal ring is locally centrally within the vestibule.

The hymen itself marks the boundary of the vagina and the vestibule. During development, the hymen forms a continuous membrane between these two regions but usually regresses via programmed cell death to various degrees such that the hymen may be round or annular with a single opening, septate with several large openings divided by thin septa, or cribriform with multiple small openings. Rarely, the hymen undergoes little to no regression and remains imperforate, requiring surgical resection prior to menarche [19]. Intravaginal tampon use, intercourse, parturition, or other mechanical trauma often tears the hymen resulting in small hymenal tags (or carunculae), which are normal.

Lateral to the vaginal introitus are the minor and major (Bartholin's) vestibular glands. The minor vestibular glands are analogous to the urethral glands of Littre in the male and consist of simple tubular glands lined by a mucus-secreting columnar epithelium. The glands open onto and merge with the stratified squamous epithelium on the vestibular surface. The paired major vestibular glands (Fig. 1.15a, b) are analogous to the bulbourethral glands in the male and exist as paired tubuloalveolar glands located within the subcutaneous tissue deep to the labia minora, vestibule, and hymen. The acini of Bartholin's glands consist of mucus-secreting columnar epithelial cells that secrete into a pair of ducts that open posterolateral to the hymen. The major efferent duct of Bartholin's gland is lined by a transitional cell epithelium. Also with the subcutaneous tissue within the vestibule lateral to the vaginal introitus are the paired erectile bulbs of the vestibule invested by slips of skeletal bulbospongiosus muscle. Small bands of erectile tissue connect the anterior ends of the vestibular bulbs to the glans clitoris. Like the clitoris, erection of the vestibular bulbs is a vascular event mediated by parasympathetic fibers originating from the lateral horns of segments S2 to S4 of the sacral spinal cord [1].

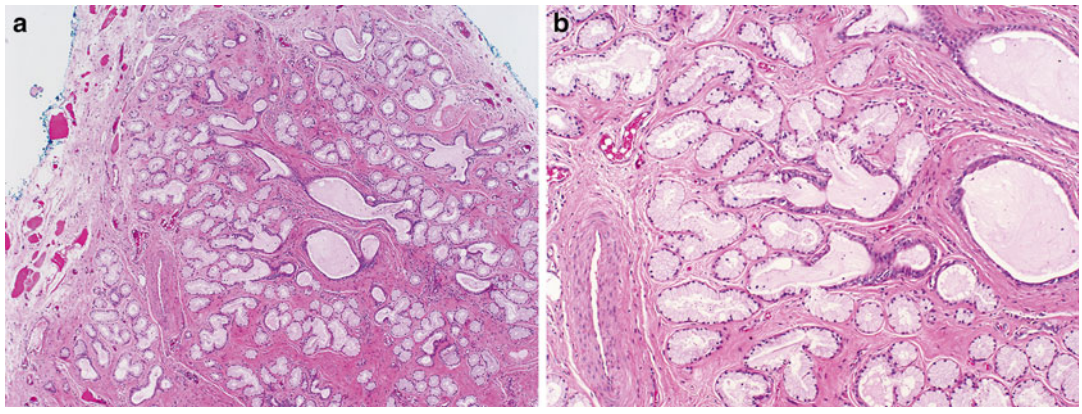


Fig. 1.15 (**a**) Major (Bartholin's) vestibular gland. (**b**) Higher power shows mucin producing glands opening to ducts lined by stratified epithelium

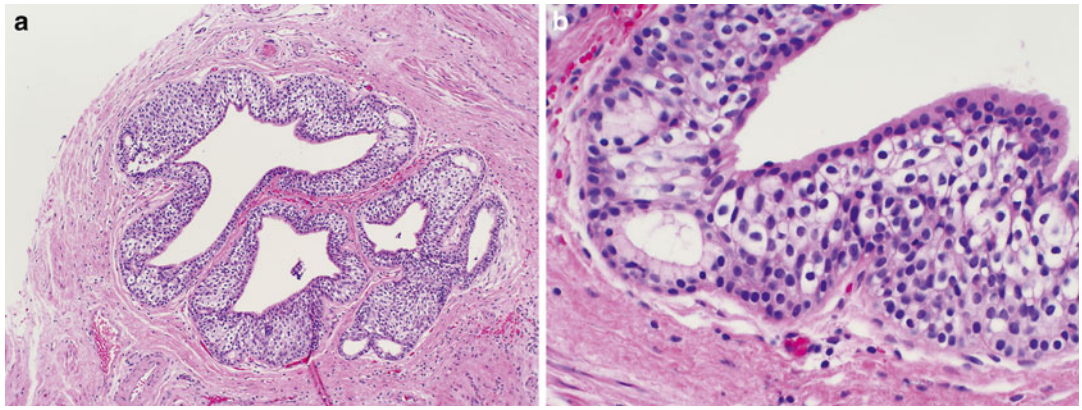


Fig. 1.16 (**a**) Periurethral (Skene's) glands, with urothelial-type lining with mucinous glands. (**b**) Detail

Anterior to the vaginal introitus is the urethral meatus which is lined by a transitional epithelium that merges with the stratified squamous epithelium of the vestibule. Throughout the urethra are multiple minor periurethral glands (of Huffman) comprised of columnar, mucinous epithelium. The major periurethral (or Skene's) glands are paired structures analogous to the male prostate. The glands are comprised of mucinous pseudostratified columnar epithelial cells that secrete into ducts lined by a transitional epithelium that merges with the vestibular squamous epithelium (Fig. 1.16a, b) [20]. Mucinous glands are often at the base. The ducts coalesce into a pair of openings found immediately posterolateral to the urethral meatus.

References

1. Drake RL, Vogl AW, Mitchell AWM. Gray's anatomy for students. 2nd ed. Philadelphia: Churchill Livingstone; 2010.
2. Sadler TW. Langman's medical embryology. 12th ed. Philadelphia: Wolters Kluwer; 2012.
3. Jaubert F, Robboy SJ, Fellous M. Embryology. In: Robboy SJ, Mutter GL, Prat J, Bentley RC, Russell P, MC A, editors. Robboy's pathology of the female reproductive tract. 2nd ed. Philadelphia: Churchill Livingstone; 2009.
4. Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, Bakke M, Zhao L, Frigeri C, Hanley NA, Stallings N, Schimmer BP. Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res. 2002;57:19–36.
5. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. Larsen's human embryology. 4th ed. Philadelphia: Churchill Livingstone; 2009.
6. Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. Development. 2007; 134(19):3401–11.
7. Payne AH, Hardy MP, Russel LD. The Leydig cell. Vienna: Cache River Press; 1996.
8. Biason-Lauber A. WNT4, RSPO1, and FOXL2 in sex development. Semin Reprod Med. 2012;30(5):387–95.
9. Carlson BM. Human embryology and developmental biology. 4th ed. Philadelphia: Mosby; 2009.
10. Krantz KE. Innervation of the human vulva and vagina; a microscopic study. Obstet Gynecol. 1958; 12(4):382–96.
11. Tanner JM. Growth at adolescence. 2nd ed. Oxford: Blackwell; 1962.
12. Putte SC. Clear cells of Toker in the developing anogenital region of male and female fetuses. Am J Dermatopathol. 2011;33(8):811–8.
13. Black CA, Murphy-Corb M. Dendritic cells in the female genital tract. In: Lotze MT, Thomson AW, editors. Dendritic cells, biology and clinical applications. 2nd ed. London: Academic; 2001. p. 412–3.
14. Bell D, Kane PB, Liang S, Conway C, Tornos C. Vulvar varices: an uncommon entity in surgical pathology. Int J Gynecol Pathol. 2007;26(1): 99–101.
15. McLean JM. Anatomy and physiology of the vulva. In: Ridley CM, editor. The vulva. New York: Churchill Livingstone; 1988. p. 39–65.
16. Burcharth J, Pedersen M, Bisgaard T, Pedersen C, Rosenberg J. Nationwide prevalence of groin hernia repair. PLoS One. 2013;8(1):e54367.
17. Amenta PS. Elias-Pauly's histology and human microanatomy. 5th ed. New York: Wiley; 1987. p. 502–3.
18. Gragasin FS, Michelakis ED, Hogan A, Moudgil R, Hashimoto K, Wu X, Bonnet S, Haromy A, Archer SL. The neurovascular mechanism of clitoral

erection: nitric oxide and cGMP-stimulated activation of BKCa channels. FASEB J. 2004;18(12): 1382–91.
19. Mor N, Merlob P, Reisner SH. Types of hymen in the newborn infant. Eur J Obstet Gynecol Reprod Biol. 1986;22(4):225–8.
20. Wilkinson EJ, Hardt NS. Vulva. In: Mills SE, editor. Histology for pathologists. 4th ed. Philadelphia: Wolters Kluwer; 2012.
21. http://en.wikipedia.org/wiki/List_of_homologues_of_the_human_reproductive_system. Accessed 15 Mar 2014.
22. Robboy SJ, Mutter GL, Shako-Levy R, Bean SM, Prat J, Bentley RC, Russell P. Cutup—gross description and processing of specimens. In: Robboy SJ, Mutter GL, Prat J, Bentley RC, Russell P, Anderson MC, editors. Robboy's pathology of the female reproductive tract. 2nd ed. Philadelphia: Churchill Livingstone; 2009.

Part II

Inflammatory Dermatoses of the Vulva

Histological Clues in Interpreting Vulvar Inflammatory and Autoimmune Dermatoses

2

Mai P. Hoang, Maria Angelica Selim, and Bruce Smoller

Introduction

This chapter will be structured in the following manner. A specific histological term that may provide a diagnostic clue will be precisely defined. Along with the histological definition, the clinical correlate will be described so that there is the beginning of the essential clinicopathologic correlation that is always necessary in order to make the best possible diagnosis. After presenting this information, the reader will be introduced to a brief discussion of some of the entities in which such histological changes might be observed. This presentation is not meant to be comprehensive, and certainly, not every situation in which one might encounter the histological change will be addressed. Rather, the intent is to provide a quick overview of the most common situations in which each finding might be identified when examining tissue from the vulva. Finally, we will discuss additional features that one might consider when observing the histological change that would enable for a more precise resolution of the differential diagnosis.

B. Smoller, M.D. (✉)
Department of Pathology, University of Rochester, School of Medicine and Dentistry, Rochester, NY, USA
e-mail: Bruce_smoller@urmc.rochester.edu

Histological Clues

Spongiosis

Spongiosis is defined as the presence of intraepidermal edema. Microscopically, this manifests as an increased space between adjacent keratinocytes and widening of intercellular spaces on routine sectioning (Fig. 2.1). In more florid cases, there may be loculations of fluid accumulation either within the epidermis or in the overlying stratum corneum. Clinically, spongiosis presents as eczematous dermatitis. This is an "oozing," wet eruption that may also present with maceration in the vulva. In a very acute process, there will be no change in the stratum corneum. However, as the process becomes more subacute or chronic, there is almost always parakeratosis. Long-standing lesions almost always demonstrate acanthosis. Spongiosis may be seen in the posttraumatic state wherein hemorrhage may be present. There is a wide range of spongiotic (eczematous) conditions that occur in the vulva, including irritant or allergic contact dermatitis, an id reaction and secondary to dermatophyte infections. The specific features useful to distinguish between these entities can be found in Chap. 3. For example, the presence of necrotic keratinocytes would suggest irritant contact dermatitis, whereas Langerhans cell microabscess would suggest allergic contact dermatitis.

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_2,

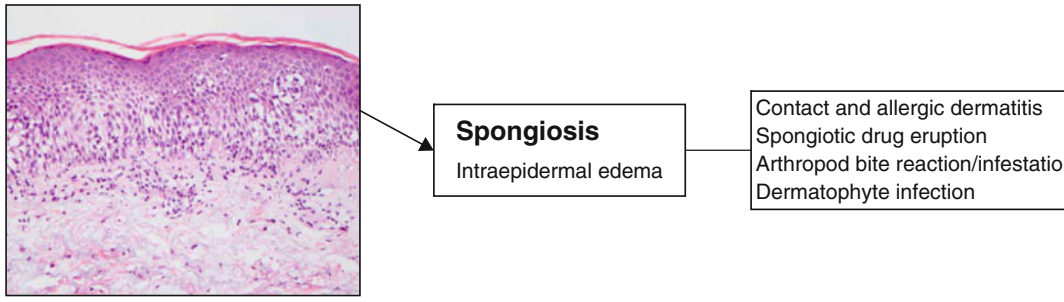


Fig. 2.1 Spongiotic pattern. Spongiosis is characterized by intraepidermal edema

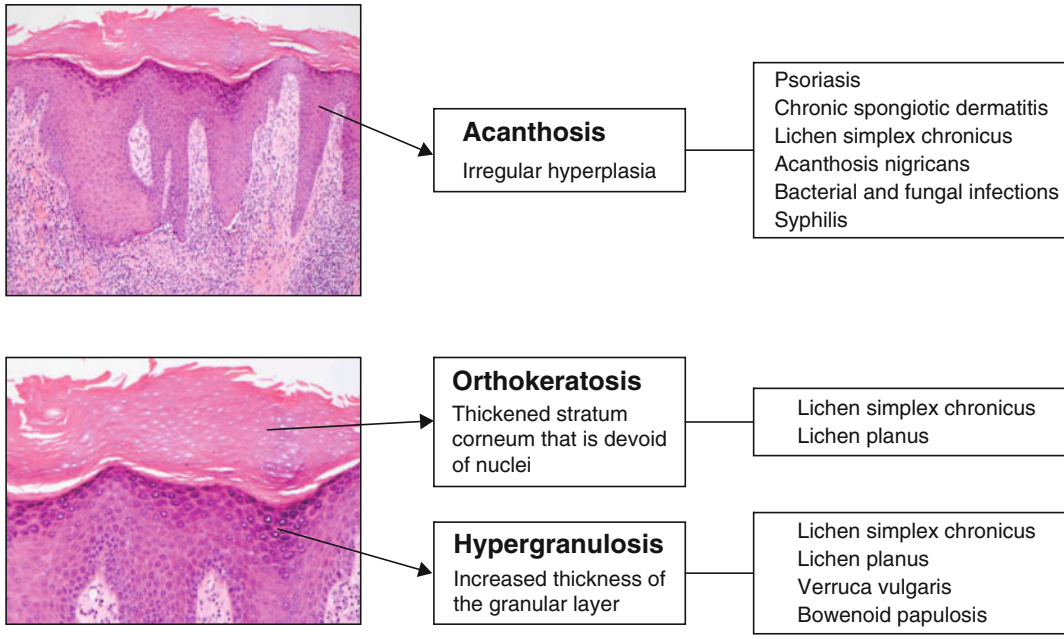


Fig. 2.2 Acanthotic pattern is characterized by irregular epidermal hyperplasia

Acanthosis

Acanthosis is defined as a thickened epidermis. Acanthosis may be "regular" with rete ridges of similar length (resembling psoriasis) or "irregular" with rete ridges with marked difference in length and width such as what is seen in pseudoepitheliomatous hyperplasia (Fig. 2.2). The clinical correlation of acanthosis is the sense of thickening of the skin upon palpation. Acanthosis is frequently associated with changes in the stratum corneum such as parakeratosis or orthokeratotic hyperkeratosis. Acanthosis is evidence of chronicity and does not occur in the acute setting. Thus, one may think of plaque-stage psoriasis that has been present for a while when there is regular acanthosis. This finding would not be expected in a relatively acute lesion of psoriasis. In addition, psoriasis of the genital site can exhibit spongiosis and only focal mounds of parakeratosis containing neutrophils. Similarly, chronic spongiotic (eczematous) processes will

demonstrate regular acanthosis, though acute spongiotic processes will not show this change. Lichen simplex chronicus may show either regular or irregular acanthosis, and this always occurs with hypergranulosis and orthokeratotic hyperkeratosis as well as vertical papillary dermal fibrosis. In acanthosis nigricans, there is frequently regular acanthosis. Regular (psoriasiform) acanthosis is a common feature of syphilis, and in these cases plasma cells are almost always seen in the inflammatory infiltrate. Many other infectious diseases including viral, bacterial, and fungal infections demonstrate irregular acanthosis, and most commonly, there is a brisk inflammatory infiltrate in these cases.

Psoriasiform Hyperplasia

See regular acanthosis above.

Parakeratosis

Parakeratosis is the preservation of nuclei into the stratum corneum (Fig. 2.2). The clinical correlate of parakeratosis is the presence of a superficial scale. Lesions that demonstrate parakeratosis are invariably described as scaly processes. This finding occurs in two basic types of conditions. It may be seen with abnormal maturation of keratinocytes such as what may be seen in squamous cell carcinoma, vulvar intraepidermal neoplasia (VIN), or bowenoid papulosis. It may also be seen in hyperproliferative states such as psoriasis and spongiotic dermatitis and with infectious processes. The observation of cytological atypia would point the diagnostician in the direction of a condition with abnormal maturation, while the presence of spongiosis and/or neutrophils might be helpful in pointing one toward a diagnosis of a spongiotic process or toward psoriasis.

Orthokeratotic Hyperkeratosis

Hyperkeratosis is defined as the excess stratum corneum for the site. Orthokeratosis describes the case when the excess keratin is devoid of nuclei (unlike parakeratosis). The excess keratin may demonstrate a basket-weaved pattern or may be compact (Fig. 2.2). The clinical correlate is that of a scale that may demonstrate a brownish color when the orthokeratosis is marked in extent. Orthokeratotic hyperkeratosis is most commonly encountered in lichen simplex chronicus and is often a clue to chronic irritation or rubbing. This is especially true when the basket-weaved pattern is lost and there is compaction of the stratum corneum. Despite the atrophic epidermis that characterizes well-developed lesions of lichen sclerosus, the stratum corneum tends to be hyperkeratotic and orthokeratotic in this condition. The difference in the thickness of the epidermis (acanthotic vs. atrophic) can be a useful additional clue to distinguish lichen simplex chronicus from lichen sclerosus, although one can see superimposed changes of lichen simplex chronicus in long-standing lichen sclerosus due to rubbing.

Hypergranulosis

Hypergranulosis is defined as a relative increase in the thickness of the granular layer (Fig. 2.2). This is site specific and one would expect to see a much thicker granular layer on acral skin than is normal on vulvar skin. Hypergranulosis often correlates with a whitish color clinically. Depending upon the extent of the hyperkeratosis, the white coloring may be focal and "lacy" (lichen planus) or diffusely white (some cases of lichen simple chronicus). When the observer notes hypergranulosis, several diagnostic entities become likely. Lichen simplex chronicus demonstrates this finding in concert with psoriasiform epidermal hyperplasia, compact orthokeratotic hyperkeratosis, and dermal fibrosis. In lichen planus, one sees hypergranulosis accentuated in eccrine acrosyringium along with a lichenoid inflammatory infiltrate. A verruca vulgaris may demonstrate hypergranulosis and frequently papillomatosis. If cytological atypia is noted, bowenoid papulosis should be considered as a diagnostic possibility.

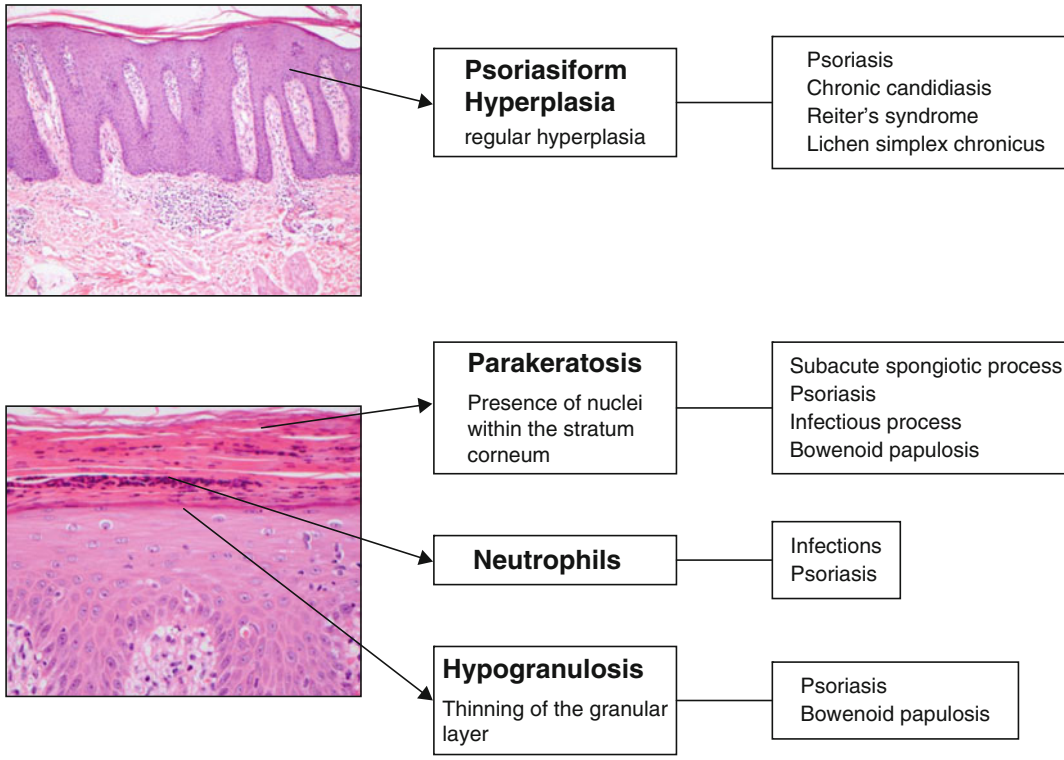


Fig. 2.3 Psoriasiform pattern is characterized by regular epidermal hyperplasia

Hypogranulosis

Hypogranulosis is defined as a relative thinning of the granular layer (Fig. 2.2). In some cases, it may be completely absent. Hypogranulosis frequently occurs in concert with parakeratosis. There is no reproducible clinical correlation to the observation of hypogranulosis. The most common situations where the observation of hypogranulosis is encountered in vulvar biopsies include psoriasis, relatively acute spongiotic dermatitis, and bowenoid papulosis. The presence of neutrophils may point toward the diagnosis of psoriasis, while the presence of spongiosis (see below) might lead to a diagnosis of some type of spongiotic (eczematous) process. Cytological atypia in the presence of hypogranulosis might favor a diagnosis of bowenoid papulosis.

Dyskeratosis

Dyskeratosis refers to the abnormal keratinization of individual cells within the lower portions or midportions of the epidermis (Fig. 2.3). This change may occur in situations with abnormal keratin production or in situations with exocytosis of lymphocytes into the epidermis. There is no clinical correlate to this finding except when it is extensive and may give rise to small vesicles. Dyskeratosis is a prominent finding in Darier's disease and in warty dyskeratoma. In both cases, there is also a prominent hyperkeratosis. Warty dyskeratoma will demonstrate a cup-shaped architecture that is not seen in Darier's disease. Dyskeratosis may be seen in inflammatory entities such as lichen planus in concert with hypergranulosis, orthokeratotic hyperkeratosis, and a

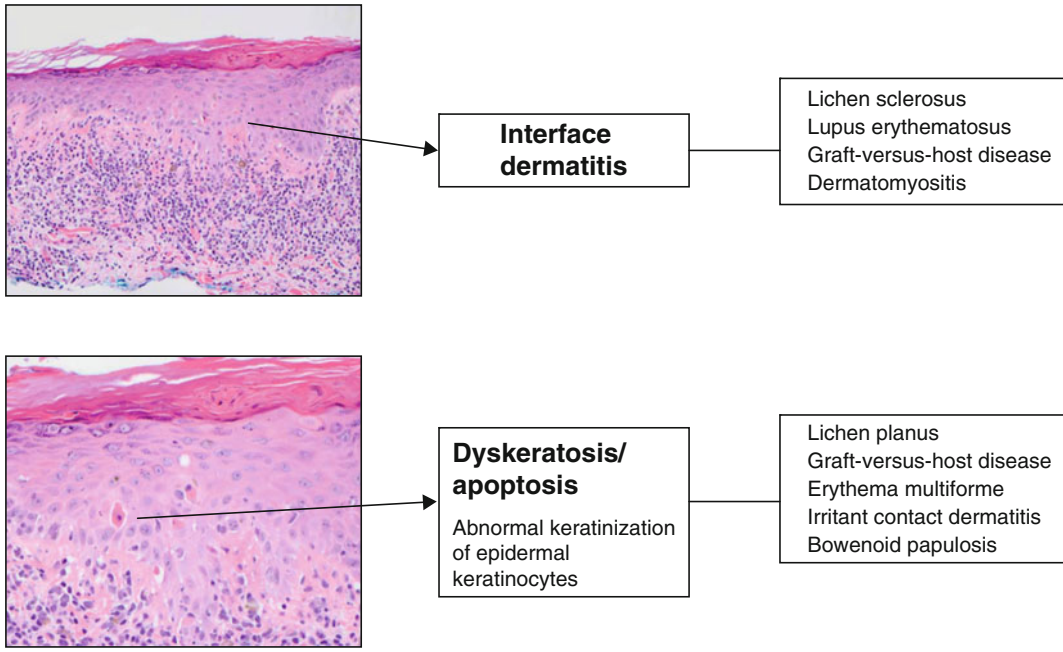


Fig. 2.4 Interface pattern is characterized by dyskeratotic or apoptotic keratinocytes

band-like inflammatory infiltrate. Similarly, dyskeratotic cells may be seen in erythema multiforme and in graft-versus-host disease, though the inflammatory infiltrate is not usually as pronounced as that seen in lichen planus. Cases of bowenoid papulosis may similarly demonstrate dyskeratosis and cytological atypia that is more diffuse than what is encountered with chemotherapeutic changes.

Pigment Incontinence

Pigment incontinence refers to the drop of melanin pigment from the basal layer of the epidermis into the papillary dermis where it is most commonly engulfed by macrophages (Fig. 2.4). When pigment incontinence is seen under a microscope, the clinicians can almost always detect anomalies in pigment distribution on the skin surface. This may result in an appearance of clinical hyperpigmentation or hypopigmentation, and the clinical difference is not always readily apparent microscopically. Pigment incontinence occurs in situations where there is destruction of basal keratinocytes and melanocytes, namely, lichenoid or interface dermatoses, as described above. It is not associated with the acute onset of an inflammatory process, but rather, it is seen as a consequence of a process that has evolved over weeks to months. It can be seen as a secondary phenomenon in each of the diseases listed above in lichenoid and interface dermatitides and is most clinically apparent and concerning in darker-skinned individuals.

Sclerosis

Sclerosis is the deposition of dense fibrous tissues within the papillary dermis that is usually characterized by the presence of wispy type III collagen fibers. Sclerosis manifests clinically as hardening of the skin and the appearance and feel of a plaque. It occurs in chronic inflammatory processes. Linear, horizontally oriented sclerosis is a hallmark feature of well-developed lesions of lichen sclerosus. It occurs concomitantly with atrophying of the epidermis and overlying orthokeratotic hyperkeratosis. In general, the more

prominent the sclerosis, the less intense the inflammatory infiltrate and the older the lesion. Sclerosis can also be seen in morphea or scleroderma; however, these cases are extremely unusual in this location, and the sclerosis is not limited to the papillary dermis but is mainly centered within the reticular dermis. A lymphoplasmacellular inflammatory infiltrate may be present in these situations. Lichen simplex chronicus also demonstrates dermal fibrosis, but in most cases, the thickened dermal collagen is present in vertical columns within the papillary dermal tips and not in a horizontal array such as what is encountered in lichen sclerosus.

Neutrophils

Neutrophils, also known as polymorphonuclear leukocytes, are white blood cells that do not normally reside in the skin but migrate into the dermis and epidermis in a variety of inflammatory conditions. When present in clusters within the epidermis (most commonly the stratum corneum) (Fig. 2.2), the clinical appearance is that of pustules, vesicles filled with "cloudy" fluid comprised of serum and neutrophils. When not in the stratum corneum, epidermal pustules may present with painful erythema. Similarly, clusters of dermal neutrophils will be erythematous and are often associated with clinical pain. Neutrophilic abscesses within the stratum corneum and the epidermis are a common feature of psoriasis. Regular psoriasiform acanthosis, parakeratosis, hypogranulosis, and vascular ectasia occur along with the neutrophilic infiltrates. Neutrophilic abscesses in the outflow tract of follicular units may be a clue to seborrheic dermatitis. In the absence of other changes, neutrophilic abscesses occurring with scattered dermal eosinophils raise the possibility of acute generalized exanthematous pustulosis. Amicrobial pustulosis of the folds may also demonstrate subcorneal neutrophilic abscesses. As with other body sites, the presence of neutrophils within a biopsy should raise the possibility of an infectious process. When purely dermal, the presence of neutrophils should prompt the search for leukocytoclastic vasculitis, Sweet's syndrome, linear IgA bullous dermatosis, and cellulitis.

Plasma Cells

Plasma cells are a subtype of B lymphocyte that is normally present in mucosal regions. Their presence in the vulva is not unexpected, and they may reside there normally in small numbers. There is no clinical correlation to the presence of scattered dermal plasma cells. Conditions in which increased plasma cells may be present include syphilis that also demonstrates psoriasiform epidermal hyperplasia and a band-like inflammatory infiltrate. Plasma cell vulvitis would have abundant dermal plasma cells as well as erythrocyte extravasation and hemosiderin deposition. A subacute folliculitis can result in the appearance of scattered plasma cells along with abundant lymphocytes and neutrophils. In lesions of lichen sclerosus, scattered plasma cells may be encountered, but the characteristic changes as described above will be detected. Plasma cells are also frequently encountered in connective tissue diseases such as lupus erythematosus, morphea, and scleroderma. These cells will be present along with the changes as described earlier in the chapter and in subsequent chapters.

Acantholysis

Acantholysis is the dyscohesion of adjacent keratinocytes caused by the dissolution of desmosomes (Fig. 2.5). This gives the microscopic appearance of an epidermis that is "falling apart." Acantholysis usually occurs within the stratum malpighium, though similar changes can occur in the stratum granulosum. There is often associated spongiosis in situations that give rise to acantholysis. The clinical correlation is that of small, flaccid blisters within the epidermis. Acantholysis occurs in situations with structural defects in desmosomal proteins such as is seen in Darier's disease and Hailey-Hailey disease. In the former, there is almost

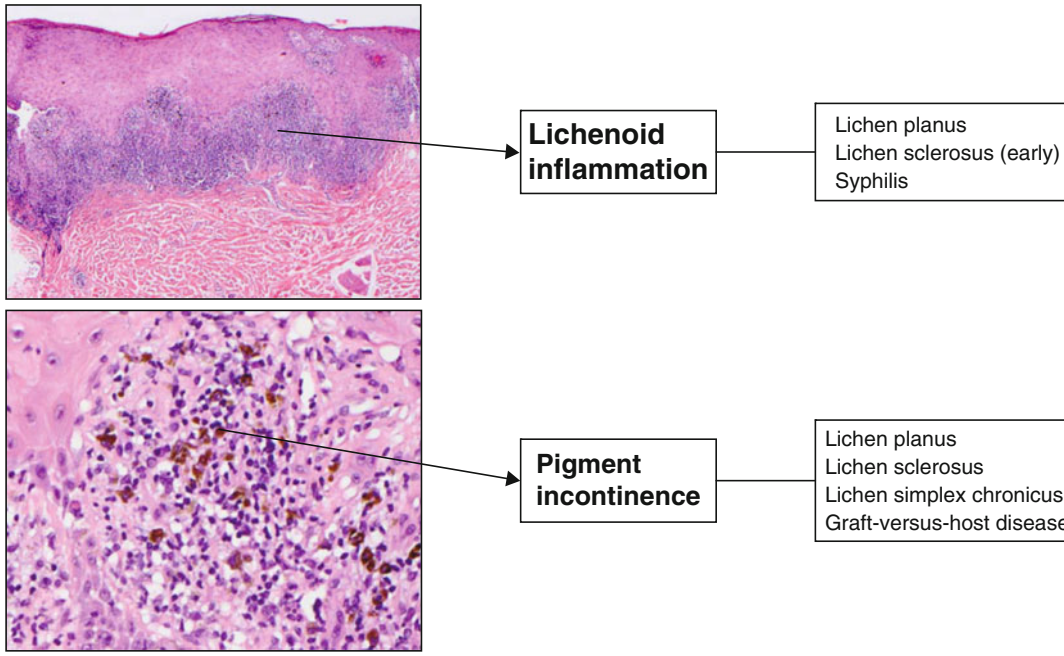


Fig. 2.5 In a lichenoid pattern, a band-like inflammation is seen in the superficial dermis

always extensive overlying orthokeratotic hyperkeratosis, while in the latter, spongiosis is usually quite prominent. Papular acantholytic dermatosis of the genitocrural area is an acantholytic disorder restricted to the genital area with a distinct clinical presentation. Acantholysis is also present in autoimmune diseases, such as in all types of pemphigus. Direct immunofluorescence is a very useful technique to establish this diagnosis. Acantholysis can also be encountered in herpetic dermatoses with other characteristic changes of viral infection. Rarely in the vulva, acantholysis may be seen in situations with cytological atypia such as in squamous cell carcinoma.

Reaction Pattern

Spongiotic Pattern

Spongiotic dermatitis is characterized by intraepidermal spongiosis (Fig. 2.1). A variety of conditions including contact or allergic dermatitis, drug eruption, and others will be discussed in Chap. 3.

Psoriasiform or Acanthotic Pattern

See acanthosis above (Fig. 2.2).

Interface Pattern

Interface dermatitis is defined as a lymphocytic infiltrate that extends into the epidermis resulting in basal vacuolization and focal necrosis of basal keratinocytes (Fig. 2.3). Focal spongiosis is often seen within the epidermis. Within the dermis, the lymphoid infiltrate involves the superficial vascular plexus. It is less confluent than is seen in lichenoid infiltrates and is associated with a different range of inflammatory disorders. Interface dermatitis does not have a discrete clinical correlate, though in extensive cases, subepidermal blistering may be present. The superficial lymphocytic infiltrate correlates with the clinical appearance of erythema. Interface dermatitis raises a differential diagnosis that includes lupus erythematosus and dermatomyositis (see Chap. 3). In lupus erythematosus, there is often orthokeratotic hyperkeratosis and a dense and peri-appendageal lymphoid infiltrate. In dermatomyositis, the inflammatory infiltrate is

generally less pronounced, not as deep and not peri-appendageal. Dermal mucin deposition may be extensive. Erythema multiforme and graft-versus-host disease may also demonstrate interface dermatitis. The presence of abundant papillary dermal edema and occasional neutrophils within the dermis may favor a fixed drug eruption. Early lesions of lichen sclerosus may demonstrate interface dermatitis or lichenoid dermatitis, often with overlying orthokeratotic hyperkeratosis.

Lichenoid Pattern

Lichenoid dermatitis is similar to interface dermatitis. It is characterized by a band-like infiltrate within the papillary dermis that extends up into the epidermis resulting in focal necrosis of basal keratinocytes, basal vacuolization, and obscuring of the dermal epidermal junction (Fig. 2.4). There is no discrete clinical correlate to this histological finding and all observed clinical changes can be attributed to the concomitant histological changes. The prototypical lichenoid dermatosis is lichen planus that occurs with hyperkeratosis, hypergranulosis, and irregular acanthosis of the epidermis (see Chap. 5). Lichen sclerosus, in its early and well-developed (but not late) stages, will demonstrate a lichenoid infiltrate, often with epidermal atrophy, overlying orthokeratotic hyperkeratosis, and dermal sclerosus. Syphilis is characterized by a band-like inflammatory infiltrate with psoriasiform epidermal hyperplasia. Usually, the other changes of a lichenoid process are not present and scattered plasma cells are present.

Acantholytic and Blister Pattern

This pattern can exhibit blister below the stratum corneum, within the epidermis, or below the epidermis:

Intraepidermal Blister

See acantholysis (Fig. 2.6).

Subepidermal Blister

A subepidermal blister is a neat separation of the epidermis from the underlying dermis (Fig. 2.7). It may occur at several levels within

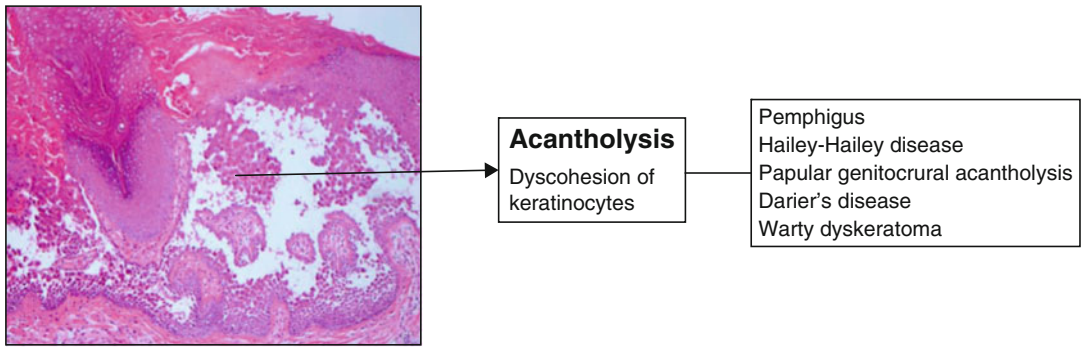


Fig. 2.6 Acantholytic pattern is an intraepidermal process

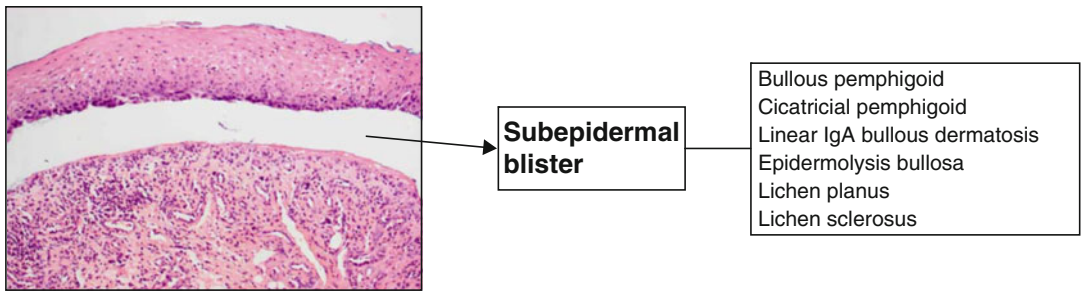


Fig. 2.7 A subepidermal blister pattern is shown here

the basement membrane zone, depending upon the pathogenesis of the blister (see Chap. 4). This separation that is evident on routine histological sections is apparent as a clinical blister. When subepidermal, blisters tend to be taut, tense, and remain intact. The presence of a non-inflammatory subepidermal blister may be suggestive of epidermolysis bullosa, an inherited mechanic-blistering process. Subepidermal blisters may occur in autoimmune blistering disorders such as IgA bullous dermatosis of childhood, cicatricial pemphigoid, and bullous pemphigoid. In these situations, there is a concomitant marked inflammatory infiltrate. Subepidermal blisters can also occur as a result of the marked destruction of basal keratinocytes such as is seen in lichen planus, lichen sclerosus, or erythema multiforme.

Inflammatory Disorders Affecting the Epidermis of the Vulva

3

Russell A. Ball, Libby Edwards, Jason C. Reutter, Kelly L. West, and Maria Angelica Selim

Introduction

Inflammatory dermatoses involving the epidermis of the vulva may exhibit unique changes not seen in other body sites. One reason is that the vulva has the unusual feature of being lined by keratinizing hair-bearing, modified mucous membrane, and classic mucous membrane surfaces. Additionally, the occlusion, friction, and moisture in this region may greatly alter the clinical and histologic findings. Lastly, even in modern times, there is often a stigma for patients to seek therapy as well as unfamiliarity of many providers with the pathology of this body region, such that biopsies are often performed after a variety of self-treatment and often-inappropriate treatment regimens. It is not uncommon for multiple pathologic and iatrogenic changes to coexist in the same biopsy specimen, posing a challenge to the pathologist.

M.A. Selim, M.D. (✉)
Department of Pathology, Duke University Medical Center, Durham, NC, USA
e-mail: Angelica.selim@duke.edu

Spongiotic Pattern

Allergic and Irritant Contact Dermatitis

Clinical Features

Contact dermatitis of the vulva, as in other anatomic locations, may be irritant (ICD) or allergic (ACD) in nature. ACD is a hypersensitivity reaction (type IV, delayed type) requiring prior exposure and sensitization, while ICD requires no prior sensitization. Both ICD and ACD manifest cutaneous signs and symptoms at the site of insult. Alternatively, vulvar dermatitis may develop in the absence of a localized allergen, as in atopic dermatitis. ICD is more common than ACD, while atopic dermatitis of the vulva is infrequently reported in the literature. Overall, vulvar contact dermatitis affects approximately 15–30 % of women [1–3]. ACD, ICD, and atopic dermatitis are forms of "eczema," as is seen on nongenital skin as well.

The clinical presentations of vulvar ICD and ACD show significant overlap, and both display variability in clinical findings dependent on the potency and duration of exposure. Acute ICD typically develops within minutes to hours after exposure, while acute ACD (a type IV hypersensitivity reaction) tends to be slower in onset, developing within a few days. Chronic contact dermatitis of either type develops over the course of months to years. In severe acute ICD, skin

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_3, © Springer Science+Business Media New York 2015

appears erythematous and edematous with vesicles, erosions, and ulcerations, most exaggerated on the delicate skin of the modified mucous membranes of the vulva [4, 5] (Fig. 3.1). Often quite painful, the cutaneous manifestations are well circumscribed and mirror the area of contact with relative sparing in protected areas such as the skin folds. Chronic ICD develops in the setting of prolonged exposure to less caustic irritants. In contrast to acute ICD, the chronic form of ICD is less well demarcated and more prone to lichenification [4]. Granuloma gluteale, pseudoverrucous papules and nodules, and Jacquet's erosive dermatitis are unusual forms of ICD often developing in the setting of urinary incontinence or benzocaine use [6–16]. While historically considered separate entities based on histopathological findings, they show considerable clinical overlap and are all essentially variants of genitocrural irritant dermatitis [17].

ACD usually presents with pruritus [3, 18–21]. Skin may be normal, erythematous, or edematous with vesicles and blisters [5]. Fine desquamation can be a sign of evolving allergic contact dermatitis [22]. In severe cases, ACD is erosive and disfiguring [23]. In its late stage, ACD may manifest as lichen simplex chronicus [24]. Of note, lichen simplex chronicus may be the end point of a variety of eczematous dermatitis or superimposed on other inflammatory processes (e.g., psoriasis, lichen sclerosus, lichen planus, etc.). Not infrequently, the process that started the itch-scratch cycle leading to lichen simplex chronicus changes may have resolved at the time of the biopsy. Of note, both ICD and ACD are not uncommonly present with minimal clinical findings or even as vulvodynia [25, 26].

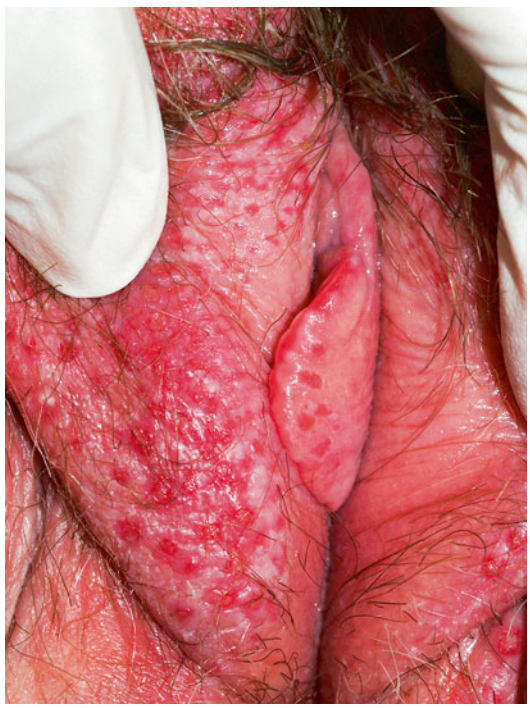


Fig. 3.1 Contact dermatitis showing eroded vesicles on all three skin types

Vulvar skin is a unique environment due to moisture, occlusion, and friction in which the effects of externally applied agents may be magnified and/or altered [27]. Numerous irritants and allergens have been implicated in vulvar dermatitis, most commonly constituents of cosmetics, preservatives, and medications (including topical steroids), as well as body fluids and overzealous hygiene practices (Table 3.1) [4, 5, 19, 20, 28, 29]. The genetics and pathophysiology of contact dermatitis are complex and multifactorial and remain poorly understood [30]. Patients may have some degree of predisposition to contact dermatitis, and an allergic/atopic history is frequently elucidated [1, 2]. Atopic dermatitis has been linked to filaggrin mutations; both may increase the risk of developing ICD [31].

Central to patient management is identification and removal of the irritant/allergen. Patch testing may prove helpful in the workup [28]; however, due to the unique vulvar environment, results of traditional patch testing on non-vulvar skin may not be entirely applicable [32, 33]. With removal

Table 3.1 Important vulvar irritants/allergens

Body fluids	Latex	Neomycin (Neosporin)
Soaps and detergents	Spermicides	Topical steroids
Perfume	Chlorhexidine (KY)	Imiquimod
Heat	Lanolin	Podophyllin
Sanitary napkins and wipes	Benzocaine (Vagisil)	Trichloracetic/ bichloracetic acid

of the inciting agent, prognosis is excellent. Often, the inciting agent is not identified, and topical and systemic therapies may be needed to control the disease. In these situations, remissions are achievable but recurrences are common—these patients requiring long-term follow-up for management.

Histopathology

Biopsy findings of both ICD and ACD vary with the age of the lesion. Early lesions may show minimal spongiosis (Fig. 3.2) with progression to spongiotic vesicles, lymphoeosinophilic infiltrate, dermal edema and serum crust (Fig. 3.3). In subacute contact dermatitis, the epidermis becomes less spongiotic with development of early psoriasiform hyperplasia. (See *Vignette 3* at the end of this chapter.) Chronic lesions will be acanthotic with hyperkeratosis and parakeratosis. With time, chronic irritation leads to the finding of lichen simplex chronicus [34] (Fig. 3.4).

ICD and ACD show significant histopathological overlap and may be impossible to differentiate from each other [35]. Dyskeratosis and balloon cell change may suggest an irritant contact dermatitis [36, 37]. Neutrophilic infiltrate may be seen as a reaction to keratinocyte death. Eosinophils may be a helpful clue to an allergic nature [25] but are also a common finding in drug reactions. Prominent spongiotic vesiculation with eosinophils, Langerhans cell microabscesses, serum crust, and a relatively spared dermis are practically pathognomonic of ACD, but in the vulva, these changes may be less well developed as in nongenital skin, requiring the pathologist to maintain a high index of suspicion. Skin biopsy may be most useful to rule out other entities in the differential diagnosis. Tissue should be sent for direct immunofluorescence (DIF) studies when the differential includes bullous disorders.

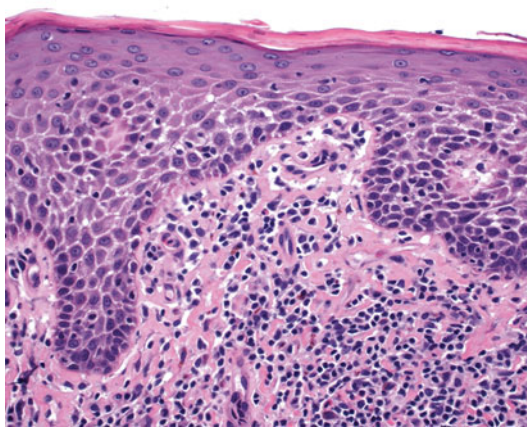


Fig. 3.2 Early spongiosis in a case of allergic contact dermatitis. Rare eosinophils, focal parakeratosis, chronic inflammation, and pigment incontinence are present

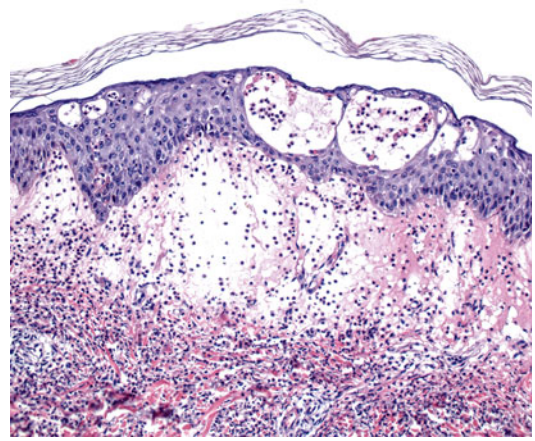


Fig. 3.3 Acute contact dermatitis with spongiotic vesicles in epidermis and papillary dermal edema with lymphoeosinophilic infiltrate

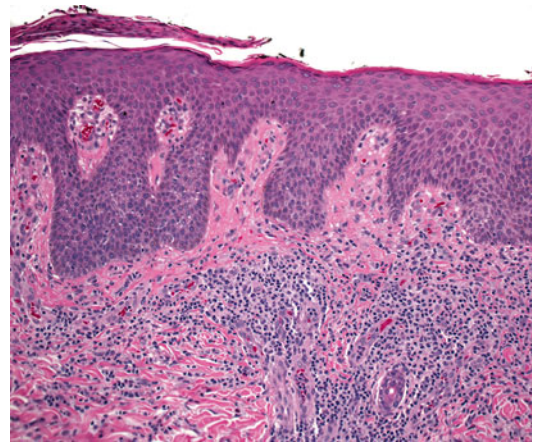


Fig. 3.4 A case of contact dermatitis with early changes of lichen simplex chronicus with elongation of rete ridges and prominent vessels of papillary dermis

Differential Diagnosis

The clinical differential diagnosis is broad and varies with the form and severity of dermatitis. Acute severe ICD and severe ACD may mimic

blistering disorders (bullous pemphigoid, mucous membrane pemphigoid, pemphigus vulgaris, bullous, or erosive lichen planus), infection (severe candidiasis, herpes virus infection), erythema multiforme, fixed drug reaction, and Hailey-Hailey disease [4, 22, 23]. Blistering disorders will usually show intraepidermal or subepidermal vesiculation and can be further evaluated with DIF. Infection can be investigated with culture as well as search for viral cytopathic effect and use of special stains. While irritant contact dermatitis may show occasional necrotic keratinocytes, more extensive keratinocyte apoptoses along with variable vacuolar interface dermatitis are clues to fixed drug reaction and erythema multiforme. Extensive acantholysis is the hallmark of Hailey-Hailey disease and is not a feature of contact dermatitis.

When the biopsy shows primarily spongiosis and inflammation, the pathological differential diagnosis includes fungal infection, insect bite reaction, drug reaction, and early lichen sclerosus [39, 40]. Special stains including periodic acid-Schiff (PAS) or Grocott's methenamine silver (GMS) for fungal infection should be performed. Although the presence of numerous eosinophils suggests insect bite and drug reactions, this finding cannot be relied upon for diagnosis since it can be seen a variety of conditions. Features of early lichen sclerosus are subtle, and its definitive diagnosis would require clinicopathologic correlation. Eosinophilic spongiosis may also be seen in early phases of immunobullous disorders [41, 42]; however, marked dermal edema, dermal eosinophils, and blister formation are seen in the developed phase of these disorders. Immunofluorescence may be helpful if a blistering disorder is suspected. The chronic form of contact dermatitis resembles psoriasis or chronic fungal infection and may evolve to lichen simplex chronicus. Neutrophils are a central component to the diagnosis of psoriasis and are frequently seen in fungal infections but are relatively less common in contact dermatitis. Ultimately, clinical correlation is required for accurate diagnosis.

The differential diagnosis for chronic contact dermatitis includes lichen simplex chronicus, psoriasis, seborrheic dermatitis, tinea cruris, erythrasma, candidiasis, Paget disease, and squamous cell carcinoma in situ [4, 22]. Psoriasis shows a unique constellation of findings: dilated papillary dermal vessels, thinning of suprapapillary plates, confluent parakeratosis with diminution of the granular layer, and neutrophilic collections. Infection should be evaluated with the aid of special stains and microbiology culture. Paget disease will show proliferation of large epithelioid cells of glandular derivation throughout the epidermis with ascent toward the stratum corneum. Squamous cell carcinoma in situ is an intraepidermal proliferation of dysplastic squamous cells. It should also be noted that contact dermatitis is often superimposed on other vulvar pathology, thus altering the clinical picture and complicating diagnosis [20, 38].

Summary

Clinical Presentation

Acute irritant/allergic contact dermatitis

- Well demarcated
- Generally confined to site of contact; as a result skin folds are relatively spared (particularly in cases of irritant contact dermatitis)
- Erythema, edema, erosions, and blistering

Chronic irritant/allergic contact dermatitis

- Pruritus (more pronounced in allergic than irritant contact dermatitis)
- Poorly demarcated
- Variable skin changes may include lichenification and pigmentary alteration
- Vulvodynia
- Pseudoverrucous papules and nodules (a unique form of chronic irritant contact dermatitis)

Histologic Features

Early lesions

- Epidermal spongiosis with variable vesicle formation
- Dyskeratosis and balloon cell degeneration in irritant reactions
- Dermal chronic inflammatory infiltrate accentuated in a perivascular pattern
- Variable exocytosis
- Dermal and epidermal eosinophils frequent in allergic reactions

(continued)

(continued)

Late lesions

- Hyperkeratosis
- Parakeratosis
- Epidermal acanthosis and hypergranulosis
- Dermal fibrosis and perivascular lymphocytic infiltrate

Differential Diagnosis

- Fungal infection
- Drug reaction
- Arthropod assault
- Bullous disorders
- Early lichen sclerosus
- Psoriasis
- Lichen simplex chronicus (often superimposed)
- Paget disease

Takeaway Essentials

Clinically Relevant Pearls

- Contact dermatitis is a common cause of vulvar symptoms and should be considered in any patient presenting with vulvar complaints, including those with minimal clinical changes and those not responding to therapy.
- The presentation of contact dermatitis is highly variable and often superimposed on other pathology, which may confound diagnosis.
- Vulvar eczema is common, but due to social stigma may often be underreported and self-medicated, which can also be a challenge to diagnosis.

Pathology Interpretation Pearls

- Biopsy findings may be nonspecific but may be very helpful in ruling out other disease processes.
- Special stains for fungal organisms or immunofluorescence studies may be useful adjuncts.
- Acute and irritant contact dermatitis may be indistinguishable on pathology.

Amicrobial Pustulosis of the Folds

Clinical Features

Amicrobial pustulosis of the folds (APF) is a recently described entity [43], with approximately 40 cases reported in the literature to date. Patients tend to be females in the third or fourth decade of life with an underlying autoimmune disturbance, most commonly lupus erythematosus [44]. Other diseases associated with APF include: idiopathic thrombocytopenic purpura [45], myasthenia gravis [45], celiac disease [46], mixed connective tissue disease [47], lupus erythematosus-scleroderma overlap [48], discoid lupus erythematosus with Sjogren's syndrome [49], IgA nephropathy with Sjogren's syndrome [50, 51], Hashimoto's thyroiditis [52, 53], Grave's disease [53], and autoimmune hepatitis [54]. Patients with APF may also have autoantibodies or elevated immunoglobulins but fail to fulfill criteria for autoimmune disease [44, 49, 55].

Lesions are ill-defined collections of papules and pustules on an erythematous base that are both follicular and non-follicular in distribution. Papules and pustules coalesce to form plaques and areas of macerated erosion. Lesions involve major and minor skin folds and are also common on the scalp, around the external ear canal, and nostrils (Fig. 3.5). Secondary impetiginization with crust-

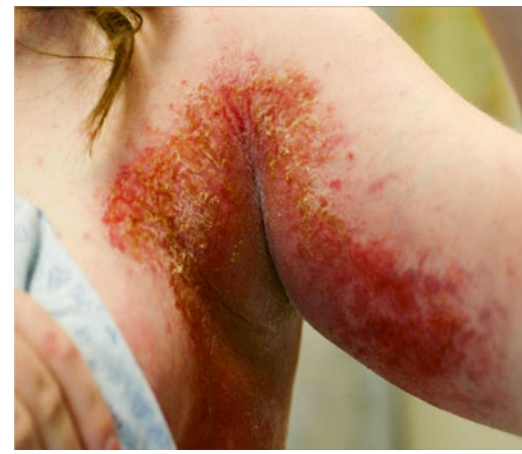


Fig. 3.5 Amicrobial pustulosis of the folds showing scaling, crusted lesions in axilla; similar lesions were also seen in inguinal folds, labia majora, and mons pubis (Photo courtesy of Neil Prose, M.D.)

ing is common. Scalp lesions may be associated with hair loss in the form of a non-scarring alopecia [47, 49, 56]. Diagnostic criteria have been proposed that include obligate and minor criteria (Table 3.2); diagnosis requires fulfillment of all obligate criteria plus one minor criteria [46]. Lesions are generally confined to the skin; however, in one case, extracutaneous oral and gastrointestinal manifestations were present [57].

APF is a chronic, relapsing disease. Patients often have a protracted history of a "rash" present for months to years prior to diagnosis. The associated autoimmune disturbance is either well known [54] or uncovered at the time of workup of APF [53]. Patients who fall short of fully developed autoimmune disease may go on to develop one in the future, necessitating close follow-up [58]. For the most part, cutaneous involvement by APF is independent of the activity of underlying autoimmune disease. However, in some cases, skin findings have resolved with resolution of the underlying disease [49, 50, 54].

The etiology and pathogenesis of APF remain unclear. Most consider APF as belonging to the category of neutrophilic dermatoses/autoinflammatory syndromes [57–62], yet it also has strong links to autoimmune disease. This raises questions of interplay between the innate and adaptive immune systems in the pathogenesis of APF [63]. In the setting of autoimmune disease, autoantibodies may erroneously activate complement within the skin, generating chemotactic factors that induce neutrophil migration or otherwise activate the innate immune system [51, 59, 61].

Table 3.2 Diagnostic criteria for amicrobial pustulosis of the folds

Obligate criteria	Minor criteria
Pustulosis involving one or more major skin folds, one or more minor skin folds, and anogenital area	Association with one or more autoimmune disorder
Histopathology showing intraepidermal spongiform pustules	Positive ANA (1:160 or higher)
Negative microbiology culture results	One or more serum autoantibodies

Data from: Marzano et al. [46]

The proinflammatory chemokine and cytokine profiles in APF tissue share similarities with those in other neutrophilic dermatoses and pustular disorders [59, 64].

Histologic Features

Intraepidermal and subcorneal spongiform pustules are the hallmark of APF (Fig. 3.6). The surrounding epidermis shows mild spongiosis with neutrophilic exocytosis. Epidermal acanthosis or psoriasiform hyperplasia is common. The stratum corneum may contain parakeratosis and neutrophilic debris. Neutrophils and lymphocytes populate the superficial to mid dermis. The dermal inflammatory infiltrate is accentuated around blood vessels, hair follicles, and sweats ducts and may be associated with nuclear dust between collagen fibers. Eosinophils and plasma cells are a less commonly reported component of the dermal inflammatory infiltrate. Papillary dermal edema and superficial dermal capillary dilation have been reported, which is important to remember when considering distinction of APF from psoriasis. The dermal mononuclear infiltrate consists primarily of CD3+ CD4+ lymphocytes, while CD15+ granulocytes are found in the spongiform pustules [48, 65]. Ki67 and Bcl-2 may be increased, accompanied by redistribution of

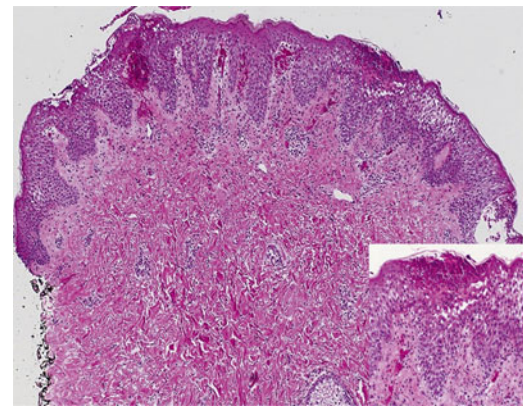


Fig. 3.6 Amicrobial pustulosis of the folds microscopically exhibits neutrophilic pustules, variable spongiosis, and mild acute and chronic inflammation in the dermis. High power {inset photo} shows a large neutrophilic pustule beneath the cornified layer and involving the upper dermis

involucrin expression to the lower portion of the stratum spinosum [65]. CD8 and p53 immunohistochemical staining may be useful in differentiating APF from psoriasis [65]; however, this remains to be substantiated with larger studies. The results of DIF/lupus band studies in the majority of cases are negative. Of note, not all APF patients have a clinical diagnosis of LE. Even among those who do, not all tested are positive in the lupus band test.

Differential Diagnosis

The differential diagnosis includes other pustular disorders such as pustular psoriasis, subcorneal pustular dermatosis (Sneddon-Wilkinson disease), and acute generalized exanthematous pustulosis (AGEP). Personal/family history and triggering events can usually be elucidated in cases of psoriasis. Subcorneal pustular dermatosis affects an older patient population, spares the face, is less erythematous, and is often associated with IgA gammopathy, inflammatory bowel disease, or pyoderma gangrenosum. AGEP is characterized by sudden onset of a widespread, non-follicular-based rash in patients who are acutely ill, with subsequent spontaneous resolution. On pathology, the above pustular disorders can have significant overlap with APF. Confluent parakeratosis with hypogranulosis may be a clue to psoriasis. The observation of follicle-centered pustulation, if present, can be helpful as this feature should not be a component of pustular psoriasis, subcorneal pustular dermatosis, or AGEP. Ultimately, the presence of spongiosis in addition to pustules, in combination with clinical data, will point to a diagnosis of APF. Infection and drug reaction must be ruled out. Cultures should be included in the workup. APF-like eruptions may be a form of anti-TNF alpha drug reactions [66]. Blistering disorders such as IgA pemphigus and pemphigus foliaceus may also be considered. The presence of acantholysis as well as positive immunofluorescence studies will be helpful in making the distinction from APF.

In summary, APF is a diagnosis of exclusion that requires a high level of suspicion and ultimately depends upon synthesis of clinical and histopathological data.

Summary

Clinical Presentation

- Folliculocentric and non-folliculocentric lesions
- Papules/pustules on erythematous base
- Coalesce into plaques (+/– crusting/erosion) with intact papules/pustules at borders
- Involvement of major and minor skin folds and anogenital area
- Microbiology cultures negative
- Associated with autoimmune disease or presence of autoantibodies

Histologic Features

- Intraepidermal and subcorneal spongiform pustules
- Epidermal spongiosis and neutrophil exocytosis
- Dermal inflammatory infiltrate of lymphocytes and neutrophils may be accentuated in a perifollicular and perivascular distribution
- Special stains for infectious organisms are negative

Differential Diagnosis

- Pustular psoriasis
- Subcorneal pustular dermatosis
- Acute generalized exanthematous pustulosis
- Infection
- Drug reaction
- IgA pemphigus

Takeaway Essentials

Clinically Relevant Pearls

- Amicrobial pustulosis of the folds is a unique clinicopathological condition but requires a high index of suspicion and ultimately is a diagnosis of exclusion.
- There is a strong correlation between amicrobial pustulosis of the folds and autoimmune disorders, so thorough screening and follow-up are recommended.

(continued)

(continued)

- Cutaneous lesions do not appear to wax and wane with the underlying autoimmune disease.

Pathology Interpretation Pearls

- Unlike most pustular disorders, amicrobial pustulosis of the folds has a spongiotic component.
- Immunofluorescence is helpful to rule out blistering disorders.

Cytotoxic/Vacuolar Interface Pattern

Fixed Drug Eruption

Clinical Features

Fixed drug eruptions are rare, and recurrent type IV hypersensitivity reactions triggered by a wide range of medications. More than 100 drugs have been implicated in this reaction, but major offenders include analgesics, antibiotics, and sedatives (Table 3.3). Several factors may complicate the identification of the culprit, such as: drug as a component of other medications (e.g., phenolphthalein in laxatives), patients with multiple medications, and the cross reactivity among various drugs. Occasionally, the offending medication cannot be identified; these cases have been called "fixed drug-like eruptions" [67]. Patients may complain of a burning or itchy sensation, but systemic symptoms are rare. The clinical appearance may differ depending on the affected anatomic location. On keratinized vulvar skin, fixed drug eruption presents as one or few well-demarcated erythematous round plaques that can evolve to blisters and erosions. This symmetric vulvitis with erosive changes may extend from labia majora to inguinal folds and the perineum. On the other hand, mucosal and modified vulvar mucous membranes commonly blister leading to erosions with irregular borders. Lesions have the tendency to occur in the same anatomic location after exposure to the medication; however, new lesions may appear in each episode. Sites of predilection besides the genitalia include face and lips. The time lapse between first exposure and the clinical lesion is 1–2 weeks and is often reduced to less than 24 h in subsequent exposures but may be as short as "minutes." Although nongenital fixed drug eruption leads to postinflammatory hyperpigmentation, vulvar lesions have the tendency to be of the nonpigmenting type [68]. The pathogenesis of fixed drug eruption has not been completely elucidated; it appears that the offending drug acts as a hapten and binds to proteins present in cells. In the nonpigmenting variant, the target antigen is believed to be dermal rather than epidermal [69]. A cellular cytotoxic response against the altered cells is elicited with cellular destruction executed by CD8+ lymphocytes mediated by Fas ligand triggering a caspase cascade [70]. The increased incidence of HLA-B22 supports a genetic susceptibility. The diagnosis of fixed drug eruption is reached after recognizing the relationship between the abrupt onset of an erythematous well-demarcated, erythematous, and symmetrical vulvitis with the ingestion of a medication known to produce this type of skin eruption.

Table 3.3 Frequent drug offenders in vulvar fixed drug eruption

Ibuprofen	NSAIDs
Cyclooxygenase-2 inhibitors	Barbituric acid
3-hydroxy-3-methyl-glutaryl-coenzyme reductase inhibitors	Tetracyclines
Pseudoephedrine	Metronidazole
Fluconazole	Griseofulvin
Trimethoprim-sulfamethoxazole	Carbamazepine

Histopathology

In its classic form, fixed drug eruption displays an interface inflammatory pattern with a superficial and deep mixed inflammatory infiltrate. Its healing phase is characterized by marked postinflammatory melanin incontinence; however, vulvar fixed drug reaction may not demonstrate classic histologic features [68]. In the vulva, fixed drug reactions commonly show epidermal spongiosis associated with a mild perivascular and interstitial lymphocytic infiltrate admixed with eosinophils and neutrophils. This predominantly dermal-based reaction lacks the classic postinflammatory pigmentary incontinence, leading to the designation as "nonpigmentary fixed drug eruption."

Differential Diagnosis

The presence of a recurrent, cutaneous, erythematous plaque in the same anatomic location in conjunction with the recent ingestion of a medication known to produce fixed drug eruption is pathognomonic. Genital lesions with focal blister formation and erosions raise the differential diagnosis of contact dermatitis, herpes simplex, pemphigus vulgaris, Behçet syndrome, and erythema multiforme/toxic epidermal necrolysis. Epidermal spongiosis can be seen in both contact dermatitis and fixed drug eruption; however, identification of Langerhans cells granulomas favors contact dermatitis. The superficial and deep perivascular inflammatory infiltrate with the presence of neutrophils favors fixed drug eruption over erythema multiforme and toxic epidermal necrolysis. The simultaneous presence of oral ulcers, documentation of pathergy, and involvement of internal organs supports the diagnosis of Behçet syndrome. Viral cytopathic changes can differentiate herpes virus infection from fixed drug eruption. If a blistering disorder is strongly suspected, a DIF test on properly fixed tissue (such as in Michel's medium) may clarify the diagnosis.

Summary

Clinical Presentation

- Hair-bearing skin: recurrent, circumscribed erythematous recurrent plaque
- Modified mucosa: recurrent, erythematous plaque with irregular erosions
- A nonpigmenting variant of fixed drug eruption is commonly seen on the vulva.
- The hallmark is the reappearance of the lesion in the same anatomic location with rechallenge by the offending drug.

Histologic Features

- Vacuolar interface dermatitis (classic)
- Superimposed spongiotic change (a common variation, especially in early lesions)
- Perivascular lymphocytic infiltrate with few neutrophils and eosinophils

Differential Diagnosis

- Contact dermatitis
- Erythema multiforme
- Toxic epidermal necrolysis

Takeaway Essentials

Clinically Relevant Pearls

- Careful history taking is essential to reach diagnosis.
- A fixed drug reaction is diagnosed when lesions subsides after cessation of the drug intake and or recur after rechallenge.
- Since hypersensitivity to the drug is lifelong, the cure is to avoid the drug.
- The dissimilarity to the classic clinical presentation of fixed drug reaction, high frequency of nonpigmentary type, makes it unlikely to be clinically suspected.
- Fixed drug reaction should be considered in any acute or recurrent vulvitis with nonspecific biopsy, negative microbiology, and unresponsiveness to treatment.

Pathology Interpretation Pearls

- The presence of a deep infiltrate is a clue to differentiate fixed drug eruption from erythema multiforme and toxic epidermal necrolysis.
- In recurrent flare-ups, histologic features of acute and chronic inflammation coexist.

Erythema Multiforme

Clinical Features

Erythema multiforme (EM) is a cutaneous hypersensitivity phenomenon characterized by acute onset of self-limited, pleomorphic lesions. The clinical episode of EM lasts from 1 week to 10 days. Commonly, it affects extremities in a symmetrical distribution. Rarely the vulva is the only affected

anatomic location, and in a review of 65 patients with recurrent EM, 25 % exhibited genital involvement [71]. Infection (e.g., herpes virus, *Streptococcus*, and *Mycoplasma*) is often the underlying cause; other stimuli include drugs and pregnancy. Medications triggering this reaction encompass antibiotics (particularly sulfonamides and their derivatives like the cyclooxygenase-2 inhibitor celecoxib), oral contraceptives, selective serotonin reuptake inhibitors, 5-fluorouracil sestamibi, and many others. The disease can affect patients with a wide range of age, but it is uncommon in childhood. Clinically, painful areas of erythema are quickly followed by the development of vulvar blisters that end in painful enlarging ulcers. The hallmark clinical presentation is the targetoid or "bull's eye" lesion (central zone of necrosis, blister, or erosion surrounded by edema and erythema) (Figs. 3.7 and 3.8). A variation of the classical targetoid lesion, known as the "atypical targetoid lesion,"

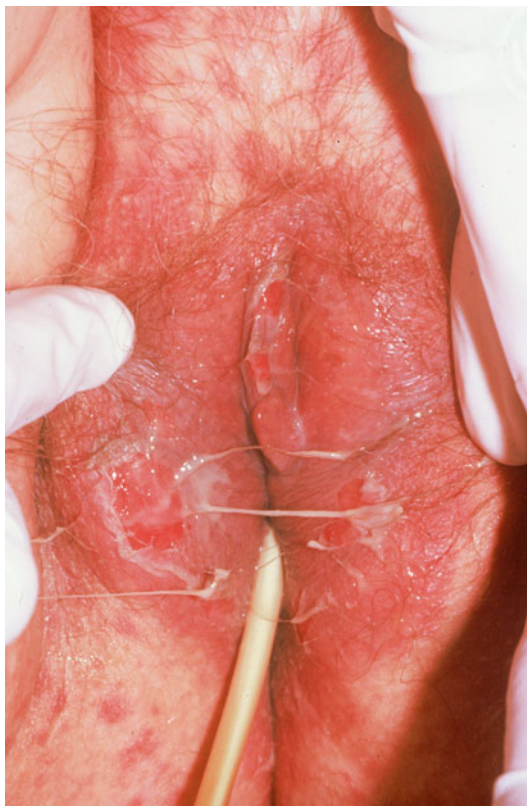


Fig. 3.7 Eroded area on labia majora from erythema multiforme

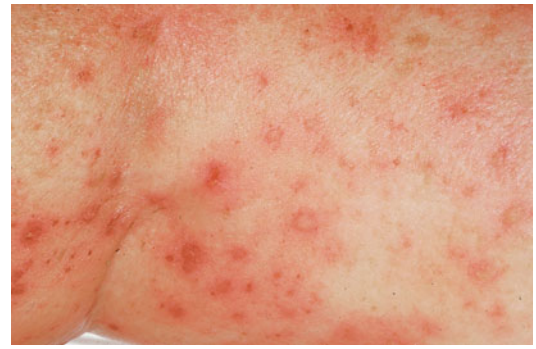


Fig. 3.8 Targetoid lesions in axilla in patient with erythema multiforme. Most patients with vulvar erythema multiforme will have lesions on other cutaneous surfaces

can also occur in the form of a round raised lesion with only two zones: central edema and an erythematous border. Two forms of EM have been delineated on the basis of frequency and time course of an episode(s): recurrent and persistent types. The persistent form is associated with malignancies and Epstein-Barr virus infection, while the recurrent type is seen in patients suffering from recurrent infections like herpes simplex virus (HSV). Recurrent attacks of EM-like eruptions secondary to herpes viral infection can be halted with use of treatment during HSV prodromal or contiguous suppressive therapy.

EM can present with mucosal involvement, fever, and systemic symptoms. Medications and infections with *Mycoplasma pneumoniae* are usually associated with these severe cases. The latter form of EM needs to be separated from Stevens-Johnson syndrome (SJS). A critical difference between these two diseases is internal organ involvement in SJS. EM has typical or raised atypical targetoid lesions on the extremities or face in a patient with less fever and milder mucosal lesions but frequent recurrences, while SJS exhibits flat atypical targetoid lesions or purpuric macules that are widespread or limited to the trunk. When bullae are present, to diagnose EM, the epidermal detachment must be less than 10 % of body surface [72].

Patients with widespread lesions are toxic and exhibit an increased risk of secondary infection and temperature instability comparable to burn patients. The mortality in these cases is around 40 % [73].

Histopathology

The histology of a lesion of EM varies depending on the clinical stage of the disease. Early lesions may show changes mainly in the dermis in the form of edema, chronic inflammation, and red cell extravasation associated with focal interface changes—a constellation of findings that correlate with the clinical appearance of purpura. The classic picture of an acute fully developed lesion includes normal stratum corneum overlying squamous epithelium with marked destruction of the basal layer by vacuolar alteration of keratinocytes and presence of dyskeratotic cells (Figs. 3.9 and 3.10). Ballooning and reticular degeneration of keratinocytes is associated with individual cell necrosis and associated fluid accumulation. Individual cell death may progress to confluent epidermal necrosis. The inflammatory cell infiltrate is comprised of lymphocytes with histiocytes located in the upper dermis. Eosinophils may be identified. Leukocytoclasis is not seen. Eventually, the epidermis detaches forming a subepidermal blister leading to an ulcer and reepithelialization. Histologic changes cannot point to a particular trigger; however, the presence of keratinocyte necrosis in the acrosyringium associated with eosinophils is most frequently seen in drug-mediated EM [74]. Histologic changes when seen in the vulvar-modified mucosa or mucosa are similar to hair-bearing skin; however, intercellular edema is more prominent and blisters more quickly evolve to erosions/ulcers. DIF shows intraepidermal Civatte bodies, staining usually with IgM and occasionally with C3. Commonly there is granular staining for C3 along the dermoepidermal junction.

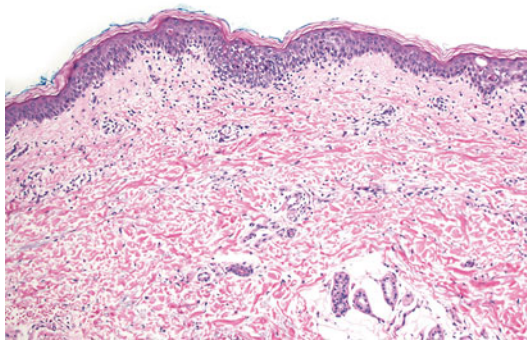


Fig. 3.9 Low power view of erythema multiforme with normal cornified layer and minimal dermal change

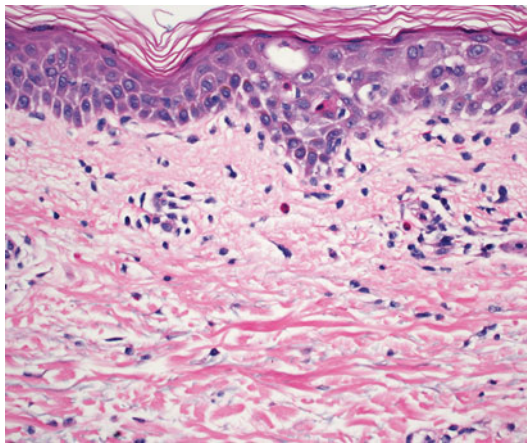


Fig. 3.10 Higher power of erythema multiforme where necrotic keratinocytes and sparse dermal inflammatory infiltrate of lymphocytes and eosinophils are seen

Differential Diagnosis

EM shares similar histologic features with SJS; however, EM shows a more inflammatory reaction, while SJS exhibits more keratinocytic necrosis. Ultimately, it is the extensive mucosal and internal organ involvement seen in SJS that separates these two disorders. EM-like changes can be seen in paraneoplastic pemphigus and in the hypersensitivity reaction to phenytoin and carbamazepine; a DIF test and the clinical presentation is essential to differentiate EM from these diseases. EM may be sometimes confused with connective tissue disease (e.g., systemic and subacute lupus erythematosus and dermatomyositis) and graft-versus-host disease (GVHD). Dermal mucin and presence of hyperkeratosis with parakeratosis, evidence of chronic disease, are associated with connective tissue disease. Satellitosis can be seen in EM and GVHD; hyperkeratosis, hypergranulosis, and hyperpigmentation of the basal layer would favor GVHD. The presence of deep dermal infiltrate can separate fixed drug reaction from EM. Importantly, none of the abovementioned findings are absolutely pathognomonic of any of the diseases, and only a clinicopathologic correlation will ensure their distinction.

Summary

Clinical Presentation

- Vulvar involvement is often accompanied by lesions on nongenital sites
- Painful erythema leading to blister and ulcer (<10 % body surface detachment)
- Targetoid lesions
- Atypical targetoid lesions
- Painful vulvar areas of erythema followed by blisters and ulcers (ulcers often worse on modified mucosa and mucosa)

Histologic Features

- Marked vacuolar basal layer degeneration and presence of necrotic keratinocytes at all levels of epidermis
- Isolated and scattered keratinocytic necrosis may become confluent.
- Subepidermal blister due to damage of basal layer can be seen.
- Prominent dermal infiltrate of lymphocytes and histiocytes
- Eosinophils can also be seen in the infiltrate.

Differential Diagnosis

- Stevens-Johnson syndrome
- Toxic epidermal necrolysis
- Fixed drug reaction
- Connective tissue disease
- Graft-versus-host disease

Takeaway Essentials

Clinically Relevant Pearls

- The diagnosis is made by the conjunction of abrupt onset and clinical appearance of the lesions in a patient recently exposed to medications or suffering from infection.
- In bullous erythema multiforme, a biopsy for direct immunofluorescence is recommended to rule out autoimmune disease as treatments drastically differ.
- The rapid onset of erythema multiforme can separate this entity from bullous disorders, while the number and anatomic distribution of lesions can help differentiate erythema multiforme from fixed drug eruptions.

Pathology Interpretation Pearls

- The presence of necrotic keratinocytes at the opening of the sweat gland and eosinophils in the dermal infiltrate are frequently associated with a drug etiology.

Toxic Epidermal Necrolysis and Stevens-Johnson Syndrome

Clinical Features

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are cutaneous eruptions that display diffuse, tender erythema rapidly evolving to blistering, and extensive shedding of skin. Fortunately, these life-threatening conditions have low incidence with 1.2–6 cases and 0.4–1.2 cases per million for SJS and TEN, respectively. When blisters remain discrete and are associated with mucous membrane involvement, the condition is classified as SJS; when blisters rupture and coalesce in areas of denuded erythema, the disease is diagnosed as TEN. A consensus paper by Bastuji-Garin and colleagues in 1993, established the clinical criteria such that detachment of more than 30 % of the body surface qualifies for the diagnosis of TEN [75]. Vulvar lesions are commonly part of the diffuse mucous-cutaneous involvement.

Clinical lesions range from blisters and erosions to atypical targetoid lesions (round lesions with central edema surrounded by erythema). In vulvar mucosal or modified mucosal involvement, the blisters have a short life span—turning rapidly into erosions (Fig. 3.11). Severe cases of SJS may lead to complications like vaginal stenosis [76] and visual impairment secondary to keratitis with conjunctival scarring. The risk of death, reportedly around 35 %, can be predicted using a quantitative "severity of illness" score (SCORTEN) [77].

Factors associated with poor prognosis are advanced age, large areas of denuded skin, deteriorating renal function, and extensive involvement of the bronchial epithelium. In the majority of cases of TEN, a drug is the culprit

Table 3.4 Most frequent drugs associated with increased risk for toxic epidermal necrolysis and Stevens-Johnson syndrome

Sulfonamides	Phenytoin	Doxycycline
Trimethoprim-sulfamethoxazole	Nonsteroidal antiinflammatory	Nucleoside reverse-transcriptase inhibitor
Phenobarbital	Oxicam	Cyclooxygenase inhibitors
Carbamazepine	Allopurinol	Corticosteroids
Nitroglycerin patch	Griseofulvin	Sustained-release bupropion

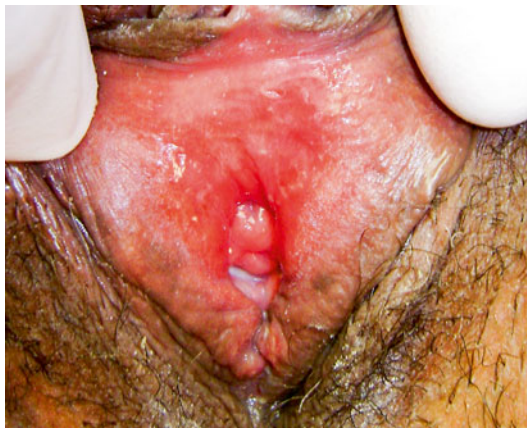


Fig. 3.11 Erythematous and eroded area at introitus in a patient with toxic epidermal necrolysis

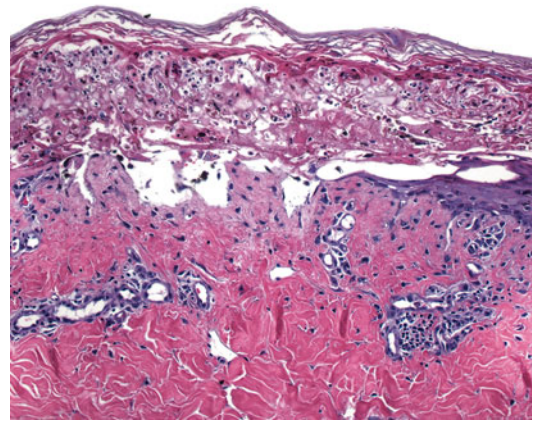


Fig. 3.12 Toxic epidermal necrolysis with vacuolar degeneration and full-thickness necrosis of epidermis with normal orthokeratin layer, signifying acute nature of process

with less than 15 % of cases associated with other stimuli like immunizations or infections. Only half of the cases of SJS are associated with a therapeutic drug. A constantly growing list of medications has been seen reported as offenders in SJS and TEN; the most common group of drugs include antibiotics, nonsteroidal antiinflammatory drugs, and anticonvulsants (Table 3.4). The epidermal destruction is mediated by factors like CD8+ lymphocytes with cytokine effectors, such as tumor necrosis factor (TNF) alpha, perforin, and granzyme B. Apoptosis of keratinocytes may also occur through activation of CD40/CD40L and Fas and Fas-ligand pathways on epidermal cells. Cytokines may explain the disparity between degree of lymphocytic infiltrate and degree of epidermal destruction [78].

Histopathology

The spectrum of histologic finding in SJS and TEN are similar to EM. All three entities display sparsely inflamed interface dermatitis with basal layer vacuolization and keratinocyte necrosis that may become confluent (Fig. 3.12). Eventually a blister is formed with necrotic epidermis as the roof. In patients in which skin is denuded, it may be "rolled" and embedded on end to examine the epidermis without performing a biopsy. The full-thickness necrosis can be readily seen (Fig. 3.13). Histologic exam may have prognostic importance, as the degree of inflammation may predict survival; in one study, inflammation and survival correlated inversely [79]. In the healing phase, milia can be seen on the hair-bearing surface of the vulva, and adenosis may be detected in the mucosa or modified mucosa of the vulva and vagina [80].

Differential Diagnosis

Staphylococcal scalded skin syndrome is an important clinical differential diagnosis (see Chap. 4). This entity is characterized by subcorneal detachment with collection of neutrophils and absence of full-thickness necrosis. On histologic grounds, EM can overlap with SJS and TEN. The presence of a marked inflammatory reaction, restriction of

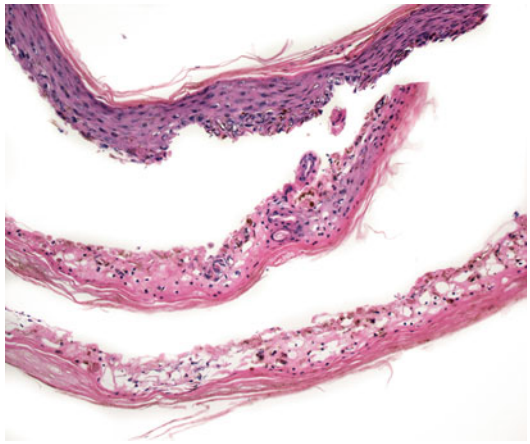


Fig. 3.13 Denuded skin from patient with toxic epidermal necrolysis, arranged in a "roll" showing changes of necrosis ranging from basal layer to full-thickness epidermis. The keratin layer shows little change, signifying the acute nature of the process

the necrosis to the lower portion of the epidermis, and erythrocyte extravasation favor EM.

Summary

Clinical Presentation

- Painful erythema, blisters, and ulcers on the vulva may be part of in diffuse disease
- Stevens-Johnson syndrome
  - Flat atypical targetoid lesions and erythematous macules
  - Blisters and erosions of one or more mucous membranes
  - Less than 10 % body surface detachment
- Toxic epidermal necrolysis
  - Flat atypical targetoid lesions and/or erythematous macules
  - More than 30 % body surface detachment
- Overlap Stevens-Johnson syndrome/toxic epidermal necrolysis
  - Flat atypical targetoid lesions and/or erythematous macules
  - 10–30 % body surface detachment

Histologic Features

- Pauci-inflammatory interface dermatitis
- Keratinocytic necrosis leading to full-thickness necrosis
- Mild superficial dermal infiltrate of lymphocytes and histiocytes
- Eosinophils may be present

Differential Diagnosis

- Erythema multiforme
- Staphylococcal scalded skin syndrome

Takeaway Essentials

Clinically Relevant Pearls

- Mucosal and conjunctival involvement is noted.

Pathology Interpretation Pearls

- When a blister is sampled, the edge of the lesion is essential to identify the primary interface process.
- Presence of dead keratinocytes in the acrosyringium and eosinophils in the dermal infiltrate favor a drug-related etiology.

Graft-Versus-Host Disease

Clinical Features

Graft-versus-host disease (GVHD) is a major multisystem complication in transplant patients—targeting the skin, gastrointestinal tract, lung, and liver. GVHD classically occurs in bone marrow transplant patients but also can follow solid organ transplantation, transfusion of nonirradiated blood or blood products in severely immunosuppressed patients, and as a complication of transplacental transfer of maternal lymphocytes in an immunodeficient fetus. The pathogenesis is extremely complex, but it essentially occurs when transplanted immunocompetent donor T-cell lymphocytes are activated and react to foreign host major histocompatibility complex (MHC)-antigens in an immunosuppressed recipient patient. Cytokines including IL-1, TNF alpha,

Table 3.5 National Institutes of Health consensus development project on criteria for chronic graft-versus-host disease

Classic acute GVHD: acute GVHD presenting within 100 days after HCT or donor leukocyte infusion
Persistent, recurrent, or late-onset acute GVHD: acute GVHD occurring more than 100 days after transplantation without chronic GVHD symptoms
Classic chronic GVHD: chronic GVHD without features of acute GHVD regardless of timing from transplantation
Overlap syndromes: both acute and chronic GVHD features present regardless of timing from transplantation

and granulocyte-monocyte colony-stimulating factor (GM-CSF) also play a role in the pathogenesis of this disease. Besides human leukocyte antigen (HLA) disparity, development of acute GVHD may be secondary to sex mismatch, increased patient age, and the development of infection. Twenty-four percent to 36 % of patients suffering from GVHD develop genital involvement [81]. Vulvar manifestations may be GVHD's first or only presentation [81]. These patients are at increased risk of infection which is a concerning cause of morbidity and mortality.

In 2005, the National Institute of Health proposed to divide GVHD into the following: classic acute, persistent, classic chronic, and overlap syndromes [82] (Table 3.5). Acute GVHD may resolve or in 35 % of cases evolve to chronic GVHD. The risk of chronic GVHD is eleven times greater if the patient has experienced acute GVHD. Chronic GVHD develops in 10 % of all patients with allogeneic bone marrow transplant with involvement of organs such as the skin, eyes, mouth, esophagus, liver, genitalia, muscle, and central and peripheral nervous system.

Vulvovaginal GVHD can be seen at any stage of the disease coexisting with manifestations of GVHD in other organs. 75 % of patients with vulvar involvement by GVHD have manifestations of chronic disease in nongenital skin [83]. The severity of genital GVHD is independent of the severity of GVHD found in other organs. Most gynecologic manifestations develop an average of 1 year after transplantation, although reported late-onset cases highlight the need of a long-term gynecologic follow-up in these patients. Symptoms include vulvar or vaginal dryness, irritation, dyspareunia, and postcoital bleeding. Examination shows

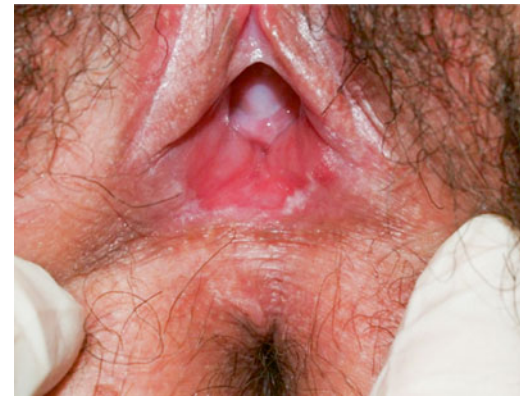


Fig. 3.14 Erythema, erosion, and exudate in a patient with graft-versus-host disease

Table 3.6 Severity grading of gynecologic graft-versus-host disease

Severity	Description
Mild	Discomfort or discharge
	Vulva and or vaginal mucosal erythema
Moderate	Genital desquamation and erosions
	Decreased vaginal elasticity
	Fibrinous exudate
Severe	Vaginal adhesions and fibrous banding
	Reduction of vaginal capacity
	Vaginal stenosis or occlusion

Modified with permission from Zantomio et al. [83]

excoriated and ulcerated erythematous patches, fissures, thickened mucosa, vestibular tenderness, and a narrowed introitus (Fig. 3.14). Scarring of the vulva and vagina presents in the form of labial fusion and vaginal closure, respectively. Early diagnosis of genital GVHD prevents progression to fibrotic stages and decreases morbidity in these patients. The clinical presentation is classified in three tiers based on the degree and extension of the gynecologic findings in the vulva and vagina (Table 3.6). Its most extreme presentation includes a TEN-like eruption. This classification better portrays the dynamic character of the diseases. The majority of the patients exhibit a mild form of genital GVHD; only 6–12 % present with severe forms. These patients are at high risk of fungal, bacterial, and viral infection. The higher risk of genital infection by HPV in these patients is underscored by the increased incidence of cervical cancer in long-term stem cell transplantation survivors compared to general population [84].

Histopathology

Classically, the acute lesion of GVHD shows vacuolar alteration of the basal layer with presence of dyskeratotic cells at all levels of the epidermis (Fig. 3.15). The presence of apoptotic keratinocytes surrounded by lymphocytes is called satellite cell necrosis (lymphocyte-associated apoptosis) and may precede prominent keratinocyte necrosis (Fig. 3.16). Perivascular lymphocytic infiltrate with exocytosis is invariably seen. The density of inflammatory cells directly correlates with the possibilities of developing more severe form of acute GVHD [85].

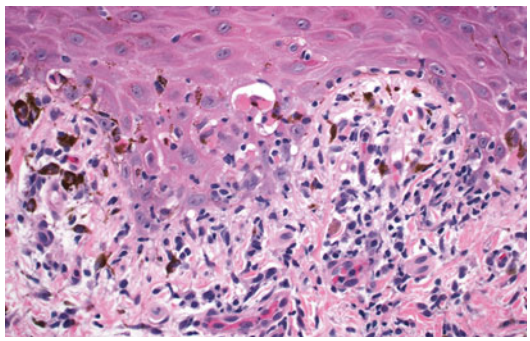


Fig. 3.15 Graft-versus-host disease with lymphocytes at the dermoepidermal junction and necrotic keratinocytes in lower epidermis

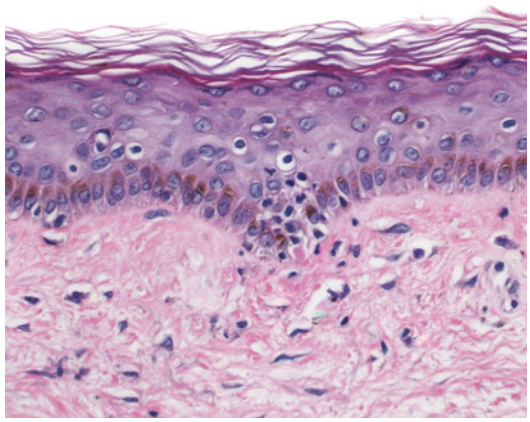


Fig. 3.16 Graft-versus-host showing lymphocytes surrounding necrotic keratinocytes at base of central rete ridge, signifying satellite cell necrosis. In early cases such as this, the keratinocyte necrosis may be limited

Eosinophils can be identified, and they do not necessarily indicate a drug reaction [86]. The inflammatory reaction extends to the hair follicle with involvement of the bulge. The presence of more than five dyskeratotic cells in adnexal structures is said to support the interpretation of GVHD. The most severe presentation of acute GVHD is TEN-like lesions present with epidermal necrosis involving the sweat glands with degenerative changes and keratinous plugging of acrosyringium.

Four grades of acute GVHD are described: grade one is characterized by focal or diffuse vacuolar alteration of the basal layer; in grade two, vacuolar alteration of the basal layer, spongiosis, and dyskeratotic cells are noted; grade three is considered when a subepidermal cleft is identified; and grade four is reached when epidermis is completely lost [87]. The lichenoid variant of chronic GVHD is nearly indistinguishable from idiopathic lichen planus and shows the classic hyperkeratosis, hypergranulosis, basal cell vacuolization, Civatte bodies, pigmentary incontinence, and a band-like lymphocytic infiltrate with scattered macrophages. Features seen only in chronic GVHD are the presence of satellitosis in early phase and plasma cells and eosinophils in the infiltrate. The late stage of chronic GVHD is characterized by epidermal atrophy with the presence of fibrosis predominantly in the upper dermis leading to obliteration of anatomic structures. Features identified in acute GVHD, such as vacuolar alteration of the basal layer, Civatte bodies, and chronic inflammation, may not be apparent. Fluorescent in situ hybridization analysis for donor lymphocytes in the skin may play a role in the diagnosis of acute graft-versus-host disease [88].

Differential Diagnosis

The histologic features seen in acute GVHD can be reproduced by eruption of lymphocyte recovery, drug reactions, chemotherapy, and viral infection. Although the presence of eosinophils can argue in favor of a drug reaction, it is not a reliable finding. Chronic GVHD may mimic lichen planus. Helpful clues to chronic GVHD

include the presence of satellitosis in early phase, a less conspicuous dermal infiltrate, and the presence of plasma cells and eosinophils in the infiltrate. In summary, there is no consistent histologic feature or constellation of findings to diagnose GVHD based on pathology alone. Clinical history of transplantation is the only reliable feature to separate acute or chronic GVHD from its mimickers. Therefore, clinical history is paramount when GVHD is a diagnostic consideration as it turns subtle or nonspecific histologic findings into predictive features.

Summary

Clinical Presentation

- Vulvar erythematous patches, fissures, thickened mucosa, vestibulitis, and narrowed introitus
- Scarring of the vulva and vagina presents as labial fusion and vagina closure

Histologic Features

- Squamous epithelium with vacuolar alteration of basal layer and dyskeratotic cells
- Satellite necrosis
- Chronic stages present as lichenoid dermatitis with plasma cells and occasionally eosinophils
- Fibrosis with obliteration of anatomic structures

Differential Diagnosis

- Chemotherapy reaction
- Viral infection
- Toxic epidermal necrolysis
- Lichen planus

Takeaway Essentials

Clinically Relevant Pearls

- Vulvar and vaginal GVHD can be confused with hypoestrogenism. The presence of erythema and absence of response to estrogen therapy supports GVHD.
- Early detection of gynecologic GVHD is key to avoiding morbidity in these patients.
- Surveillance of these patients is recommended to detect early HPV-related neoplasms.

Pathology Interpretation Pearls

- Clinical history is paramount when GVHD is a diagnostic consideration as it turns subtle or nonspecific histologic findings into predictive features.

Lupus Erythematosus

Clinical Features

Lupus erythematosus (LE) is typically a multiorgan disease (systemic) that may or may not have skin manifestations. It may also be limited to the skin (cutaneous). While the incidence of vulvar LE is unknown, most experts agree that genital lupus is extremely rare. Studies have reported vulvar LE occurring in the setting of both chronic cutaneous [89] and systemic LE [90].

Little is written about vulvar LE; but in the few studies reported, vulvar lesions are typically on the mucosal skin of labia minora and vaginal introitus, often with erosions or ulcers [91, 92]. Lesions may be bullous [90]. Hair loss may occur.

Herein, three clinical presentations affecting the vulva are discussed: discoid lupus erythematosus (DLE), systemic lupus erythematosus (SLE) with skin involvement, and bullous lupus erythematosus [93]. In all forms of LE, scaling patches may be seen. DLE more commonly shows clinical evidence of hyperkeratosis at the site of follicles (follicular plugging). Bullous LE additionally shows vesicles and/or bullae. SLE is the least likely to be scaly and may be erythematous with occasional poikilodermatous changes, secondary to atrophy in long-standing cases.

As an autoimmune disease, a variety of autoantigens, immune mediators, and environmental causes have implicated in the pathogenesis of LE [94], which may be associated with other autoimmune diseases. Viruses, hormones,

hereditary factors, and ultraviolet (UV) light have also been implicated. As UV light (photosensitivity) is well known to promote and exacerbate lesions of LE, the rarity of LE on genital skin may be in part explained by its protection from the sun.

While it is believed to follow the course of the nongenital forms of the disease, due to its rarity, the prognosis of vulvar LE is still unknown. DLE is often isolated to the skin; however, SLE is typically a chronic disease process with a waxing and waning course. The morbidity in systemic lupus is particularly high when renal or intracranial vessels are involved. In studies of non-vulvar sites, progression of discoid lupus to systemic lupus occurs in up to one-third of patients [95].

Rare cases of vulvar LE have been associated with squamous cell carcinoma of the vulva and vulvar intraepithelial neoplasia, raising the question of whether the immune dysregulation or therapy with immunosuppressive drugs can be a risk factor for the development of malignancy on the vulva [96, 97].

Histopathology

In DLE, there is irregular acanthosis alternating with epidermal atrophy associated with vacuolar alteration of the basal layer and perivascular chronic inflammatory infiltrate (Fig. 3.17). Civatte bodies (apoptotic keratinocytes) are common along the basal layer. The basement membrane is usually thickened and may be accentuated by a PAS stain. Hyperkeratosis at the follicular orifice (follicular plugging) is often present (Fig. 3.18). The dermis exhibits a perivascular chronic inflammatory infiltrate that may extend throughout the dermis and into the subcutaneous tissue. Peri-eccrine inflammation is another histologic finding in this disease. Scarring is common, especially in the upper dermis, and early manifestations may be better detected using elastic stains. Increased mucin may be present in the dermis in long-standing lesions, which may be highlighted by the use of colloidal iron or Alcian blue stains. Interface changes and chronic inflammation often affect follicles, explaining the alopecia seen in some cases.

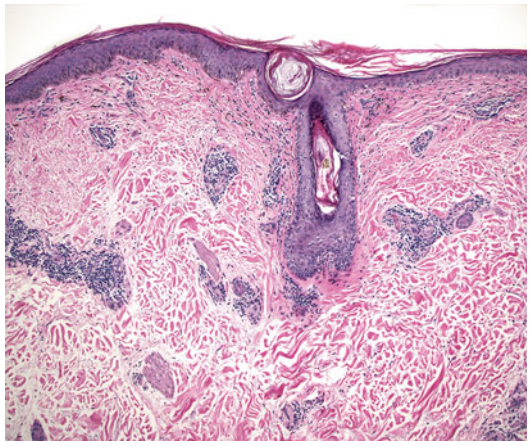


Fig. 3.17 Discoid lupus erythematosus showing variable hyperkeratosis, acanthosis, and an infiltrate of lymphocytes focally along the dermal-epidermal junction, the follicular interface, and around vessels

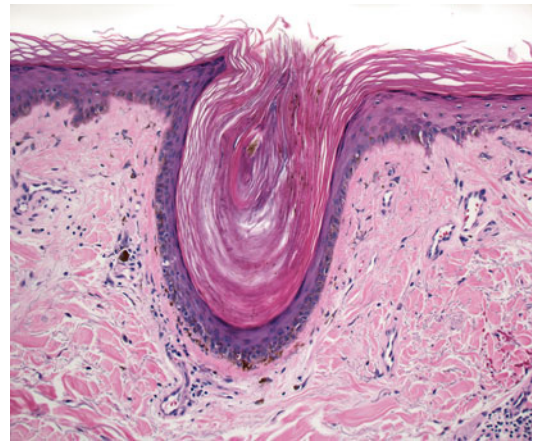


Fig. 3.18 High power view of discoid lupus erythematosus with follicular hyperkeratosis, corresponding to the follicular plugging seen clinically

SLE also demonstrates an interface dermatitis, but the histologic changes in the epidermis and dermis as compared to DLE are much more subtle and easily missed. Epidermal atrophy is the rule. Basement membrane thickening is variable and Civatte bodies are rare (Figs. 3.19 and 3.20). In early lesions, scattered neutrophils may be present in the upper dermis, but in developed lesions, the inflammation is predominantly lymphoplasmacytic. When neutrophils collect in the papillary dermis, the changes may mimic immunobullous disease such as dermatitis herpetiformis (Fig. 3.21). Increased mucin may be pronounced in some instances.

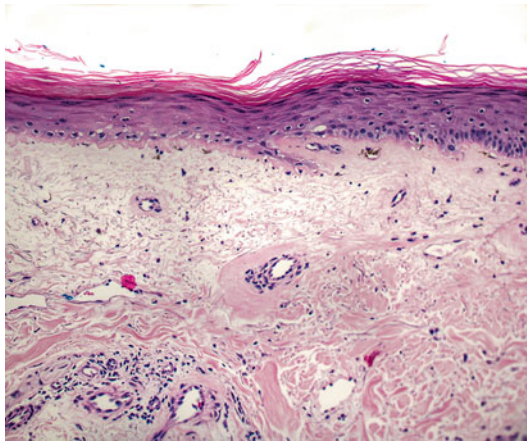


Fig. 3.19 Systemic lupus erythematosus showing variable epidermal atrophy, vacuolar change along the dermal-epidermal junction, thickening of the basement membrane at the dermal-epidermal junction, and around vessels as well as a sparse perivascular lymphoid infiltrate

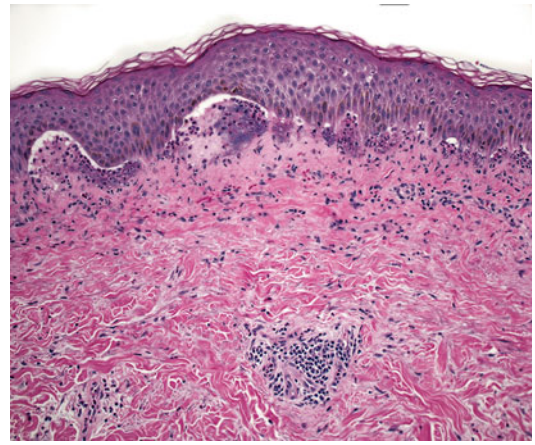


Fig. 3.21 Lupus erythematosus may show collections of neutrophils in the papillary dermis mimicking the changes seen in early immunobullous disorders such as dermatitis herpetiformis

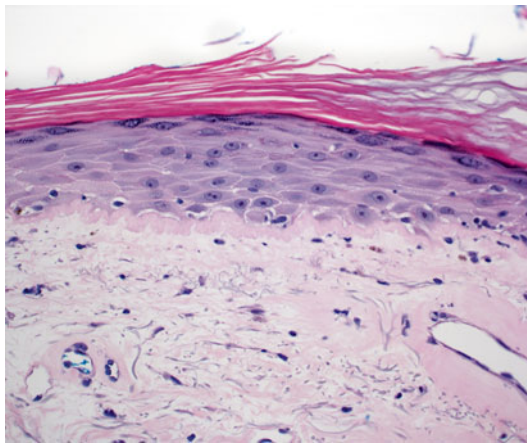


Fig. 3.20 Systemic lupus erythematosus with high power view demonstrating thickening of basement membrane at dermoepidermal junction and around vessels

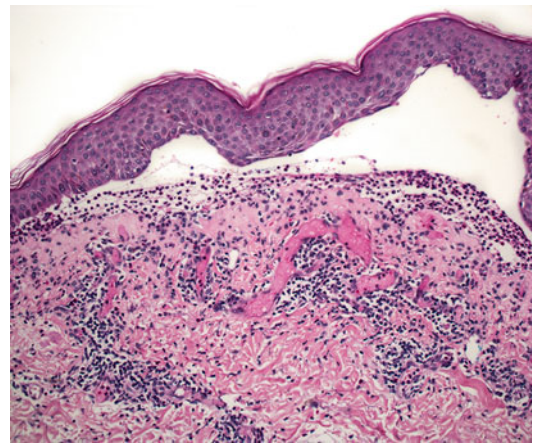


Fig. 3.22 Extensive degeneration of the basal epidermis leads to separation of the epidermis from dermis with lymphoid and neutrophils in dermis in bullous lupus erythematosus

Bullous LE shares most of the features of SLE, but the hallmark is separation of the epidermis form the dermis, causing formation of a vesicle or bulla. Ulceration and scarring may ensue (Fig. 3.22).

DIF performed on the patient's frozen biopsy tissue (lupus band test) may be helpful in difficult cases to confirm the diagnosis, typically showing a "full-house" pattern with deposition of IgM, IgA, IgM, and IgG in a granular pattern at the dermal-epidermal junction. A positive DIF result is more common in DLE than SLE, but in all types, false-negatives are not infrequent. False-positives may occur in nongenital cases, and it is seen predominantly in chronically light-exposed skin.

Differential Diagnosis

The differential diagnosis for the DLE form includes other interface/lichenoid dermatitides. Lichen planus is the primary consideration and usually can be excluded by the lack of basement membrane thickening and dermal mucin and the presence of a "sawtooth" pattern of acanthosis

surmounting chronic inflammation that is more dense and confluent than in DLE.

In SLE, all other forms of vacuolar interface dermatitis should be excluded, such as drug eruptions, graft-versus-host disease (GVHD), and rare viral exanthems. Drug eruptions typically show an infiltrate of eosinophils. GVHD often exhibits satellite cell necrosis of the epidermis in the acute form that most commonly mimics vulvar LE. Viral exanthems may produce a vacuolar interface dermatitis but are often accompanied by lesions elsewhere on the body typical of a viral rash. Moreover, well-developed lesions of SLE will exhibit pronounced basement membrane thickening and mucin in the dermis not seen in cases of in drug eruptions, GVHD, or viral exanthems. Dermatomyositis may be indistinguishable from SLE, but fortunately, it is even rarer than LE on the vulva, and in this situation, clinical correlation may be essential to differentiate the two.

Changes in bullous LE may clinically and histologically mimic other bullous diseases. Unlike bullous disease such as dermatitis herpetiformis, vulvar LE will often show some perivascular or periadnexal inflammation with variable basement membrane thickening. In some instances of bullous LE, clinicopathologic correlation and DIF studies may be essential to confirm the diagnosis and exclude other immunobullous disorders, e.g., dermatitis herpetiformis will show clusters of IgA in the papillary dermis, while bullous LE will show deposition of all immunoglobulins along the dermoepidermal junction.

Summary

Clinical Features

- Discoid lupus erythematosus
  - Erythematous plaque with variable scale
  - Follicular plugging
- Systemic lupus erythematosus
  - Erythematous macule with variable scale
  - Possible atrophy with poikilodermatous change
- Bullous lupus erythematosus
  - Variable scale
  - Vesicular and/or bullous lesions

Histologic Features

- Discoid lupus erythematosus
  - Interface dermatitis at dermoepidermal junction and follicles
  - Hyperkeratosis with follicular accentuation
  - Basement membrane thickening variable
  - Perivascular and periadnexal chronic inflammation
  - Mucin in dermis frequent
- Systemic lupus erythematosus
  - Interface dermatitis mostly of dermoepidermal junction with less involvement of follicles
  - Basement membrane thickening variable
  - Less chronic inflammation than typically seen in DLE
  - Mucin present in long-standing cases
- Bullous lupus erythematosus
  - Interface dermatitis may be subtle in areas of vesicle formation.
  - Infiltrate often composed of neutrophils, sometimes in clusters in the papillary dermis
  - Chronic inflammation often not seen in early lesions
  - Mucin deposition is rare.

Differential Diagnosis

- Lichen planus
- Drug eruption
- Dermatomyositis
- Graft-versus-host disease
- Viral exanthem

Takeaway Essentials

Clinically Relevant Pearls

- Incidence and natural history are unknown due to rare presentation on the vulva.
- Up to one-third of discoid lupus erythematous cases may progress to systemic lupus.
- Scarring has been seen.

(continued)

(continued)

Pathology Interpretation Pearls

- Histology may vary depending on the form of lupus erythematosus.
- Diagnosis may require DIF and serologic testing.

Acanthotic Pattern

Lichen Simplex Chronicus

Clinical Features

Lichen simplex chronicus (LSC) of the vulva is a nonspecific cutaneous reaction pattern rather than a disease sui generis and may be induced by any condition that causes the patient to rub or scratch [98]. Not limited to the vulva, any body site may be affected. LSC of the scrotum is considered to be the male equivalent.

Lesions may be solitary or multiple, including bilateral. They are characterized as erythematous, lichenified papules to plaques, with scale that is variable due to vulvar moisture. LSC affects the labia majora, mons pubis, and perianal regions, rarely extending to other areas of glabrous/mucosal skin, such as the labia minora and vaginal introitus (Fig. 3.23).

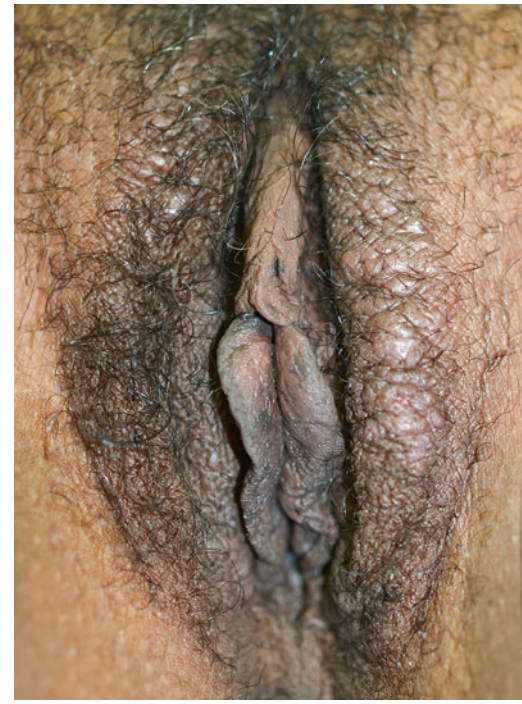


Fig. 3.23 Lichen simplex chronicus, more pronounced on the left side of labia majora with shiny, flat-topped plaque (lichenification)

The etiology of LSC is diverse. It may be primary (idiopathic) or secondary to a variety of causes. The primary form is considered by many to be a vulvar presentation of an atopic/neurodermatitis. In this regard, most patients report a history of an atopic disposition including asthma, allergic rhinitis, and eczema. Since all lesions are self-induced, when occurring on normal-appearing skin, LSC has been nicknamed "the itch that rashes" [99]. The secondary form may be caused by essentially anything that may be pruritic, such as local infections (especially dermatophytosis or candidiasis), underlying neoplasia, or a wide variety of other dermatitides [100, 101]. Regardless of etiology, pruritus is the common denominator in all patients [102].

LSC may be a diagnostic problem for both the clinician and the pathologist. If LSC is suspected, the clinician should first attempt to stop the patient from scratching in order to prevent the obscuring of an underlying lesion by the LSC. Clinically, this may be a challenge, as patients often scratch while in their sleep. Left untreated, the chronic pruritus may become severe enough to induce excoriations, large ulcerations, and even suicidal ideation. Interestingly, the scratching of lesions of LSC can also cause intense pleasure, described by some as erotic in nature, and rare patients have been known to miss the pruritus after treatment. Regardless of cause, it is well known that the more the patient rubs or scratches, the more the individual lesions itch, setting the patient up for a what is known as a vicious "itch-scratch cycle." In the primary/idiopathic form, also termed "neurodermatitis," it is speculated that some neurologic impulse, whether central or peripheral, induces the pruritus, whereby, the physical trauma of the skin may cause cutaneous nerves to become more sensitized, exacerbating the pruritus.

In addition to the avoidance of scratching, topical medications, such as high-potency steroids or calcineurin inhibitors, are used in therapy. Many clinicians caution patients that they

may not be cured of LSC, as it often remits and recurs, but that with careful management, their symptoms may be greatly controlled.

Despite careful history and examination, due to the nonspecific or poorly developed features, many cases are difficult to diagnose by clinical means alone. In these cases, biopsy is often required. It may be essential to stop therapy for a period of weeks before biopsy to prevent the masking of any underlying pathologic process.

Histopathology

LSC of the vulva is histologically similar to that on seen other body sites. All cases exhibit some degree of epidermal change to include hyperkeratosis, hypergranulosis, and irregular acanthosis. Nearly all cases exhibit fibrosis of the papillary dermis, the characteristic pattern of which is vertically oriented collagen fibers within the dermal papillae. Likewise, prominent dermal capillaries also show a vertical orientation. In our experience, this vertical pattern of fibrosis and vascularity are the best clues to the diagnosis.

Parakeratosis is a variable finding and should encourage the pathologist to carefully examine the tissue sections for foci of spongiosis, and when accompanied by a dermal infiltrate of eosinophils, the etiology of a chronic contact dermatitis should be suggested in the diagnostic report. A nonspecific chronic inflammation is often observed, but in cases with severe pruritus, erosions and ulcerations may be present, resulting in foci of necrotic debris and a neutrophilic infiltrate—the latter making identification of an underlying pathologic process even more problematic. In these instances, it behooves the pathologist to suggest to the clinician to re-biopsy non-ulcerated lesions if at all possible. It is also best to biopsy lesions that have not been treated with topical therapies as they may also mask the underlying changes (Figs. 3.24 and 3.25).

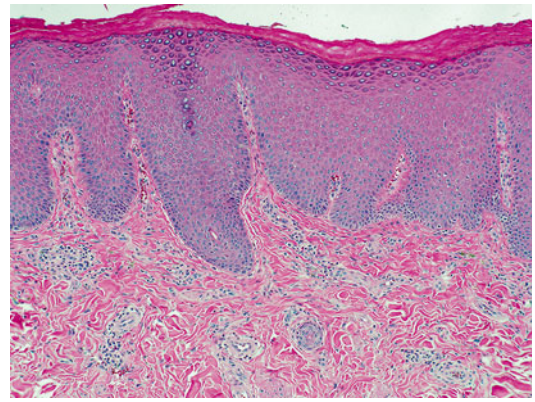


Fig. 3.24 Low power view of lichen simplex chronicus showing irregular epidermal hyperplasia, spanning the punch biopsy

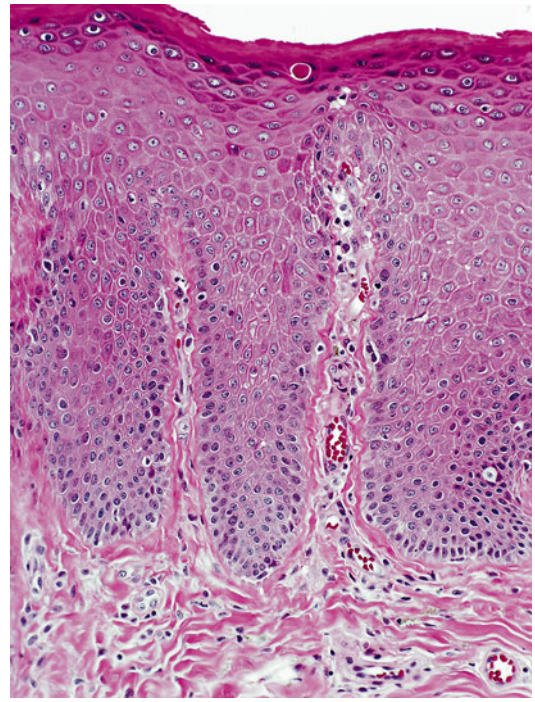


Fig. 3.25 High power view of lichen simplex chronicus. On the vulva, keratin layer may be less pronounced. This case demonstrates confluent orthokeratosis, focal hypergranulosis, vertically oriented collagen, and vessels in the papillary dermis and sparse chronic inflammation

Differential Diagnosis

Infectious processes (such as Candida and dermatophytosis), other skin disease (such as lichen sclerosus), and neoplasia (such as carcinoma and Paget disease) must be histologically excluded. A PAS stain should be performed to rule out fungal infection. Evaluation for epidermal atypia is also warranted, and this may be particularly difficult as some cases exhibit reactive atypia. Regarding this, some authors have sug-

gested that LSC may be a premalignant condition and a precursor to verrucous carcinoma [103]. Like these authors, we also believe that the atypia in some cases is of questionable importance, but at this time, there is no definite evidence that LSC is a precursor to malignancy. For the pathologist, it is often impossible to be certain that a biopsy sample is representative of the entire clinical lesion, so when atypia is identified, we do suggest that it be mentioned in the diagnostic report to ensure that the patient receive careful clinical follow-up. Psoriasis can often be excluded due to the irregular acanthosis, non-confluent parakeratosis, lack of neutrophilic epidermal abscesses, and a lack of thinning of the suprapapillary epidermal plates. Human papillomavirus (HPV)-related lesions usually show some degree of koilocytosis that is not seen in LSC, but distinction can be impossible, especially in biopsies of irritated lesions. Unlike lichen sclerosus, there is more acanthosis and an absence of hyalinization (sclerosis) of the papillary dermis. Squamous cell carcinoma in situ may exhibit subtle atypia, so careful examination of multiple levels may be required for definite diagnosis. Moreover, all forms of neoplasia must be excluded, such as Paget disease which may be exceedingly subtle; in any suspected case, we suggest a battery of immunohistochemical stains be considered to detect malignant Paget cells within the epidermis, e.g., cytokeratin (CK) 7 and CAM 5.2 will detect the majority of cases. Pseudoepitheliomatous hyperplasia (PEH) is often superimposed on LSC, which may make distinction from squamous cell carcinoma more difficult. In this regard, PEH may exhibit slight cytologic atypia and mitotic activity, and clues to PEH are derivation from the adnexal epithelium and a sharply pointed base in the upper to mid-reticular dermis [104].

Summary

Clinical Presentation

- Lichenified papules and plaques
- Most common on labia majora and mons
- Severe cases accompanied by linear erosions/ulcerations

Histologic Features

- Irregular epidermal acanthosis
- Variable spongiosis associated with chronic inflammation and/or eosinophilic infiltrate
- Vertically oriented papillary dermal collagen fibers and capillaries

Differential Diagnosis

- Idiopathic ("neurodermatitis")
- Chronic contact dermatitis
- Neoplasia with secondary irritation
- Any pruritic dermatitis with secondary irritation

Takeaway Essentials

Clinically Relevant Pearls

- Since it may mimic carcinoma, any suspicious areas, e.g., ulcerated, nonhealing, or enlarging areas, should be biopsied.
- Ulcerated or excoriated areas should be avoided when biopsy takes place; biopsy at the edge of an ulcer is more often helpful than the ulcer bed.
- In rare cases, it may be necessary to stop all therapies for a period of time before taking biopsy to prevent masking of underlying disease processes.

Pathology Interpretation Pearls

- Irregular epidermal acanthosis and vertically oriented collagen fibers and capillaries are the best clues to the diagnosis.
- Fungal stains, such as PAS, should be performed to exclude occult dermatophyte or *Candida*.

Psoriasis

Clinical Features

Psoriasis is a chronic skin condition that affects between 1 % and 2 % of the US population [105]. Vulvar psoriasis accounts for about 5 % of patients presenting with persistent vulvar itching

Table 3.7 Comparison of features of female genital psoriasis depending on location

Location	Mons pubis	Labia majora	Intertriginous
Clinical	Silvery scale	Variable scale	Often no scale
	Raised plaques	Plaques to patches	Large patches
	Salmon-colored	Salmon-colored to erythematous	Bright red and shiny ("glazed")
	Surface typically intact	Erosions, fissures, and ulceration possible	Erosions, fissures, and ulceration common
Histopathology	Marked epidermal hyperplasia	Variable epidermal hyperplasia	Epidermal hyperplasia may be absent
	Confluent parakeratosis	Minimal to absent parakeratosis	Minimal to absent parakeratosis
	Spongiform pustules in cornified layers and within epidermis	Pustulation may be absent in early lesions	Pustulation may be absent in early lesions
	Thinning of suprapapillary dermal plates common	Thinning of suprapapillary dermal plates may be absent	Thinning of suprapapillary dermal plates may be absent
	Spongiosis rare	Variable spongiosis (more common in early lesions)	Spongiosis not uncommon
	Erosions rare	Erosions not uncommon	Erosions common

or burning [106]. It may be associated with psoriasis elsewhere on the body or be isolated to the vulva. The incidence of vulvar psoriasis in patients with psoriasis at other body sites is still unknown. In patients with inverse psoriasis, a clinical variant involving skin folds, up to 79 % of patients have genital involvement [107]. Vulvar psoriasis is most common in adults but can also affect prepubertal girls [108]. Despite its prevalence, one reviewer could identify only eight textbooks and five articles in peer-reviewed journals specifically referring to a "definition and description" of vulvar psoriasis [109].

Clinically, vulvar lesions of psoriasis typically appear as erythematous unilateral or bilateral plaques affecting the labia majora, mons, and perianal areas. The clinical appearance of vulvar psoriasis differs from classic psoriasis at other body sites (Table 3.7): notably moisture, warmth, and friction may cause fissuring and maceration while the classic silvery scale of psoriatic lesions is often absent. Thus, the classic form is mainly limited to the mons pubis (Fig. 3.26). In the flexures such as the genitocrural folds and on the labia, scale is often absent, and the lesions are more often red, flat, and variably eroded or ulcerated (Fig. 3.27). Symptoms include itching, burning, pain, and rawness, with decreased quality of life in severe cases. The gluteal cleft may be the only site affected other than the vulva and may be a clue to the diagnosis.

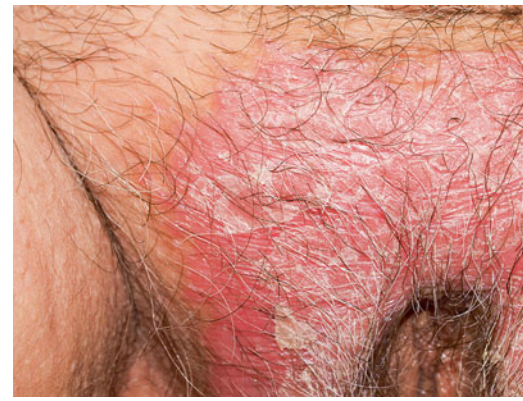


Fig. 3.26 Psoriasis on mons pubis, showing classic features of salmon-colored plaque with silvery scale

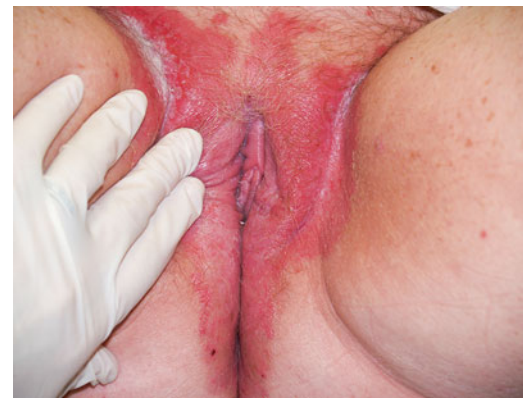


Fig. 3.27 Psoriasis involving the labia majora shows a plaque variable scale, but intertriginous lesions show brightly erythematous patches with no scale and focal erosions with exudates

There is no unifying theory as to the etiology of psoriasis. Current evidence supports a complex interplay of polygenetic, autoimmune, and environmental factors. As an example, late-onset psoriasis (after age 50) is not associated with the *PSORS1* gene, while acute onset (typically in adolescence) is strongly associated with this gene [110]. Immunity in psoriasis has been studied extensively, and multiple cytokines (especially interferons) and inflammatory cells (especially T cells) are involved in both the initiation and continuation of the disease. Trauma may induce psoriasis on uninvolved skin in approximately 25 % of cases ("Koebnerization") [111]. Thus, the pruritus often accompanying vulvar cases, causing rubbing/scratching, may aggravate the condition.

Regardless of the pathogenic mechanism, the final common pathway is the accelerated cell cycling of the epidermis (hyperproliferation) and inflammation, resulting in the visible papulosquamous eruption.

Cutaneous psoriasis may be associated with musculoskeletal and cardiovascular disease [112]. There is a risk of secondary infection in cases with excoriation and ulceration. Thus prognosis and therapy depend on associated comorbidities. Despite a wide variety of treatment options, psoriasis remains a chronic, noncurable condition. Careful treatment and follow-up are required due to the potentially severe impact on a patient's psychological, physical, and social well-being [113].

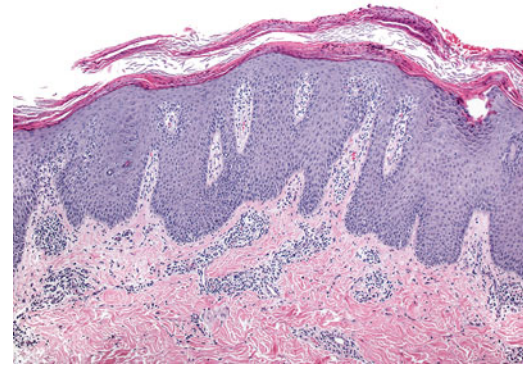


Fig. 3.28 Psoriasis on mons pubis, showing classic features of epidermal hyperplasia, loss of the granular layer, confluent parakeratosis, and sparse dermal chronic inflammation

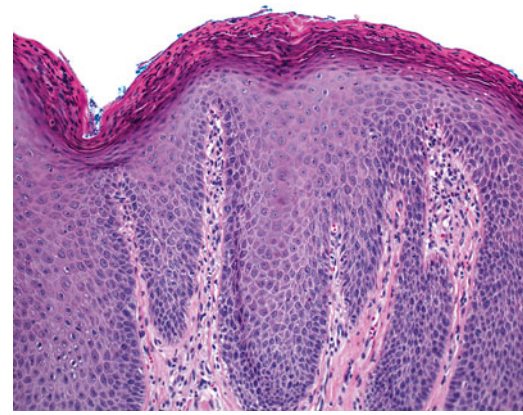


Fig. 3.29 High power view of psoriasis on mons pubis with neutrophilic abscesses in the cornified layer, proliferation of papillary dermal vessels, and thinning of the suprapapillary epidermal plates

Histopathology

Early lesions of psoriasis are often nondiagnostic, and the histologic changes are limited to nonspecific dermal chronic inflammation and prominent capillaries. In developed lesions, the principal histologic features include variable epidermal hyperplasia with elongation of the rete ridges in a regular, club-shaped pattern; alternatively, there may be thinning of the suprapapillary epidermal plates, which may be a diagnostic clue in poorly developed cases (Figs. 3.28 and 3.29). Premature keratinization of the epidermis results confluent parakeratosis and diminishment of the granular layer. Dilated and tortuous blood vessels are present in the papillary dermis. Neutrophilic infiltrates and/or variable chronic inflammation may be present in the upper dermis. In the epidermis, neutrophils may form microabscesses in the stratum corneum (Munro microabscesses) or within the viable layers of the epidermis (spongiform pustules of Kogoj). Eosinophils are very rare, except in cases of childhood or eruptive psoriasis. Spongiosis is also rare except in very early lesions or lesions from intertriginous areas. In advanced lesions, epidermal changes mimic lichen simplex

chronicus. It should be noted that on the vulva (similar to the penis), there may be less epidermal hyperplasia than is seen at other body sites. This is more pronounced in the intertriginous areas, where neutrophilic abscesses and epidermal hyperplasia may be minimal and spongiosis evident. (See *Vignette 1* at the end of this chapter.)

Differential Diagnosis

Ultimately, psoriasis on the vulva and perianal area may have differing clinical and histologic features than lesions elsewhere, thus complicating the differential diagnosis (Table 3.8). The presence of neutrophils may raise suspicion of dermatophyte or Candida infection, necessitating fungal staining (e.g., PAS or GMS). If spongiosis is present, a chronic eczematous dermatitis may be difficult to exclude: prominent spongiosis or the presence of significant eosinophils favors an eczematous process. Lichen simplex chronicus (LSC) shares the feature of epidermal acanthosis, but LSC exhibits more irregular acanthosis with hypergranulosis, non-confluent parakeratosis, vertically oriented collagen fibers, and capillaries in the papillary dermis with the absence of neutrophilic pustules. In poorly developed lesions, distinction between the two may be impossible. Moreover, as vulvar psoriasis is frequently pruritic, LSC may become superimposed. (See *Vignette 2* at the end of this chapter.) Accurate diagnosis may rely on multiple biopsies with careful clinicopathologic correlation. Reiter's disease is thought by many to be a variant of psoriasis and is discussed later in this chapter. Reiter's syndrome may be indistinguishable by histologic grounds alone.

Summary

Clinical Presentation

- Erythematous, glazed plaques with less silvery scale than nongenital psoriasis
- Erosions, fissures, and ulceration may be present.
- Mons pubis exhibits features more similar to nongenital sites.

Histologic Features

- Epidermal hyperplasia with club-shaped rete ridges
- Thinning of suprapapillary epidermal plates
- Confluent parakeratosis and diminishment of granular layer
- Munro microabscesses and spongiform pustules of Kogoj
- Dilated, tortuous papillary dermal vessels
- Variable chronic inflammation of upper dermis

Differential Diagnosis

- Chronic eczema
- Chronic contact dermatitis
- Lichen simplex chronicus
- Fungal infection with dermatophyte or Candida
- Reiter's syndrome

Takeaway Essentials

Clinically Relevant Pearls

- Lesions on the vulva differ from those on mons pubis and nongenital sites; mons shows classic salmon-colored patches with silvery scale, while inguinal creases and labia show brightly erythematous patches without scale and variable erosion and ulceration.
- Involvement of gluteal cleft may be only site affected other than the vulva and may be a clue to the diagnosis.

Pathology interpretation pearls

- Confluent parakeratosis, regular acanthosis, epidermal microabscesses, and thinning of the suprapapillary dermal plates are the most specific histologic clues.

Table 3.8 Differential diagnosis for psoriasis on the vulva and perianal area, which may have differing clinical and histologic features than lesions elsewhere, thus complicating the differential diagnosis

	Fixed drug eruption	Erythema multiforme	Stevens-Johnson syndrome/ toxic epidermal necrolysis	Graft-versus-host disease	Lupus erythematosus
Vulvar location	Hair bearing skin and mucosa	Hair bearing skin and mucosa	Hair bearing skin and mucosa	Hair bearing skin and mucosa	Hair bearing skin
Classic clinical presentation	Well demarcated erythematous plaque	Vulvar is affected as part of a diffuse disease	Vulva is affected as part of a diffuse disease	Vulva erythematous patches, fissures, thickened mucosa, vestibulitis, and narrowed introitus	Variable from erythema to plaques with follicular plugging, hair loss, erosions and ulcers. May involve hair-bearing skin, modified mucous membranes and vagina
	Mucosal lesions with irregular surface erosions	Painful erythema leading to blister and ulcer (<10 % body surface detachment)	Painful erythema leading to blister and ulcer (10–30 % body surface detachment)	Vulva and vagina stenosis are end stage complications	
	Frequent absence of post-inflammatory hyperpigmentation	Typical and atypical targetoid lesions	Atypical targetoid lesions Mucous membrane and internal organ involvement		
Presentation	Acute/recurrent	Acute/recurrent	Acute	Acute/chronic	Acute/chronic
Stratum corneum	Basket-weave	Basket-weave	Basket-weave	Hyperparakeratosis	Hyperkeratosis and follicular plugging
Epidermis	Spongiosis	Vacuolar alteration of basal layer	Vacuolar alteration of basal layer	Vacuolar alteration of basal layer	Vacuolar alteration of basal layer
		Dyskeratotic cells	Dyskeratotic cells	Dyskeratotic cells	Thickening of basement membrane
		Subepidermal blister with partial to full thickness necrosis	Subepidermal blister with full thickness necrosis	Formation of subepidermal blister Epidermal atrophy and hypergranulosis can be seen	Variable follicular plugging
Superficial dermis	Perivascular lymphocytic infiltrate with few neutrophils and eosinophils	Perivascular lymphocytic infiltrate with occasionally eosinophils	Perivascular lymphocytic infiltrate with occasionally eosinophils	Perivascular or band-like lymphocytic and histiocytic infiltrate, with occasionally eosinophils	Perivascular lymphocytes and variable dermal mucin Periadnexal inflammation Eosinophils in drug-related cases
Deep dermis	Perivascular chronic inflammation	Minimal	Minimal	Minimal	Chronic inflammation may extend into subcutis
Hallmark	Recurrence after challenge of offender drug	Targetoid lesions	Mucosal involvement	Clinical history of transplantation	Localized or part of systemic disease

Reiter's Syndrome

Clinical Features

Reiter's syndrome is typically a disease of young adult males that presents with the triad of arthritis, conjunctivitis, and urethritis. Reports in females are less common [114], and vulvar involvement by Reiter's syndrome is even more rare with only five cases reported [115–118].

In both sexes, skin may also be involved—appearing as crusted, erythematous papules and plaques on the scalp, soles of the feet, buttocks, and extremities. Pustular lesions often resemble pustular psoriasis; this cutaneous manifestation of Reiter's syndrome has been termed "keratoderma blenorrhagica."

In the few cases of reported vulvar involvement, clinical appearance is quite variable with linear ulcers, verrucous lesions, pustules, red/crusted plaques, and circinate erosions. Other clinical features also may be variable. For example, in the case reported by Edwards and Hansen [115], the mucocutaneous features preceded the arthritis by 4 years. The same authors demonstrated involvement of both the cutaneous and mucosal surfaces with plaques on the labia majora and papules on the labia minora.

The precise etiology of Reiter's disease is unknown. It is evident that a genetic predisposition (most commonly HLA-B27 positivity) in concert with an infectious process is predisposing. Genital infections by *Chlamydia*, *Ureaplasma*, *and Mycoplasma* and gastrointestinal infections by *Shigella*, *Yersinia*, and *Campylobacter* have been associated. An immune complex vasculitis induced by deposition of bacterial antigens in cutaneous vessels has been implicated, and *Chlamydia*-specific antigens have been identified in the cutaneous lesions [119].

Histopathology

Classic histology on nongenital skin is morphologically similar to pustular psoriasis, including psoriasiform epidermal hyperplasia with loss of granular layer, parakeratosis, and intraepidermal microabscesses (pustules). In the vulvar lesion reported by Lotery et al. [118], some areas of the biopsy also showed pseudoepitheliomatous hyperplasia and deep dermal microabscesses.

Differential Diagnosis

The primary consideration in the differential diagnosis is pustular psoriasis. As the histologic findings may be identical, without clinicopathologic correlation, distinction may be impossible. Complicating matters, some authors believe that Reiter's syndrome is actually a form of psoriasis.

Summary

Clinical Presentation

- Involvement of cutaneous and mucosal surfaces
- Erythematous and crusted papules and plaques
- Erosions and ulcers

Histologic Features

- Psoriasiform epidermal hyperplasia with pustules

Differential Diagnosis

- Pustular psoriasis

Takeaway Essentials

Clinically Relevant Pearls

- Exceedingly rare on vulva
- Erosions may be similar to those seen on the penis in men.

Pathology Interpretation Pearls

- May be impossible to distinguish from pustular psoriasis on histologic grounds alone

Case Vignettes

Vignette 1

Clinical history: The patient was a 65-year-old female with erythema of the vulva, inguinal creases, and abdominal pannus. In the flexural areas, the scale was less pronounced and demonstrated glazed erythema (Figs. 3.30 and 3.31). Patches and plaques with scale were also evident on upper thighs (Fig. 3.32). The patient was initially managed with antibiotics with worsening of lesions. All cultures were negative. A biopsy was performed to rule out pustular psoriasis.

Microscopic description: The initial biopsy (Fig. 3.33) revealed a slightly acanthotic epidermis with mild spongiosis. The granular layer was only focally attenuated with focal mild parakeratosis. Rare neutrophils were present in the stratum corneum. The dermis contained occasional lymphocytes and rare eosinophils. Five months later, a second biopsy (Fig. 3.34) was taken from the perineum. This biopsy demonstrated spongiosis, but showed classic spongiform pustules of Kogoj. A fungal stain was negative on both biopsies.

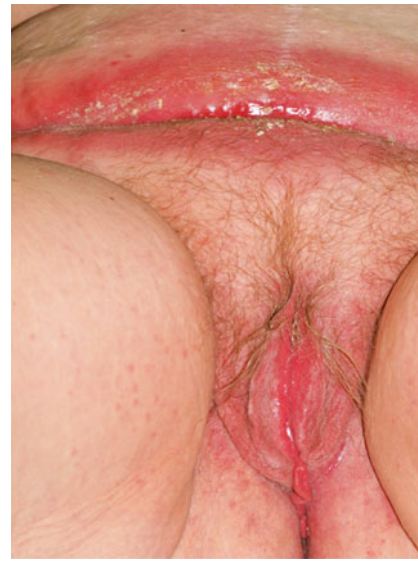


Fig. 3.30 Vignette 1. Erythematous plaques with flexures and folds

(continued)

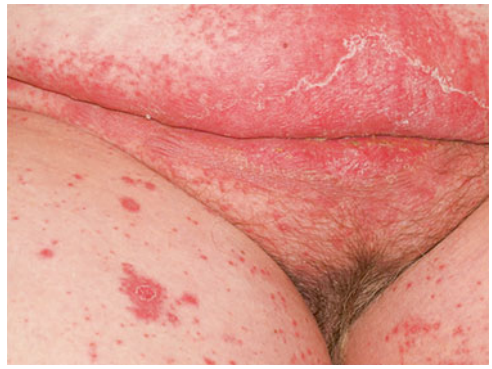


Fig. 3.31 Vignette 1. Some scale is noted on lesion of the lower abdomen

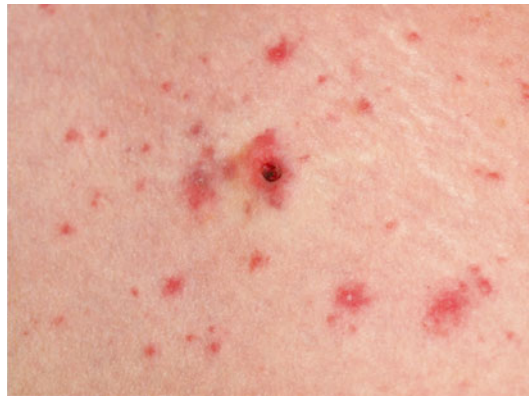


Fig. 3.32 Vignette 1. On upper legs, there are erythematous papules with focal pustulation

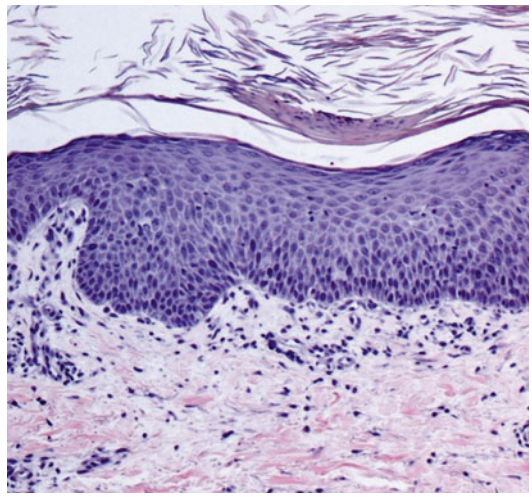


Fig. 3.33 Vignette 1. The initial biopsy showed a slightly acanthotic epidermis with mild spongiosis. The granular layer was only focally attenuated with focal mild parakeratosis with rare neutrophils and eosinophils

(continued)

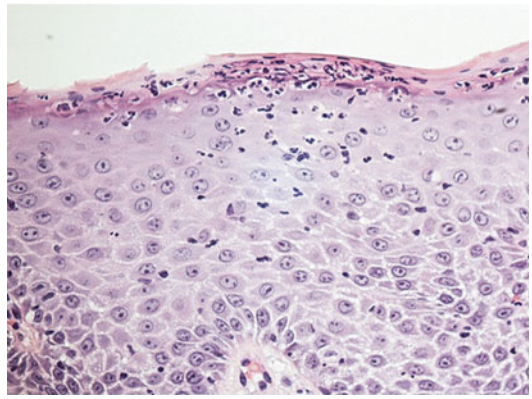


Fig. 3.34 Vignette 1. 5 months later, a second biopsy was taken from the perineum. This biopsy demonstrated spongiosis, but showed classic spongiform pustules of Kogoj

Diagnosis: Inverse psoriasis

Discussion: The diagnosis of inverse psoriasis can be difficult because classic architectural features of psoriasis such as regular psoriasiform hyperplasia, loss of granular layer, and confluent parakeratosis may be absent in the vulva, especially in flexural areas. Furthermore, spongiosis and eosinophils may be seen, leading to a confusing picture to the unsuspecting pathologist. This patient responded to topical corticosteroids.

Vignette 2

Clinical history: The patient was a 35-year-old female with a history of lichen sclerosus and psoriasis. She presented with white, lichenified plaques on bilateral labia majora and minora as well as inguinal creases, clinically suspicious for lichen simplex chronicus (Fig. 3.35).

Microscopic description: A biopsy was performed (Fig. 3.36), revealing regular, psoriasiform hyperplasia and attenuation of the granular layer of the epidermis. Focally, neutrophils were noted in the superficial stratum spinosum and in the stratum corneum (Fig. 3.37). Parakeratosis is not prominent. For completeness, a PAS stain for fungus was obtained, revealing hyphae within the cornified cells (Figs. 3.38).

(continued)

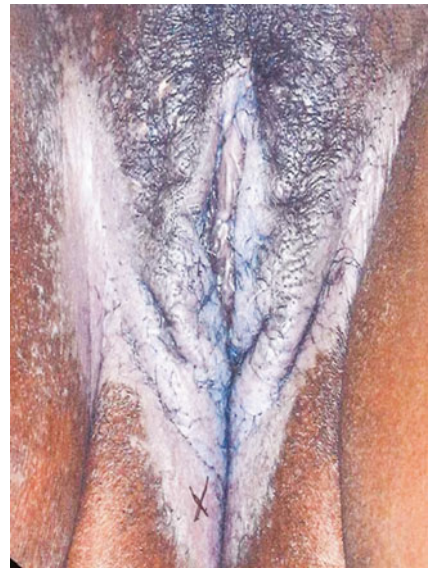


Fig. 3.35 Vignette 2. White, lichenified plaques on bilateral labia majora and minora as well as inguinal creases, clinically suspicious for lichen simplex chronicus, are seen

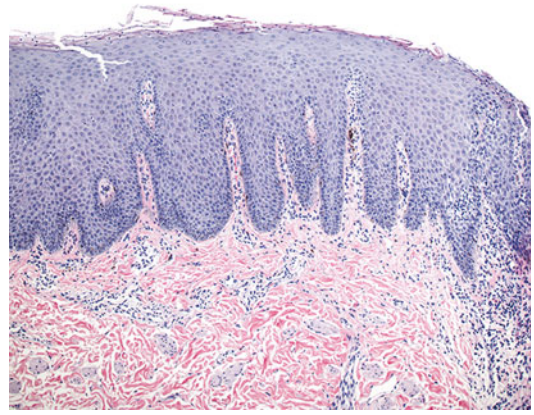


Fig. 3.36 Vignette 2. A biopsy was performed revealing regular, psoriasiform hyperplasia and attenuation of the granular layer of the epidermis. Not uncommon in the vulva, parakeratosis is less pronounced than psoriasis seen at other body sites

(continued)

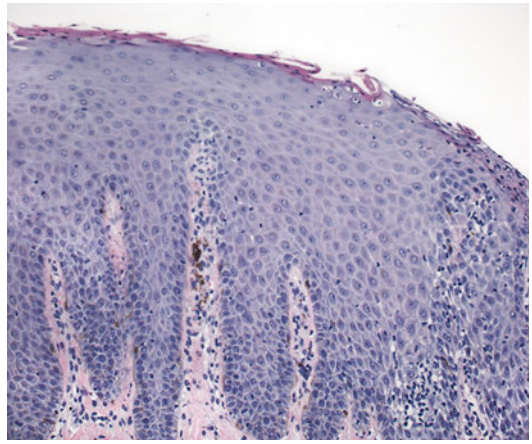


Fig. 3.37 Vignette 2. Focally, neutrophils were noted in the superficial stratum spinosum and in the stratum corneum

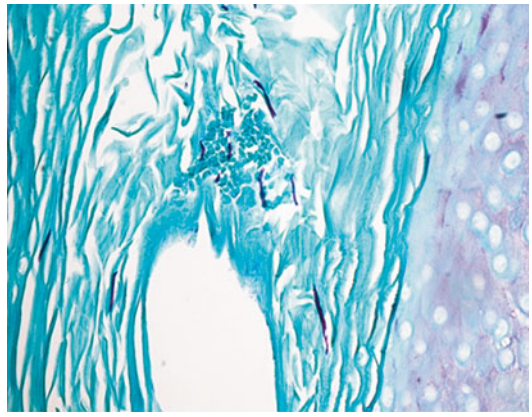


Fig. 3.38 Vignette 2. A PAS stain for fungus was obtained, revealing hyphae

Diagnosis: Psoriasis and superimposed dermatophyte infection

Discussion: This case illustrates that two processes may be responsible for the histologic changes. This is common in the vulva. In this case, psoriasis and dermatophyte infection are present within the same lesion. Without identification of the fungus, this patient's clinical lesion would have likely worsened despite the compelling histologic findings of psoriasis and appropriate therapy for psoriasis. Not uncommon, parakeratosis is less pronounced in vulvar psoriasis than nongenital psoriasis.

Vignette 3

Clinical history: The patient was a 50-year-old female who presented with the chief complaint of itching, burning, and "rawness" of the vulva (Fig. 3.39). Complete history revealed use of over-the-counter topical agents, e.g., Vagisil.

Microscopic description: A biopsy showed parakeratosis, mild epidermal spongiosis, and a lymphoeosinophilic infiltrate in the superficial dermis (Fig. 3.40). Spongiosis is seen focally in this biopsy (Fig. 3.41).

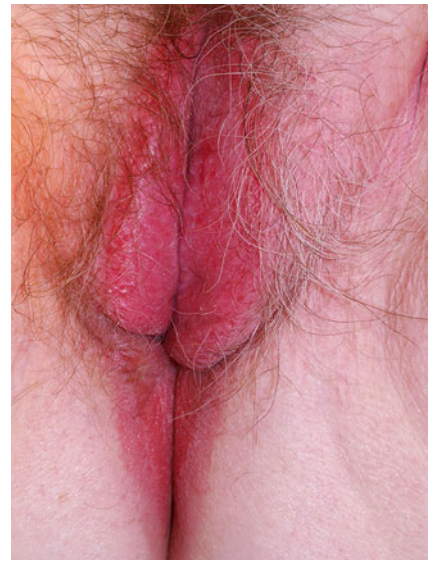


Fig. 3.39 Vignette 3. The sharply demarcated erythema correlates with contact points with the allergic agent

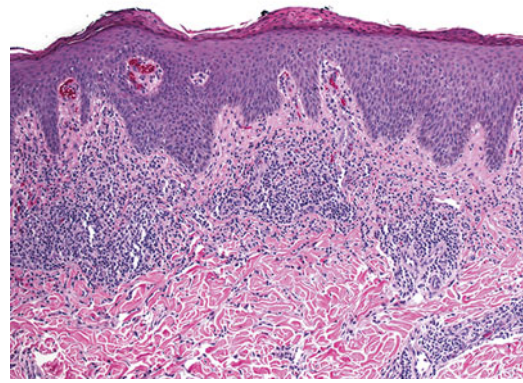


Fig. 3.40 Vignette 3. Low power view reveals focal parakeratosis, mild spongiosis, and an inflammatory infiltrate

(continued)

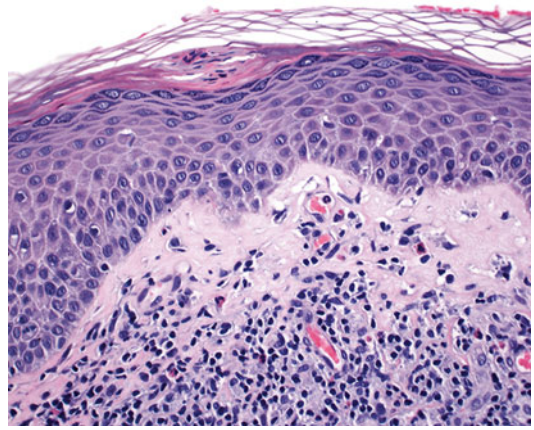


Fig. 3.41 Vignette 3. Higher power reveals a mound of parakeratosis, consistent with the subacute nature of the process. The inflammatory infiltrate in the dermis is composed of lymphocytes and eosinophils

Diagnosis: Allergic contact dermatitis

Discussion: Many patients use topical therapies prior to being seen for vulvar disorders. Although specifically marketed for the female genitalia, several of these may cause pronounced irritant or allergic contact dermatitis or both when used inappropriately. This case illustrates the contact dermatitis induced by using over-the-counter therapies. Parakeratosis surmounting a slightly spongiotic dermatitis heralds that the lesion is early, also called by some pathologists as "subacute" when these changes are noted.

Abbreviations

ACD	Allergic contact dermatitis
APF	Amicrobial pustulosis of the folds
DLE	Discoid lupus erythematous
EM	Erythema multiforme
GVHD	Graft-versus-host disease
HSV	Herpes simplex virus
ICD	Irritant contact dermatitis
LE	Lupus erythematous
LSC	Lichen simplex chronicus
PEH	Pseudoepitheliomatous hyperplasia
SJS	Stevens-Johnson syndrome
SLE	Systemic lupus erythematous
TEN	Toxic epidermal necrolysis

References

1. Nyirjesy P, Peyton C, Weitz MV, Mathew L, Culhane JF. Causes of chronic vaginitis: analysis of a prospective database of affected women. Obstet Gynecol. 2006;108(5):1185–91.
2. Crone AM, Stewart EJ, Wojnarowska F, Powell SM. Aetiological factors in vulvar dermatitis. J Eur Acad Dermatol Venereol. 2000;14(3):181–6.
3. Brenan JA, Dennerstein GJ, Sfameni SF, Drinkwater P, Marin G, Scurry JP. Evaluation of patch testing in patients with chronic vulvar symptoms. Australas J Dermatol. 1996;37(1):40–3.
4. Schlosser BJ. Contact dermatitis of the vulva. Dermatol Clin. 2010;28(4):697–706.
5. Amankwah Y, Haefner H. Vulvar edema. Dermatol Clin. 2010;28(4):765–77.
6. Garrido-Ruiz MC, Rosales B, Luis Rodríguez-Peralto J. Vulvar pseudoverrucous papules and nod-

ules secondary to a urethral–vaginal fistula. Am J Dermatopathol. 2011;33(4):410–2.
7. Fujita M, Ohno S, Danno K, Miyachi Y. Two cases of diaper area granuloma of the adult. J Dermatol. 1991;18(11):671–5.
8. Maekawa Y, Sakazaki Y, Hayashibara T. Diaper area granuloma of the aged. Arch Dermatol. 1978;114(3): 382–3.
9. Dytoc MT, Fiorillo L, Liao J, Krol AL. Granuloma gluteale adultorum associated with use of topical benzocaine preparations: case report and literature review. J Cutan Med Surg. 2002;6(3):221–5.
10. Coppo P, Salomone R. Pseudoverrucous papules: an aspect of incontinence in children. J Eur Acad Dermatol Venereol. 2002;16(4):409–10.
11. Rodríguez Cano L, García-Patos Briones V, Pedragosa Jové R, Castells Rodellas A. Perianal pseudoverrucous papules and nodules after surgery for Hirschsprung disease. J Pediatr. 1994;125(6 Pt 1):914–6.
12. Goldberg NS, Esterly NB, Rothman KF, Fallon JD, Cropley TG, Szaniawski W, et al. Perianal pseudoverrucous papules and nodules in children. Arch Dermatol. 1992;128(2):240–2.
13. Bergman B, Knutson F, Lincoln K, Löwhagen GB, Mobacken H, Wåhlén P. Chronic papillomatous dermatitis as a peristomal complication in conduit urinary diversion. Scand J Urol Nephrol. 1979;13(2):201–4.
14. Borglund E, Nordström G, Nyman CR. Classification of peristomal skin changes in patients with urostomy. J Am Acad Dermatol. 1988;19(4):623–8.
15. Hara M, Watanabe M, Tagami H. Jacquet erosive diaper dermatitis in a young girl with urinary incontinence. Pediatr Dermatol. 1991;8(2):160–1.
16. Virgili A, Corazza M, Califano A. Diaper dermatitis in an adult. A case of erythema papuloerosive of Sevestre and Jacquet. J Reprod Med. 1998;43(11): 949–51.
17. Robson KJ, Maughan JA, Purcell SD, Petersen MJ, Haefner HK, Lowe L. Erosive papulonodular dermatosis associated with topical benzocaine: a report of two cases and evidence that granuloma gluteale, pseudoverrucous papules, and Jacquet's erosive dermatitis are a disease spectrum. J Am Acad Dermatol. 2006;55(5 Suppl):S74–80.
18. Marren P, Wojnarowska F, Powell S. Allergic contact dermatitis and vulvar dermatoses. Br J Dermatol. 1992;126(1):52–6.
19. Lewis FM, Shah M, Gawkrodger DJ. Contact sensitivity in pruritus vulvae: patch test results and clinical outcome. Am J Contact Dermat. 1997;8(3):137–40.
20. Utaş S, Ferahbaş A, Yildiz S. Patients with vulval pruritus: patch test results. Contact Dermat. 2008; 58(5):296–8.
21. Haverhoek E, Reid C, Gordon L, Marshman G, Wood J, Selva-Nayagam P. Prospective study of patch testing in patients with vulval pruritus. Australas J Dermatol. 2008;49(2):80–5.
22. Trager JDK. What's your diagnosis? Acute vulvar erythema, edema, and pruritus in a young woman. J Pediatr Adolesc Gynecol. 2005;18(4):275–80.
23. Warner N, Salvaggio HL, Zaenglein AL. JAAD grand rounds quiz. An erosive vulvar rash. J Am Acad Dermatol. 2013;68(2):350–2.
24. Virgili A, Bacilieri S, Corazza M. Evaluation of contact sensitization in vulvar lichen simplex chronicus. A proposal for a battery of selected allergens. J Reprod Med. 2003;48(1):33–6.
25. Bowen AR, Vester A, Marsden L, Florell SR, Sharp H, Summers P. The role of vulvar skin biopsy in the evaluation of chronic vulvar pain. Am J Obstet Gynecol. 2008;199(5):467.e1–6.
26. O'Hare PM, Sherertz EF. Vulvodynia: a dermatologist's perspective with emphasis on an irritant contact dermatitis component. J Womens Health Gend Based Med. 2000;9(5):565–9.
27. Farage MA, Miller KW, Berardesca E, Maibach HI. Incontinence in the aged: contact dermatitis and other cutaneous consequences. Contact Dermat. 2007;57(4):211–7.
28. Beecker J. Therapeutic principles in vulvovaginal dermatology. Dermatol Clin. 2010;28(4):639–48.
29. O'Gorman SM, Hara M, Torgerson RR, Watanabe M, Tagami H. Allergic contact dermatitis of the vulva. Dermatitis. 2013;24(2):64–72.
30. Schnuch A, Westphal G, Mössner R, Uter W, Reich K. Genetic factors in contact allergy–review and future goals. Contact Dermat. 2011;64(1):2–23.
31. Visser MJ, Landeck L, Campbell LE, McLean WHI, Weidinger S, Calkoen F, et al. Impact of atopic dermatitis and loss-of-function mutations in the filaggrin gene on the development of occupational irritant contact dermatitis. Br J Dermatol. 2013;168(2):326–32.
32. Wakashin K. Sanitary napkin contact dermatitis of the vulva: location-dependent differences in skin surface conditions may play a role in negative patch test results. J Dermatol. 2007;34(12):834–7.
33. Farage M, Maibach HI. The vulvar epithelium differs from the skin: implications for cutaneous testing to address topical vulvar exposures. Contact Dermat. 2004;51(4):201–9.
34. Burrows LJ, Shaw HA, Goldstein AT. The vulvar dermatoses. J Sex Med. 2008;5(2):276–83.
35. Willis CM, Young E, Brandon DR, Wilkinson JD. Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. Br J Dermatol. 1986;115(3):305–16.
36. Kanerva L. Electron microscopic observations of dyskeratosis, apoptosis, colloid bodies and fibrillar degeneration after skin irritation with dithranol. J Cutan Pathol. 1990;17(1):37–44.
37. Willis CM, Stephens CJ, Wilkinson JD. Epidermal damage induced by irritants in man: a light and electron microscopic study. J Invest Dermatol. 1989; 93(5):695–9.
38. Danby CS, Margesson LJ. Approach to the diagnosis and treatment of vulvar pain. Dermatol Ther. 2010;23(5):485–504.
39. Kiyohara T, Satoh S, Kumakiri M. Eosinophilic spongiosis in vulvar lichen sclerosus. J Dermatol. 2013;40(2):148–9.

40. Carlson J, Lamb P, Malfetano J, Ambros R, Mihm MJ. Clinicopathologic comparison of vulvar and extragenital lichen sclerosus: histologic variants, evolving lesions, and etiology of 141 cases. Mod Pathol. 1998;11(9):844–54.
41. Ruiz E, Deng JS, Abell EA. Eosinophilic spongiosis: a clinical, histologic, and immunopathologic study. J Am Acad Dermatol. 1994;30(6):973–6.
42. Crotty C, Pittelkow M, Muller SA. Eosinophilic spongiosis: a clinicopathologic review of seventy-one cases. J Am Acad Dermatol. 1983;8(3):337–43.
43. Crickx B, Diego ML, Guillevin L, Picard C, Grossin M. Pustulose amicrobienne et lupus érythémateux systémique. Communication no. 11. Journées Dermatologiques de Paris. París; 1991.
44. Márquez-Balbás G, Iglesias M, Herrera-Acosta E, Vidal-Olmo I, Guilabert A, Mascaró-Galy JM, et al. Amicrobial pustulosis of the folds: report of a new case and review of the literature. Actas Dermo-Sifiliográficas (Engl Ed). 2009;100(8):710–4. Elsevier.
45. Lagrange S, Chosidow O, Piette JC, Wechsler B, Godeau P, Frances C. A peculiar form of amicrobial pustulosis of the folds associated with systemic lupus erythematosus and other auto-immune diseases. Lupus. 1997;6(6):514–20.
46. Marzano AV, Capsoni F, Berti E, Gasparini G, Bottelli S, Caputo R. Amicrobial pustular dermatosis of cutaneous folds associated with autoimmune disorders: a new entity? Dermatol (Basel). 1996; 193(2):88–93.
47. Bénéton N, Wolkenstein P, Bagot M, Cosnes A, Wechsler J, Roujeau JC, et al. Amicrobial pustulosis associated with autoimmune diseases: healing with zinc supplementation. Br J Dermatol. 2000;143(6): 1306–10.
48. Stefanidou MP, Kanavaros PE, Stefanaki KS, Tosca AD. Amicrobial pustulosis of the folds. A cutaneous manifestation associated with connective tissue disease. Dermatol (Basel). 1998;197(4):394–6.
49. Kuyama M, Fujimoto W, Kambara H, Egusa M, Saitoh M, Yamasaki O, et al. Amicrobial pustular dermatosis in two patients with immunological abnormalities. Clin Exp Dermatol. 2002;27(4):286–9.
50. Natsuga K, Sawamura D, Homma E, Nomura T, Abe M, Muramatsu R, et al. Amicrobial pustulosis associated with IgA nephropathy and Sjögren's syndrome. J Am Acad Dermatol. 2007;57(3):523–6.
51. Lim YL, Ng SK, Lian TY. Amicrobial pustulosis associated with autoimmune disease in a patient with Sjögren syndrome and IgA nephropathy. Clin Exp Dermatol. 2012;37(4):374–8.
52. López-Navarro N, Alcaide A, Gallego E, Herrera-Acosta E, Gallardo M, Bosch RJ, et al. Amicrobial pustulosis of the folds associated with Hashimoto's thyroiditis. Clin Exp Dermatol. 2009;34(8): e561–3.
53. Claeys A, Bessis D, Cheikhrouhou H, Pouaha J, Cuny J-F, Truchetet F. Amicrobial pustulosis of the folds revealing asymptomatic autoimmune thyroiditis. Eur J Dermatol. 2011;21(4):641–2.
54. Méndez-Flores S, Charli-Joseph Y, Saeb-Lima M, Orozco-Topete R, Fernández Sánchez M. Amicrobial pustulosis of the folds associated with autoimmune disorders: systemic lupus erythematosus case series and first report on the association with autoimmune hepatitis. Dermatology. 2013;226(1):1–4.
55. Okuyama R, Masu T, Kumasaka N, Aiba S, Tagami H. Amicrobial pustulosis of the folds affecting a young male without any accompanying autoimmune diseases. Dermatology. 2008;217(2):121–3.
56. Inui S, Azukizawa H, Asada H, Itami S. Amicrobial pustulosis with antinuclear antibodies and rheumatoid factor. Br J Dermatol. 2006;154(3):568–9.
57. Kerl K, Masouyé I, Lesavre P, Saurat J-H, Borradori L. A case of amicrobial pustulosis of the folds associated with neutrophilic gastrointestinal involvement in systemic lupus erythematosus. Dermatol (Basel). 2005;211(4):356–9.
58. Marzano AV, Ramoni S, Caputo R. Amicrobial pustulosis of the folds. Report of 6 cases and a literature review. Dermatology. 2008;216(4):305–11.
59. Marzano AV, Cugno M, Trevisan V, Lazzari R, Fanoni D, Berti E, et al. Inflammatory cells, cytokines and matrix metalloproteinases in amicrobial pustulosis of the folds and other neutrophilic dermatoses. Int J Immunopathol Pharmacol. 2011;24(2): 451–60.
60. Marzano AV, Ishak RS, Saibeni S, Crosti C, Meroni PL, Cugno M. Autoinflammatory skin disorders in inflammatory bowel diseases, pyoderma gangrenosum and Sweet's syndrome: a comprehensive review and disease classification criteria. Clin Rev Allergy Immunol. 2013;45(2):202–10.
61. Boms S, Gambichler T. Review of literature on amicrobial pustulosis of the folds associated with autoimmune disorders. Am J Clin Dermatol. 2006;7(6):369–74.
62. Pena-Robichaux V, Hasan A, McHargue C. Amicrobial pustulosis of the folds. J Rheumatol. 2013;40(7):1228–9.
63. Lipsker D, Saurat J-H. Neutrophilic cutaneous lupus erythematosus. At the edge between innate and acquired immunity? Dermatology. 2008; 216(4):283–6.
64. Antille C, Frei M, Sorg O, Tran C, Kaya G, Masouyé I, et al. Amicrobial pustulosis of the folds associated with auto-immune disorders. A case report with an analysis of cytokine expression profile in skin lesions of cutaneous neutrophilic lupus. Dermatology. 2008; 216(4):324–9.
65. Gambichler T, Boms S, Hochdorfer B, Altmeyer P, Kreuter A. Immunohistology of amicrobial pustulosis of the folds. Clin Exp Dermatol. 2007;32(2):155–8.
66. Lee HY, Pelivani N, Beltraminelli H, Hegyi I, Yawalkar N, Borradori L. Amicrobial pustulosis-like rash in a patient with Crohn's disease under anti-TNF-alpha blocker. Dermatology. 2011;222(4): 304–10.
67. George AO, Ogunbiyi AO. Fixed drug eruption and fixed drug-like eruption. Int J Dermatol. 2005;44(4): 349–50.

68. Fischer G. Vulvar fixed drug eruption. A report of 13 cases. J Reprod Med. 2007;52(2):81–6.
69. Drummond C, Fischer G. Vulval fixed drug eruption due to paracetamol. Australas J Dermatol. 2009; 50(2):118–20.
70. Choi HJ, Ku JK, Kim MY, Kang H, Cho SH, Kim HO, et al. Possible role of Fas/Fas ligand-mediated apoptosis in the pathogenesis of fixed drug eruption. Br J Dermatol. 2006;154(3):419–25.
71. Schofield JK, Tatnall FM, Leigh IM. Recurrent erythema multiforme: clinical features and treatment in a large series of patients. Br J Dermatol. 1993;128(5): 542–5.
72. Côté B, Wechsler J, Bastuji-Garin S, Assier H, Revuz J, Roujeau JC. Clinicopathologic correlation in erythema multiforme and Stevens-Johnson syndrome. Arch Dermatol. 1995;131(11):1268–72.
73. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. N Engl J Med. 1994;331(19): 1272–85.
74. Zohdi-Mofid M, Horn TD. Acrosyringeal concentration of necrotic keratinocytes in erythema multiforme: a clue to drug etiology. Clinicopathologic review of 29 cases. J Cutan Pathol. 1997;24(4):235–40.
75. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. Arch Dermatol. 1993;129(1):92–6.
76. Graham-Brown RA, Cochrane GW, Swinhoe JR, Sarkany I, Epsztejn LJ. Vaginal stenosis due to bullous erythema multiforme (Stevens-Johnson syndrome). Case report. Br J Obstet Gynaecol. 1981; 88(11):1156–7.
77. Bastuji-Garin S, Fouchard N, Bertocchi M, Roujeau JC, Revuz J, Wolkenstein P. SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol. 2000;115(2):149–53.
78. Paquet P, Paquet F, Saleh Al W, Reper P, Vanderkelen A, Piérard GE. Immunoregulatory effector cells in drug-induced toxic epidermal necrolysis. Am J Dermatopathol. 2000;22(5):413–7.
79. Quinn AM, Brown K, Bonish BK, Curry J, Gordon KB, Sinacore J, et al. Uncovering histologic criteria with prognostic significance in toxic epidermal necrolysis. Arch Dermatol. 2005;141(6):683–7.
80. Bonafe JL, Thibaut I, Hoff J. Introital adenosis associated with the Stevens-Johnson syndrome. Clin Exp Dermatol. 1990;15(5):356–7.
81. Spinelli S, Chiodi S, Costantini S, Van Lint MT, Raiola AM, Ravera GB, et al. Female genital tract graft-versus-host disease following allogeneic bone marrow transplantation. Haematologica. 2003;88(10): 1163–8.
82. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945–56.
83. Zantomio D, Grigg AP, MacGregor L, Panek-Hudson Y, Szer J, Ayton R. Female genital tract graft-versus-host disease: incidence, risk factors and recommendations for management. Bone Marrow Transplant. 2006;38(8):567–72.
84. Lara LADS, De Andrade JM, Mauad LMQ, Ferrarese SR, Marana HRC, Tiezzi DG, et al. Genital manifestation of graft-vs-host disease: a series of case reports. J Sex Med. 2010;7(9):3216–25.
85. Hymes SR, Farmer ER, Lewis PG, Tutschka PJ, Santos GW. Cutaneous graft-versus-host reaction: prognostic features seen by light microscopy. J Am Acad Dermatol. 1985;12(3):468–74.
86. Marra DE, McKee PH, Nghiem P. Tissue eosinophils and the perils of using skin biopsy specimens to distinguish between drug hypersensitivity and cutaneous graft-versus-host disease. J Am Acad Dermatol. 2004;51(4):543–6.
87. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. Transplant Proc. 1974;6(4):367–71.
88. Meves A, el-Azhary RA, Talwalkar JA, Moore SB, Brewer JD, Motsonelidze C, et al. Acute graft-versus-host disease after liver transplantation diagnosed by fluorescent in situ hybridization testing of skin biopsy specimens. J Am Acad Dermatol. 2006; 55(4):642–6.
89. Burge SM, Frith PA, Juniper RP, Wojnarowska F. Mucosal involvement in systemic and chronic cutaneous lupus erythematosus. Br J Dermatol. 1989;121(6):727–41.
90. Kettler AH, Bean SF, Duffy JO, Gammon WR. Systemic lupus erythematosus presenting as a bullous eruption in a child. Arch Dermatol. 1988; 124(7):1083–7.
91. Bilenchi R, Pisani C, Poggiali S, Andreassi A, de Padova LA, di Perrit T. Discoid lupus erythematosus of the vulva. Lupus. 2004;13(10):815–6.
92. Pipkin C. Erosive diseases of the vulva. Dermatol Clin. 2010;28(4):737–51.
93. Privette ED, Werth VP. Update on pathogenesis and treatment of CLE. Curr Opin Rheumatol. 2013;25(5): 584–90.
94. Yu C, Chang C, Zhang J. Immunologic and genetic considerations of cutaneous lupus erythematosus: a comprehensive review. J Autoimmun. 2013;41:34–45.
95. Chong BF, Song J, Olsen NJ. Determining risk factors for developing systemic lupus erythematosus in patients with discoid lupus erythematosus. Br J Dermatol. 2012;166(1):29–35.
96. Piura B, Rabinovich A, Shaco-Levy R, Sukenik S. Vulvar invasive squamous cell carcinoma occurring in a young woman with systemic lupus erythematosus. Eur J Gynaecol Oncol. 2005;26(1):103–5.
97. Stepanić V, Corusić A, Matković V, Sentić M, Bosnić D, Mahovlić V. Vulvar intraepithelial neoplasia in a young woman with systemic lupus erythematosus: a case report. Lupus. 2010;19(1):96–9.

98. Lynch PJ. Lichen simplex chronicus (atopic/neurodermatitis) of the anogenital region. Dermatol Ther. 2004;17(1):8–19.
99. Edwards L, Lynch PJ, Neill SM. Genital dermatology atlas. Philadelphia: Lippincott Williams & Wilkins; 2011.
100. Rimoin LP, Kwatra SG, Yosipovitch G. Female-specific pruritus from childhood to postmenopause: clinical features, hormonal factors, and treatment considerations. Dermatol Ther. 2013;26(2):157–67.
101. Zamirska A, Reich A, Berny-Moreno J, Salomon J, Szepietowski JC. Vulvar pruritus and burning sensation in women with psoriasis. Acta Derm Venereol. 2008;88(2):132–5.
102. Stewart KMA. Clinical care of vulvar pruritus, with emphasis on one common cause, lichen simplex chronicus. Dermatol Clin. 2010;28(4): 669–80.
103. Nascimento AF, Granter SR, Cviko A, Yuan L, Hecht JL, Crum CP. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinoma? Am J Surg Pathol. 2004;28(5):638–43.
104. Zayour M, Zayour M, Lazova R, Lazova R. Pseudoepitheliomatous hyperplasia: a review. Am J Dermatopathol. 2011;33(2):112–22. quiz123–6.
105. Gudjonsson JE, Elder JT. Psoriasis: epidemiology. Clin Dermatol. 2007;25(6):535–46.
106. Fischer GO. The commonest causes of symptomatic vulvar disease: a dermatologist's perspective. Australas J Dermatol. 1996;37(1):12–8.
107. Guglielmetti A, Conlledo R, Bedoya J, Ianiszewski F, Correa J. Inverse psoriasis involving genital skin folds: successful therapy with dapsone. Dermatol Ther (Heidelb). 2012;2(1):15.
108. Morris A, Rogers M, Fischer G, Williams K. Childhood psoriasis: a clinical review of 1262 cases. Pediatr Dermatol. 2001;18(3):188–98. Blackwell Science Inc.
109. Kapila S, Bradford J, Fischer G. Vulvar psoriasis in adults and children: a clinical audit of 194 cases and review of the literature. J Low Genit Tract Dis. 2012; 16(4):364–71.
110. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009;361(5):496–509.
111. Weiss G, Shemer A, Trau H. The Koebner phenomenon: review of the literature. J Eur Acad Dermatol Venereol. 2002;16(3):241–8.
112. Griffiths CEM, Barker JNWN. Pathogenesis and clinical features of psoriasis. Lancet. 2007;370(9583): 263–71.
113. de Korte J, Sprangers MA, Mombers FM, Bos JD. Quality of life in patients with psoriasis: a systematic literature review. J Investig Dermatol Symp Proc. 2004;9(2):140–7.
114. Yli-Kerttula UI. Clinical characteristics in male and female uro-arthritis or Reiter's syndrome. Clin Rheumatol. 1984;3(3):351–60.
115. Edwards L, Hansen RC. Reiter's syndrome of the vulva. The psoriasis spectrum. Arch Dermatol. 1992; 128(6):811–4.
116. Thambar IV, Dunlop R, Thin RN, Huskisson EC. Circinate vulvitis in Reiter's syndrome. Br J Vener Dis. 1977;53(4):260–2.
117. Daunt SO, Kotowski KE, O'Reilly AP, Richardson AT. Ulcerative vulvitis in Reiter's syndrome. A case report. Br J Vener Dis. 1982;58(6):405–7.
118. Lotery HE, Galask RP, Stone MS, Sontheimer RD. Ulcerative vulvitis in atypical Reiter's syndrome. J Am Acad Dermatol. 2003;48(4):613–6.
119. Magro CM, Crowson AN, Peeling R. Vasculitis as the basis of cutaneous lesions in Reiter's disease. Hum Pathol. 1995;26(6):633–8.

4 Blistering Disorders and Acantholytic Processes Affecting the Epidermis of the Vulva

Mai P. Hoang, María Teresa Fernández-Figueras, and Martin C. Mihm Jr.

Introduction

Blistering and acantholytic disorders are a heterogeneous group of diseases that predominantly result from a defect, inherited or acquired, in the adhesion of keratinocytes. Abnormal structures in the basement membrane and superficial dermis can also lead to blister formation. Two criteria are seminal in the classification of these disorders: the level of separation and the type of inflammation observed. Blisters can develop in any of four anatomic levels: the split may occur at the subcorneal level like in staphylococcal scalded skin syndrome and pemphigus foliaceus, or within the epidermis such as pemphigus vulgaris, papular acantholytic dyskeratosis, Hailey–Hailey disease, and Darier's disease. The separation is at the dermal–epidermal junction for bullous pemphigoid, cicatricial pemphigoid, pemphigoid gestationis, linear IgA disease, and epidermolysis bullosa (Tables 4.1 and 4.2). Cases with subepidermal separation rarely affect the genitalia. Workup of a patient with a blistering disorder includes a skin biopsy, preferentially from the edge of the blister, and a second sample from the non-affected skin for direct immunofluorescence (DIF) examination.

M.P. Hoang (✉)
Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: mhoang@mgh.harvard.edu

Subcorneal Blister

Staphylococcal Scalded Skin Syndrome

Staphylococcal scalded skin syndrome (SSSS) is caused by exfoliative (or epidermolytic) toxins produced by coagulase-positive group II *Staphylococcus aureus* (especially strain 71) [1]. These organisms are responsible for a preceding conjunctivitis or upper respiratory tract infection. Most cases of SSSS are associated with exfoliative toxins A and B [2]. The exfoliative toxins (ETs) induce epidermal blistering through the cleavage of desmoglein-1, the cell–cell adhesion molecule that is expressed by keratinocytes in the stratum granulosum [2]. Currently, the role of exfoliative toxins in the pathogenesis of SSSS is not well defined [3].

Clinical Features

SSSS often starts as skin tenderness and erythema in flexural skin and with mucous membrane sparing. Vulva was the presenting site in one rare case of SSSS in a child [4]. Subsequently large flaccid blisters with positive Nikolsky's sign develop which rupture readily. Widespread exfoliation which can involve most of the body surface (Ritter's disease) is caused by toxins produced from infections at distant site and reaches the cutaneous target via hematogenous

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_4,

Table 4.1 Classification of blistering disorders and acantholytic processes affecting the epidermis

Subcorneal blister
Staphylococcal scalded skin syndrome
A microbial pustulosis of the folds (Chap. 3)
Pemphigus foliaceus
Intraepidermal blister
Pemphigus vulgaris
Papular acantholytic dyskeratosis of vulvocrural area
Hailey–Hailey disease
Darier's disease
Erythema multiforme/Stevens–Johnson syndrome (Chap. 3)
Herpesvirus infection (Chap. 6)
Subepidermal blister
Bullous pemphigoid and mucous membrane (cicatricial) pemphigoid
Pemphigoid gestationis
Linear IgA disease/chronic bullous disease of childhood
Epidermolysis bullosa
Bullous lichen planus (Chap. 5)
Bullous lichen sclerosus (Chap. 5)

spread. Significant skin sloughing can result in poor thermoregulation, delicate fluid balance, and susceptibility to superinfection. SSSS mainly affects neonates and children less than 6 years of age likely due to an immature immune system and less effective renal toxin clearance [5]. In children spontaneous healing occurs after several days with mortality less than 5 %. In contrast, staphylococcal septicemia may develop in adult patients with mortality up to 60 % [6].

Histopathology and Differential Diagnosis

Subcorneal blister is present containing occasional acantholytic cells and few neutrophils (Fig. 4.1). On frozen section of sloughed skin, one would see only the granular cells attached to the stratum corneum (Fig. 4.2). Only a sparse dermal infiltrate is seen, in contrast to a heavier infiltrate in bullous impetigo and pemphigus foliaceus. DIF studies are negative.

Table 4.2 Summary of clinical and histopathologic features

	Genital involvement	Mucosal involvement	Age group commonly involved	Level of separation	Type of inflammatory infiltrate	DIF
Pemphigus foliaceus			Adults	Subcorneal		Intercellular IgG and C3
Pemphigus vulgaris	80 %		Adults	Intraepidermal		Intercellular IgG and C3
Bullous pemphigoid	9 %	Uncommon	Elderly, rarely in children	Subepidermal	Eosinophils	Linear IgG and C3 at BMZ
Mucous membrane pemphigoid	55 %	Always with associated scarring	Adults	Subepidermal	Neutrophils and eosinophils	Linear IgG and C3 at BMZ
Pemphigoid gestationis	5 %	Rarely		Subepidermal	Eosinophils	Linear C3 at BMZ
Linear IgA disease	60 %	Common	Children and adults	Subepidermal	Neutrophils	Linear IgA at BMZ
Epidermolysis bullosa	Rarely	Rarely	Infants	Subepidermal	Minimal	
SSSS		Rarely	Children	Subcorneal	Minimal	Negative
Papular acantholytic dyskeratosis			Adults	Intraepidermal	Minimal	Negative
Hailey–Hailey disease	Common		Adults	Intraepidermal	Neutrophils	Negative
Darier's disease			Adults	Intraepidermal	Minimal	Negative

DIF direct immunofluorescence examination, *SSSS* Staphylococcal scalded skin syndrome, *BMZ* basement membrane zone

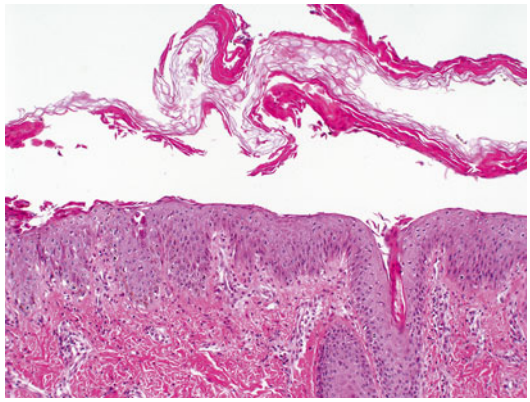


Fig. 4.1 Staphylococcal scalded skin syndrome. A subcorneal blister with minimal inflammatory infiltrate is seen (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

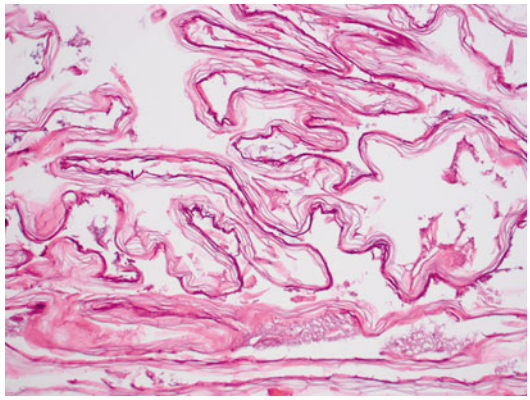


Fig. 4.2 Staphylococcal scalded skin syndrome. Stratum corneum and granular cells comprised the blister roof

Summary

Clinical Presentation

- Skin tenderness and erythema in flexural skin, then widespread exfoliation
- Caused by infection of exfoliative toxin-producing *Staphylococcus aureus*

Histologic Features

- The exfoliative toxin causes subcorneal blister at the granular layer.

Differential Diagnosis

- Bullous impetigo
- Pemphigus foliaceus

Takeaway Essentials

Clinically Relevant Pearls

- The exfoliative toxins induce epidermal blistering through the cleavage of desmoglein-1 that is expressed by keratinocytes in the stratum granulosum.
- Diagnosis may be reached by identifying the detached epidermis in frozen section analysis; the presence of stratum corneum and superficial epidermis confirms the diagnosis of SSSS.

Pathology Interpretation Pearls

- Subcorneal blister at the granular layer
- Minimal dermal inflammation
- Minimal acantholysis in contrast to bullous impetigo

Pemphigus Foliaceus and Pemphigus Vulgaris

Pemphigus vulgaris, a rare acquired immunobullous disorder of the skin and mucosa, and pemphigus foliaceus are caused by autoantibodies against desmoglein 3 [7] and desmoglein 1 [8], respectively. Recently, endemic pemphigus foliaceus (*fogo selvagem*) has been suggested to be triggered by environmental antigen with molecular mimicry with desmoglein 1 [9].

Clinical Features

Vulvar lesions were the second most frequent site of mucosal pemphigus vulgaris, with a frequency of approximately 22–51 % [8, 10], affecting labia majora and minora followed by vagina. Rarely genital lesions can be the sole manifestation of the disease especially in young age [10–12]. The two variants of pemphigus vulgaris described in the pediatric population, childhood, and juvenile types have increased incidence of genital lesions than the adult counterpart. Involvement of the uterine cervix and vagina is not uncommon in genital pemphigus vulgaris [10]. These patients often present with painful mucosal erosions (Fig. 4.3)

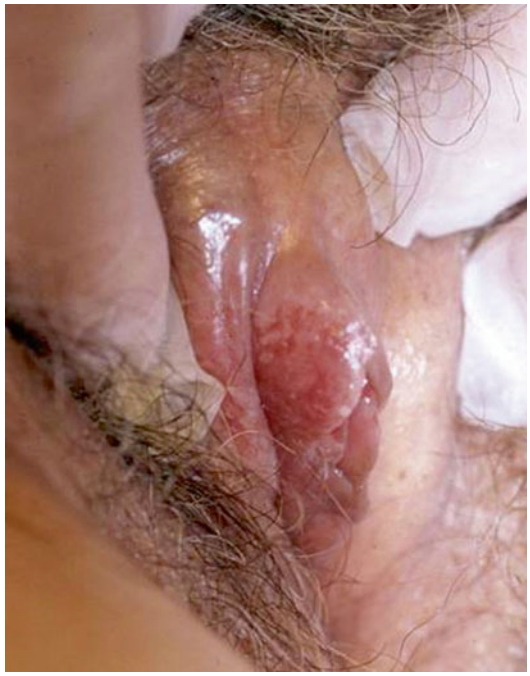


Fig. 4.3 Pemphigus vulgaris. Superficial ulcers seen on an erythematous base (Courtesy of Dr. Agustin Alomar, Department of Dermatology, DERMADEX, Instituto Universitario Dexeus, Barcelona, Spain)

and/or flaccid bullae (Fig. 4.4) involving the mouth or nasal mucosa, associated with round to oval bullae in non-erythematous skin with predilection for scalp, face, axillae, and groins. In the vulvar, as in non-genital skin, blisters have a short duration leading to superficial ulcers and erosions [8, 12]. Nikolsky's sign is present. Rarely the clinical, histologic, and antibody profile may change from pemphigus vulgaris to pemphigus foliaceus [13]. In pemphigus vegetans, a vegetative variant of pemphigus vulgaris, hypertrophic or warty vegetations develop (Fig. 4.5) in association with the bullae in inguinal, perianal, umbilical, mammary, axillary, and scalp [14]. The localized and self-limited character of pemphigus vegetans distinguishes it from pemphigus vulgaris.

Pemphigus foliaceus presents with recurrent crops of flaccid bullae that rupture readily resulting in shallow erosions and crusted erythematous plaques. Mucous involvement is rare. Of interest, a recent series from Brazil documented genital involvement by pemphigus foliaceus for the first time in 4/15 (27 %) patients [8].

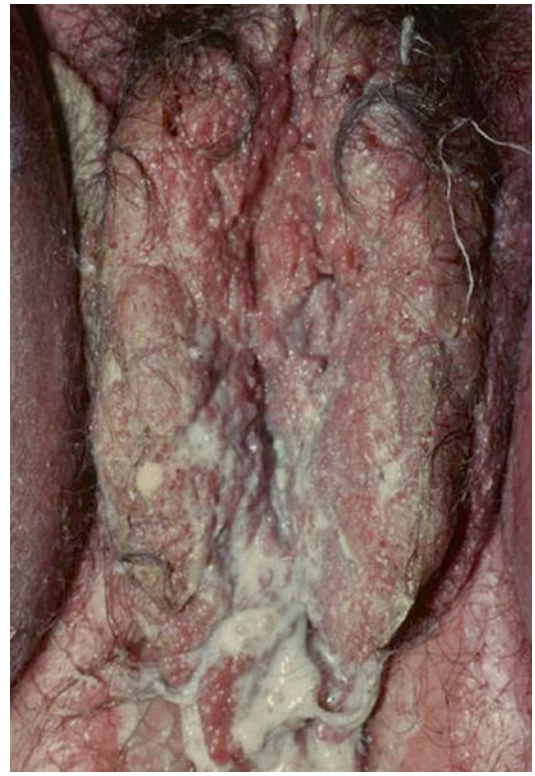


Fig. 4.4 Pemphigus vulgaris. Flaccid bullae and abundant secretions related to vaginal involvement [12] (Courtesy of Dr. Carlos Ferrándiz, Department of Dermatology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain)

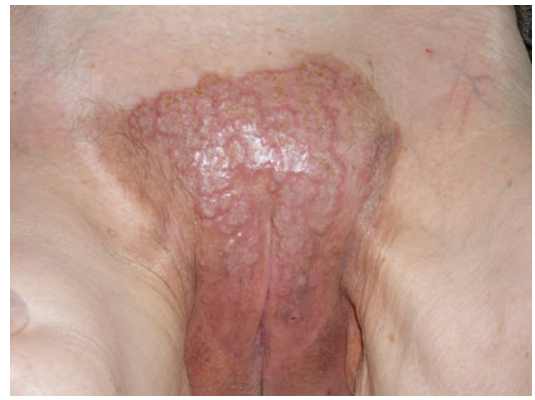


Fig. 4.5 Pemphigus vegetans. Slightly raised plaques and small ulcerations (Courtesy of Dr. Isabel Bielsa, Department of Dermatology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain)

Histopathology

In pemphigus vulgaris, acantholysis results in intraepidermal blister with basilar cells aligned at

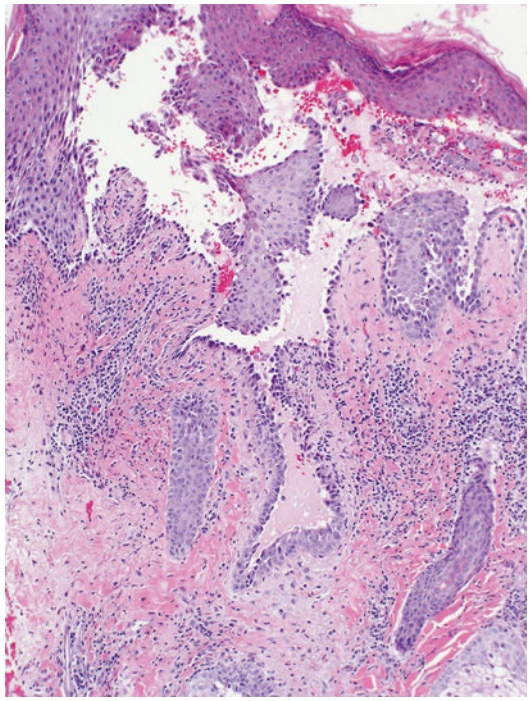


Fig. 4.6 Pemphigus vulgaris. Suprabasilar acantholysis is noted (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

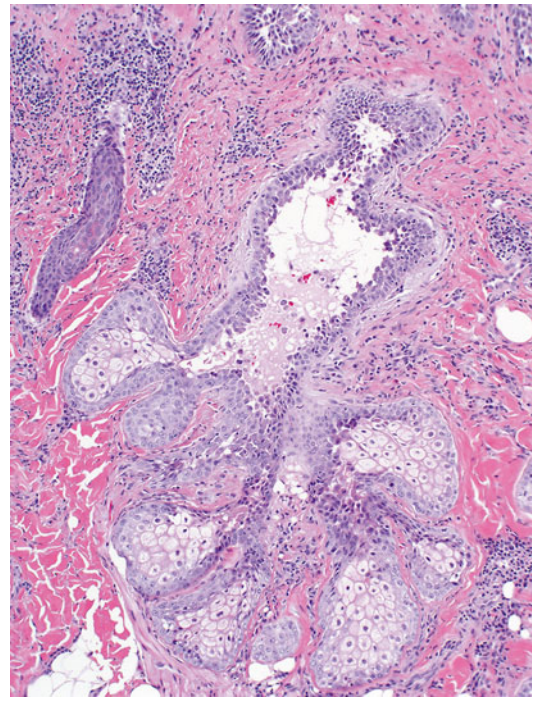


Fig. 4.7 Pemphigus vulgaris. Acantholysis can be prominent within follicular epithelium (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

the floor of the blister resembling a row of "tombstones" (Figs. 4.6 and 4.7). There are no brightly eosinophilic dyskeratotic cells. The roof of the blister shows scalloping of cells due to acantholysis. In addition to the mentioned histologic features, verrucous epidermal hyperplasia, intraepidermal eosinophilic microabscesses containing acantholytic cells, and dermal infiltrate of lymphocytes and eosinophils are also seen in pemphigus vegetans (Figs. 4.8a, b) [15]. Acantholysis is noted only at the granular layer in pemphigus foliaceus (Fig. 4.9). The characteristic cell is a dyskeratotic granular layer cell. In some cases there is a mid-epidermal cleft with acantholytic cells. However, there is no evidence of suprabasilar acantholysis, ruling out a variant of pemphigus vulgaris. A moderately dense and mixed infiltrate comprising of lymphocytes, neutrophils, and scattered eosinophils is seen around the dermal blood vessels and also interstitially in both pemphigus foliaceus and pemphigus vulgaris. Neutrophils can be prominent in both lesions. For pemphigus vulgaris and pemphigus vegetans, acantholysis can be prominent within follicular epithelium; however, eosinophils would be prominent within the inflammatory infiltrate for pemphigus vegetans (Fig. 4.8).

For all three types of pemphigus, intercellular IgG and C3 depositions are seen on direct immunofluorescence (DIF) studies (Fig. 4.10). Circulating autoantibodies to epidermal intracellular spaces can be detected with indirect immunofluorescence (IIF) method against monkey esophageal epithelium; and the titer correlates with the clinical activity of the disease. Not long ago, IgG4 immunostaining has been shown to be a sensitive and specific test for diagnosing pemphigus in the setting of active lesions when additional frozen tissue is not available for DIF examination [16]. In addition, it has recently been reported that DIF can be performed on plucked hair follicles as an alternative test [17].

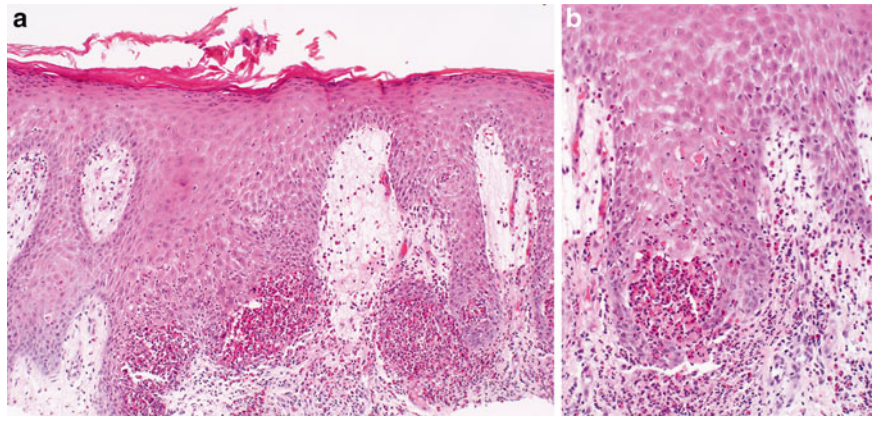


Fig. 4.8 Pemphigus vegetans. (**a**) Marked epidermal hyperplasia resembling a verrucous process. (**b**) Eosinophilic microabscesses containing acantholytic cells are seen within the epidermis (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

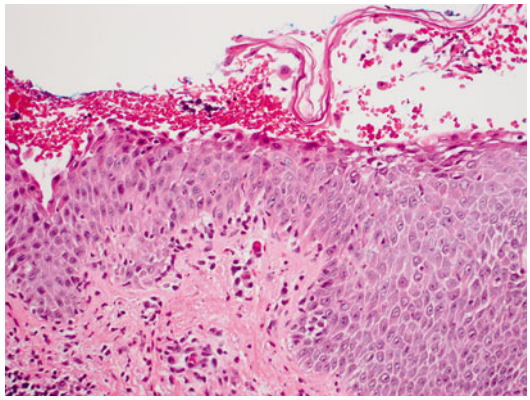


Fig. 4.9 Pemphigus foliaceus. Subcorneal separation and acantholysis are seen

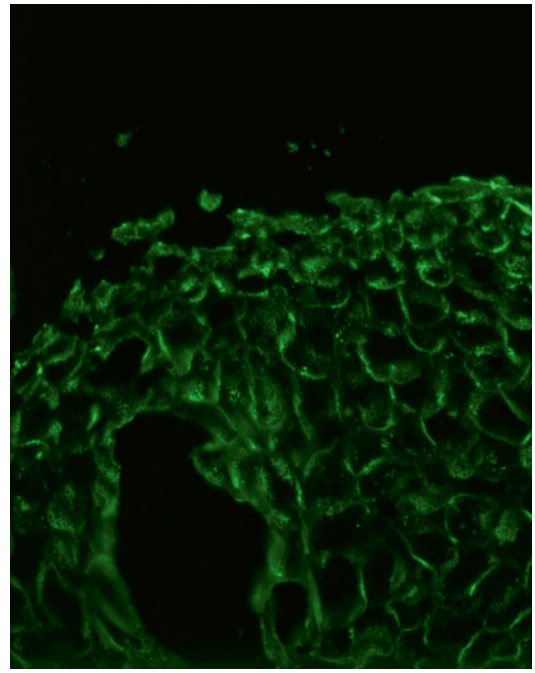


Fig. 4.10 Pemphigus vulgaris. Direct immunofluorescence examination demonstrates intercellular IgG deposition within the epidermis

Differential Diagnosis

Although Hailey–Hailey disease, Darier's disease, and acantholysis of the vulvocrural area are in the histologic differential diagnosis for pemphigus vulgaris, the DIF studies are negative in these entities. The follicular involvement noted in pemphigus vulgaris is not seen in these other intraepidermal acantholytic disorders; whereas, dyskeratosis is not a feature of pemphigus vulgaris. Marked dermal inflammation would be seen in Hailey–Hailey disease in contrast to the paucity of it in pemphigus vulgaris. Infection is in the differential diagnosis for pemphigus vegetans due to the marked epidermal hyperplasia and inflammation. Special stains, cultures for microorganisms and mmunofluorescent tests are useful tools to differentiate these two diseases. DIF studies would be negative in bullous impetigo, dermatophytosis, and SSSS—entities with similar histologic features to pemphigus foliaceus. In addition, Gram and periodic acid–Schiff (PAS) stains would highlight the intracorneal bacteria and fungal hyphae in bullous impetigo and dermatophytosis,

respectively. An intercellular IgA deposition on DIF would be seen within the epidermis for IgA pemphigus. Of interest, intraepidermal subcorneal and suprabasal acantholysis can be observed on histologic sections due to EMLA (eutectic mixture of local anesthetics) application, and these changes can pose a potential diagnostic pitfall [18].

Summary

Clinical Presentation

- Pemphigus vulgaris often presents with cutaneous flaccid bullae in association with painful mucosal erosions and/or flaccid bullae in the mouth or nasal mucosa.
- Vulvar superficial ulcers and erosions are the second most frequent form of mucosal disease.
- Pemphigus foliaceus presents with recurrent crops of flaccid bullae that rupture readily resulting in shallow erosions and crusted erythematous plaques.
- Pemphigus vegetans characteristic warty erythemaous plaques affect the labia and they are associated with extragenital bullae.

Histologic Features

- Acantholysis at the granular layer in pemphigus foliaceus
- Intraepidermal blister with suprabasilar acantholysis in pemphigus vulgaris
- Verrucous epidermal hyperplasia and intraepidermal eosinophilic microabscesses containing acantholytic cells in pemphigus vegetans
- Intercellular IgG and C3 depositions are seen on DIF studies for both pemphigus foliaceus and vulgaris

Differential Diagnosis

- For pemphigus foliaceus: bullous impetigo, staphylococcal scalded skin syndrome, dermatophytosis, and IgA pemphigus
- For pemphigus vulgaris: Hailey–Hailey disease, Darier's disease, and acantholysis of the vulvocrural area

Takeaway Essentials

Clinically Relevant Pearls

- Endemic pemphigus foliaceus is triggered by environmental antigen with molecular mimicry with desmoglein 1.

Pathology Interpretation Pearls

- Acantholysis noted in hair follicular epithelium for pemphigus vulgaris and vegetans but not pemphigus foliaceus
- In 20 % of pemphigus foliaceus, direct immunofluorescence staining is seen in the upper third of the epidermis.
- Intraepidermal subcorneal and suprabasal acantholysis can be seen on histologic sections due to EMLA (eutectic mixture of local anesthetics) application.

Immunohistochemical/Molecular Findings

- IgG4 immunostaining on paraffin-embedded tissue may be a new sensitive and specific test.

Intraepidermal Blister

Papular Acantholytic Dyskeratosis of the Vulvocrural Area

Clinical Features

Papular acantholytic dyskeratosis of the vulvocrural area or papular genitocrural acantholysis often presents as solitary or grouped, flesh-colored papules on the vulva and perianal region, with extension to the thigh and perineum in some cases [19, 20]. However, a spectrum of clinical presentations includes vesicles, bullae, patches, and plaque [20, 21]. A rare case in pediatric age group has been reported [22]. These lesions are generally asymptomatic, but pruritus and burning sensation can be the symptoms in some cases [23].

It has been suggested that some cases of papular genitocrural acantholysis is an allelic variant

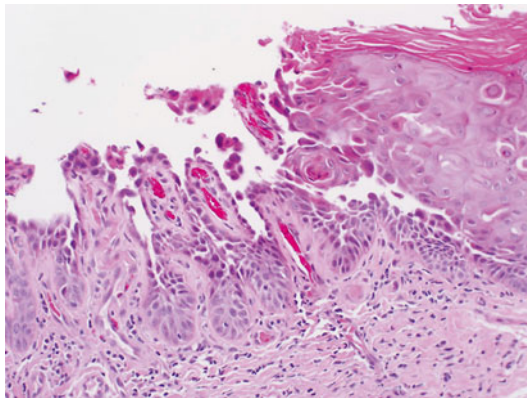


Fig. 4.11 Papular acantholytic dyskeratosis. Acantholytic, dyskeratotic cells including corps ronds and grains are seen within the epidermis (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

of Hailey–Hailey disease since heterozygous mutations in intron 5 and exon 24 of the *ATP2C1* gene have been reported [24, 25].

Histopathology

Histologic sections show hyperkeratosis, hypergranulosis, epidermal acanthosis, and acantholysis in the spinous zone (Fig. 4.11) [26]. The histologic features of intraepidermal acantholytic, dyskeratotic cells including corps ronds and grains can be indistinguishable from those of Darier's disease and Hailey–Hailey disease. Corps ronds are cells with pyknotic nuclei with surrounding clear halo in the granular layer of epidermis. Grains are elongated cells with scant cytoplasm and abundant keratohyalin granules. DIF examination is negative.

Differential Diagnosis

While Darier's and Hailey–Hailey diseases are familial, acantholytic dermatosis of the vulvocrural area is often sporadic. Discrete columns of parakeratosis overlying suprabasilar acantholysis are seen in Darier's disease. A broad zone of acantholysis is present in Hailey–Hailey disease rather than focal area as seen in papular acantholytic dyskeratosis of genitocrural area.

Summary

Clinical Presentation

- Solitary or grouped, flesh-colored papules

Histologic Features

- Intraepidermal acantholysis, dyskeratotic cells including corps ronds and grains

Differential Diagnosis

- Darier's disease
- Hailey–Hailey disease

Takeaway Essentials

Clinically Relevant Pearls

- Consider diagnosis in any case of asymptomatic and eruptive genitocrural papules

Pathology Interpretation Pearls

- Presence of corps ronds and grains rules out pemphigus.
- A broad zone of acantholysis is present in Hailey–Hailey disease rather than focal area as seen in papular acantholytic dyskeratosis of genitocrural area.

Immunohistochemical/Molecular Findings

- Negative direct immunofluorescence studies also speak against immunobullous disease.

Hailey–Hailey Disease

Hailey–Hailey disease (benign familial pemphigus) is an autosomal dominant acantholytic genodermatosis whose pathogenesis is thought to involve mutations in a calcium pump, *ATP2C1*, which encodes the human secretory pathway Ca^{2+}/Mn^{2+} APTase protein 1. The latter is a part of an adhesion complex between desmosomal proteins and tonofilaments [27]. The mutation is thought to weaken the intracellular junctions. Although the disease is familial, up to one-third of patients have no family history of the disease.

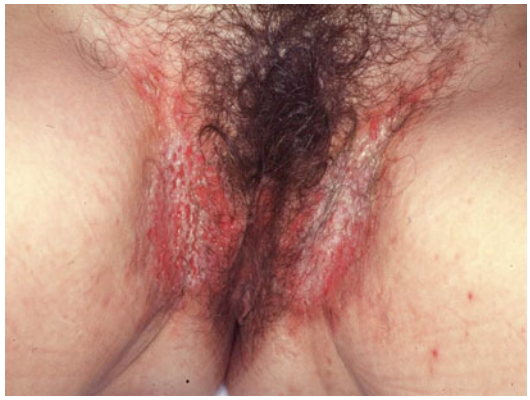


Fig. 4.12 Hailey–Hailey disease. Eroded and crusted whitish plaques involving the genital area and inguinal folds (Courtesy of Dr. Carlos Ferrándiz, Department of Dermatology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain)

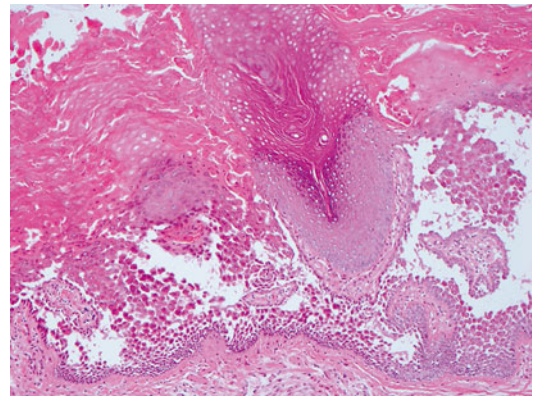


Fig. 4.13 Hailey–Hailey disease. Acantholysis involving the entire epidermis is seen

Hailey–Hailey disease often occurs during the second and fourth decades with frequent remissions and relapses and tends to improve with age.

Clinical Features

There is predilection of the disease for flexural or intertriginous areas such as neck, axillae, inframammary region, inguinal folds, and perianal and genital region (Fig. 4.12) [27]. Isolated vulvar involvement has been reported [28, 29]. The lesions start as pruritic erosions that spread centrifugally to surrounding skin with associated crust and foul odor. Resolution can result in hypopigmentation but not scarring. More than 70 % have asymptomatic longitudinal white bands affecting the nails [27]. Superinfection with *Candida albicans*, *Staphylococcus aureus*, and herpesvirus can be seen, frequently explaining the foul order in these patients [27, 30]. Rarely, squamous cell carcinoma can arise in the setting of Hailey–Hailey disease [31, 32]. Risk factors for development of squamous cell carcinoma such as sun exposure, irradiation, and exposure to carcinogenic agents were not identified in these patients [32].

Histopathology

There is a suprabasilar cleft with at least half of the overlying epidermis showing acantholysis and mild dyskeratosis. The marked acantholysis gives an appearance of "dilapidated brick wall" with irregular spaces between keratinocytes (Fig. 4.13). Surface crust and prominent dermal inflammation are seen. DIF studies are negative in contrast to positive findings in pemphigus vulgaris.

Differential Diagnosis

The main histologic differential diagnosis would be pemphigus vulgaris which has absence of scale crust, normal thickness epidermis, suprabasilar acantholysis, follicular epithelium involvement, and minimal dermal inflammation. In contrast to Darier's disease, corps ronds and grains are infrequently identified.

Summary

Clinical Presentation

- Predilection of the disease for flexural or intertriginous areas such as neck, axillae, and groins
- Start as pruritic erosions that spread centrifugally to surrounding skin with associated crust and foul odor

Histologic Features

- Intraepidermal acantholysis involving at least half of the epidermis above a suprabasilar cleft
- Marked surface crust, epidermal acanthosis, and dermal inflammation
- Negative direct immunofluorescence studies

Differential Diagnosis

- Pemphigus vulgaris

Takeaway Essentials

Clinically Relevant Pearls

- Crusts predominate with some small peripheral vesicles.

Pathology Interpretation Pearls

- Diffuse acantholysis and mild dyskeratosis are characteristic.
- Corps ronds and grains are infrequent.

Immunohistochemical/Molecular Findings

- Negative DIF studies rule out pemphigus group of disorders.

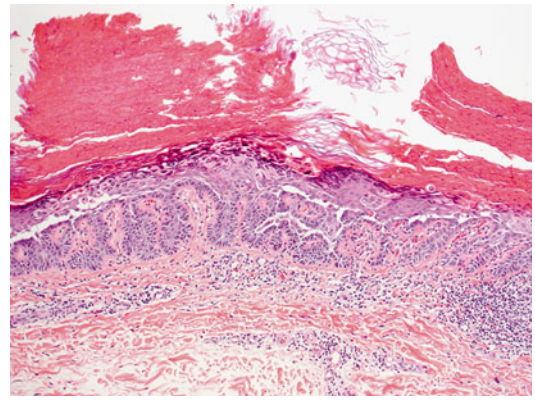


Fig. 4.14 Darier's disease. Columns of parakeratosis, suprabasilar acantholysis, and marked dyskeratosis are seen

Darier's Disease

Darier's disease in an uncommon genodermatosis whose pathogenesis is thought to be due to mutation of a gene on chromosome 12q encoding a calcium pump protein, *ATP2A2* [33]. Although it can be autosomal dominant, only half of the cases in a large series have a positive family history [34].

Clinical Features

The disorder often presents at puberty, but can appear later in life, with numerous hyperkeratotic papules usually distributed over the trunk and a "sandpaper" feel. The disease has a seborrheic distribution (chest, neck, back, ears, and groin) and frequently involves the vulva. Localized vulvar presentation has been described [35, 36] and rarely can be associated with squamous cell carcinoma [37]. The associated rash often has a distinct odor and is worsened by humidity, heat, stress, and sunlight. The nails tend to be thin, brittle, and dystrophic and sometimes with characteristic vertical striations. Associated palmar pitting can also be seen. These findings are often helpful clues in diagnosis of the disease. Superinfection with bacteria, virus, or fungus is common.

Histopathology

Histologic sections show columns of parakeratosis and suprabasilar acantholysis and marked dyskeratosis within an acanthotic epidermis (Fig. 4.14). Discrete columns of parakeratosis represent the keratotic papules seen clinically, and they overlie the acantholytic and dyskeratotic cells above a suprabasilar cleft. The dyskeratotic cells have two forms. One exhibits a bright pink oval cytoplasm with a hyperchromatic nucleus, called grains, and is present above the basal layer extending to the surface. The other form called corps ronds is present at the edge of the granular cell layer with prominent round nuclei and a basophilic and amphophilic cytoplasm. There is typically only sparse dermal inflammation.

Differential Diagnosis

In the histologic differential diagnosis, warty dyskeratoma is a solitary lesion [38]. Prominent dermal inflammatory infiltrate containing occasional eosinophils would be seen in Grover's disease. Papular acantholytic dyskeratosis of the genitocrural area would have histologic features resembling both Darier's disease and Hailey–Hailey disease. Hailey–Hailey disease has more extensive acantholysis rather than discrete foci as seen in Darier's disease, and acantholytic cells do not present in the granular layer and stratum corneum.

Summary

Clinical Presentation

- Numerous hyperkeratotic papules usually distributed over the trunk frequently involve the vulva.
- Onset often during puberty.

Histologic Features

- Columns of parakeratosis and suprabasilar acantholysis within an acanthotic epidermis with special corps grains and ronds

Differential Diagnosis

- Warty dyskeratoma
- Darier-like Grover's disease
- Papular acantholytic dyskeratosis of the genitocrural area
- Hailey–Hailey disease

Takeaway Essentials

Clinically Relevant Pearls

- The distribution resembles seborrheic dermatitis of the trunk.
- Sandpaper feel.
- The associated rash often has a distinct odor and is worsened by humidity, heat, stress, and sunlight.
- The nails tend to be thin, brittle, and dystrophic and sometimes with characteristic vertical striations.
- Palmar pitting.

Pathology Interpretation Pearls

- Columns of parakeratosis
- Focal suprabasilar cleft with dyskeratotic grains and corps ronds

Subepidermal Blister

Bullous Pemphigoid and Mucous Membrane Pemphigoid (Cicatricial Pemphigoid)

Bullous pemphigoid is the most frequent autoimmune blistering disorder in adults as well as the most common one within the pemphigoid group [4]. Its incidence is approximately 10/1,000,000 inhabitants, increasing parallel to age. Bullous pemphigoid in children has been separated into infantile and childhood variants. The infantile variant is more frequently seen in boys of less than 1 year and localized to acral location, while the childhood type of bullous pemphigoid commonly is diagnosed in girls around 8 years of age with typical genital involvement, sometimes as the only manifestation [39].

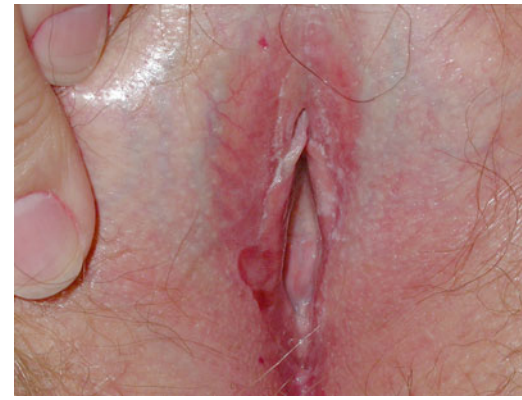


Fig. 4.15 Bullous pemphigoid. Initial stage is characterized by minimal blistering. The small erosions can be misinterpreted as excoriations in the setting of an erythematous and pruritic vulvar dermatosis (Courtesy of Dr. Luis Puig, Department of Dermatology, Hospital de Sant Pau, Barcelona, Spain)

The cutaneous presentation of bullous pemphigoid is very polymorphic with the initial nonbullous phase with associated generalized pruritus that can mimic many inflammatory dermatoses including eczema and urticaria [40, 41]. This initial nonspecific presentation (Fig. 4.15) can be the only sign of the disease in rare cases [42]. The bullous stage presents initially as tense blisters on erythematous base without Nikolsky's sign (detachment of the superficial epidermis from the underlying dermis when the examining finger is slid over the skin surface). Erythema and urticaria can be prodromal changes. Trunk, groin, and flexural areas are commonly affected sites [43, 44]. Mucosal involvement is seen in 10–30 % of patients. Radiation therapy, ultraviolet radiation, thermal or electrical burns, and surgical procedures have been reported as inducers [42].

Mucous membrane pemphigoid (cicatricial pemphigoid) has a predilection for mucosal sites such as conjunctivae and oral mucosa. The incidence has been estimated to be 1/1,000,000 inhabitants affecting most frequently women in

the sixth to seventh decade of life. Vulvar involvement can be the only manifestation of cicatricial pemphigoid in children [45]. On the contrary in adult women, vulva is more commonly affected by mucous membrane pemphigoid than bullous pemphigoid [46], 50 % versus 8 % respectively [47, 48]. Clinical presentation can be misdiagnosed as lichen sclerosus [49] and can raise false concern for abuse when occur in children [49, 50]. The labia majora and minora are frequently affected by bullae similar to those of bullous pemphigoid with the exception of associated scarring [51, 52]. In advanced disease extensive fibrosis and scarring lead to narrowing of the vagina and stenosis of the urethral orifice. Nikolsky's sign is seen.

Both are caused by autoantibodies against components of hemidesmosome (230-kD BPAg1 and 180-kD BPAg2) and type VII collagen resulting in damage of the basement membrane and subsequent loss of adhesion between the dermis and epidermis [53]. In rare cases there are overlapping features between linear IgA and the childhood form of bullous pemphigoid, since autoantibodies against 120 kD have been reported in these cases [54, 55]. Although other targeted antigens including 230-kD BPAg1, alpha3 chain of laminin 5, beta4 integrin, 168-kD protein, and 450-kD protein have been reported, BPAg2 is the most frequently involved in mucous membrane pemphigoid [56, 57]. Both bullous pemphigoid and mucous membrane pemphigoid can be the result of a drug hypersensitivity reaction [58].

Histopathology

In both types of pemphigoid, the histology is characterized by a subepidermal vesiculation containing serum, fibrin, and variable numbers of eosinophils and neutrophils. Bullous pemphigoid shows papillary dermis with preserved architecture and edema and perivascular and interstitial eosinophils admixed with a minor component of neutrophils, lymphocytes, and histiocytes (Fig. 4.16). Subepithelial scarring is only seen in cicatricial pemphigoid (Fig. 4.17). Inflammatory cell-poor lesions are rarely seen. DIF studies usually demonstrate a linear IgG and C3 deposition at the basement membrane zone (Fig. 4.18).

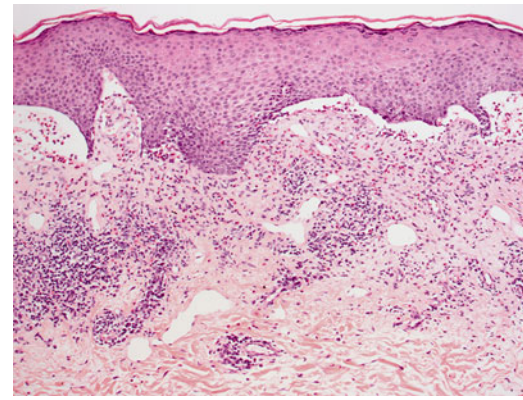


Fig. 4.16 Bullous pemphigoid. A subepidermal blister containing many eosinophils is seen

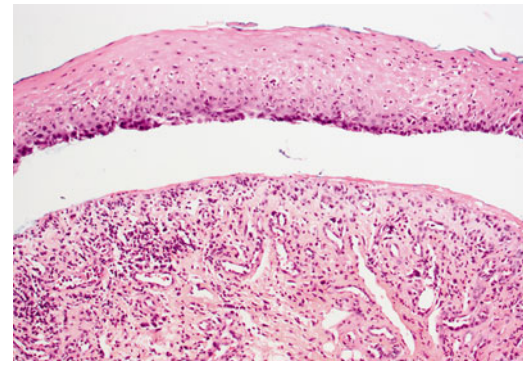


Fig. 4.17 Cicatricial pemphigoid. A subepidermal blister containing minimal inflammatory cells and underlying fibrotic dermis

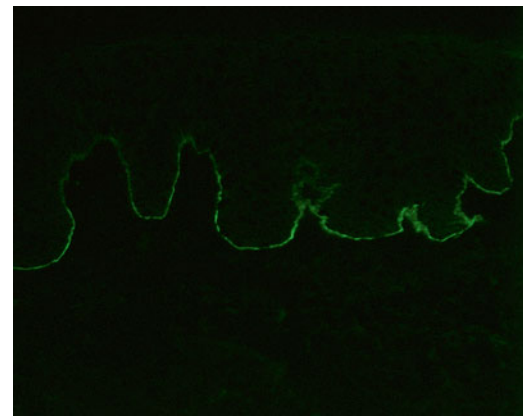


Fig. 4.18 Bullous pemphigoid. Direct immunofluorescence examination shows linear C3 deposition at the basement membrane zone

When fresh tissue is not available for DIF, C3d immunostaining has been shown to be helpful [59]. Deposition of IgA and/or IgM may or may not be present [60]. Indirect immunofluorescence (IIF) studies performed on salt-split skin usually demonstrate that autoantibodies from the patient's serum are associated with the roof or epidermal side of the blister. Recently DIF findings around hair follicles have been reported to be a helpful clue [61]. Serum autoantibodies to the 230-kD BPAg1 and 180-kD BPAg2 can be demonstrated by immunoblotting, but this is usually not necessary [62].

Differential Diagnosis

The main differential diagnosis is pemphigoid gestationis, another subepidermal bullous dermatosis with linear C3 deposition seen at the basement membrane zone on DIF, and epidermolysis bullosa. (See Vignette 1 at the end of this chapter.)

Pemphigoid gestationis is an autoimmune vesiculobullous dermatosis of pregnancy, with a strong association with HLA-DR3 and HLA-DR4 and with an incidence of 1 in 50,000–60,000 pregnancies [63, 64]. The initial presentation, usually in the second or third trimester of pregnancy or in the immediate postpartum period, includes severe pruritus and polymorphous papulovesicular eruptions that lead to blistering and superficial ulcerations. The legs are the most frequently involved site, followed by the trunk. The vulva and pubic area may be involved in 10 % of cases [65]. Spontaneous resolution follows soon after delivery, although it may occur during the late third trimester. There is a tendency for pemphigoid gestationis to recur with subsequent pregnancies, sometimes with an earlier onset and a more severe course [64]. Recognition of pemphigoid gestationis is important due to its association with intrauterine growth retardation [64].

When the histologic sections show a subepidermal vesiculation with associated eosinophilic spongiosis and superficial dermal infiltrate of lymphocytes and eosinophils, the diagnosis of pemphigoid gestationis can be reached readily. However, the histology varies greatly depending on the timing of the biopsy and the nature of the primary lesion; thus, nonspecific dermal inflammation, subepidermal vacuolization with perivascular inflammation, and epidermal ulceration are often seen [65]. DIF studies demonstrate linear C3 deposition at the basement membrane zone in 7/7 cases and linear IgG in 1/7 cases and have been shown to have the highest diagnostic sensitivity [65]. Only linear C3 is seen on DIF in the classic presentation.

Summary

Clinical Presentation

- Bullous pemphigoid in children has been separated into infantile and childhood variants.
- Bullous pemphigoid: polymorphic lesions with the initial non-bullous phase that can mimic an inflammatory dermatosis.
- Mucous membrane pemphigoid (cicatricial pemphigoid) has a predilection for mucosal sites such as conjunctivae and oral mucosa.
- Vulvar involvement can be the only manifestation of cicatricial pemphigoid in children.
- In adult women, vulva is more commonly affected by mucous membrane pemphigoid than bullous pemphigoid.
- Autoantibodies to the 230-kD BPAg1 and 180-kD BPAg2.

Histologic Features

- Subepidermal bullous dermatosis with eosinophils
- Presence of eosinophils at the dermal epidermal junction in pre-bullous stage of bullous pemphigoid
- Subepithelial scarring seen only in cicatricial pemphigoid
- DIF: linear IgG and C3 deposition at the basement membrane zone

Differential Diagnosis

- Epidermolysis bullosa
- Pemphigoid gestationis

Takeaway Essentials

Clinically Relevant Pearls

- Think of bullous pemphigoid when you have a pediatric patient with a blistering disorder.
- Child abuse is a clinical pitfall.
- In adult women early detection and treatment would avoid marked vulvar scarring.

Pathology Interpretation Pearls

- Eosinophilic spongiosis and eosinophils at the dermal epidermal junction are clues for pre-bullous or urticarial stage of bullous pemphigoid.

Immunohistochemical/Molecular Findings

- Direct immunofluorescence findings around hair follicles can be diagnostic clues.

Linear IgA Disease

Characterized as an entity in 1979 by Chorzelski [66], linear IgA disease or linear IgA bullous dermatosis affects all ages, and specifically in children, it is known as chronic bullous dermatosis of childhood or linear IgA disease of childhood. While the adult linear IgA disease affects females between the ages of 60 and 65 years old, the childhood form most often occurs in children of preschool age with a peak incidence at 4.5 years [67]. The antigen is a 97-kD molecule, identical to the carboxyl terminal of 180 kD (BPAg2), located at the basement membrane [68].

Clinical Features

It presents as clusters of annular lesions or "clusters of jewels" on the lower abdomen, pelvic, inguinal, and genital areas. They are usually pruritic and become ulcerated and crusted within 24 h. The eruption can be preceded by a bacterial or viral infection [67, 69], or it is drug induced, often by vancomycin [70].

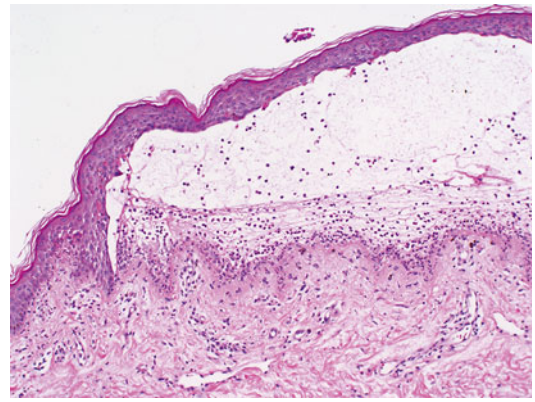


Fig. 4.19 Linear IgA disease. A subepidermal blister and prominent dermal neutrophils (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

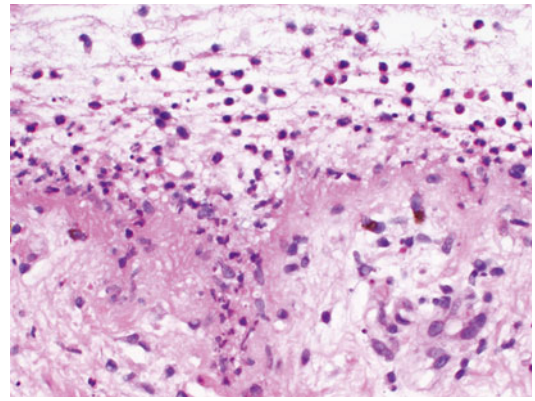


Fig. 4.20 Linear IgA disease. An infiltrate of neutrophils is seen at the dermal epidermal junction in an early blister (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

Histopathology

A subepidermal blister containing predominantly neutrophils, sometimes with admixed eosinophils, is seen (Figs. 4.19 and 4.20). The predominance of neutrophils helps to distinguish this entity from bullous pemphigoid, though eosinophils have been reported to be more prominent in drug-induced linear IgA disease. DIF examination of lesional and perilesional skin demonstrates a linear IgA deposition at the basement membrane zone.

Differential Diagnosis

The histologic findings can be similar between linear IgA disease and dermatitis herpetiformis; however, dermatitis herpetiformis rarely involves the vulvar skin [71]. Neutrophils within the superficial dermis may form microabscesses in papillary dermis reminiscent of dermatitis herpetiformis, but in linear IgA disease the neutrophils tend to be more evenly distributed along the basement membrane zone. In addition, the histologic picture of fibrin within the dermal papillae is more often seen in dermatitis herpetiformis cases. While DIF shows linear IgA deposition at the basement membrane zone in linear IgA disease, granular and papillary dermal IgA deposition would be seen in dermatitis herpetiformis.

Summary

Clinical Presentation

- Present as clusters of annular lesions or "clusters of jewels" on the lower abdomen, pelvic, inguinal, and genital areas

Histologic Features

- Subepidermal bullous dermatosis containing predominantly neutrophils

Differential Diagnosis

- Dermatitis herpetiformis

Takeaway Essentials

Clinically Relevant Pearls

- Although over the past 30 years, many drugs have been alleged to induce LAD, drug as an etiology has been proven conclusively ONLY in a few cases.

Pathology Interpretation Pearls

- More widespread neutrophil infiltration seen in linear IgA in contrast to sparing of the rete tips seen in dermatitis herpetiformis

Immunohistochemical/Molecular Findings

- DIF is the main test to distinguish linear IgA from dermatitis herpetiformis due to their overlapping histologic features.

Case Vignettes

Vignette 1

Clinical History: A 36-year-old woman of Ashkenazi Jewish descent presented with several vulvar and perineal ulcerations for the past 4 months. No extragenital lesions were noted. She was born with congenital skin blisters; however, she has not had classic bullae nor mouth blisters for the past 15 years. A definitive diagnosis has not been made since birth. No family history of blistering disorder was noted. Dystrophic nails were seen on her hands and feet. Patchy hair loss was noted over 65 % of her scalp. She reported improvement with oral corticosteroids; however, the side effects, especially the weight gain, were bothersome.

Microscopic Description: A punch biopsy from the labia majora shows a subepidermal blister (Fig. 4.21). Vascular proliferation, fibrosis, and chronic inflammatory infiltrate are seen in the floor of the blister (Fig. 4.22). No significant inflammation was appreciated. Ultrastructural examination revealed the separation to be below the lamina densa (Fig. 4.23).

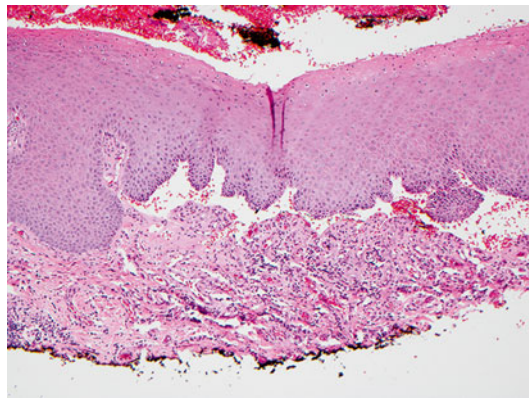


Fig. 4.21 Vignette 1. A subepidermal vesiculation is seen

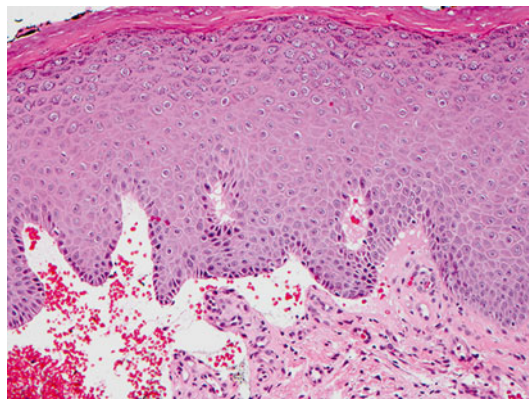


Fig. 4.22 Vignette 1. Vascular proliferation and minimal inflammatory infiltrate are seen in the dermis below the blister

(continued)

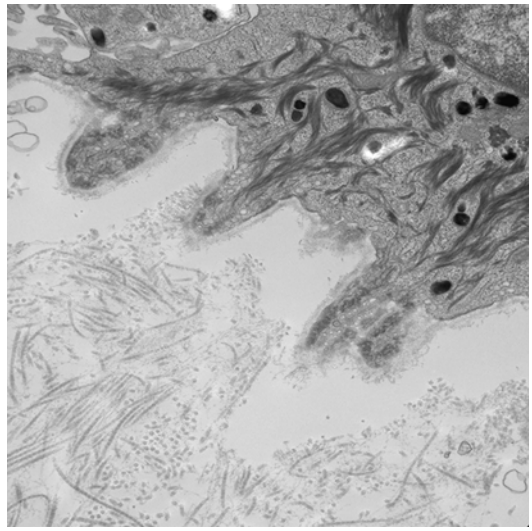


Fig. 4.23 Vignette 1. Electron microscopy demonstrated that the lamina densa is seen attached to the roof of the blister, and only collagen bundles comprised the floor of the blister; thus, the separation occurs below the lamina densa

Diagnosis: Molecular testing revealed G2031S mutation of *COL7A1* gene. These findings are supportive of the diagnosis of dominant dystrophic epidermolysis bullosa (DDEB).

Discussion: This is an unusual case. There has been only a rare case reporting vulvar involvement in the setting of epidermolysis bullosa [72]. Epidermolysis bullosa (EB), a group of hereditary bullous disorders, is classified based on the level of the blister. The separation is within the basal keratinocytes in EB simplex (EBS) and lamina lucida in junctional EB (JEB) and below the lamina densa in the dystrophic EB (DEB). Keratin 5 or 14 mutations are the cause of the three major subtypes of EBS: severe Dowling–Meara form, moderate generalized form, and mild localized form [73, 74]. The three subtypes of JEB-Herlitz, non-Herlitz, and JEB with pyloric atresia are autosomal recessive and caused by complete absence of laminin 332, reduction in laminin 332 or complete absence of collagen XVII, and mutation in the main receptor for laminin 332, respectively [75, 76]. Mutations of collagen VII gene cause DEB [77]. The phenotype of dominant DEB is milder than that of recessive DEB. Over 200 different mutations in the *COL7A1* gene are seen in DEB subtypes, both recessive and dominant ones [78]. The presented patient had a mutation in exon 73 of *COL7A1*, resulting in a substitution of glycine by a serine at the amino acid position 2031, or G2031S.

Electron microscopy remains important in the classification of EB. In contrast to ultrastructural findings of this case, the separation would be within basal keratinocytes for EBS and within the lamina lucida for JEB.

Vignette 2

Clinical History: A 22-month-old girl presented with a history of a perioral rash which was assumed to be impetigo. Over a course of several days, she developed generalized erythema that progressed to skin desquamation. Lesions were seen over her forehead, eyes, neck, back, arms, and perianal and genital (Fig. 4.24) region.

Microscopic Description: A punch biopsy of skin revealed a blister right at the granular layer of the epidermis (Fig. 4.25). There is a minimal inflammation in the dermis.

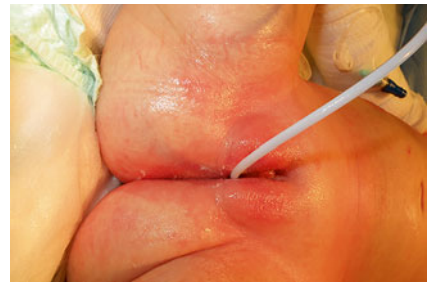


Fig. 4.24 Vignette 2. Erythema and superficial desquamation are seen (Courtesy of Dr. Carol E. Cheng, Department of Dermatology, Massachusetts General Hospital, Boston, MA)

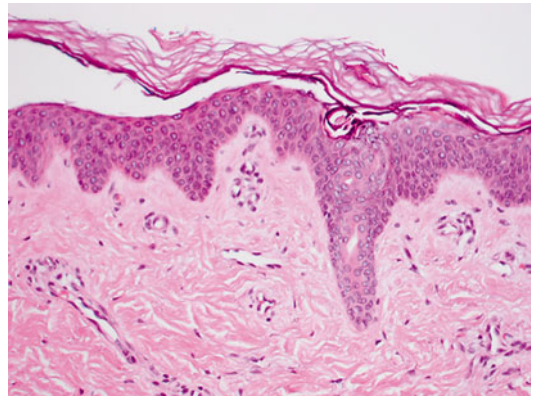


Fig. 4.25 Vignette 2. A subcorneal blister is seen

Diagnosis: Staphylococcal scalded skin syndrome

Discussion: The main histologic differential diagnosis of this case would include bullous impetigo and pemphigus foliaceus. An infiltrate of neutrophils would be prominent in bullous impetigo, and clusters of cocci would be seen on the Gram stain. Direct immunofluorescence studies would be positive in the setting of pemphigus foliaceus. The clinical differential diagnosis would include Stevens–Johnson syndrome/toxic epidermal necrolysis, and often frozen section would be performed on sloughed skin to rule out this possibility.

Vignette 3

Clinical History: A 64-year-old woman presented with small pustules on her vulva that has developed into a widespread lesion. Physical examination revealed vegetating plaques on her entire vulva that clinically were concerning for condylomata acuminata (Fig. 4.26).

Microscopic Description: Histologic sections of the skin biopsy revealed marked epidermal acanthosis and papillomatosis (Fig. 4.27). Inflammation was noted in the superficial dermis and within the epidermis (Fig. 4.28). At high magnification, the inflammatory infiltrate within the epidermis was comprised of prominent eosinophils admixed with neutrophils (Fig. 4.29). Direct immunofluorescence examination demonstrated intercellular IgG and C3 deposition within the epidermis.

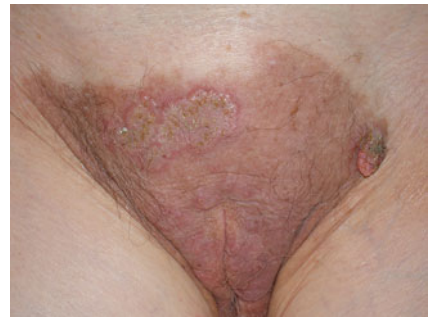


Fig. 4.26 Vignette 3. Slightly raised plaques and vegetating excrescences (Courtesy of Dr. Isabel Bielsa, Department of Dermatology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain)

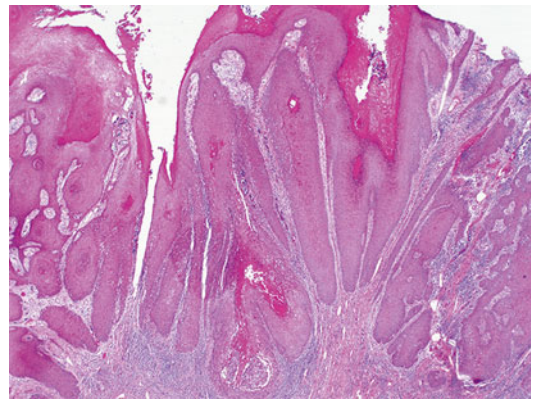


Fig. 4.27 Vignette 3. Marked epidermal acanthosis and papillomatosis

(continued)

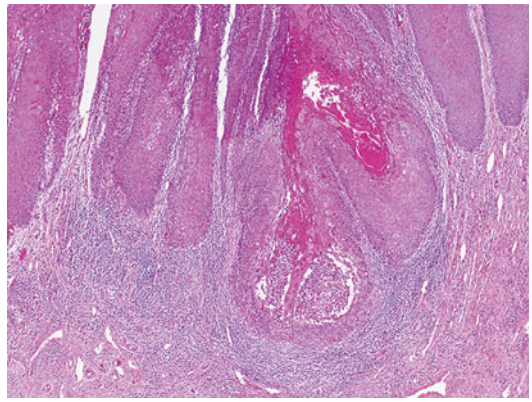


Fig. 4.28 Vignette 3. An inflammatory infiltrate is seen in the superficial dermis and within the epidermis

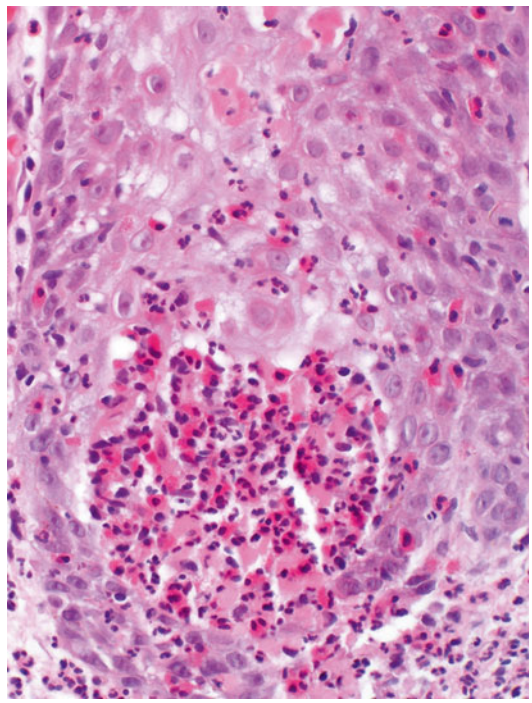


Fig. 4.29 Vignette 3. Collection of neutrophils and prominent eosinophils is noted within the epidermis

(continued)

Diagnosis: Pemphigus vegetans

Discussion: Pemphigus vegetans is a variant of pemphigus vulgaris that is characterized clinically by hypertrophic and vegetative skin lesions predominantly in the intertriginous areas. Oral involvement is common [79]. Historically, pemphigus vegetans has been separated into the Hallopeau type which has a benign course and the Neumann type which has extensive lesions often refractory to treatment [80]. Proliferative and verrucous lesions develop at sites of bullae that erode, grow into vegetating masses with sizes up to several centimeters, and can simulate clinically as warts [14]. The histologic findings are epidermal hyperplasia, suprabasilar acantholysis, and intraepidermal microabscesses comprised of eosinophils and neutrophils. The direct immunofluorescence findings would be similar to those of pemphigus vulgaris. The main autoantigen in pemphigus vegetans is desmoglein 3, although occasionally also desmoglein 1 and desmocollin 3 [14, 81]. Systemic steroids are still the main treatment for pemphigus vegetans. For refractory cases, adjuvant immunosuppressants such as cyclophosphamide, cyclosporine, azathioprine, and dapsone might be considered [14].

The main differential diagnosis is pyodermatitis–pyostomatitis vegetans, an inflammatory mucocutaneous dermatosis that is associated with inflammatory bowel disease [82, 83]. This entity shares the clinical and histologic findings with pemphigus vegetans yet with negative DIF results [82, 83]. One must be aware that in some rare cases C3, IgG, and IgA deposits can be seen at the basement membrane zone or within the epidermis, attributed to epithelial damage, and this reportedly has been resulted in a misdiagnosis as IgA pemphigus [84].

References

1. Patel NN, Patel DN. Staphylococcal scalded skin syndrome. Am J Med. 2010;123(6):505–7. doi:10.1016/j.amjmed.2009.09.041.
2. Amagai M, Matsuyoshi N, Wang ZH, Andl C, Stanley JR. Toxin is bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nat Med. 2000;6(11):1275–7.
3. Monday SR, Vath GM, Ferens WA, Deobald C, Rago JV, Gahr PJ, et al. Unique superantigen activity of staphylococcal exfoliative toxins. J Immunol. 1999;162(8):4550–9.
4. Fischer G, Rogers M. Vulvar disease in children: a clinical audit of 130 cases. Pediatr Dermatol. 2000; 17(1):1–6.
5. Ladhani S. Understanding the mechanism of action of the exfoliative toxins of *Staphylococcus aureus*. FEMS Immunol Med Microbiol. 2003;39(2):181–9.
6. Patel GK, Finlay AY. Staphylococcal scalded skin syndrome: diagnosis and management. Am J Clin Dermatol. 2003;4(3):165–75.
7. Amagai M. Pemphigus: autoimmunity to epidermal cell adhesion molecules. Adv Dermatol. 1996;11:319–52.
8. Barbosa NDF, de Aguiar LM, Maruta CW, Aoki V, Sotto MN, Labinas GH, et al. Vulvo-cervico-vaginal manifestations and evaluation of Papanicolaou smears in pemphigus vulgaris and pemphigus foliaceus. J Am Acad Dermatol. 2012;67(3):409–16.
9. Qian Y, Jeong JS, Maldonado M, Valenzuela JG, Gomes R, Teixeira C, et al. Cutting edge: Brazilian pemphigus foliaceus anti-desmoglein 1 autoantibodies cross-react with sand fly salivary LJM11 antigen. J Immunol. 2012;189(4):1535–9.
10. Akhyani M, Chams-Davatchi C, Naraghi Z, Daneshpazhooh M, Toosi S, Asgari M, Malekhami F. Cervicovaginal involvement in pemphigus vulgaris: a clinical study of 77 cases. Br J Dermatol. 2008; 158(3):478–82.
11. Malik M, Ahmed AR. Involvement of the female genital tract in pemphigus vulgaris. Obstet Gynecol. 2005;106(5 Pt 1):1005–12.
12. Batta K, Munday PE, Tatnall FM. Pemphigus vulgaris localized to the vagina presenting as chronic vaginal discharge. Br J Dermatol. 1999;140(5):945–7.

13. Kanawa S, Hashimoto T, Nishikawa T, Nishiyama S. Changes in clinical features, histologic findings, and antigen profiles with development of pemphigus foliaceus from pemphigus vulgaris. Arch Dermatol. 1994;130(12):1534–8.
14. Zaraa I, Sellami A, Bouguerra C, Sellami MK, Chelly I, Zitouna M, et al. Pemphigus vegetans: a clinical, histological, immunopathological and prognostic study. J Eur Acad Dermatol Venereol. 2011; 25(10):1160–7.
15. Wong KT, Wong KK. A case of acantholytic dermatosis of the vulva with features of pemphigus vegetans. J Cutan Pathol. 1994;21(5):453–6.
16. Zhang X, Hyjek E, Soltani K, Petronic-Rosic V, Shea CR. Immunohistochemistry for immunoglobulin G4 on paraffin sections for the diagnosis of pemphigus. Arch Pathol Lab Med. 2012;136(11):1402–7.
17. Alexandru A, Zurac S, Salavastru CM, Andrei R, Tebeica T, Staniceanu F, Tiplica GS. Direct immunofluorescence on hair follicles – present and future perspectives. Am J Dermatopathol. 2013;35(4):472–6.
18. Lewis FM, Agarwal A, Neill SM, Calonje JE, Stefanato CM. The spectrum of histopathologic patterns secondary to the application of EMLA on vulvar epithelium: clinicopathologic correlation in three cases. J Cutan Pathol. 2013;40(8):708–13.
19. Chorzelski TP, Kudejko J, Jablonska S. Is papular acantholytic dyskeratosis of the vulva a new entity? Arch Dermatol. 1984;6(6):557–60.
20. Cooper PH. Acantholytic dermatosis localized to the vulvocrural area. J Cutan Pathol. 1989;16(2):81–4.
21. Pestereli HE, Karaveli S, Oztekin S, Zorlu G. Benign persistent papular acantholytic and dyskeratotic eruption of the vulva: a case report. Int J Gynecol Pathol. 2000;19(4):374–6.
22. Saenz AM, Cirocco A, Avendano M, Gonzalez F, Sardi JR. Papular acantholytic dyskeratosis of the vulva. Pediatr Dermatol. 2005;22(3):237–9.
23. Coppola G, Muscardin LM, Piazza P. Papular acantholytic dyskeratosis. Am J Dermatopathol. 1986; 8(4):364–5.
24. Pernet C, Bessis D, Savignac M, Tron E, Guillot B, Hovnanian A. Genitoperineal papular acantholytic dyskeratosis is allelic to Hailey-Hailey disease. Br J Dermatol. 2012;167(1):210–2.
25. Lipoff JB, Mudgil AV, Young S, Chu P, Cohen SR. Acantholytic dermatosis of the crural folds with ATP2C1 mutation is a possible variant of Hailey-Hailey disease. J Cutan Med Surg. 2009;13(3): 151–4.
26. Logan E, Baxter ML, Walsh NM. White papules and plaques on the vulvar and perineal areas: challenge. Am J Dermatopathol. 2013;35(2):235–6.
27. Burge SM. Hailey-Hailey disease: the clinical presentation, response to treatment and prognosis. Br J Dermatol. 1992;126(3):275–82.
28. Evron S, Leviatan A, Okon E. Familial benign chronic pemphigus appearing as leukoplakia of the vulva. Int J Dermatol. 1984;23(8):556–7.
29. Wieselthier JS, Pincus SH. Hailey-Hailey disease of the vulva. Arch Dermatol. 1993;129(10):1344–5.
30. Misra R, Raman M, Singh N, Agarwal N. Hailey-Hailey disease masquerading as candidiasis. Int J Gynecol Obstet. 1993;42(1):51–2.
31. Cockayne SE, Rassl DM, Thomas SE. Squamous cell carcinoma arising in Hailey-Hailey disease of the vulva. Br J Dermatol. 2000;142(3):540–2.
32. Holst VA, Fair KP, Wilson BB, Patterson JW. Squamous cell carcinoma arising in Hailey-Hailey disease. J Am Acad Dermatol. 2000;43(2 Pt 2): 368–71.
33. Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S, et al. Mutation in ATP2A2, encoding a Ca^{2+} pump, cause Darier disease. Nat Genet. 1999;21(3):271–7.
34. Burge SM, Wilkinson JD. Darier-White disease: a review of the clinical presentation of 193 patients. J Am Acad Dermatol. 1992;27(1):40–50.
35. Barrett JFR, Murray LA, MacDonald HN. Darier's disease localized to the vulva. Case report Br J Obstet Gynecol. 1989;96(8):997–9.
36. Ridley CM, Buckley CH. Darier's disease localized to the vulva (correspondence). Br J Dermatol. 1991; 98(1):112.
37. Vazquez J, Morales C, Gonzalez LO, Lamelas ML, Ribas A. Vulval squamous cell carcinoma arising in localized Darier's disease. Eur J Obstet Gynecol Reprod Biol. 2002;102(2):206–8.
38. Duray PH, Merino MJ, Axiotis C. Warty dyskeratoma of the vulva. Int J Gynecol Pathol. 1983;2(3):286–93.
39. Waisbourd-Zinman O, Ben-Amitai D, Cohen AD, Feinmesser M, Mimouni D, Adir-Shani A, et al. Bullous pemphigoid in infancy: clinical and epidemiological characteristics. J Am Acad Dermatol. 2008;58(1):41–8.
40. Hertl M, Schmidt T. Underrecognition of the heterogeneous clinical spectrum of bullous pemphigoid. JAMA Dermatol. 2013;26:1–2.
41. Bakker CV, Terra JB, Pas HH, Jonkman MF. Bullous pemphigoid as pruritus in the elderly: a common presentation. JAMA Dermatol. 2013;26:1–5.
42. Schiavo AL, Ruocco E, Brancaccio G, Caccavale S, Ruocco V, Wolf R. Bullous pemphigoid: etiology, pathogenesis, and inducing factors: facts and controversies. Clin Dermatol. 2013;31(4):391–9.
43. Marren P, Wojnarowska F, Venning V, Wilson C, Nayar M. Vulvar involvement in auto-immune bullous diseases. J Reprod Med. 1993;38(2):101–7.
44. Urano S. Localized bullous pemphigoid of the vulva. J Dermatol. 1996;23(8):580–2.
45. Kharfi M, Khaled A, Anane R, Fazaa B, Karnoun MR. Early onset childhood cicatricial pemphigoid: a case report and review of the literature. Pediatr Dermatol. 2010;27(2):119–24.
46. Ridley CM. Cicatricial pemphigoid of the vulva. Am J Obstet Gynecol. 1985;152(7 Pt 1):916–7.
47. Marren P, Walkden V, Mallon E, Wojnarowska F. Vulval cicatricial pemphigoid may mimic lichen sclerosus. Br J Dermatol. 1996;134(3):522–4.
48. Farrell AM, Kirtschig G, Dalziel KL, Allen J, Dootson G, Edwards S, Wojnarowska F. Childhood genital pemphigoid: a clinical and immunopatho-

logical study of five patients. Br J Dermatol. 1999;140(2):308–12.
49. Trueb RM, Didierjean L, Fellas A, Elias A, Borradori L. Childhood bullous pemphigoid. Report of a case with characterization of the targeted antigens. J Am Acad Dermatol. 1999;40(2 Pt 2):338–44.
50. Levine V, Sanchez M, Nestor M. Localised vulvar pemphigoid in a child misdiagnosed as sexual abuse. Arch Dermatol. 1992;128(6):804–6.
51. Frith P, Charnock M, Wojnarowska F. Cicatricial pemphigoid diagnosed from ocular features in recurrent severe vulval scarring. Two case reports. Br J Obstet Gynaecol. 1991;98(5):482–4.
52. Chan LS, Ahmed AR, Anhalt GJ, Bernauer W, Cooper KD, Elder MJ, et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenetic factors, medical treatment and prognostic indicators. Arch Dermatol. 2002;138(3):370–9.
53. Liu Z, Diaz LA. Bullous pemphigoid: end of the century overview. J Dermatol. 2001;28(11):647–50.
54. Arechalde A, Braun RP, Calza AM, Hertl M, Didierjean L, Saurat JH, Borradori L. Childhood bullous pemphigoid associated with IgA antibodies against BP180 or BP230 antigens. Br J Dermatol. 1999;140(1):112–8.
55. Collier P, Wojnarowska F, Allen J, Kirtschiq G. Molecular overlap of the IgA target antigens in the subepidermal blistering diseases. Dermatology. 1994;189 Suppl 1:105–7.
56. Leverkus M, Bhol K, Hirako Y, Pas H, Sitaru C, Baier G, et al. Cicatricial pemphigoid with circulating autoantibodies to beta4 integrin, bullous pemphigoid 180 and bullous pemphigoid 230. Br J Dermatol. 2001; 145(6):998–1004.
57. Ghohestani RF, Nicolas JF, Rouselle P, Claudy AL. Identification of 168-kDa mucosal antigen in a subset of patients with cicatricial pemphigoid. J Invest Dermatol. 1996;107(1):136–9.
58. Vassileva S. Drug-induced pemphigoid: bullous and cicatricial. Clin Dermatol. 1998;16(3):379–87.
59. Pfaltz K, Mertz K, Rose C, Scheidegger P, Pfaltz M, Kempf W. C3d immunohistochemistry on formalin-fixed tissue is a valuable tool in the diagnosis of bullous pemphigoid of the skin. J Cutan Pathol. 2010; 37(6):654–8.
60. Korman NJ. Bullous pemphigoid. The latest in diagnosis, prognosis, and therapy. Arch Dermatol. 1998;134(9):1137–41.
61. Lehman JS, Carnilleri MJ. Diagnostic utility of direct immunofluorescence findings around hair follicles and sweat glands in immunobullous disease. J Cutan Pathol. 2013;40(2):230–5.
62. Lim HW, Bystryn JC. Evaluation and management of disease of the vulva: bullous diseases. Clin Obstet Gynecol. 1978;21(4):1007–22.
63. Lipozencic J, Ljubojevic S, Bukvic-Mokos Z. Pemphigoid gestationis. Clin Dermatol. 2012;30(1): 51–5.
64. Semkova K, Black M. Pemphigoid gestationis: current insights into pathogenesis and treatment. Eur J Obstet Reprod Biol. 2009;145(2):138–44.
65. Castro LA, Lundell RB, Krause PK, Gibson LE. Clinical experience in pemphigoid gestationis: report of 10 cases. J Am Acad Dermatol. 2006;55(5):823–8.
66. Chorzelski TP, Jablonska S. IgA linear dermatosis of childhood (chronic bullous disease of childhood). Br J Dermatol. 1979;101(5):535–42.
67. Egan CA, Zone JJ. Linear IgA bullous dermatosis. Int J Dermatol. 1999;38(11):818–27.
68. Ishiko A, Shimizu H, Masunaga T, Yancey KB, Giudice GJ, Zone JJ, Nishikawa T. 97-kDa linear IgA bullous dermatosis antigen localizes in the lamina lucida between the NC16A and carboxyl terminal domain of the 180 kDa bullous pemphigoid antigen. J Invest Dermatol. 1998;111(1):93–6.
69. Wojnarowska F, Frith P. Linear IgA disease. Dev Ophthalmol. 1997;28:64–72.
70. Fortuna G, Salas-Alanis JC, Guidetti E, Marinkovich MP. A critical reappraisal of the current data on drug-induced linear immunoglobulin A bullous dermatosis: a real and separate nosological entity? J Am Acad Dermatol. 2012;66(6):988–94.
71. Leonard JN, Haffenden GP, Ring NP, McMinn RM, Sidqwick A, Mowbray JF, et al. Linear IgA disease in adults. Br J Dermatol. 1982;107(3):301–16.
72. Petersen CS, Brocks K, Weismann K, Kobayasi T, Thomsen HK. Pretibial epidermolysis bullosa with vulvar involvement. Acta Derm Venereol. 1996; 76(1):80–1.
73. Irvine A, McLean W. Human keratin diseases: the increasing spectrum of disease and subtlety of the phenotype-genotype correlation. Br J Dermatol. 1999;140(5):815–28.
74. Ishida-Yamamoto A, McGrath J, Chapman S, Leigh IM, Lane EB, Eady RA. Epidermolysis bullosa simplex (Dowling-Meara type) is a genetic disease characterized by an abnormal keratin-filament network involving keratins K5 and K14. J Invest Dermatol. 1991;97(6):959–68.
75. McGrath J, Kivirikko S, Ciatti S, Moss C, Dunnill GS, Eady RA, et al. A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in Herlitz junctional epidermolysis bullosa: prenatal exclusion in a fetus at risk. Genomics. 1995;29(1):282–4.
76. Vidal F, Aberdam D, Miquel C, Christiano AM, Pulkkinen L, Uitto J, et al. Integrin beta 4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. Nat Genet. 1995;10(2):229–34.
77. Christiano A, Greenspan D, Hoffman G, Zhang X, Tamai Y, Lin AN, et al. A missense mutation in type VII collagen in two affected siblings with recessive dystrophic epidermolysis bullosa. Nat Genet. 1993;4(1):62–6.
78. Nordal EJ, Mecklenbeck S, Hausser I, Skranes J, Bruckner-Tuderman L, Gedde-Dahl Jr T. Generalized dystrophic epidermolysis bullosa: identification of a novel, homozygous glycine substitution, G2031S, in

exon 73 of COL7A1 in monozygous triplets. Br J Dermatol. 2001;144(1):151–7.
79. Apalla Z, Sotiriou E, Lazaridou E, Manousari A, Trigoni A, Papagarifallou I, Ioannides D. Pemphigus vegetans of the tongue: a diagnostic and therapeutic challenge. Int J Dermatol. 2013;53(3):350–1.
80. Ahmed AR, Blose DA. Pemphigus vegetans. Neumann type and hallopeau type. Int J Dermatol. 1984;23:135–41.
81. Saruta H, Ishii N, Teye K, Ono F, Ohyama B, Koga H, et al. Two cases of pemphigus vegetans with IgG anti-desmocollin 3 antibodies. JAMA Dermatol. 2013; 149(10):1209–13.
82. Storwick SG, Prihoda MB, Fulton JR, et al. Pyodermatitis-pyostomatitis vegetans: a specific marker for inflammatory bowel disease. J Am Acad Dermatol. 1994;31:336–42.
83. Mascarenhas R, Fernandes B, Reis JP, Tellechea O, Figueiredo A. Pemphigus vulgaris with nail involvement presenting with vegetating and verrucous lesions. Dermatol Online J. 2003;9:14.
84. Abellaneda C, Mascaro Jr JM, Vazquez MG, Pablo IM, Iranzo P. All that glitters is not pemphigus: pyodermatitis-pyostomatitis vegetans misdiagnosed as IgA pemphigus for 8 years. Am J Dermatopathol. 2011;33(1):e1–6.

5 Inflammatory Dermatoses Affecting the Dermis or Both the Epidermis and Dermis of the Vulva

Maria Teresa Fernández-Figueras

Introduction

Inflammatory dermatoses affecting the dermis or both the epidermis and the dermis are a heterogeneous group of entities with different inflammatory patterns. They include conditions such as vulvodynia and vestibulodynia, with minimal objective changes from a clinical and histopathological standpoint, but a marked impact on the quality of life. Conversely, in the group of lichenoid dermatoses, lesions of lichen planus and lichen sclerosus present clear-cut histopathological characteristics with noticeable clinical presentation, being in some cases the cause of severe anatomical deformities. Other inflammatory conditions in this group like granulomatous and vasculitic diseases can be restricted to the vulva (foreign body reaction, simple aphthosis, or Zoon vulvitis), occur in association with extragenital lesions in a limited number of locations (complex aphthosis, Crohn disease), or be part of a multisystem disease (sarcoidosis, Behçet disease). This chapter will discuss in-depth entities with lichenoid, granulomatous, and vasculopathic inflammatory patterns with emphasis on diagnostic clues and focus on differentiating among them. Our present understanding of vulvar pain syndromes is also presented.

M.T. Fernández-Figueras, M.D., Ph.D. (✉)
Department of Anatomic Pathology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
e-mail: maiteffig@gmail.com

Lichenoid Pattern

Lichen Planus

Clinical Features

Lichen planus (LP) is a chronic inflammatory autoimmune disorder that can affect any mucocutaneous surface [1]. Vulvar LP is more common in women between 30 and 60 years old [1] with a peak of incidence in the fifth decade [1, 2]. The patients complain of pain, itching, burning, dysuria, and dyspareunia with frequent postcoital bleeding and yellowish discharge that indicates vaginal involvement with presence of desquamative inflammatory vaginitis [1, 3, 4]. The most common finding in vulvar LP is the presence of introital erythema that can extend to the vagina (Fig. 5.1); less frequently these patients present with whitish slightly hyperkeratotic lesions containing the characteristic Wickham striae (Fig. 5.2) [5].

Vulvar LP can adopt three distinctive clinical forms: the most common is erosive LP, accounting from 74 % [6] to 95 % [7] of cases, followed by papulosquamous LP that usually appears in the setting of generalized disease, whereas the hypertrophic variant of LP is quite rare [1].

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_5,

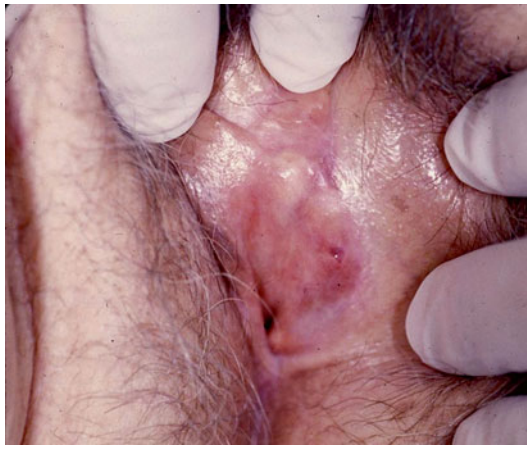


Fig. 5.1 Lichen planus. Introital erythema with small foci of ulceration showing well-demarcated borders (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

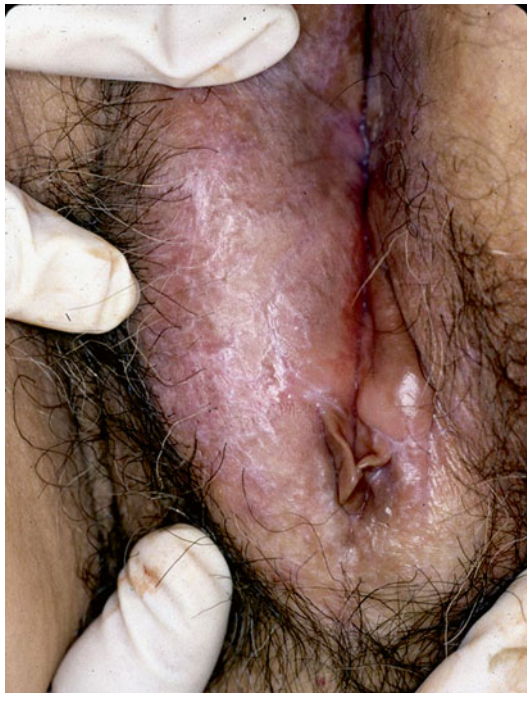


Fig. 5.2 Lichen planus. Whitish hyperkeratotic lesions with conspicuous Wickham striae (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

Erosive lichen planus is characterized by mucosal fragility with quite well-demarcated areas of erosion and ulceration (Fig. 5.1). In up to 70 % of these patients, the vaginal mucosa is also involved [1, 8]. Hemorrhage and scarring can lead to synechiae, introital stenosis, or vaginal obliteration. Papulosquamous LP appears clinically as pruritic keratotic papules on a slightly hyperkeratotic background. These papules are usually pinkish and poorly circumscribed rather than being small, well delimited, and violaceous as in cutaneous surfaces [8]. In hypertrophic LP the lesions are thick hyperkeratotic plaques that may resemble a vulvar squamous cell carcinoma (SCC) [8].

The incidence of LP in women is approximately 1 %. The most common location is the oral mucosa [9]. Twenty-five percent to 65 % of women with oral disease also present with vulvar and vaginal LP, which is often asymptomatic [10–12], constituting a triad known as vulvovaginal-gingival syndrome or plurimucosal LP [8, 13]. Vulvovaginal involvement is also common in women with cutaneous LP [14] and LP cicatricial alopecia [15]. In a study from the United Kingdom, 19.2 % of patients with vulvar LP also presented with LP alopecia [15]. Frontal fibrosing alopecia, a variant of lichen planopilaris, was the most common variant and it was found in a proportion similar to that of the general population [16]. Interestingly, all patients with vulvar LP and frontal fibrosing alopecia also presented with oral involvement [15]. Visceral mucosal surfaces can also be affected by LP; 42 % of individuals with esophageal LP also presented with vulvar disease in one study [17], and there is one report of LP in the uterine cervix [18].

Autoimmune diseases are frequently present in patients with vulvar LP; this association was found in 29 % of patients of a series [19], the most common comorbidity being autoimmune thyroiditis (15 %), followed by vitiligo (5.5 %), alopecia areata (3.9 %), and celiac disease (2.4 %), whereas chronic active hepatitis accounted for less than 1 % of cases.

Some women present with hybrid forms of LP and lichen sclerosus, and superposition of the two entities may be observed. It has been suggested that LP may act as a triggering factor in the development of LS [20].

Etiology and/or Pathogenesis

Although the exact pathogenesis of vulvar LP is not known, genetic and autoimmune factors are clearly implicated. Familial cases of LP are rather common, often in association with HLA class II antigens; specifically, the *HLADQBI 0201* allele is associated with some cases of vulvovaginal-gingival syndrome [21]. The association of LP with other autoimmune diseases and the presence of circulating autoantibodies in a frequency higher than controls are arguments in favor of an autoimmune etiology [19, 22]. Furthermore, the lymphocytic infiltrate in LP exhibits an autoimmune phenotype, corresponding to a Th1 interferon (IFN) gamma-induced immune response [23] with activated T cells targeting basal keratinocytes. Indirect immunofluorescence using sera from 56 individuals with a definitive clinical diagnosis of erosive genital LP showed epidermal-binding and basement membrane zone antibodies, which were also present in the sera of 61 % of these patients [24]. Recent evidences also support an association between beta-blockers and nonsteroidal anti-inflammatory treatments and the presence of vulvar LP [25].

Prognosis or Course

Vulvar LP is a long-lasting inflammatory condition with great variation in severity of symptoms and response to treatment. The course is in general more prolonged than in the nongenital cutaneous counterpart, but the risk of malignant transformation is moderately low. Combining the results of four case series published in the literature, Simpson et al. [26] found infiltrating vulvar SCC in 1.3 % of patients. A multicenter case study yielded a similarly low percentage of 2.3 % [27]. At any rate, cases of aggressive malignant transformation exist [28], thus making periodical control of these patients and histopathological examination of suspicious lesions advisable.

Histopathology

The histologic appearance of LP in the vulva is similar to that in other locations although, often, changes displayed in mucosal LP are subtler than in its cutaneous counterpart. Thus, the findings can be quite equivocal for those who do not routinely review this type of pathology under the microscope. The most consistent finding in all cases of vulvar LP is a lymphocytic infiltrate forming a band in the superficial connective tissue that obscures the basal epithelial layer (Fig. 5.3), but the intensity varies greatly from case to case and can be limited to a small number of cells sprinkling the mucosal basal layers (Fig. 5.4). Plasma cells can also be abundant, as in any

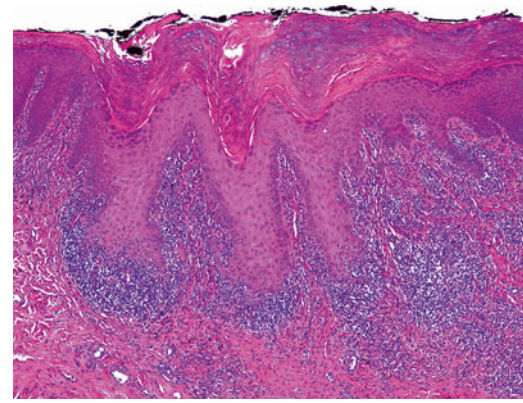


Fig. 5.3 Lichen planus. Epithelial hyperplasia with dense lymphocytic infiltrate at the basal layer and compact hyperkeratosis with small foci of parakeratosis

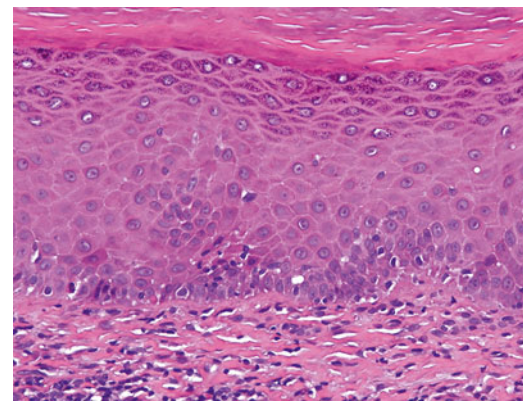


Fig. 5.4 Lichen planus. The biopsy shows scant lymphocytic infiltrate at the basal layer. Nevertheless, the presence of hypergranulosis supports the diagnosis of lichen planus

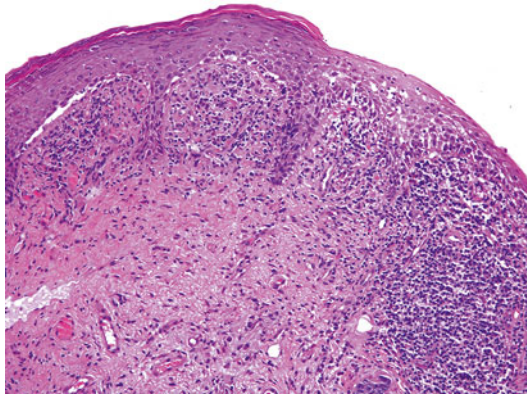


Fig. 5.5 Papulonodular lichen planus. Acanthosis with sawtooth silhouette, hypergranulosis, lichenoid lymphocytic infiltrate, basal vacuolar change, and dyskeratosis

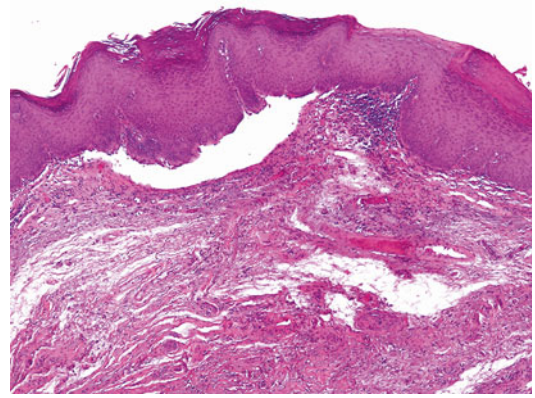


Fig. 5.7 Lichen planus. Small subepidermal blister (Max Joseph space)

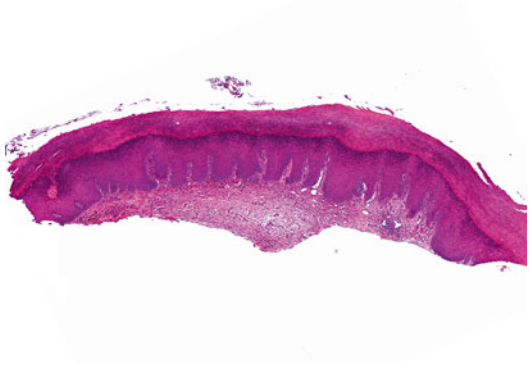


Fig. 5.6 Hypertrophic lichen planus. Epithelial hyperplasia with a thick horny layer

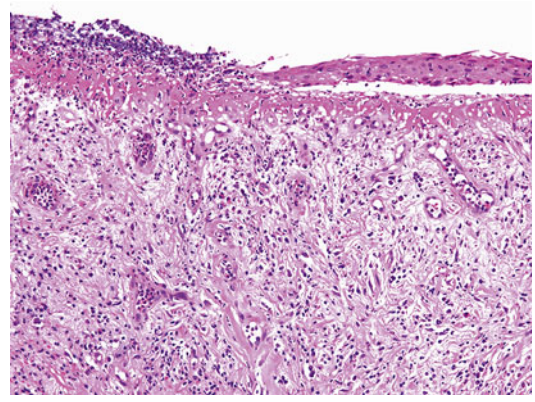


Fig. 5.8 Erosive lichen planus with epithelial detachment and extensive ulceration

mucosal inflammation. The epidermis may be either atrophic or hypertrophic with the distinctive sawtooth silhouette of rete ridges (Fig. 5.5), although it is not always present, and can be observed only focally. It is common to find hypergranulosis, some degree of hyperkeratosis with occasional areas of parakeratosis (Fig. 5.3). An intense thickening of the horny layer is the hallmark of the hyperkeratotic variant of vulvar LP or the presence of lichen simplex chronicus changes due to rubbing (Fig. 5.6).

Basal epithelial cells show vacuolization or cytological alterations that can be misinterpreted as atypical, but are secondary to the inflammatory damage. Small subepidermal blisters (Fig. 5.7) can also be observed, in some cases leading to epithelial detachment that is especially prominent in erosive LP (Fig. 5.8). Dyskeratotic apoptotic cells (the so-called Civatte bodies) are usually scarce and their identification often requires a careful search. Direct immunofluorescence (DIF) studies may show shaggy fibrinogen staining of the basement membrane and IgM-positive apoptotic keratinocytes within the basal layer or in the upper portion of the dermis [29].

Immunophenotype

The inflammatory infiltrate is fundamentally constituted by T lymphocytes with CD4+, CD8+, and FOXP3+ phenotypes. An increased expression of the proinflammatory cytokines corresponding to Th1 immune response is also present [23].

Differential Diagnosis

The main clinical and histopathological differential diagnosis is to be made with lichen sclerosus (LS). A correct identification of the disease has clinical importance, but it can be difficult because both entities share many clinicopathological features and can even seldom coexist. Nonetheless, there are some helpful clues to tell them apart. The main differential features are summarized in Table 5.1. Vulvar lesions of LP are often painful and their surface is keratotic or ulcerated, instead of itchy and atrophic like in LS. In addition, LP rarely spreads to the vaginal or perianal areas and introital stenosis is a rare event, occurring only in the advanced stages. For patients with extragenital lesions, the distinction between both entities is even easier. Cutaneous involvement is more common in LP than in LS and consists of polygonal flat-top papules, whereas LS is characterized by the development of atrophic macules. Furthermore, oral lesions are frequent in LP but extremely rare in LS, and nail lesions have only been described in LP. Histopathological differentiation between LP and LS in doubtful cases may require a careful study of many step sections; the main feature in favor of LP is the serrated silhouette of the epithelium that, in most cases, is present at least focally. However, to ensure the diagnosis of LP, it is even more important to rule out the presence of distinguishing features of LS, such as basal membrane thickening that can be highlighted by a PAS staining, submucosal edema with ectatic blood vessels, hints of fresh or old hemorrhage, and, principally, the homogeneous hyalinization of the collagen characteristic of LS. Although chronic lesions of LP may show a band of sclerotic collagen at the base, it is never as wide as in LS.

Table 5.1 Main clinical and histopathological differential features between lichen planus and lichen sclerosus

	Lichen planus	Lichen sclerosus
Clinical features		
Cutaneous involvement	Frequent	Infrequent
Vaginal involvement	Present, frequent in the erosive variant	Extremely rare
Perianal involvement	Rare	Frequent
Oral involvement	Frequent	Extremely rare
Nail involvement	Infrequent	Absent
Presenting symptoms	Sores and pain	Pruritus
Introital stenosis	Frequent	Only in the advanced stage
Clinical hallmarks	Keratosis and ulceration	Mucosal thinning and hemorrhage
Histologic features		
Serrated epidermis	Frequently focal	Extremely rare
Thickening of the basal membrane	Extremely rare	Frequent
Submucosal edema	Extremely rare	Frequent
Ectatic blood vessels	Extremely rare	Frequent
Hemorrhage or siderophages	Rare	Frequent
Collagen hyalinization	Rare	Frequent

Other simulators of LP are the lichenoid drug reactions; their distinction is further complicated by the fact that some drugs can precipitate an LP eruption. The presence of many eosinophils in the infiltrate and sometimes vascular damage can contribute to its recognition. Erosive LP may resemble clinically and microscopically cicatricial pemphigoid and pemphigus. The absence of a significant number of eosinophils and the presence of dyskeratotic cells are features in favor of LP. In case of doubt, a DIF examination can contribute to its distinction. This technique is especially useful in the case of paraneoplastic pemphigus also known as paraneoplastic autoimmune multiorgan syndrome (PAMS). The reason of its utility is that the first manifestations of PAMS can arise in the oral and genital area and they can be microscopically indistinguishable from LP. The presence of immunoglobulins and complement deposition in the intercellular spaces and along the basal membrane of PAMS is definitive to reveal the correct diagnosis.

An international electronic-Delphi consensus on clinicopathological criteria for diagnosis of erosive LP has been recently published [30], but

its utility remains to be validated in a large series of cases. The authors propose that three out of the following nine criteria can be sufficient to make the diagnosis of erosive LP:

- Well-demarcated erosions or erythematous areas at the vaginal introitus
- Presence of hyperkeratosis and/or Wickham striae at the periphery
- Symptoms of pain/burning
- Scarring or loss of normal architecture
- Vaginal inflammation
- Involvement of other mucosal surfaces
- Presence of a well-defined inflammatory band involving the dermoepidermal junction
- Presence of an inflammatory band consisting predominantly of lymphocytes
- Signs of basal layer degeneration

A careful clinicopathological correlation is mandatory for a definite categorization of each case, and when reaching a definite diagnosis is not feasible, the best approach is to provide symptomatic treatment and wait for the appearance of more specific findings, rather than trying to fit the case into a wrong entity.

Takeaway Essentials

Clinical Relevant Pearls

- Vaginal involvement favors the diagnosis of lichen planus versus lichen sclerosus.
- Look for extragenital lesions like lichen planopilaris.
- The high incidence of autoimmune diseases in these patients makes it advisable to perform a complete workup. Autoimmune thyroiditis is the most frequent among them.

Pathology Interpretation Pearls

- The abundance of plasma cells in the infiltrate can be misleading, but almost always they account for less than 50 % of the cellularity.
- Isolated atypical squamous cells at the basal layer are often reactive and do not necessarily indicate malignant transformation. Step sections can help to establish or rule it out.

Summary

Clinical Presentation

- Introital erythema extending to the vagina
- Less frequently hyperkeratosis with Wickham striae
- Three clinical forms: erosive, papulosquamous, and hypertrophic

Histologic Features

- Band-like lymphocytic infiltrate at the basal epithelial layer
- Basal cell vacuolization and occasional dyskeratosis

Differential Diagnosis

- Lichen sclerosus
- Zoon vulvitis
- Squamous cell carcinoma (hypertrophic variant)

Zoon Vulvitis

Clinical Features

Zoon vulvitis (ZV), also known as plasma cell vulvitis or vulvitis plasmacellularis, is an inflammatory disease quite rare in female and much more frequent in male genitalia [31]. Most cases arise in adult women [32], although ZV has been occasionally reported in prepubertal girls [33]. Some patients are asymptomatic, whereas others present with pruritus that can be intense [34], soreness, pain, burning, or bleeding [32]. Dyspareunia and dysuria are habitual consequences.

Clinically, lesions of ZV tend to occur in the labia minora and introitus and consist of sharply demarcated macules or papules that rarely ulcerate. Their surface is shiny red or brown red. The clitoral glans is rarely involved [32, 35–37].

The presence of ZV is not related to other medical disorders, although there are isolated reports of ZV in association with autoimmune

polyglandular syndrome [38], LS [39], herpes simplex virus type II [40], and acquired immunodeficiency syndrome (AIDS) [41].

Etiology and/or Pathogenesis

Whereas Zoon balanitis is a clear-cut entity, probably a reactive condition secondary to maceration and chronic irritation in uncircumcised men [31], the existence of its feminine counterpart as an independent entity has been questioned [42]. Nevertheless, vulvar cases with clinicopathological features identical to those in men exist, and the same causes can be accountable as etiological factors, since poor hygiene, sweating, trauma, and persistent friction can also occur in the vulvar mucosa.

Prognosis or Course

In some patients the lesions slowly evolve to spontaneous resolution. For the rest of them, none of the therapeutic approaches that have been tried has provided consistently satisfactory results [37]. The existence of ZV does not carry an increased risk of vulvar SCC.

Histopathology

A plasma cell infiltrate is the hallmark of ZV. However, the infiltrate is not necessarily composed exclusively of plasma cells. Lymphocytes, eosinophils, and neutrophils can also be abundant. A proportion of plasma cells representing 50 % or more of the infiltrate seem to be sufficient for the diagnosis (Fig. 5.9); when the percentage drops to 25–50 %, the coexistence of other characteristic findings of ZV such as epithelial atrophy, vascular proliferation, dilated capillaries, superficial dermal fibrosis, erythrocyte extravasation, and hemosiderin deposition allows recognition of ZV. If plasma cells account for less than 25 % of the infiltrate, a nonspecific plasma cell mucosal reaction cannot be excluded [43].

The presence of "lozenge-shaped" or "diamond-shaped" keratinocytes with horizontal axes longer than vertical axes in the suprabasal epithelial layers has been considered a characteristic finding of ZV and balanitis, but this is a rather subjective microscopic finding, and thus its feasibility as a diagnostic clue is questionable.

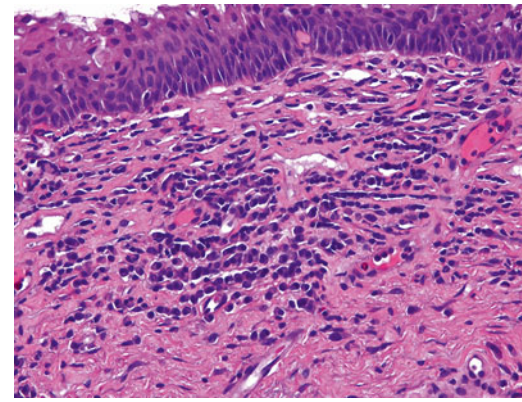


Fig. 5.9 Zoon vulvitis. Inflammatory infiltrate composed mainly of plasma cells. Some lozenge keratinocytes can be appreciated in the left portion of the epithelium

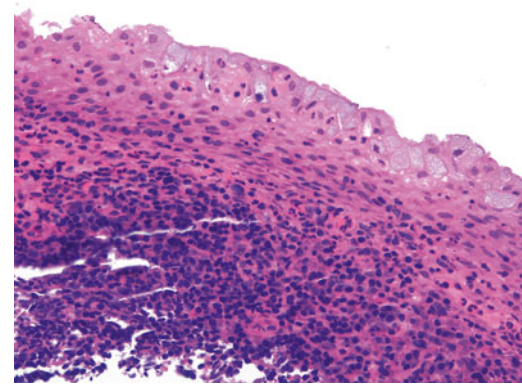


Fig. 5.10 Zoon vulvitis. Mucinous metaplasia of the superficial epithelium

Differential Diagnosis

Many entities can be included in the clinical differential diagnosis of ZV. Especially important is the distinction from vulvar SCC, Bowen disease, and extramammary Paget disease. A biopsy can easily provide help in this differentiation. Nevertheless, it is important to keep in mind that the presence of areas of mucinous metaplasia has been described in ZV (Fig. 5.10) [44], and care should be taken in not making a wrong diagnosis of extramammary Paget disease. In addition, cases of ZV overlap clinicopathologically with lichen aureus [45]. Other entities included in the differential diagnosis of ZV are lichen planus, contact dermatitis, lupus erythematosus, candidiasis,

herpes simplex virus or human papillomavirus infections, bullous disorders, and fixed drug eruption. Especially important is the distinction from syphilis, also characterized by a dense plasma cell infiltrate. The best way to rule out this possibility is performing an immunohistochemical staining against *Treponema pallidum*; additionally, primary chancres tend to ulcerate, and both primary and secondary syphilis lesions often present with an associated granulomatous infiltrate, plasma cells, vasculitis, and lichenoid or psoriasiform features. ZV in girls may simulate sexual abuse [33], a possibility that at any rate should never be disregarded without further investigation.

Summary

Clinical Presentation

- Usually on the labia minora and introitus
- Shiny red or rusty, sharply demarcated macules or papules

Histologic Features

- Inflammatory infiltrate rich in plasma cells (≥50 % or between 25 % and 50 % with a clinically typical presentation)

Differential Diagnosis

- Syphilis
- Lichen aureus (overlap cases exist)

Takeaway Essentials

Clinical Relevant Pearls

- Ulceration is rare and more suggestive of syphilis or other conditions.
- The glans clitoris is almost never involved.

Pathology Interpretation Pearls

- Plasma cells are common in the inflammatory infiltrate of any inflamed mucosa and can obscure the underlying pathology. Before making a diagnosis of Zoon vulvitis, it is necessary to search actively for infections or features that point toward other dermatoses
- Zoon vulvitis can show mucinous metaplasia that does not indicate extramammary Paget disease.

Immunohistochemical Findings

- An immunostaining with antibodies against *Treponema pallidum* is the best way to rule out syphilis.

Lichen Sclerosus

Clinical Features

Lichen sclerosus (LS), known in the past as lichen sclerosus et atrophicus or kraurosis vulvae, is a chronic autoimmune mucocutaneous disorder that usually involves the anogenital region of women. It can also occur in male genitalia and extragenital locations. LS is one of the most common vulvar dermatoses [14], with a predilection for Caucasian females with two peaks of incidence in prepubertal and postmenopausal women. The prevalence of vulvar LS has been reported to be 0.11 % for girls [46] and 1.7 % for adult women [47].

Lesions of LS usually begin around the clitoris with secondary involvement of the interlabial sulcus (Fig. 5.11) and posterior spread to the labia minora and majora, the perineum, and the perianal skin (Fig. 5.12), with a typical eight-shaped figure. In contrast with LP, vaginal involvement in LS is extremely rare [48], but episiotomy scars and areas with genital jewelry can be involved due to a Koebner phenomenon. Extragenital lesions may develop in up to 11 % of patients [49, 50].

Vulvar soreness and itching are the commonest symptoms in girls. Adult patients may also report burning sensation or pain, and dyspareunia and dysuria are common complaints, especially when fissures and ulcers develop.

On clinical examination, the earliest changes include erythema, mucosal thinning and wrinkling, focal lichenification, erosion, purpura, abnormal

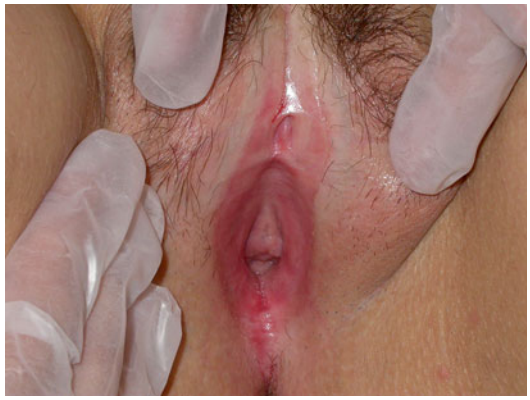


Fig. 5.11 Lichen sclerosus. The clitoris and interlabial sulcus showing erythema, mucosal thinning, focal pigmentation, and a small fissure (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

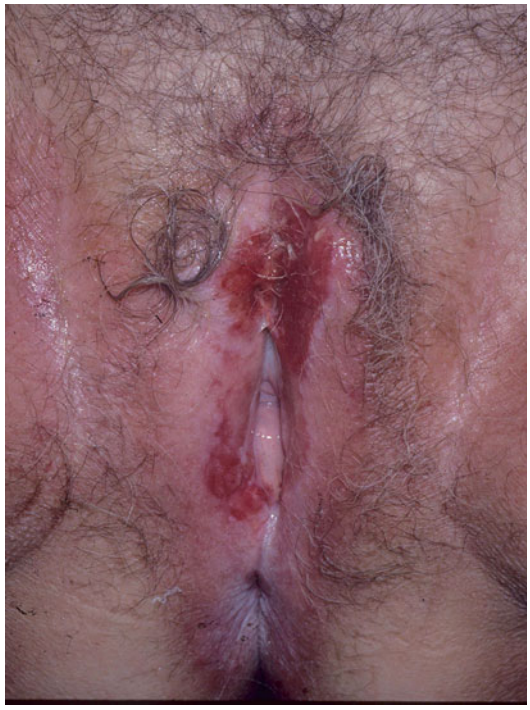


Fig. 5.12 Lichen sclerosus. Lesion involving the vulva, perineum, and perianal skin (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

pigmentation, and hypotrichosis (Fig. 5.11) [51]. Later on, mucosal pallor, atrophy, or hyperkeratosis becomes more evident, and pale indurated papules and plaques with areas of telangiectasia and

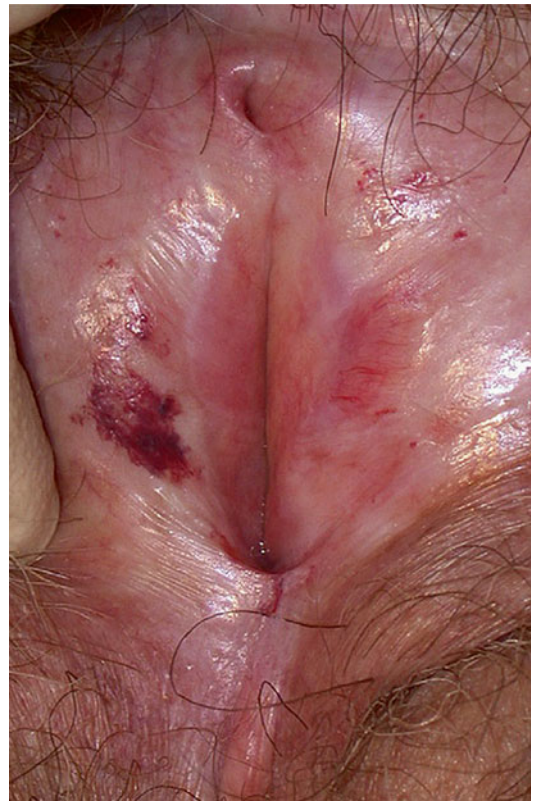


Fig. 5.13 Lichen sclerosus. Atrophic mucosa with telangiectasia and hemorrhage (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

hemorrhage develop (Figs. 5.12 and 5.13). In the advanced stage, the lesions are lichenified white-porcelain papules and plaques with superficial crinkling or cellophane-like atrophic skin (Figs. 5.14 and 5.15). Ulcerations, fissures, blisters, ecchymoses, and subepidermal hemorrhage are frequent (Figs. 5.11 and 5.12) [51]. Chronic inflammation may lead to scarring with adhesions of the clitoral hood, resorption and fusions of the labia minora, pseudocyst formation, and stenosis of the vaginal introitus. Since there is no synchronicity among the individual lesions of LS, areas with early and late changes can occur simultaneously in different vulvar zones from the same patient [52].

The typical dermoscopic features of LS include patchy structureless white to yellowish areas, linear anastomosing telangiectasia, and

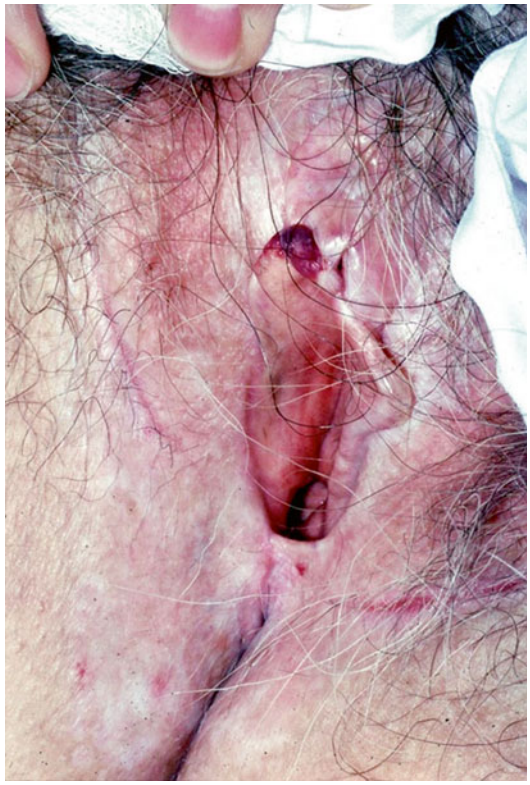


Fig. 5.14 Lichen sclerosus. Intense atrophy with indurated white-porcelain areas (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

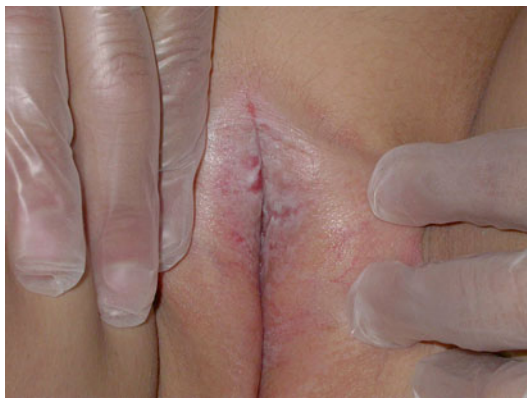


Fig. 5.15 Lichen sclerosus. Atrophic mucosa with multiple erosions (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

randomly arranged red dotted areas corresponding to loose aggregates of blood vessels [53].

Autoimmune disorders are frequent in patients with LS [19, 54]; they have been reported to be present in at least 18.9 % of cases [54]. This association is less common in the male population with genital LS. In two case series autoimmune thyroiditis was the most frequent autoimmune comorbidity, occurring in 12–16 % of patients; vitiligo was the second most frequent in one of the studies [19], whereas morphea represented only 1.5–1.7 % of the associated autoimmune disorders.

LS has been considered to carry an increased risk of developing vulvar SCC in adult patients [55, 56]. In a cohort of symptomatic vulvar LS patients, malignant transformation was described in 21 % of cases [56]. Nevertheless, the mechanisms and real incidence of malignant transformation are subject to debate. Some authors contend that even though atypical epithelial changes are frequently found in close contact to invasive SCC [55, 57], only a small proportion of them are typical areas of LS, whereas most cases correspond to differentiated vulvar intraepithelial neoplasia (dVIN) [58], a nonhuman papillomavirus (HPV)-related dysplasia that mainly affects elderly patients [59]. In summary, even though LS has been considered a potential precursor of dVIN, the risk of LS malignant transformation might have been overestimated. A counterargument underscores the possibility of asymptomatic LS patients and complete replacement of a precursor lesion by tumor as potential reasons to consider the reported incidence of malignant transformation an underestimate.

Abnormal pigmentation is frequent in LS, the most common alterations being melanosis and lentiginosis [60]. Melanocytic nevi occurring in areas of LS may acquire features of persistent melanocytic nevi and lead to a misdiagnosis of malignant melanoma, especially in children [61]. However, rare cases of malignant melanoma associated with vulvar LS have been reported in the literature [62, 63], and in the presence of atypical pigmented areas, a biopsy is mandatory [64].

There are also individual reports of Langerhans cells histiocytosis, basal cell carcinoma, and Merkel cell carcinoma [64, 65] appearing in the setting of LS.

Etiology and/or Pathogenesis

Autoimmunity and genetic factors play a major role in the development of LS. On one side, the

high incidence of familial cases and the association with several HLA haplotypes favor the existence of an inherited susceptibility [66]. On the other side, the close association between LS and autoimmune diseases demonstrated by several studies [54, 67] and the existence of autoimmune antibodies in 42 % of LS patients [67] strongly support autoimmune mechanisms. Furthermore, the inflammatory infiltrate in LS displays an autoimmune phenotype corresponding to a Th1 response with high expression of microRNA-155, like in LP [18]. Another interesting observation in this regard is the presence of IgG circulating autoantibodies against extracellular matrix protein 1 (ECM1) in approximately 75 % of patients with vulvar LS [68], whereas these antibodies are absent in patients with other lichenoid dermatoses [68]. Furthermore, mutations in the *ECM1* gene are responsible for lipoid proteinosis, which presents histopathological similarities with LS; this has led some authors to suggest that ECM1 could act as an autoantigen [68]. However, recent studies have shown that the autoreactivity to this antigen increases with the progress and extent of the disease, indicating that it is a secondary pathogenetic event that probably contributes to LS progression, rather than being involved in the initiation of the process [69]. Furthermore, high titers of autoantibodies targeting the basement membrane zone have been found in several studies [70, 71]. This finding has not been confirmed using more accurate detection techniques [71]. Nevertheless, a relationship seems to exist, at least in some cases, between LS and immunobullous diseases [72].

Another element that might facilitate the development of LS would be chronic exposure to urine of a susceptible epithelium under occlusion or an alteration of the epithelial barrier, as in atopic dermatitis [73, 74]. Hormonal factors might also influence the development of the disease, since in a series of 44 pediatric cases, both Turner syndrome (2 cases) and renal disease (2 cases) were overrepresented [75]. Finally, the altered methylation and hydroxymethylation status present in vulvar LS (either isolated or associated with vulvar SCC) may contribute to enhance the autoimmune and inflammatory response [76, 77], whereas the influence of infectious agents remains uncertain [60].

Prognosis or Course

The disease can be progressive or follow a relapsing and remitting course [60]. There is no correlation between the duration of the disease, the degree of clinical and histopathological involvement, and the severity of symptoms [52, 60]. In a large series of newly diagnosed LS in adult women, up to 58 % of cases were asymptomatic, although in many patients some scarring of the clitoral prepuce or resorption of the labia minora was clinically evident [47].

Most remissions occur in the first 3 months after treatment [62]. Spontaneous healing is rare, even in prepubertal cases where there can be some improvement with age, but resolution is especially unlikely if the disease remains active after menarche [78, 79].

Histopathology

The typical appearance of a fully developed lesion of LS shows mucosal atrophy with vacuolar degeneration at the basal layer, basal membrane hyalinization (Fig. 5.16), and occasional dyskeratosis. Subepithelial edema is commonly present and can be so intense as to cause pseudovesiculation. The edema merges imperceptibly with the dermal collagen, which shows a characteristic homogeneous ground-glass appearance (Fig. 5.17). This sclerotic collagen forms a wide band as the lesion progresses, entrapping dilated capillary vessels (Fig. 5.18) that bleed easily. An inflammatory infiltrate of variable intensity is often present at the interface between the altered

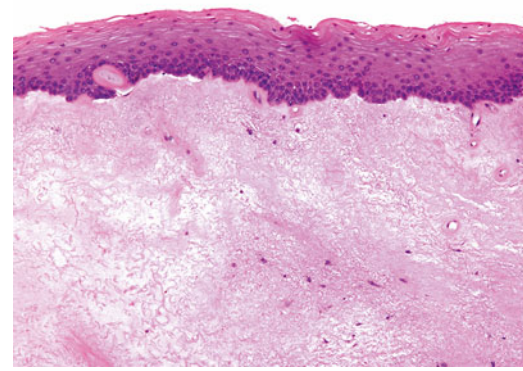


Fig. 5.16 Lichen sclerosus. Hyalinization at the basal membrane zone and intense subepithelial edema

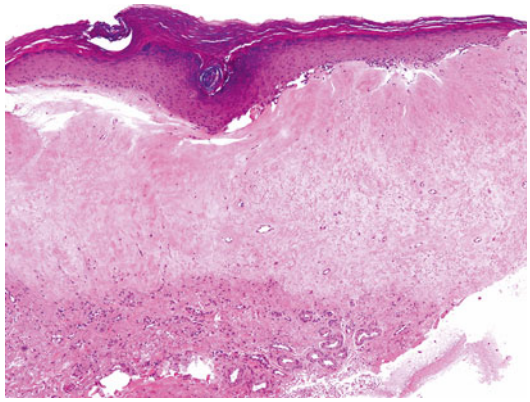


Fig. 5.17 Lichen sclerosus. Homogeneous sclerosis of the collagen in the upper dermis with superficial edema

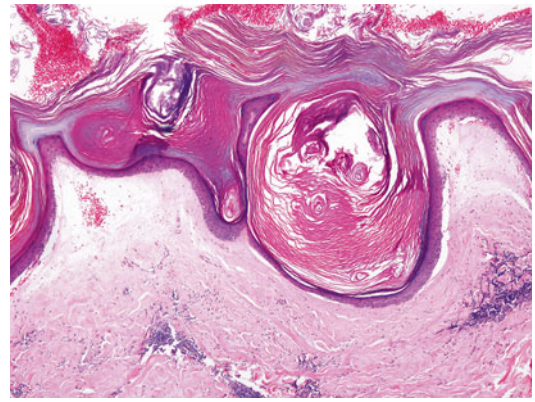


Fig. 5.19 Lichen sclerosus. Epithelial atrophy with keratin plugs

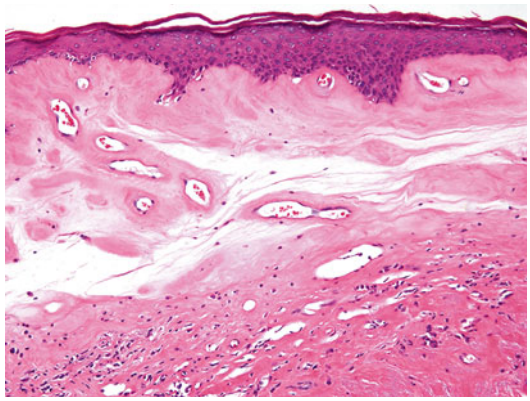


Fig. 5.18 Lichen sclerosus. Hyalinized collagen containing dilated capillary vessels

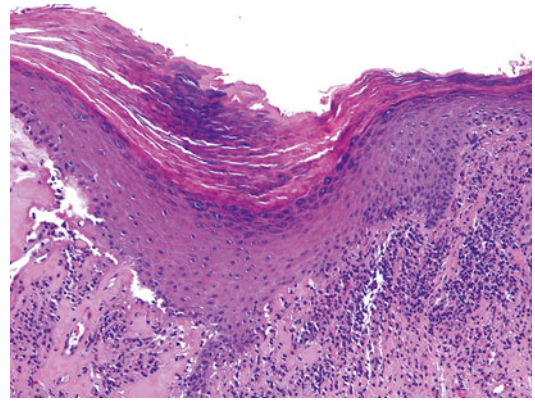


Fig. 5.20 Lichen sclerosus. Focus of early involvement showing a lichenoid pattern with lymphocytic infiltrate obscuring the basal layer, hypergranulosis, and hyperkeratosis. In close contact with this area, the biopsy shows features of advanced stage with the characteristic hyaline sclerosis of the collagen

collagen and the uninvolved connective tissue. Hair follicles with corneal plugs, dystrophic hairs, and hyperkeratotic acrosyringia can also be observed at this stage (Fig. 5.19).

Unlike in advanced stages, the earliest changes of LS are subtle and can be easily missed or misleading. The epithelium may display psoriasiform hyperplasia and the lymphocytic infiltrate can be dense or sparse, with lichenoid, interstitial, epitheliotropic, or vasculitic pattern (Fig. 5.20) [80, 81]. When the sclerosis is still not prominent, the absence of cytological atypia at the lower epithelial layers helps to rule out dVIN [82]. The key for diagnosis at this early stage can be found looking for the typical features of LS around the adnexal structures, where the lesion is more evident.

Lichenification due to chronic scratching can modify the usual appearance of LS with epithelial acanthosis, presence of dyskeratotic cells in all layers of the epidermis, hypergranulosis, and hyperkeratosis that can be compact and orthokeratotic or parakeratotic [82]. Some cases show large areas of subepithelial hemorrhage. The accumulation of melanophages as a result of basal destruction is rarely prominent, but in some instances can

cause areas of striking atypical pigmentation. Aggregates of elastotic material can be also found. Mucinous metaplasia is another rare finding and has to be differentiated from Paget disease [83].

Immunophenotype

Lymphocytes present within the infiltrate are mostly T cells, CD4+, CD8+, and FOXP3+ cells [18]. Overexpression of MIB1 and p53 may help to identify cases with higher risk to evolve to vulvar SCC [84], but p53 immunoreactivity involving only the basal cell layer can also occur in LS due to ischemic stress [85].

Differential Diagnosis

Sexual abuse is the main clinical differential diagnosis in girls. In one study this possibility was considered in 77 % of pediatric cases. Nevertheless, this possibility has to be always considered since both conditions may coexist [86]. The earliest clinical changes in adults can be subtle and simulate vitiligo or *Candida* infection. In more advanced stages the differential diagnosis includes LP, dVIN, lichen simplex chronicus, psoriasis, mucosal pemphigus, cicatricial pemphigoid, extramammary Paget disease, and vulvar SCC [60, 80, 87]. Vaginal involvement does not rule out LS, but is strongly in favor of other possibilities [48].

Histopathologically the main differential diagnosis is lichen planus. The most important differential features between the two entities are summarized in Table 5.1. It is also important to differentiate LS from dVIN; even though both diseases can coexist in the same patient, the latter should be diagnosed only in the presence of obvious basal atypia. Some cases with prominent blistering may simulate pemphigoid, and the presence of epithelial atrophy with a lymphocytic infiltrate at the dermoepidermal junction and rows of lymphocytes within the basal layer of the epidermis may resemble early lesions of mycosis fungoides (MF). A differential feature is the presence of homogenized collagen in LS, whereas in MF the dermis contains coarse bundles of collagen. Nonetheless, such an important differential diagnosis cannot be solely based on this inconstant feature. Furthermore, in some cases it may be necessary to study step sections looking for the typical features of LS, or even perform additional biopsies from different areas, in order to clarify the diagnosis. An additional obstacle for the distinction is that in a single biopsy from early MF, neither the immunophenotype, nor a clonal T cell receptor gene rearrangement (TCR) are reliable techniques to separate benign from malignant infiltrates.

Loss of dermal elastic fibers in LS but not in LP has been proposed as a clue for their distinction [88], but subsequent studies have failed to confirm these differences [89].

Overexpression of p53 and MIB1 contributes to the identification of lesions with high risk of vulvar SCC transformation [84]. However, the prognostic implications are not conclusive when p53 expression is limited to the basal layer [85]. Additionally, alterations in the basal membrane can be highlighted using periodic acid-Schiff (PAS) staining, which at the same time allows ruling out *Candida* infection.

Summary

Clinical Presentation

- Early lesions: erythema and mucosal thinning on the clitoris and interlabial sulcus
- Late lesions: white-porcelain papules and plaques with cellophane-like atrophic skin and figure-of-eight distribution (complete vulvar involvement, narrowed at the perineum and surrounding the anal region)

Histologic Features

- Mucosal atrophy with vacuolar damage
- Edema between the epithelium and a band of sclerotic "glassy" collagen

Differential Diagnosis

- Lichen planus
- Differentiated VIN

Takeaway Essentials

Clinical Relevant Pearls

- Look for extragenital lesions (present in 11 % of cases).
- The high incidence of autoimmune diseases in these patients makes it advisable to perform a complete workup. Autoimmune thyroiditis is the most frequent among them.

Pathology Interpretation Pearls

- In the early lesions look for typical changes around adnexal structures.
- In a small or fragmented biopsy specimen, basement membrane hyalinization is a clue for the diagnosis of lichen sclerosus.
- Melanocytic nevi within the lesion can acquire features that simulate melanoma.
- For vulvar lesions resembling early mycosis fungoides, consider the diagnosis of lichen sclerosus and look for its typical features performing additional sections, if necessary, or ask for a new biopsy from a different area.

Histochemical Findings

- A PAS staining can highlight the basal membrane thickening.
- A staining for elastic fibers can illustrate the absence of elastic fibers at the base of the lesions that, although nonspecific, points toward the diagnosis of lichen sclerosus.

Immunohistochemical Findings

- Overexpression of p53 limited to the basal layer can be reactive and does not indicate dVIN.

Granulomatous Diseases

Crohn Disease

Clinical Features

Crohn disease (CD) is a chronic relapsing granulomatous disorder that can affect any portion of the gastrointestinal tract. It is characterized by transmural bowel inflammation with marked tendency to form fistulas to adjacent structures and sometimes to the skin [90]. Mild gynecological alterations can occur in up to 24 % of female patients [91] since pediatric age [92], but serious gynecological complications such as fistulas or metastatic CD are less frequent. In a series of patients with CD, genital fistulization was present in 3.8 % of women; in most cases the site of drainage was the vagina and less frequently the vulva [93]. Conversely, the vulva is the gynecological location most susceptible to develop metastatic CD [94], defined as the presence of non-caseating granulomatous inflammation discontinuous to gastrointestinal tract involvement. Metastatic and intestinal CD may occur simultaneously; nonetheless, metastatic lesions can also precede or appear subsequently to intestinal involvement [91]. More than half of metastatic CD cases occur in gynecological locations [95]; the average age of presentation is 34 years, but there are reports in children as young as 8 years old [96, 97].

The commonest clinical manifestations of CD in the vulva are local edema with labial swelling, ulcerations (that tend to be linear but can adopt other configurations) [97, 98], abscesses, draining sinuses, and hypertrophic exophytic lesions with a "bulbous" or condylomatous appearance (Fig. 5.21) [95, 97–101]. An increased incidence of malignancies has also been reported [98].

Table 5.2 Granulomatous diseases of the vulva

	Metastatic Crohn disease	*Sarcoidosis*	*Foreign body reaction*	*Granulomatous infections*
Vulvar clinical presentation	Edema	Pruriginous patches, papules, or nodules	Erythema and tumefaction	Swelling
	Ulceration	Ulceration is rare		Ulceration
	Draining sinuses			Abscessification
	Exophytic lesions			
Local pain	Moderate	Rare (one case)	Moderate	Usually intense
Associated clinical findings	Anal fissures and exophytic lesions	Simultaneous inguinal, perineal, and perianal involvement	Related to the foreign body origin (such as recent surgery or a neoplasm)	Fever
	Gastrointestinal symptoms	Facial lesions		Malaise
		Systemic involvement		
Epidermis	Frequently ulcerated	Rarely ulcerated	Usually normal	Variable: Normal Ulceration Epidermal hyperplasia with parakeratosis
	Occasional lichenoid features			
Dermis	Granulomatous dermatitis with mixed cell inflammatory infiltrate	Non-necrotizing granulomas with scant tendency to fuse	Palisades of mononucleated histiocytes and multinucleated giant cells	Necrotizing granulomas
	Small abscesses of neutrophils	Sparse lymphocytic infiltrate and absence of acute inflammation	Foreign material in the center of the granulomas or within the cytoplasm of giant cells	Neutrophils and plasma cells depending on the type of infection
Type of granulomas	Necrotizing and non-necrotizing	Sarcoid	Foreign body type	Necrotizing
Hallmark	Granulomatous inflammation with ulceration	Non-necrotizing epithelioid granulomas that do not tend to fuse	Granulomas with multinucleated foreign body-type giant cells	Necrotizing granulomas
	Non-caseating granulomas in more than one-third of cases	Scant lymphocytic infiltrate		
Nonconstant additional microscopic findings	Eosinophils	Scar tissue and foreign bodies can trigger sarcoidosis and can be focally identified	Eosinophils	Infectious agents. Usually identified with the contribution of special techniques
	Granulomatous vasculitis		Abscesses of neutrophils	
	Dilated lymphatic spaces		Fibrosis	
	Necrobiosis			

Etiology and/or Pathogenesis

The exact pathogenesis of metastatic CD is not known, but the dysregulated proinflammatory response has been classically attributed to the migration to the skin of intestinal antigens or immune complexes, probably related to the gut microbial flora [102].

Prognosis or Course

The severity of non-intestinal manifestations of CD may not parallel the intestinal disease [90], but many metastatic lesions resolve spontaneously or with the same treatment used for the control of the intestinal process [103]. Nevertheless, surgical repair can be required due to delays or failure of medical treatment.

Histopathology

Fistulization of Crohn disease can be easily diagnosed on clinical grounds alone, but a biopsy may be required to rule out malignant transformation. Non-caseating granulomas have been considered the most distinctive feature of metastatic CD (Fig. 5.22). In some cases these granulomas are

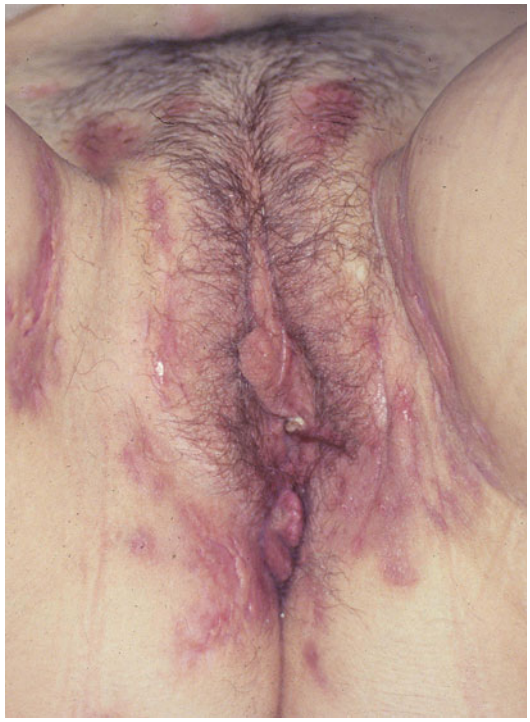


Fig. 5.21 Crohn disease. Vulvar edema associated with linear erythematous lesions in the inguinal region and a bulbous perianal protuberance (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

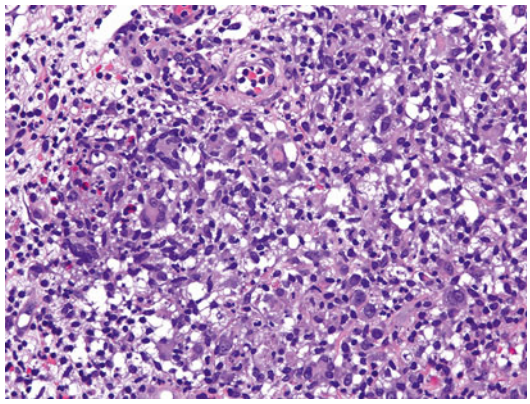


Fig. 5.22 Crohn disease. Granulomatous inflammation with multinucleated giant cells. Eosinophils are noticeable

well developed, with presence of multinucleated giant cells of Langhans and foreign body types [94, 104], but this is not a constant feature; definite granulomas were present in only 38 % of cases in a series of vulvar CD [98]. Thus, relying

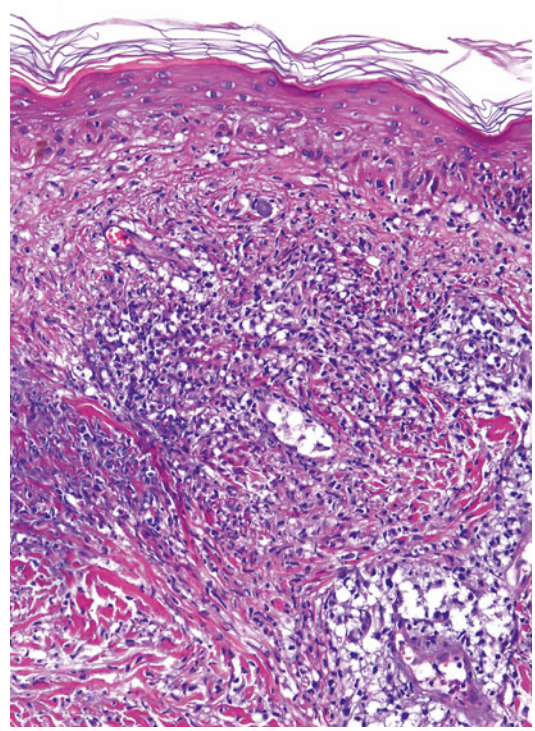


Fig. 5.23 Crohn disease. Granulomatous vasculitis and focus of necrobiosis

exclusively in this criterion would utterly impair the sensitivity of microscopic diagnosis. In the same series granulomas were not always well developed, and ulceration was observed in the same proportion as granulomatous inflammation [98]. Two other findings that have been described in metastatic CD are the combination of lichenoid and granulomatous dermatitis and the presence of granulomatous vasculitis (Fig. 5.23) [94]. Dilated lymphatic spaces were observed by Foo et al. [98] in 31 % of biopsy specimens, leading in some cases to an initial diagnosis of lymphangioma or lymphangiokeratoma. It has been hypothesized that lymphatic ectasia and proliferation might appear as a consequence of the fibrosis due to surgical procedures or chronic inflammation [98]. Remarkably, an image similar to an intralymphatic histiocytosis can be appreciated in one paper [105], in which it was labeled as lymphangitis. The presence of aggregates of neutrophils and focal eosinophilia (Fig. 5.22) is also a feature typical of CD, and necrobiosis (Fig. 5.23) is an additional finding that favors this diagnosis.

Differential Diagnosis

The clinicopathological differential diagnosis of vulvar CD includes hidradenitis suppurativa that may cause draining sinuses and areas of abscessification. Hidradenitis can be differentiated from CD because of the abundance of neutrophils with a tendency to abscessification and the likelihood of the infiltrate to be centered in the apocrine glands and hair follicles. Cystic hair follicles are also frequent, as well as epidermal cysts. It is common to find areas of granulomatous reaction, but it tends to be foreign body type, secondary to keratin leakage from the ruptured cysts.

Pyoderma gangrenosum (PG) is a neutrophilic autoinflammatory dermatosis that frequently occurs in relation with systemic processes, with inflammatory bowel disease being one of the most common triggering factors. Genital involvement by PG in a patient with CD is rather unusual, but this has to be considered in the differential diagnosis of metastatic CD [106]. Microscopically, well-developed lesions of PG are characterized by neutrophilic infiltrate that is in general more suppurative than vulvar CD. In addition, in PG the granulomatous inflammation is always necrotizing and foci of neutrophilic or lymphocytic vasculitis can be found.

Infections should also be differentiated from vulvar CD. The most important are tuberculosis, actinomycosis, and lymphogranuloma venereum. Clinically, the coexistence of perianal lesions such as skin tags, anal fissures, ulcers, fistulas, perianal abscesses, and anorectal strictures favors the diagnosis of vulvar CD. In addition, the absence of pain or tenderness in the vulvar swelling militates against an infectious etiology [107]. In tuberculosis the granulomas are noticeable, confluent, and caseating. In actinomycosis the main microscopic finding is the presence of fistulous tracts filled by neutrophilic infiltrate that usually contain large collections of actinomyces. The hallmark of lymphogranuloma venereum is the presence of lymphadenopathies that can contain stellate areas of necrosis leading to draining sinuses and their complete destruction. Microscopically, the dermal findings are usually nonspecific, and the main involvement is located in the subcutaneous tissue where the lymph nodes can be completely effaced due to the necroinflammatory process. In case of doubt, histochemical, immunohistochemical, and molecular techniques, as well as microbiological cultures, are advisable to rule out an infectious process.

Vulvar sarcoidosis can also be considered in the differential diagnosis of CD; the presence of an abundant inflammatory infiltrate rich in plasma cells and eosinophils, ulceration, and lymphatic dilatation are features that favor the diagnosis of CD. Vulvitis granulomatosa is the genital counterpart of cheilitis granulomatosa and can overlap with metastatic CD [108], but cases unrelated to CD also occur and this possibility should be taken into consideration.

Finally, factitial dermatitis and sexual abuse can also simulate a vulvar CD. In both cases the lesions are often externally initiated; superficial excoriations are the most common finding, and, in general, the inflammatory infiltrate is limited to the tissues closer to the area of where the injury was inflicted. The remaining tissues are free of pathology although sometimes they may show scars or hints of previous involvement. However, it is important to keep in mind that the damage can be caused by a wide range of means such as the suction effect, thermal injuries, or injection of foreign materials and, therefore, can lead to a host of inexplicable changes. Thus, factitial dermatitis and sexual abuse are possibilities that always have to be taken into consideration when facing unusual clinicopathological findings. The main differential features among CD, granulomatous infections, foreign body reactions, and sarcoidosis are summarized in Table 5.2.

Foreign Body Reaction

Clinical Features

Foreign body reactions appear clinically as localized areas of tumefaction and erythema. The most common causes in the vulva are keratinous material released to the interstitium by a follicle-associated process and the rupture of an epidermal inclusion cyst. Other possible causes are the presence of retained suture material from a previous

Summary

Clinical Presentation

- Local edema with labial swelling
- Ulceration (often linear), abscesses, and draining sinuses
- Exophytic swollen lesions, especially perianal

Histologic Features

- Non-caseating granulomas or mixed granulomatous infiltrate with eosinophils
- Lichenoid and granulomatous dermatitis with vascular damage

Differential Diagnosis

- Hidradenitis suppurativa
- Sarcoidosis
- Infections

Takeaway Essentials

Clinical Relevant Pearls

- Anal lesions, even fissures or skin tags, should alert about the possibility of vulvar Crohn disease.
- Vulvar Crohn disease can be the first manifestation of the process (also in children). Nevertheless, endoscopy can detect asymptomatic gastrointestinal involvement and aid in confirming the diagnosis.

Pathology Interpretation Pearls

- Granulomas are not always well formed; frequently CD presents as a loose collection of histiocytes.
- Dilated lymphatic spaces can be misinterpreted as tumoral or malformative lesion, yet they are a clue for diagnosis.

surgical procedure and the existence of tattoos and genital jewelry. Much rare is the accidental implantation of sand, splinters, and other materials or the anecdotal cases of migration of liquid silicone injected in the breasts or buttocks for augmentation purposes [109]. Extensive talcum powder use to mitigate pruritus can produce

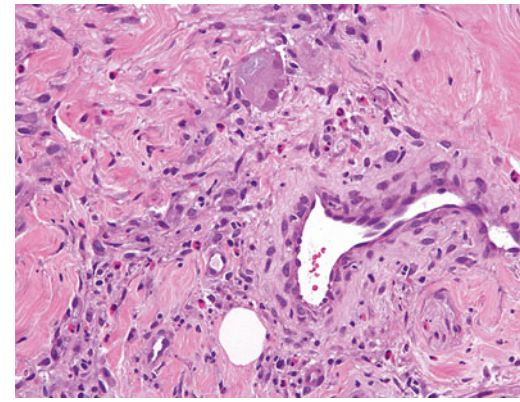


Fig. 5.24 Foreign body reaction. A giant cell containing phagocytosed surgical material and many eosinophils in an area of induration developed after a gynecological surgical procedure

foreign body granulomas; histologic study and directed clinical history can clarify this diagnosis [110]. Another rare cause of foreign body reaction is the presence of inert extracellular material that originated from a neoplasm, such as keratin from a cystic teratoma or mucopolysaccharides from an adenocarcinoma or a myxoid neoplasm [111].

Histopathology

The typical histologic appearance in all cases is that of a foreign body reaction with mononucleated macrophages that tend to form palisades around the causative material and/or foreign body-type multinucleated giant cells that may contain phagocytosed foreign materials in their cytoplasm; eosinophils and neutrophils can be abundant (Fig. 5.24). Examination under polarized light microscopy is recommended to highlight the foreign material and can contribute to its identification, as in the case of talcum powder, which displays a characteristic Maltese cross appearance.

Differential Diagnosis

The differential diagnosis includes other granulomatous vulvar processes such as CD or sarcoidosis; granulomatous infections, especially granuloma inguinale, and deep fungal infections; and primary immunodeficiencies. Granulomas in sarcoidosis and CD can contain occasional multinucleated giant cells, but never as numerous as in a foreign body reaction. Palisades of histiocytes are also absent in both processes, and

although small fragments of foreign material can be occasionally detected in the cytoplasm of giant cells, they are not present in a significant degree, but rather as an incidental finding.

Granulomatous infections including tuberculosis, deep mycosis, and granuloma inguinale should be ruled out. In tuberculous granulomas giant cells are mostly Langerhans cell type and necrosis is caseous. Deep mycotic infections are characterized by the association of granulomas and abscesses of neutrophils, similar to foreign body reaction. The cause can be the accidental implantation of a splinter or any other contaminated foreign material, making a PAS staining mandatory to exclude this possibility. Vulvar lesions of granuloma inguinale are often ulcerated and the infiltrate contains many plasma cells in addition to numerous histiocytes. These histiocytes often contain many elements of *Klebsiella* granulomatis (Donovan bodies) in their cytoplasm that are better appreciated using a Giemsa staining.

In primary immunodeficiencies, there is not only an increased risk of infections but also relapsing episodes of noninfectious chronic granulomatous inflammation. Bacterial, fungal, or viral tests on the skin biopsy are often helpful to exclude an infection. The main differential features among CD, granulomatous infections, foreign body reactions, and sarcoidosis are summarized in Table 5.2.

Summary

Clinical Presentation

- Erythema and tumefaction

Histologic Features

- Granulomatous infiltrate with foreign body giant cells (5–15 nuclei that can be centrally placed or polarized in the portion of the cytoplasm closest to the foreign material)
- Presence of foreign material (keratin, talcum powder, or others)

Differential Diagnosis

- Crohn disease
- Sarcoidosis
- Granulomatous infections

Takeaway Essentials

Clinical Relevant Pearls

- Consider step section to investigate the possibility of a neoplasm when seeing a foreign body reaction to mucinous material (adenocarcinoma or myxoid neoplasm) or keratin (teratoma).
- Detailed clinical history is paramount for a definitive diagnosis.

Pathology Interpretation Pearls

- To search for foreign bodies, study the sections under polarized light microscopy, and look for tattoo pigments.
- The presence of small amounts of foreign material does not rule out sarcoidosis.

Sarcoidosis

Clinical Features

Sarcoidosis is a multisystem granulomatous disorder that most commonly affects young adults, with a slight female predominance [112]; it can occur in any part of the body, but the lungs and mediastinal lymph nodes are most commonly affected.

Different susceptibility genes could explain the marked geographical differences on the incidence and behavior of sarcoidosis. African descendants are more frequently and severely affected than those from other ethnic background [113], being sarcoidosis an important cause of premature death in this population [114]. The prevalence of sarcoidosis is also relatively high in persons of northern European descent, whereas the incidence is quite low in Asian, South American, and the Mediterranean countries [113]. Familial clustering of cases has been described, mainly in Caucasian individuals [113]. In Asia and Europe there is a peak of incidence around 50 years in women [113], which is less evident among those of sub-Saharan origin.

Skin lesions are often the first manifestation of the disease and eventually its only site of involvement. They can be specific (granulomatous) or nonspecific (usually erythema nodosum) [115] and are present in 20–30 % of patients [116]. The most frequent presentation for specific lesions is papules on the face, but any cutaneous surface can be affected, and the disease can adopt such a wide variety of forms that it is considered to be one of the "great imitators" [116]. Mucosal involvement is an infrequent manifestation of sarcoidosis, and vulvar involvement is even more rare [116–124]. In the scarce published cases, the most common symptom was pruritus, with perineal pain in one case [117–124]. The clinical appearance is variable: atrophic white patches, erythematous papules, infiltrated violaceous plaques, and even a small "soybean-sized" submucosal nodule [118]. Ulceration seems to be less common in vulvar lesions than in masculine genital sarcoidosis [116]. Simultaneous inguinal, perineal, and perianal involvement is not rare, and in one case the patient presented erythematous scaly plaques on the mons pubis in association with vulvar sarcoidosis [121]. Vulvar and facial lesions coexist in many patients, either associated with systemic disease or as the only manifestation of sarcoidosis [122–124].

Etiology and/or Pathogenesis

The etiology of sarcoidosis is unknown, but it is assumed to result from an exacerbated immune response to an infectious or environmental factor in a genetically susceptible individual. The association of sarcoidosis with autoimmune diseases has led to the hypothesis that it is a consequence of Th1 lymphocyte prevalence [125]. An association with type IV hypersensitivity has also been suggested [126]. TNF-α seems to be an important factor in the development of the granulomatous response, and, in severe cases, treatment with TNF-α-inhibitors can provide some benefit [127].

Prognosis or Course

The course of sarcoidosis is chronic; treatment is not necessary for all patients because the disease can remain stable or remit spontaneously [128]. Yet, spontaneous resolution is less likely in African descent individuals [116]. The prognosis of cutaneous sarcoidosis greatly depends on the predominant type of lesion: maculopapular eruptions and subcutaneous nodules tend to disappear in less than 2 years, whereas plaques and lupus pernio are markers of chronic systemic disease [115]. The small number of vulvar cases does not allow drawing conclusions regarding evolution of the disease in this location.

Histopathology

Sarcoidosis is the prototype of non-caseating granulomatous inflammation. Sarcoid granulomas are characterized by well-defined outlines (Fig. 5.25); even though they can be in close contact, they do not tend to fuse (Fig. 5.26). They are made up of epithelioid mononucleated histiocytes with some multinucleated giant cells that occasionally contain asteroid bodies (Fig. 5.25) and Schaumann bodies (Fig. 5.27). Other inflammatory cells are typically absent or scarce (that is why they are called "naked granulomas") and mainly correspond to lymphocytes and plasma cells. Polarizable material or small areas of fibrinoid necrosis in the center of the granulomas are considered unusual findings in sarcoidosis, but they are not so rare in the skin [129]. The most striking finding among the few reported cases of vulvar sarcoidosis was the existence of

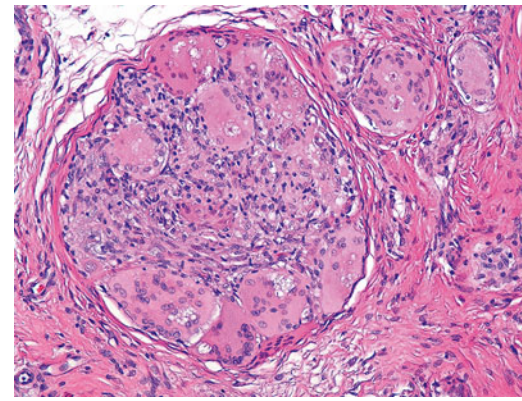


Fig. 5.25 Sarcoidosis. Sharply demarcated non-caseating granuloma made up of epithelioid histiocytes and multinucleated giant cells. Many asteroid bodies are present in the cytoplasm of the giant cells

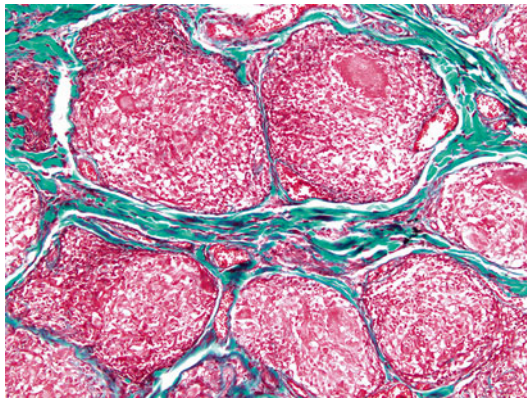


Fig. 5.26 Sarcoidosis. Sarcoid granulomas are typically well circumscribed and do not tend to fuse. These characteristics can be highlighted using Masson's like in this image

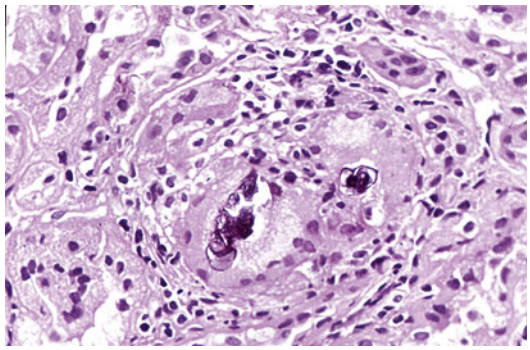


Fig. 5.27 Sarcoidosis. Calcified Schaumann bodies in the cytoplasm of two giant cells

transepidermal elimination in one case [121]; this phenomenon might be more common in cutaneous sarcoidosis than is generally believed.

Immunohistochemically, the histiocytes predominantly present in the granulomas express CD68 and PGM1 but are negative for S100 and CD1a. The scant lymphocytic infiltrate associated with the granulomatous inflammation shows a predominance of CD4+ T lymphocytes, with a CD4-CD8 ratio in the skin greater than 2:1 [130].

Differential Diagnosis

The clinical differential diagnosis includes Crohn disease, idiopathic granulomatous vulvitis, foreign body reactions, and infectious processes such as syphilis, tuberculosis, or lymphogranuloma venereum.

Microscopically, the presence of non-caseating granulomas narrows down the differential diagnosis to Crohn disease, idiopathic granulomatous vulvitis, foreign body reactions, and a large number of infectious processes. A primary immunodeficiency disorder is another possible differential diagnosis from a histologic standpoint [130]. Histochemical and immunohistochemical stainings are mandatory to rule out infections, and in some cases microbiological culture and PCR are advisable. Primary immunodeficiency disorders are characterized by a low CD4-CD8 ratio, as opposed to cutaneous sarcoidosis [130]. The presence of polarizable material does not rule out sarcoidosis but should elicit the need of a detailed clinical history to rule out granulomatous inflammation to foreign material. If Crohn disease is a strong clinical consideration, close follow-up is recommended as cutaneous CD can precede for years the gastrointestinal symptoms.

The main differential features among CD, granulomatous infections, foreign body reactions, and sarcoidosis are summarized in Table 5.2.

Summary

Clinical Presentation

- Patches, papules, plaques, or nodules that can be pruritic or painful

Histologic Features

- Non-caseating granulomas than can lie close but do not tend to fuse
- Scant inflammatory infiltrate rich in CD4+ T lymphocytes

Differential Diagnosis

- Foreign body reaction
- Crohn disease
- Granulomatous infection

Takeaway Essentials

Clinical Relevant Pearls

- Look for other sites of involvement, especially on the face and old scars.
- The presence of erythema nodosum favors the diagnosis of sarcoidosis.

Pathology Interpretation Pearls

- The presence of small amounts of fibrinoid necrosis or refractile material does not rule out sarcoidosis.

Immunohistochemical Findings

- An elevated CD4-CD8 ratio is typical of sarcoidosis.

Vasculopathic Pattern

Aphthous Ulcer

Clinical Features

Aphthous ulcers are nonsexually acquired painful ulcerations on the genital mucosa that almost always occur in prepubertal girls and young women. There are few studies addressing the incidence of vulvar aphthae, and the frequency of this condition has probably been underestimated [131]. The onset is typically acute and the most common site is the inner aspect of the labia minora. The lesions have a well-defined border with irregular outline, an even depth of 1–2 mm, and perilesional edema and erythema (Fig. 5.28). Identical lesions are frequently present on the oral mucosa. In many cases the lesions can be precipitated by viral infections or sexual intercourse [131].

There are three types of oral and vulvar aphthous ulcers:

- Minor: smaller than 10 mm, tend to heal within 1–2 weeks without scarring, and are most common
- Major: larger than 10 mm, usually last for weeks or months causing extreme pain, and heal with scarring
- Herpetiform: multiple grouped tiny ulcers, are more common on the tongue than on the vulva, and heal within a few weeks without scarring

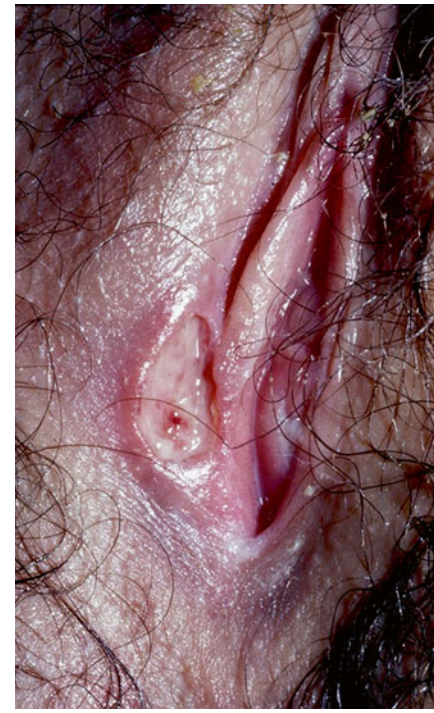


Fig. 5.28 Aphthous ulcer. Sharply demarcated ulcer in the inner aspect of the labia majora showing an irregular outline and perilesional edema and erythema (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

Oral and vulvar aphthous ulcers are usually recurrent and depending on their course can also be classified as:

- Simple aphthosis: several episodes per year, separated by disease-free periods.
- Complex aphthosis: virtually continuous presence of three or more ulcers or periodic episodes of oral and genital aphthae not explained by other specific causes. In the vulva it is also known as "nonsexually acquired genital ulceration" (NSAGU) and can be associated with intense edema of the labia minora [132].

Lipschütz ulcer (ulcus vulvae acutum) is a special variant of vulvar aphthae characterized by the development of one or more painful ulcers in the context of fever and systemic symptoms. The Lipschütz ulcers are usually located on the midportion of the labia minora, and the existence of a mirrored symmetrical lesion on the opposite

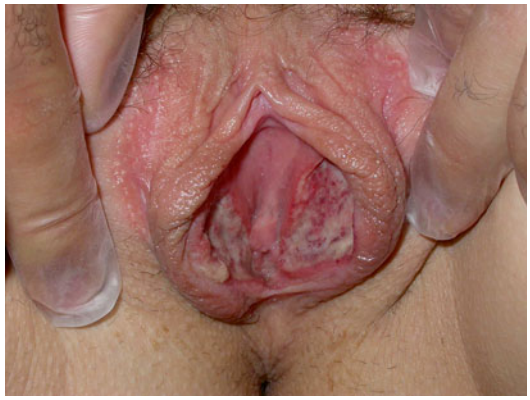


Fig. 5.29 Lipschütz ulcers (ulcus vulvae acutum) with a mirrored kissing pattern in the inner aspect of the labia minora (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

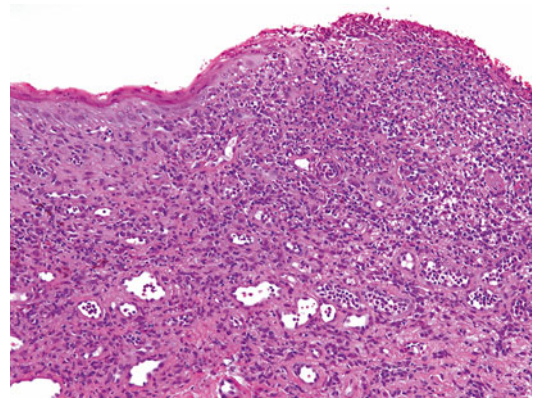


Fig. 5.30 Aphthous ulcer. Superficial neutrophilic infiltrate, predominantly perivascular, with leukocytoclastic vasculitis

side (kissing pattern) is highly characteristic (Fig. 5.29). Their borders are distinct and slightly raised and their surface is covered by a pseudomembranous exudate or an eschar [133].

Etiology and/or Pathogenesis

The cause of this condition is unclear although multiple factors seem to be involved. Some cases are temporally associated with a viral infection, but infectious agents are absent in the injured tissue [134]. Oral and vulvar aphthae seem to be the consequence of an alteration in the innate and/or acquired immunity that can be initiated by an infection or a local stress in a genetically predisposed female [134, 135]. In the case of Lipschütz ulcer, the most common triggering factor is Epstein-Barr virus infection, but some cases are related to other infections [133, 136, 137].

Prognosis or Course

By definition this systemic involvement is lacking in this condition and the main problem is discomfort and pain. Acute vulvar aphthous ulcers associated with a flu-like prodrome (Lipschütz ulcers) are unlikely to recur [138, 139]. Simple aphthosis is characterized by intermittent episodes of self-limited lesions that can be managed with symptomatic therapy, whereas the almost constant presence of vulvar ulcers in complex aphthosis, often combined with oral lesions, has a marked effect on quality of life [138].

Histologic Features

Histologic descriptions of vulvar aphthous ulcers are scarce, since these lesions are rarely subject to biopsy. The microscopic findings are identical to those in the oral mucosa and rather nonspecific. The initial stage shows a dense superficial neutrophilic infiltrate that tends to concentrate around superficial capillaries with features of leukocytoclastic vasculitis (Fig. 5.30) [140].

Superficial ulceration quickly develops and becomes covered by a crust of fibrinohemorrhagic and leukocytoclastic material containing cellular debris. After a variable period of time, T lymphocytes and plasma cells appear at the base and the borders of the ulcer and replace the acute inflammatory infiltrate as the process of reepithelialization begins. The presence of granulation tissue, fibroblastic proliferation, and scarring depends on the extension and depth of the ulceration [141]. The main role of a biopsy is to rule out other causes of vulvar ulceration [138, 142].

Differential Diagnosis

The clinical differential diagnosis [138] includes herpes virus infection, Behçet disease, Crohn disease, erosive lichen planus, fixed drug eruption, and erythema multiforme. The absence of general symptoms does not rule out a systemic disease, since vulvar involvement can be its initial manifestation [95].

Microscopically, aphthous ulcers have more superficial involvement without the primary vasculitic component or granulomatous reaction as seen in cases of Behçet disease and Crohn disease, respectively. The presence of a lichenoid inflammatory reaction at the edge of the ulcer points toward erosive lichen planus. Aphthous ulcers lack the eosinophilic infiltrate characteristic of fixed drug eruption and the epidermal apoptosis of erythema multiforme. Before making a histologic diagnosis of aphthae, it is necessary to exclude a herpes virus infection. Both lesions can be almost indistinguishable, and a helpful clue is to look for the characteristic cytopathic features (ground-glass nuclei or deeply eosinophilic intranuclear inclusions) in the epithelial cells at the border of the ulcers or in the endothelial cells of vessel walls, performing step sections if necessary.

Factitial dermatitis is another possibility that must be taken into consideration. Self-inflicted ulcerations are characterized by the presence of scant inflammatory infiltrate at the base of the ulcer and signs of manipulation such as erosions at the border and irregular detachment of the superficial crust.

Summary

Clinical Presentation

- Inner aspects of the labia minora of young women and prepubertal girls
- Acute onset after a precipitating event (viral infection or sexual intercourse)
- Ulcers with regular depth (1–2 mm), well-defined borders, and perilesional edema and erythema

Histologic Features

- Early lesions: superficial ulcers with predominantly perivascular neutrophilic infiltrate at the base and random leukocytoclastic vasculitis
- Late lesions: lymphoplasmacytic infiltrate with granulation tissue, fibroblastic proliferation, and reepithelialization

Differential Diagnosis

- Herpes virus infection
- Behçet disease
- Erosive lichen planus
- Crohn disease

Takeaway Essentials

Clinical Relevant Pearls

- Look for or ask about a history of oral sores. Their presence favors the diagnosis of aphthous ulcer and helps to classify the type of involvement.
- Acute ulcers associated with fever and systemic symptoms, especially if they have a symmetrical disposition (Lipschütz ulcers), do not tend to recur.

Pathology Interpretation Pearls

- The presence of leukocytoclastic vasculitis helps to rule out Behçet disease.

Behçet Disease

Clinical Features

Behçet disease (BD) is a multisystem inflammatory disorder with a relapsing chronic course. It is characterized by the triad of oral, genital, and ocular lesions [143]. Both sexes are usually equally affected. Most patients are individuals in their 30s with an ethnic background from geographical regions that correspond to the ancient Silk Road [144], extending from Japan and China to Turkey, where the prevalence is especially high with figures up to 370–421 cases per 100,000 population in several series [145, 146]. Nevertheless, sporadic cases can occur at any age and in any racial group [146]. One of the peculiarities of BD is the great variability of involvement among different individuals that depends in part on the geographical area and also on interindividual variability; this explains why some indi-

viduals present with only mild symptoms, whereas others develop a rapidly aggressive disease [145].

Mouth sores are the hallmark of the disease; they are present in most patients and very often are the first manifestation [146, 147]. Genital lesions consisting of painful aphthous ulcers appear in 50–93 % of cases, being especially common in Turkish patients; this illustrates the fact that regional variations influence not only the prevalence of the disease but also its manifestations [146, 148, 149]. Ocular involvement in the form of anterior or posterior uveitis is also rather frequent, especially in men from India, Iran, and Japan, where it is a cause of blindness in one-quarter of the patients [150]. In addition, the disease can affect almost any part of the body, probably because the underlying mechanism of BD is a systemic vasculitis that can encompass vessels of all types and sizes [146]. Other frequent sites of involvement are the skin (acne-like lesions, superficial thrombophlebitis, or erythema nodosum) and gastrointestinal tract (Crohn-like symptoms). Arthralgia is a frequent complaint of these patients, who can also present cardiopulmonary, vascular, renal, and urinary tract lesions as well as central nervous system involvement. This constitutes one of the most dangerous consequences of the disease with a wide range of manifestations ranging from headache and confusion to aseptic meningitis; pyramidal, cerebellar, or sensory symptoms; and rarely dementia [146, 151].

Genital involvement by BD in women occurs in the vulva and femoro-inguinal regions. The patients develop papulo-pustules, necrotic areas, or painful deep ulcers with sharply demarcated borders surrounded by edematous tissue (Fig. 5.31). Ulcers heal spontaneously in 2–4 weeks, but those located in the labia majora and femoro-inguinal region frequently cause scarring. Vaginal or cervical involvement is rare [146].

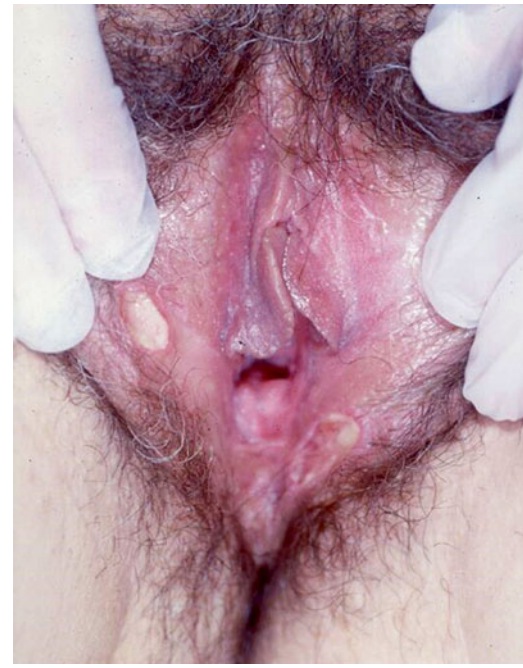


Fig. 5.31 Behçet disease. Necrotic ulcers with sharply demarcated borders surrounded by edematous tissue (Courtesy of Department of Dermatology, Hospital de la Santa Creu i Sant Pau, Badalona, Spain)

Etiology and/or Pathogenesis

BD is considered as an autoimmune reaction to environmental factors, probably infections in individuals predisposed by their hormonal status and especially by their genetic background. The most important influencing genes are those related to HLA-B51 or B5 genotype, which are more frequent in countries with a high prevalence of the disease [152, 153].

The demonstration of susceptibility loci for BD in a region that determines the secretion of H antigen has shed some light to its pathogenesis [154]. The altered secretion of the H antigen (precursor of the ABO blood group antigens) in body fluids and the intestinal mucosa would disturb mucosal glycosylation facilitating dysbiosis, determine an increased susceptibility to infections, and cause a boosted antigenic stimulation in the early life that explains the propensity of these patients to develop the autoimmune process of BD.

Prognosis or Course

The prognosis largely depends on the location and severity of the lesions, but, in general, patients with an early age of onset are those who follow a more aggressive course [155]. Loss of

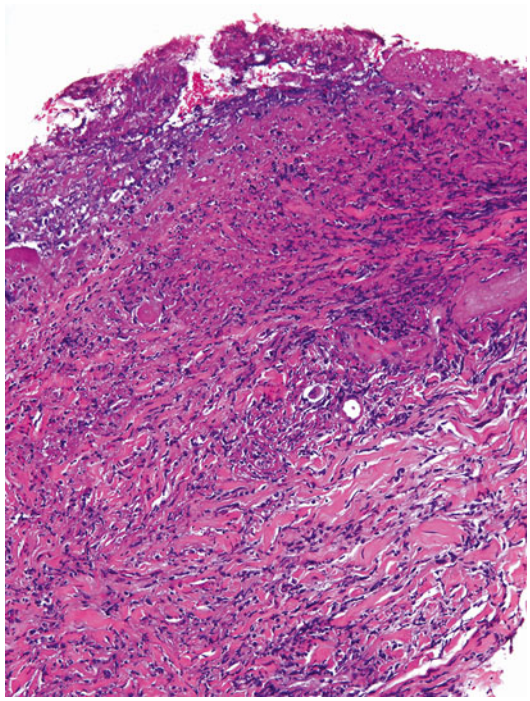


Fig. 5.32 Behçet disease. Ulcer with superficial fibrinoid necrosis and lymphohistiocytic infiltrate at the base

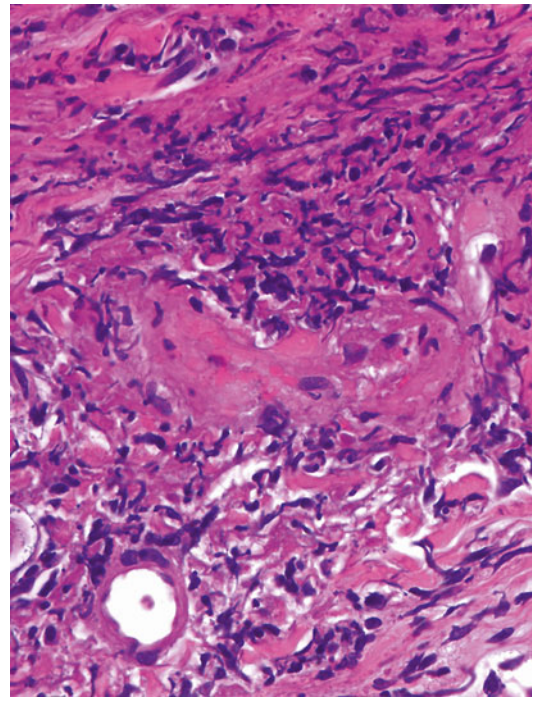


Fig. 5.33 Behçet disease. Lymphocytic vasculitis

visual acuity and neurological disease are the leading causes of morbidity and can leave irreversible disabilities, whereas massive hemorrhage due to the rupture of vascular aneurysms in the gastrointestinal tract and large vascular systems is the main cause of death [156]. The most severe symptoms usually develop during the first 5 years of the disease, and with the passage of time, symptoms tend to improve, with a decrease in mortality rate [157, 158]. The absence of diagnostic tests for the diagnosis of BD is the cause of diagnostic delays [145] that can lead to a significant increase in morbidity and mortality.

Histopathology

The histopathological picture of genital ulcers in BD is similar to that of other genital and oral aphthae. In the early stage there is a predominance of neutrophils with superficial necrosis and a progressive increase in the number of lymphocytes, histiocytes, and plasma cells as the lesions evolve (Fig. 5.32). Lymphocytic vasculitis can be seen in 50 % of cases (Fig. 5.33), but leukocytoclastic vasculitis is rare [145, 146]. Papular and pustular lesions in the vulva are similar to those in the skin, with a mixed cell infiltrate rich in neutrophils that tends to concentrate around vessels and can cause abscess formation and necrosis.

Differential Diagnosis

The differential diagnosis of the genital ulcers in BD includes nonspecific aphthous ulcerations, herpes virus infections, erosive lichen planus, autoimmune bullous diseases, and erythema multiforme. Vulvar aphthae closely resemble BD ulcerations, whereas leukocytoclastic vasculitis is common in the former and is rare in BD. Conversely, lymphocytic vasculitis is frequently seen in BD and its combination with ulceration may mimic a herpes infection. An added problem for the distinction between these two entities is that viral hyaline nuclear inclusions and multinucleated giant cells with nuclear molding are often scant and difficult to identify. Performing

step sections is mandatory to rule out herpes infection. Additionally, immunohistochemical staining with antibodies against herpes virus types 1 and 2 can also contribute to this diagnosis; this staining is especially useful in the presence of cells with altered features suggestive of viral infection but without conclusive characteristics. It is important to keep in mind that herpetic inclusions can be present not only in the superficial epithelium but also within the adnexal structures and endothelial cells.

Erosive lichen planus can also be the cause of large areas of ulceration. The presence of lichenoid damage in the epithelium at the border of the ulcers is the best clue for its recognition. Finally, CD can also cause areas of vulvar ulceration. They are usually recognizable under the microscope by the existence of numerous histiocytes within the infiltrate, even in the absence of clear-cut granulomas.

A definitive diagnosis of BD often requires a long-term follow-up and should be based on the presence of characteristic symptoms, the histopathological demonstration of lymphocytic vasculitis (whenever possible), and the exclusion of other alternative diagnoses such as inflammatory bowel disease, Crohn disease, and ulcerative colitis, which can be almost indistinguishable from BD and in some cases overlap [146, 159].

In 1990, the International Study Group for Behçet's Disease [160] proposed diagnostic criteria providing a 91 % sensitivity and 96 % specificity, based on the presence of minor or major aphthous or herpetiform oral ulcers that must recur more than three times per year and two of the following criteria: recurrent genital ulceration, anterior or posterior uveitis, cutaneous lesions (acneiform or erythema nodosum), and positive pathergy test (development of a pustule or an erythematous papule on the forearm, 24–48 h after being pricked with a small sterile needle). Although none of these findings is specific by itself, the association of several of them is highly characteristic of the disease [160].

Obtaining a biopsy specimen when BD is suspected can contribute to diagnosis, especially when images of vasculitis are evident, but fundamentally serve to rule out other possibilities.

Summary

Clinical Presentation

- Painful and deep vulvar ulcers with sharply demarcated borders, surrounded by edematous tissue
- Frequent papulo-pustules and femoro-inguinal involvement

Histologic Features

- Early lesions: mixed inflammatory infiltrate with predominance of neutrophils
- Late lesions: lymphoplasmacytic infiltrate with histiocytes and some neutrophils

Differential Diagnosis

- Herpes virus infection
- Aphthous ulcers
- Erosive lichen planus
- Crohn disease

Takeaway Essentials

Clinical Relevant Pearls

- There is an increased incidence in individuals from the geographical areas corresponding to the Silk Road. Yet, sporadic cases can occur in any part of the world.
- Mouth sores are present in almost all cases.
- Pathergy test (development of a pustule or an erythematous papule on the forearm, 24–48 h after being pricked with a small sterile needle) can contribute to the diagnosis but has a moderate specificity and sensitivity.

Pathology Interpretation Pearls

- Biopsy specimens of Behçet disease may show lymphocytic vasculitis, but leukocytoclastic vasculitis is rare and more suggestive of aphthous ulcers or herpes infection.

Vulvar Pain Syndromes

Dysesthetic (Essential) Vulvodynia

Clinical Features

The term dysesthetic or essential vulvodynia is used for vulvar pain that lasts for more than 3 months without other dermatological or gynecological causes [161, 162]. Unexplained vulvar pain is a common phenomenon that has been underestimated, in part because of the unwillingness of some women to look for help [161]. Nevertheless, vulvodynia has a great impact in the quality of life, limiting physical activities, causing sexual dysfunction, and often resulting in psychological distress. The lifetime prevalence of vulvar pain ranges from 7 % to 28 % in different studies [161, 162]. Vulvodynia affects women from 16 to 80 years and appears to be more frequent between 20 and 50 years. Vulvar pain at pediatric age is usually related to specific conditions and thus cannot be included in the concept of essential vulvodynia [163].

Pain is the most frequent complaint, but patients also report burning, tingling, and stinging sensations or a feeling of irritation that can be exacerbated by inappropriate therapies. Dyspareunia is also frequently present. These symptoms usually have an acute onset, in some cases following an episode of vaginitis or local therapeutic procedures. In many patients, vulvodynia becomes a chronic problem lasting for months or years. The condition can worsen before or during menses and symptoms can increase during sexual intercourse or after it. Nonetheless, the patient may also be symptom free for several days. In adolescents and young women, vulvar pain tends to be associated with sexual intercourse or early tampon use [163]. As a rule, pruritus is absent and its presence points toward another infectious or dermatological condition [164–167].

Some women present with vulvar erythema and edema or a slight increase in vaginal discharge, whereas in other cases there are no visible changes. During the exploration patients often complain of discomfort at the separation of the labia minora and tenderness at the orifices of the Skene and Bartholin's glands [164–167].

Etiology and/or Pathogenesis

The etiology of essential vulvodynia is enigmatic; nevertheless, all evidences suggest that several mechanisms are implicated, with a different impact on each patient. One possible cause is an alteration in the threshold of pain sensitivity, due to a sensory central pain processing disorder [168, 169]. A second possibility is that a local inflammatory process, such as *Candida* infection, or any other vaginal insult causes the release of inflammatory mediators that would be responsible for a local increase in nociceptors and a proliferation of peripheral nerves [170–173]. The fact that clinically relevant dermatoses are common in patients with vulvodynia provides further support to this hypothesis [174]. Nociceptive pain would be produced by the damage caused by the intense or repetitive stimulation of pain receptors or sensory neurons. When nociceptive vulvar pain becomes neuropathic, the patients complain of continuous burning, hyperalgesia, and allodynia (pain due to stimuli that would not normally provoke pain) [172].

A tendency to excessive immuno-inflammatory response may also play a role in the development of vulvodynia. Approximately 55 % of the patients presented another chronic health condition in one series [167], and predisposition to develop allergic reactions has been related with the development of vulvodynia [175].

The possibility of a somatization disorder [176] has also been proposed. In many cases, the features of the disease are consistent with functional pain, and the coexistence of other unexplained clinical processes such as chronic fatigue syndrome and irritable bowel syndrome is quite common [177]; thus, it is advisable to include a psychological and sexual evaluation in the study of these patients [176].

Finally, diabetes mellitus can contribute to vulvar pain by way of vulvar infections, which are common in diabetic patients and might trigger nociceptive pain through persistent inflammation, and diabetic neuropathy, which can appear either isolated or in the context of other neuropathic abnormalities [178, 179].

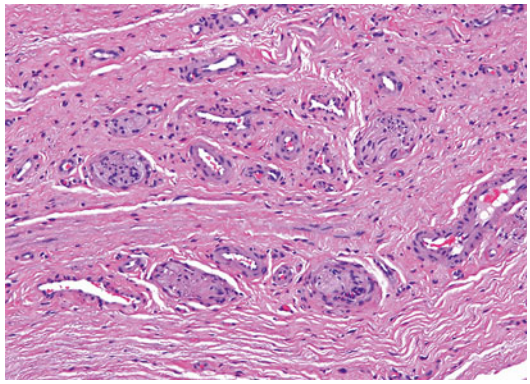


Fig. 5.34 Vestibulodynia. Aggregate of peripheral nerve fibers. A marked increase in the number of nerves is typical of vulvodynia and vestibulodynia

Summary

Clinical Presentation

- Unexplained vulvar pain that lasts for more than 3 months. Some patients also experience burning, tingling, stinging sensations or a feeling of irritation.
- Frequently the condition has an acute onset after a vaginal insult and symptoms can worsen in relation to menses or sexual intercourse.

Histologic Features

- Marked increase in number of peripheral nerves

Differential Diagnosis

- HPV vulvitis
- Chronic yeast infection

Prognosis or Course

This is a chronic disorder in which an individual prognosis regarding the duration of symptoms cannot be made. No effective treatment exists, but there are many symptomatic therapies that can improve the quality of life of these patients.

Histopathology

The most important histopathological feature of vulvodynia is an increase in the number of peripheral nerve fibers (Fig. 5.34), probably as a consequence of the continuous stimulation by an inflammatory process. Concomitant vulvar diseases are common in patients with refractory vulvodynia, and thus it is not uncommon to find features of LS, allergic or irritant dermatitis, and lichen planus in their biopsy specimens [173].

Differential Diagnosis

The differential diagnosis of essential vulvodynia includes all causes of chronic primary vulvar pain, namely, dermatological, infectious, and neoplastic conditions, including HPV vulvitis [180]. One of the leading causes of vulvodynia is chronic yeast infection, and in some patients its eradication can be sufficient to suppress the symptoms.

Takeaway Essentials

Clinical Relevant Pearls

- Pruritus is rare in essential vulvodynia and suggestive of other etiologies.
- Essential vulvodynia is extremely rare in pediatric age; vulvar pain in girls usually has a specific cause.
- Comorbidities are not uncommon in these patients; consequently the presence of intermittent infections or dermatoses does not rule out the diagnosis.

Pathology Interpretation Pearls

- It is mandatory to search for an increased number of nerve endings whenever the clinical setting suggests essential vulvodynia, even though other causes of vulvar pain can be found.

Immunohistochemical Findings

- Immunostaining with S100 can contribute to highlight the increase in number of nerve endings. For evaluation compare the findings with those of biopsy specimens corresponding to other conditions and taken from the same region.

Vestibulodynia and Focal Vulvodynia

Clinical Features

Vestibulodynia is considered one of the most frequent causes of dyspareunia in young women [181]. It differs from dysesthetic (essential) vulvodynia in the location of pain, which is limited to the vestibule. In some patients, pain is focused at two points (located at 5 and 7 h); in others pain occurs at four points (located at 5, 7, 1, and 11 h) and can include the glands of Bartholin and Skene. The only visible change in vestibulodynia is a mild erythema on the vestibular area. Subjective symptoms can vary greatly from one woman to another, ranging from a tolerable discomfort to an intense pain following light pressure [182].

Etiology and/or Pathogenesis

As in essential vulvodynia, an overgrowth of nerve fibers has been considered to be one of the most likely causes. The proliferation and spread of nerve fibers in the stroma could be facilitated by heparanase released by mast cells, which are abundant in the painful areas, [183, 184] maybe in relation to vaginal infections such as candidiasis and vaginosis, which are important triggers and risk factors of the disease [185].

Another possible cause is increased pain sensitivity. A high percentage of patients with vestibulodynia have other members of their family with similar symptoms. This could be explained by alterations in pain sensibility mediated by the *MCR1* gene [186]. Finally, some cases of vestibulodynia might be explained by cutaneous sensory disorders secondary to somatization and dissociation disorders [187].

Prognosis or Course

The severity of vestibulodynia may significantly decrease over time. In a study on 239 women followed for 2 years, most patients reported a clinically significant improvement in pain, even in the absence of any treatment [188]. Treatment should be individualized looking for the best therapeutic options, which in some cases can result in a complete relief of symptoms [188]. In other cases significant improvement can be obtained using a multidisciplinary approach that includes symptomatic treatments, physical therapies, a wide variety of drugs, and psychological support. Vestibulectomy may provide an important benefit for recalcitrant cases refractory to conservative management [189].

Histopathology (Immunophenotype, Molecular Genetics)

All histopathological studies are coincident in finding a local increase in nerve fiber density (Fig. 5.34) that according to one study is ten times higher in patients with vestibulitis than in controls [184, 190]. The relevance of the mast cell infiltrate and the existence of an inflammatory process are not so clear. Bornstein et al. [184] found a significant increase in the number of mast cells and degranulated mast cells in vestibulitis as compared to normal controls, as well as a significant positive correlation between the total nerve fiber area and the number of mast cells. He proposed the presence of eight or more mast cells per 10×10 microscopic fields as a diagnostic histopathological criterion for vestibulitis [184]. However, later studies have failed to show any significant differences in the mast cell infiltrate between primary and secondary vestibulitis [190] or a significant increase in number of mast cells or the presence of active inflammation in vestibular tissue compared to controls [191]. Some inflammation can be present around the minor vestibular glands, with exocytosis into the secretory epithelium and their excretory duct (Fig. 5.35) [184, 192].

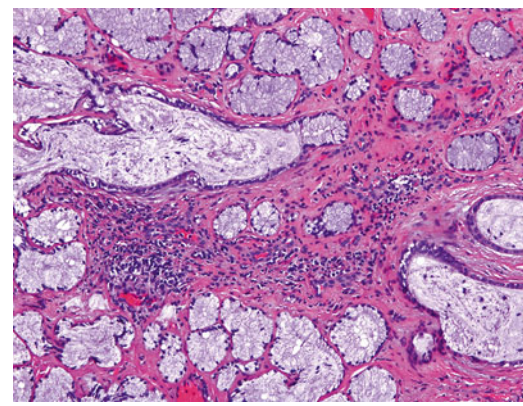


Fig. 5.35 Vestibulodynia. Lymphocytic infiltrate in a minor vestibular gland with occasional exocytosis into the epithelium and their excretory duct

An increased progesterone receptor nuclear immunostaining has been found in primary vestibulodynia compared with secondary vestibulodynia and a greater expression of estrogen receptor α in primary vestibulodynia of less than 5 years evolution [193]. Neural hyperplasia and progesterone receptor overexpression seem to be less intense in postmenopausal women [194].

Differential Diagnosis

The diagnosis of vestibulodynia has to be made after clinical exclusion of other inflammatory, infectious, or neoplastic conditions. A dermatological evaluation is necessary to rule out the typical features of other diseases and a biopsy is mandatory in case of any doubt [194].

Summary

Clinical Presentation

- Vestibular and vulvar pain and tenderness can be focused at two points (located at 5 and 7 h) or four points (located at 5, 7, 1 and 11 h). Sometimes affects the glands of Bartholin and Skene.

Histologic Features

- Local increase in nerve fiber density and presence of abundant mast cells
- Inflammatory infiltrate around minor vestibular glands, with exocytosis into their epithelium

Differential Diagnosis

- Neoplasia
- Infections

Takeaway Essentials

Clinical Relevant Pearls

- It is important to rule out referred pain from neoplasia.

Pathology Interpretation Pearls

- A significant increase in the number of mast cells and nerve endings is highly suggestive of this diagnosis.

Immunohistochemical Findings

- Immunostaining with S100 and CD117 (c-Kit) can highlight the increase in nerve endings and mast cells, respectively. During evaluation compare the findings with those of biopsy specimens corresponding to other conditions and taken from the same region.

Case Vignettes

Vignette 1

Clinical history: A 66-year-old woman with hyperkeratosis and atrophy of the labia minora and around the introitus. She presents with severe frontal alopecia and has a past history of hypothyroidism.

Microscopic description: The biopsy shows regular acanthosis of the squamous epithelium with well-developed stratum granulosum and thick horny layer. Occasional groups of dyskeratotic cells are present in the upper layers of the epithelium. There is also an abundant inflammatory infiltrate in the submucosa with a tendency to exocytosis to all layers of the epithelium. The submucosa shows thick sclerotic collagen bundles (Fig. 5.36). Focal spongiosis can be appreciated in many areas, but basal vacuolization or basal dyskeratosis and a definite lymphocytic infiltrate are absent (Fig. 5.37). At higher magnification (Fig. 5.38), the presence of dyskeratotic cells at different layers of the epidermis and the horny layer can be better appreciated.

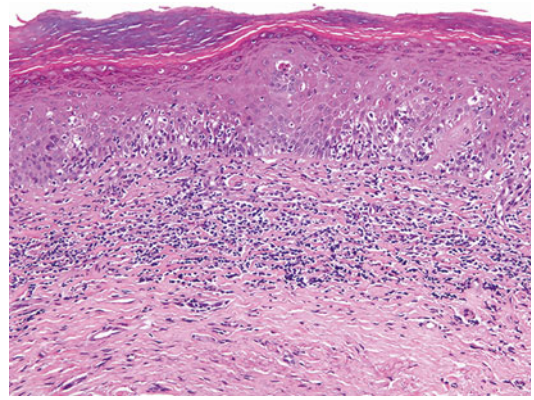


Fig. 5.36 Vignette 1. The original mucosal surface of the labia minora has been replaced by keratinizing squamous epithelium with a thick granular layer that shows occasional dyskeratosis. Lymphocytic infiltrate is present forming a band within the dermoepidermal junction and the upper dermis among dense collagen fibers

(continued)

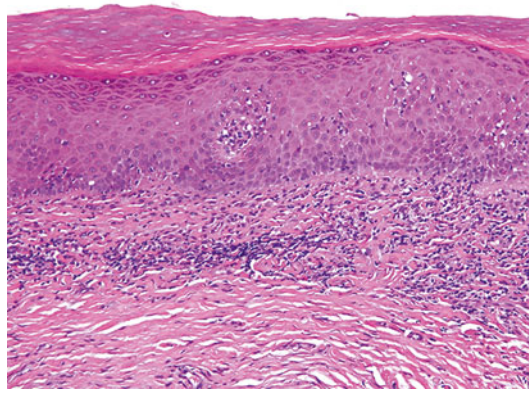


Fig. 5.37 Vignette 1. The characteristic basal lichenoid damage can be absent from many areas. Nevertheless, a thick granular layer with orthokeratosis is the clue for the diagnosis

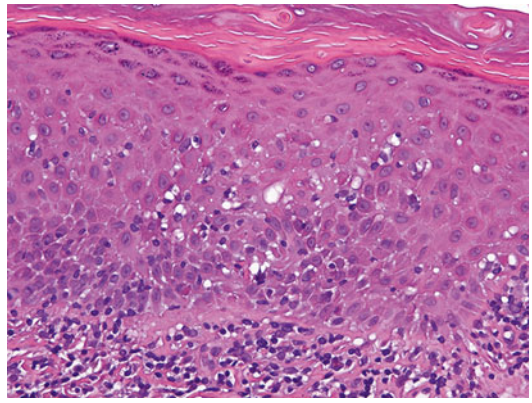


Fig. 5.38 Vignette 1. Slight spongiosis and many necrotic keratinocytes at different levels of the epidermis

Diagnosis: Lichen planus (LP)

Discussion: The biopsy shows a combination of histologic findings that are consistent with LP but not totally specific. The absence of definite basal damage and the presence of dyskeratotic cells at different layers of the epidermis as well as some features of lichenification make the diagnosis more difficult. In cases like this, it is especially important to correlate the histopathological findings with the clinical presentation. The presence of introital involvement is typical of LP. In addition, the frontal alopecia is likely to correspond to frontal fibrosing alopecia and the clinical history of hypothyroidism suggests an autoimmune disease, both processes typically associated with vulvar LP.

Vignette 2

Clinical history: *An* 8-year-old girl with small red vulvar papules in an erythematous and edematous background. The clinical diagnosis was traumatized angiokeratomas, but the possibility of child abuse was also considered.

Microscopic description: The biopsy shows marked mucosal atrophy and intense submucosal edema with well-demarcated foci of hemorrhage (Fig. 5.39). At higher magnification (Fig. 5.40), it is possible to appreciate a thick hyaline band in the area corresponding to the basal membrane. In the nonhemorrhagic area (Fig. 5.41) additional features of the disease (basal epithelial vacuolization, dilatation of capillary vessels, and homogeneous sclerosis of the collagen) are clearly displayed.

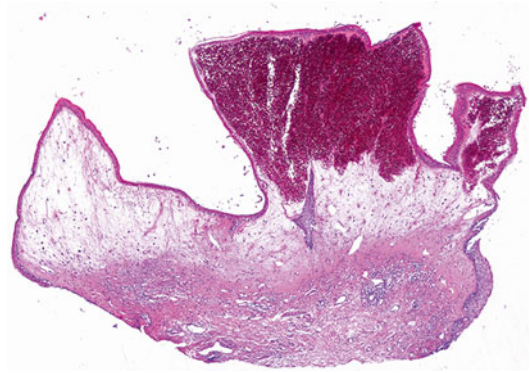


Fig. 5.39 Vignette 2. The presence of quite sharply demarcated areas of hemorrhage can simulate vascular ectasias. However, the intense subepidermal edema and the sclerosis in the upper dermis are clues for the diagnosis

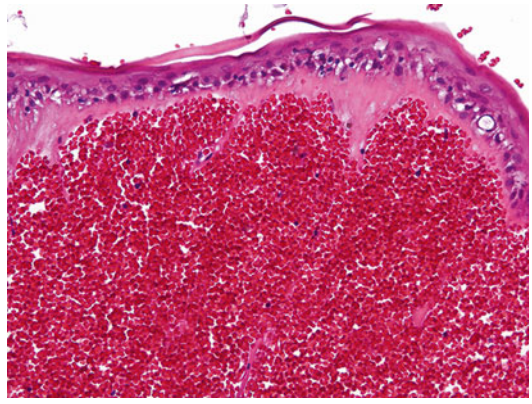


Fig. 5.40 Vignette 2. The thickened basal membrane is present between the hemorrhagic area from the epidermis that lacks an endothelial wall

(continued)

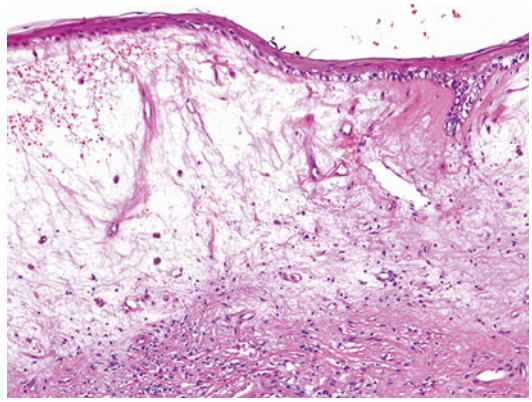


Fig. 5.41 Vignette 2. The presence of siderophages within the subepidermal edema indicates the existence of previous hemorrhage

Diagnosis: Lichen sclerosus (LS)

Discussion: In this case, the peculiar disposition of the areas of hemorrhage led to a wrong clinical diagnosis of angiokeratoma. From a histologic standpoint, the diagnosis is much easier. The biopsy displays all the characteristics typical of LS, including thick hyalinization at the basal membrane, which is a very useful clue for the diagnosis, even in small superficial shave biopsies from hemorrhagic areas. At any rate, the possibility of child abuse was only ruled out after careful evaluation of the girl and her family.

Vignette 3

Clinical history: *A* 9-year-old girl, otherwise healthy, presented with 1-year history of vulvar lesions.

Microscopic description: The biopsy specimen shows an area of epidermal ulceration with a dense and deep dermal inflammatory infiltrate (Fig. 5.42). The infiltrate is composed of loose aggregates of histiocytes forming ill-defined granulomas with multinucleated giant cells (Fig. 5.43). The granulomatous infiltrate is intermixed with lymphocytes, plasma cells, many eosinophils, and some neutrophils (Fig. 5.44). A CD68 immunostain highlights the abundance of macrophages in the infiltrate (Fig. 5.45). Special techniques to rule out infection were negative.

(continued)

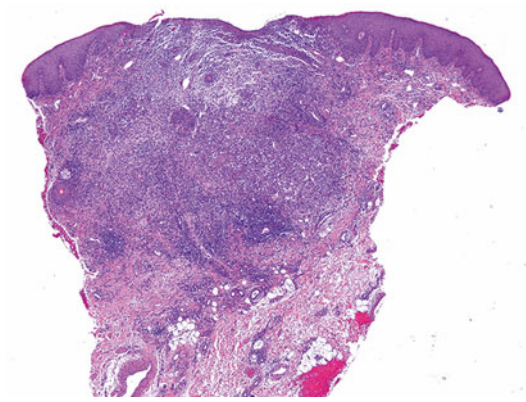


Fig. 5.42 Vignette 3. Dense nodule of inflammatory infiltrate with epidermal ulceration

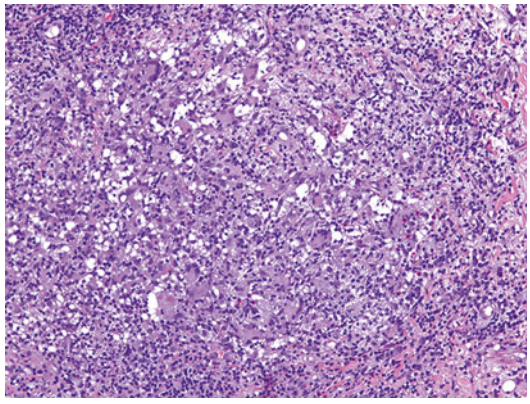


Fig. 5.43 Vignette 3. Granulomatous infiltrate containing occasional multinucleated giant cells and eosinophils

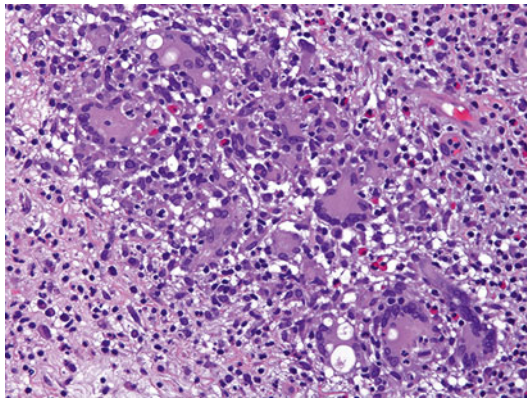


Fig. 5.44 Vignette 3. Aggregate of multinucleated giant cells in a background of lymphocytes, plasma cells, and some eosinophils

(continued)

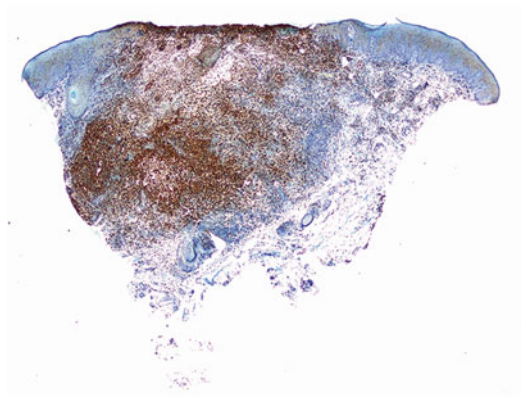


Fig. 5.45 Vignette 3. Immuno-histochemical staining for CD68 highlighting the granulomatous nature of the dermal inflammatory infiltrate

Diagnosis: Metastatic Crohn disease (CD)

Discussion: In a prepubertal girl, the presence of a dense and ill-defined granulomatous infiltrate with eosinophils in the absence of necrosis, vasculitis, or infectious agents should raise the possibility of metastatic CD. In spite of her being free of gastrointestinal symptoms, an endoscopy was performed which demonstrated mild colitis and non-caseating granulomas that supported the diagnosis of CD.

Abbreviations

BD	Behçet disease
CD	Crohn disease
dVIN	differentiated vulvar intraepithelial Neoplasia
ECM1	Extracellular matrix protein 1
LP	Lichen planus
LS	Lichen sclerosus
VSCC	Vulvar squamous cell carcinoma
ZV	Zoon vulvitis

References

1. Goldstein AT, Metz A. Vulvar lichen planus. Clin Obstet Gynecol. 2005;48(4):818–23.
2. Lewis FM. Vulval lichen planus. Br J Dermatol. 1998;138(4):569–75.
3. Murphy R, Edwards L. Desquamative inflammatory vaginitis: what is it? J Reprod Med. 2008;53(2):124–8.
4. Bradford J, Fischer G. Desquamative inflammatory vaginitis: differential diagnosis and alternate diagnostic criteria. J Low Genit Tract Dis. 2010;14(4):306–10.
5. Barchino-Ortiz L, Suárez-Fernández R, Lázaro-Ochaita P. Vulvar inflammatory dermatoses. Actas Dermosifiliogr. 2012;103(4):260–75.
6. Cooper SM, Wojnarowska F. Influence of treatment of erosive lichen planus of the vulva on its prognosis. Arch Dermatol. 2006;142(3):289–94.
7. Margesson LJ. Vulvar disease pearls. Dermatol Clin. 2006;24(2):145–55.
8. O'Connell TX, Nathan LS, Satmary WA, Goldstein AT. Non-neoplastic epithelial disorders of the vulva. Am Fam Physician. 2008;77(3):321–6.
9. Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: a study of 723 patients. J Am Acad Dermatol. 2002;46(2):207–14.
10. Di Fede O, Belfiore P, Cabibi D, De Cantis S, Maresi E, Kerr AR, et al. Unexpectedly high frequency of genital involvement in women with clinical and histological features of oral lichen planus. Acta Derm Venereol. 2006;86(5):433–8.
11. Belfiore P, Di Fede O, Cabibi D, Campisi G, Amarù GS, De Cantis S, et al. Prevalence of vulval lichen

planus in a cohort of women with oral lichen planus: an interdisciplinary study. Br J Dermatol. 2006; 155(5):994–8.
12. Eisen D. The evaluation of cutaneous, genital, scalp, nail, esophageal, and ocular involvement in patients with oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;88(4):431–6.
13. Panagiotopoulou N, Wong CS, Winter-Roach B. Vulvovaginal-gingival syndrome. J Obstet Gynaecol. 2010;30(3):226–30.
14. Ball SB, Wojnarowska F. Vulvar dermatoses: lichen sclerosus, lichen planus, and vulval dermatitis/lichen simplex chronicus. Semin Cutan Med Surg. 1998;17(3):182–8.
15. Chew A, Stefanato C, Savarese I, Neill S, Fenton D, Lewis F. Clinical patterns of lichen planopilaris in patients with vulval lichen planus. Br J Dermatol. 2014;170(1):218–20.
16. Griffin LL, Michaelides C, Griffiths CEM, Paus R, Harries MJ. Primary cicatricial alopecias: a UK survey. Br J Dermatol. 2012;167(3):692–705.
17. Fox LP, Lightdale CJ, Grossman ME. Lichen planus of the esophagus: what dermatologists need to know. J Am Acad Dermatol. 2011;65(1):175–83.
18. Gupta R, Bansal B, Singh S, Yadav I, Gupta K, Kudesia M. Lichen planus of uterine cervix – the first report of a novel site of occurrence: a case report. Cases J. 2009;2:9306. doi:10.1186/1757-1626-2-9306.
19. Cooper SM, Ali I, Baldo M, Wojnarowska F. The association of lichen sclerosus and erosive lichen planus of the vulva with autoimmune disease: a case–control study. Arch Dermatol. 2008;144(11): 1432–5.
20. Carlson JA, Lamb P, Malfetano J, Ambros RA, Mihm Jr MC. Clinicopathologic comparisons of vulvar and extragenital lichen sclerosus: histologic variants, evolving lesions and etiology of 141 cases. Mod Pathol. 1998;11(9):844–54.
21. Setterfield JF, Neill S, Shirlaw PJ, Theron J, Vaughan R, Escudier M, et al. The vulvovaginal gingival syndrome: a severe subgroup of lichen planus with characteristic clinical features and a novel association with the class II HLA DQB1*0201 allele. J Am Acad Dermatol. 2006;55(1):98–113.
22. Danielsson K, Boldrup L, Rentoft M, Coates PJ, Ebrahimi M, Nylander E, et al. Autoantibodies and decreased expression of the transcription factor ELF-3 together with increased chemokine pathways support an autoimmune phenotype and altered differentiation in lichen planus located in oral mucosa. J Eur Acad Dermatol Venereol. 2013;27(11):1410–6.
23. Terlou A, Santegoets LA, van der Meijden WI, Heijmans-Antonissen C, Swagemakers SM, van der Spek PJ, et al. An autoimmune phenotype in vulvar lichen sclerosus and lichen planus: a Th1 response and high levels of microRNA-155. J Invest Dermatol. 2012;132(3 Pt 1):658–66.
24. Cooper SM, Dean D, Allen J, Kirtschig G, Wojnarowska F. Erosive lichen planus of the vulva: weak circulating basement membrane zone antibodies are present. Clin Exp Dermatol. 2005;30(5): 551–6.
25. Clayton R, Chaudhry S, Ali I, Cooper S, Hodgson T, Wojnarowska F. Mucosal (oral and vulval) lichen planus in women: are angiotensin-converting enzyme inhibitors protective, and beta-blockers and non-steroidal anti-inflammatory drugs associated with the condition? Clin Exp Dermatol. 2010; 35(4):384–7.
26. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition? Arch Dermatol. 2012;148(11):1314–6.
27. Simpson RC, Littlewood SM, Cooper SM, Cruickshank ME, Green CM, Derrick E, et al. Real-life experience of managing vulval erosive lichen planus: a case-based review and U.K. multicentre case note audit. Br J Dermatol. 2012;167(1):85–91.
28. Chiu TL, Jones RW. Multifocal multicentric squamous cell carcinomas arising in vulvovaginal lichen planus. J Low Genit Tract Dis. 2011;15(3):246–7.
29. Helander SD, Rogers 3rd RS. The sensitivity and specificity of direct immunofluorescence testing in disorders of mucous membranes. J Am Acad Dermatol. 1994;30(1):65–75.
30. Simpson RC, Thomas KS, Leighton P, Murphy R. Diagnostic criteria for erosive lichen planus affecting the vulva: an international electronic-Delphi consensus exercise. Br J Dermatol. 2013; 169(2):337–43.
31. Weyers W, Ende Y, Schalla W, Diaz-Cascajo C. Balanitis of Zoon: a clinicopathologic study of 45 cases. Am J Dermatopathol. 2002;24(6):459–67.
32. Neri I, Patrizi A, Marzaduri S, Marini R, Negosanti M. Vulvitis plasmacellularis: two new cases. Genitourin Med. 1995;71(5):311–3.
33. Albers SE, Taylor G, Huyer D, Oliver G, Krafchik BR. Vulvitis circumscripta plasmacellularis mimicking child abuse. J Am Acad Dermatol. 2000;42(6): 1078–80.
34. Goldstein AT, Christopher K, Burrows LJ. Plasma cell vulvitis: a rare cause of intractable vulvar pruritus. Arch Dermatol. 2005;141(6):789–90.
35. Kouloubis N, Noordhoek Hegt V, van Praag MC. A woman with cayenne pepper spots. Ned Tijdschr Geneeskd. 2013;157(7):A5685.
36. Solt I, Lowenstein L, Amit A, Bergman R, Kerner H. Ulcerative vulvitis circumscripta plasmacellularis. Isr Med Assoc J. 2004;6(2):117–8.
37. Toeima E, Sule M, Warren R, Igali L. Diagnosis and treatment of Zoon's vulvitis. J Obstet Gynaecol. 2011;31(6):473–5.
38. Salopek TG, Siminoski K. Vulvitis circumscripta plasmacellularis (Zoon's vulvitis) associated with autoimmune polyglandular endocrine failure. Br J Dermatol. 1996;135(6):991–4.
39. van Kessel MA, van Lingen RG, Bovenschen HJ. Vulvitis plasmacellularis circumscripta in pre-existing lichen sclerosus: treatment with imiquimod 5% cream. J Am Acad Dermatol. 2010;63(1):e11–3.
40. Kuniyuki S, Asada T, Yasumoto R. A case of vulvitis circumscripta plasmacellularis positive for herpes

simplex type II antigen. Clin Exp Dermatol. 1998; 23(5):230–1.
41. dos Reis HL, de Vargas PR, Lucas E, Camporez T, Ferreira Dde C. Zoon vulvitis as a differential diagnosis in an HIV-infected patient: a short report. J Int Assoc Provid AIDS Care. 2013;12(3):159–61.
42. Wojnarowska F, Cooper SM. Anogenital (non-venereal) disease. In: Bolognia JL, Jorizzo JL, Rapini R, editors. Dermatology. 1st ed. London: Elsevier; 2003.
43. Virgili A, Levratti A, Marzola A, Corazza M. Retrospective histopathologic reevaluation of 18 cases of plasma cell vulvitis. J Reprod Med. 2005; 50(1):3–7.
44. Thomson MA, Carr RA, Ganesan R, Humphreys F. Extensive mucinous metaplasia of the vulva arising within Zoon's vulvitis. Br J Dermatol. 2007;156(4):750–2.
45. Li Q, Leoplod K, Carlson JA. Chronic vulvar purpura: persistent pigmented purpuric dermatitis (lichen aureus) of the vulva or plasma cell (Zoon's) vulvitis? J Cutan Pathol. 2003;30(9):572–6.
46. Powell J, Wojnarowska F. Childhood vulvar lichen sclerosus: an increasingly common problem. J Am Acad Dermatol. 2001;44(5):803–6.
47. Goldstein AT, Marinoff SC, Christopher K, Srodon M. Prevalence of vulvar lichen sclerosus in a general gynecology practice. J Reprod Med. 2005;50(7): 477–80.
48. Zendell K, Edwards L. Lichen sclerosus with vaginal involvement: report of 2 cases and review of the literature. JAMA Dermatol. 2013;149(10):1199–202.
49. Powell JJ, Wojnarowska F. Lichen sclerosus. Lancet. 1999;353(9166):1777–83.
50. Cooper SM, Gao XH, Powell JJ, et al. Does treatment of vulvar lichen sclerosus influence its prognosis? Arch Dermatol. 2004;140(6):702–6.
51. Regauer S, Liegl B, Reich O. Early vulvar lichen sclerosus: a histopathological challenge. Histopathology. 2005;47(4):340–7.
52. Marren P, Millard PR, Wojnarowska F. Vulval lichen sclerosus: lack of correlation between duration of clinical symptoms and histological appearances. J Eur Acad Dermatol Venereol. 1997;8:212–6.
53. Larre Borges A, Tiodorovic-Zivkovic D, Lallas A, Moscarella E, Gurgitano S, Capurro M, et al. Clinical, dermoscopic and histopathologic features of genital and extragenital lichen sclerosus. J Eur Acad Dermatol Venereol. 2013;27(11):1433–9.
54. Kreuter A, Kryvosheyeva Y, Terras S, Moritz R, Möllenhoff K, Altmeyer P, et al. Association of autoimmune diseases with lichen sclerosus in 532 male and female patients. Acta Derm Venereol. 2013;93(2):238–41.
55. van Seters M, ten Kate FJ, van Beurden M, Verheijen RH, Meijer CJ, Burger MP, et al. In the absence of (early) invasive carcinoma, vulvar intraepithelial neoplasia associated with lichen sclerosus is mainly of undifferentiated type: new insights in histology and aetiology. J Clin Pathol. 2007;60(5):504–9.
56. Carlson JA, Ambros R, Malfetano J, Ross J, Grabowski R, Lamb P, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. Hum Pathol. 1998;29(9):932–48.
57. Del Pino M, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. Histopathology. 2013; 62(1):161–75.
58. Kokka F, Singh N, Faruqi A, Gibbon K, Rosenthal AN. Is differentiated vulval intraepithelial neoplasia the precursor lesion of human papillomavirus-negative vulval squamous cell carcinoma? Int J Gynecol Cancer. 2011;21(7):1297–305.
59. McCluggage WG. Premalignant lesions of the lower female genital tract: cervix, vagina and vulva. Pathology. 2013;45(3):214–28.
60. Fistarol SK, Itin PH. Diagnosis and treatment of lichen sclerosus: an update. Am J Clin Dermatol. 2013;14(1):27–47.
61. Carlson JA, Mu XC, Slominski A, Weismann K, Crowson AN, Malfetano J, et al. Melanocytic proliferations associated with lichen sclerosus. Arch Dermatol. 2002;138(1):77–87.
62. Rosamilia LL, Schwartz JL, Lowe L, Gruber SB, Quint EH, Johnson TM, et al. Vulvar melanoma in a 10-year-old girl in association with lichen sclerosus. J Am Acad Dermatol. 2006;54(2 Suppl):S52–3.
63. Hassanein AM, Mrstik ME, Hardt NS, Morgan LA, Wilkinson EJ. Malignant melanoma associated with lichen sclerosus in the vulva of a 10-year-old. Pediatr Dermatol. 2004;21(4):473–6.
64. Neill SM, Lewis FM, Tatnall FM, Cox NH, British Association of Dermatologists. British Association of Dermatologists' guidelines for the management of lichen sclerosus 2010. Br J Dermatol. 2010;163(4):672–82.
65. Chang JC, Blake DG, Leung BV, Plaza JA. Langerhans cell histiocytosis associated with lichen sclerosus of the vulva: case report and review of the literature. J Cutan Pathol. 2013;40(2):279–83.
66. Sherman V, McPherson T, Baldo M, Salim A, Gao XH, Wojnarowska F. The high rate of familial lichen sclerosus suggests a genetic contribution: an observational cohort study. J Eur Acad Dermatol Venereol. 2010;24(9):1031–4.
67. Meyrick Thomas RH, Ridley CM, McGibbon DH, Black MM. Lichen sclerosus et atrophicus and autoimmunity: a study of 350 women. Br J Dermatol. 1988;118(1):41–6.
68. Oyama N, Chan I, Neill SM, Hamada T, South AP, Wessagowit V, et al. Autoantibodies to extracellular matrix protein 1 in lichen sclerosus. Lancet. 2003;362(9378):118–23.
69. Edmonds EV, Oyama N, Chan I, Francis N, McGrath JA, Bunker CB. Extracellular matrix protein 1 autoantibodies in male genital lichen sclerosus. Br J Dermatol. 2011;165(1):218–9.

70. Baldo M, Bailey A, Bhogal B, Groves RW, Ogg G, Wojnarowska F. T cells reactive with the NC16A domain of BP180 are present in vulval lichen sclerosus and lichen planus. J Eur Acad Dermatol Venereol. 2010;24(2):186–90.
71. Gambichler T, Höxtermann S, Skrygan M, Eberz B, Regauer S, Scola N, et al. Occurrence of circulating anti-bullous pemphigoid antibodies in patients with lichen sclerosus. J Eur Acad Dermatol Venereol. 2011;25(3):369–70.
72. Walsh ML, Leonard N, Shawki H, Bell HK. Lichen sclerosus and immunobullous disease. J Low Genit Tract Dis. 2012;16(4):468–70.
73. Bunker CB, Patel N, Shim TN. Urinary voiding symptomatology (micro-incontinence) in male genital lichen sclerosus (MGLSc). Acta Derm Venereol. 2013;93(2):246–8.
74. Becker K, Meissner V, Farwick W, Bauer R, Gaiser MR. Lichen sclerosus and atopy in boys: coincidence or correlation? Br J Dermatol. 2013; 168(2):362–6.
75. Lagerstedt M, Karvinen K, Joki-Erkkilä M, Huotari-Orava R, Snellman E, Laasanen SL. Childhood lichen sclerosus-a challenge for clinicians. Pediatr Dermatol. 2013;30(4):444–50.
76. Guerrero D, Guarch R, Ojer A, Casas JM, Méndez-Meca C, Esteller M, et al. Differential hypermethylation of genes in vulvar cancer and lichen sclerosus coexisting or not with vulvar cancer. Int J Cancer. 2011;128(12):2853–64.
77. Gambichler T, Terras S, Kreuter A, Skrygan M. Altered global methylation and hydroxymethylation status in vulvar lichen sclerosus – further support for epigenetic mechanisms. Br J Dermatol. 2014;170(3):687–93.
78. Dendrinos ML, Quint EH. Lichen sclerosus in children and adolescents. Curr Opin Obstet Gynecol. 2013;25(5):370–4.
79. Focseneanu MA, Gupta M, Squires KC, Bayliss SJ, Berk D, Merritt DF. The course of lichen sclerosus diagnosed prior to puberty. J Pediatr Adolesc Gynecol. 2013;26(3):153–5.
80. Regauer S, Liegl B, Reich O. Early vulvar lichen sclerosus: a histopathological challenge. Histopathology. 2007;50(3):388–9.
81. Regauer S, Liegl B, Reich O, Beham-Schmid C. Vasculitis is lichen sclerosus: an under recognised feature. Histopathology. 2004;45(3):237–44.
82. Weyers W. Hypertrophic lichen sclerosus with dyskeratosis and parakeratosis–a common presentation of vulvar lichen sclerosus not associated with a significant risk of malignancy. Am J Dermatopathol. 2013;35(7):713–21.
83. Rakha E, Mayne C, Brown L. Mucinous metaplasia of the vulva in a case of lichen sclerosus. A case report. J Clin Pathol. 2005;58(11):1217–8.
84. Raspollini MR, Asirelli G, Moncini D, Taddei GL. A comparative analysis of lichen sclerosus of the vulva and lichen sclerosus that evolves to vulvar squamous cell carcinoma. Am J Obstet Gynecol. 2007;197(6): 592.e1–5.
85. Liegl B, Regauer S. p53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). Histopathology. 2006;48(3):268–74.
86. Powell J, Wojnarowska F. Childhood vulval lichen sclerosus and sexual abuse are not mutually exclusive diagnoses. BMJ. 2000;320(7230):311.
87. Marren P, Walkden V, Mallon E, Wojnarowska F. Vulval cicatricial pemphigoid may mimic lichen sclerosus. Br J Dermatol. 1996;134(3):522–4.
88. Fung MA, LeBoit PE. Light microscopic criteria for the diagnosis of early vulvar lichen sclerosus, a comparison with lichen planus. Am J Surg Pathol. 1998;22(4):473–8.
89. Niamh L, Naveen S, Hazel B. Diagnosis of vulval inflammatory dermatoses: a pathological study with clinical correlation. Int J Gynecol Pathol. 2009; 28(6):554–8.
90. Gregory B, Ho VC. Cutaneous manifestations of gastrointestinal disorders. Part II. J Am Acad Dermatol. 1992;26(3 Pt 2):371–83.
91. Donaldson LB. Crohn's disease: "Its gynecologic aspect". Am J Obstet Gynecol. 1978;131(2): 196–202.
92. Markowitz J, Grancher K, Rosa J, Simpser E, Aiges H, Daum F. Highly destructive perianal disease in children with Crohn's disease. J Pediatr Gastroenterol Nutr. 1995;21(2):149–53.
93. de la Poza G, López-Sanroman A, Taxonera C, Marín-Jimenez I, Gisbert JP, et al. Genital fistulas in female Crohn's disease patients.: clinical characteristics and response to therapy. J Crohns Colitis. 2012;6(3):276–80.
94. Emanuel PO, Phelps RG. Metastatic Crohn's disease: a histopathologic study of 12 cases. J Cutan Pathol. 2008;35(5):457–61.
95. Barret M, de Parades V, Battistella M, Sokol H, Lemarchand N, Marteau P. Crohn's disease of the vulva. J Crohns Colitis. 2014;8(7):563–70.
96. Ploysangam T, Heubi JE, Eisen D, Balistreri WF, Lucky AW. Cutaneous Crohn's disease in children. J Am Acad Dermatol. 1997;36(5 Pt 1):697–704.
97. Corbett SL, Walsh CM, Spitzer RF, Ngan BY, Kives S, Zachos M. Vulvar inflammation as the only clinical manifestation of Crohn disease in an 8-year-old girl. Pediatrics. 2010;125(6):e1518–22.
98. Foo WC, Papalas JA, Robboy SJ, Selim MA. Vulvar manifestations of Crohn's disease. Am J Dermatopathol. 2011;33(6):588–93.
99. Andreani SM, Ratnasingham K, Dang HH, Gravante G, Giordano P. Crohn's disease of the vulva. Int J Surg. 2010;8(1):2–5.
100. Guerrieri C, Ohlsson E, Ryden G, Westermark P. Vulvitis granulomatosa: a cryptogenic chronic inflammatory hypertrophy of vulvar labia related to cheilitis granulomatosa and Crohn's disease. Int J Gynecol Pathol. 1995;14(4):352–9.

101. Khaled A, Ezzine-Sebai N, Fazaa B, Zeglaoui F, Zermani R, Kamoun MR. Chronic linear ulcerations of the inguino-crural and buttocks folds. Indian J Dermatol. 2011;56(1):101–3.
102. Burgdorf W. Cutaneous manifestations of Crohn's disease. J Am Acad Dermatol. 1981;5(6):689–95.
103. Makhija S, Trotter M, Wagner E, Coderre S, Panaccione R. Refractory Crohn's disease of the vulva treated with infliximab: a case report. Can J Gastroenterol. 2007;21(12):835–7.
104. Hackzell-Bradley M, Hedblad MA, Stephansson EA. Metastatic Crohn's disease. Report of 3 cases with special reference to histopathologic findings. Arch Dermatol. 1996;132(8):928–32.
105. Ishida M, Iwai M, Yoshida K, Kagotani A, Okabe H. Metastatic Crohn's disease accompanying granulomatous vasculitis and lymphangitis in the vulva. Int J Clin Exp Pathol. 2013;6(10):2263–6.
106. Borum ML, Cannava M, Myrie-Williams C. Refractory, disfiguring vulvar pyoderma gangrenosum and Crohn's disease. Dig Dis Sci. 1998; 43(4):720–2.
107. Mun JH, Kim SH, Jung DS, Ko HC, Kim MB, Kwon KS. Unilateral, non-tender, vulvar swelling as the presenting sign of Crohn's disease: a case report and our suggestion for early diagnosis. J Dermatol. 2011;38(3):303–7.
108. Wickramasinghe N, Gunasekara CN, Fernando WS, Hewavisenthi J, de Silva HJ. Vulvitis granulomatosa, Melkersson-Rosenthal syndrome, and Crohn's disease: dramatic response to infliximab therapy. Int J Dermatol. 2012;51(8):966–8.
109. Jeng CJ, Ko ML, Wang TH, Huang SH. Vulvar siliconoma migrating from injected silicone breast augmentation. BJOG. 2005;112(12):1659–60.
110. Pereda O, de la Cruz S, Ramos C, Cortez F, Carayhua D, Sanz M. Vulvar erosive lichen planus associated with silica granuloma: case report. Folia Dermatol Peru. 2009;20(3):159–62.
111. Zhou J, Ha BK, Schubeck D, Chung-Park M. Myxoid epithelioid leiomyoma of the vulva: a case report. Gynecol Oncol. 2006;103(1):342–5.
112. Rybicki BA, Maliarik MJ, Major M, et al. Epidemiology, demographics, and genetics of sarcoidosis. Semin Respir Infect. 1998;13(3):166–73.
113. Petit A, Dadzie OE. Multisystemic diseases and ethnicity: a focus on lupus erythematosus, systemic sclerosis, sarcoidosis and Behçet disease. Br J Dermatol. 2013;169 Suppl 3:1–10.
114. Tukey MH, Berman JS, Boggs DA, et al. Mortality among African American women with sarcoidosis: data from the Black Women's Health Study. Sarcoidosis Vasc Diffuse Lung Dis. 2013;30(2): 128–33.
115. Mañá J, Marcoval J. Skin manifestations of sarcoidosis. Presse Med. 2012;41(6 Pt 2):e355–74.
116. Fernandez-Faith E, McDonnell J. Cutaneous sarcoidosis: differential diagnosis. Clin Dermatol. 2007;25(3):276–87.
117. Decavalas G, Adonakis G, Androutsopoulos G, Gkermpesi M, Kourounis G. Sarcoidosis of the vulva: a case report. Arch Gynecol Obstet. 2007; 275(3):203–5.
118. Xu F, Cheng Y, Diao R, Zhou X, Wang X, Ma Y, et al. Sarcoidosis: vaginal wall and vulvar involvement. Sarcoidosis Vasc Diffuse Lung Dis. 2012; 29(2):151–4.
119. Tatnall FM, Barnes HM, Sarkany I. Sarcoidosis of the vulva. Clin Exp Dermatol. 1985;10(4):384–5.
120. Vera C, Funaro D, Bouffard D. Vulvar sarcoidosis: case report and review of the literature. J Cutan Med Surg. 2013;17(4):287–90.
121. Ismail A, Beckum K, McKay K. Transepithelial elimination in sarcoidosis: a frequent finding. J Cutan Pathol. 2014;41(1):22–7.
122. Klein PA, Appel J, Callen JP. Sarcoidosis of the vulva: a rare cutaneous manifestation. J Am Acad Dermatol. 1998;39(2 Pt 1):281–3.
123. de Oliveira Neto MP. Sarcoidosis with vulvar lesions. Rev Bras Med. 1972;29(3):134–9.
124. Ezughah FI, Ghaly AF, Evans A, Green CM. Vulval sarcoid: a systemic presentation of sarcoidosis. J Obstet Gynaecol. 2005;25(7):730–2.
125. Ten Berge B, Paats MS, Bergen IM, van den Blink B, Hoogsteden HC, Lambrecht BN, et al. Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. Rheumatology (Oxford). 2012;51(1):37–46.
126. Wollina U, Gruner M, Schönlebe J. Granulomatous tattoo reaction and erythema nodosum in a young woman: common cause or coincidence? J Cosmet Dermatol. 2008;7(2):84–8.
127. Tu J, Chan J. Cutaneous sarcoidosis and infliximab: evidence for efficacy in refractory disease. Australas J Dermatol. 2013. doi:10.1111/ajd.12056 [Epub ahead of print].
128. Haimovic A, Sanchez M, Judson MA, Prystowsky S. Sarcoidosis: a comprehensive review and update for the dermatologist: part I. Cutaneous disease. J Am Acad Dermatol. 2012;66(5):699.e1–18.
129. Mangas C, Fernández-Figueras MT, Fité E, Fernández-Chico N, Sàbat M, Ferrándiz C. Clinical spectrum and histological analysis of 32 cases of specific cutaneous sarcoidosis. J Cutan Pathol. 2006;33(12):772–7.
130. de Jager M, Blokx W, Warris A, Bergers M, Link M, Weemaes C, Seyger M. Immunohistochemical features of cutaneous granulomas in primary immunodeficiency disorders: a comparison with cutaneous sarcoidosis. J Cutan Pathol. 2008;35(5):467–72.
131. Bandow GD. Diagnosis and management of vulvar ulcers. Dermatol Clin. 2010;28(4):753–63.
132. Lehman JS, Bruce AJ, Wetter DA, Ferguson SB, Rogers III RS. Reactive nonsexually related acute genital ulcers: review of cases evaluated at Mayo Clinic. J Am Acad Dermatol. 2010;63(1):44–51.
133. Sárdy M, Wollenberg A, Niedermeier A, Flaig MJ. Genital ulcers associated with Epstein-Barr virus

infection (ulcus vulvae acutum). Acta Derm Venereol. 2011;91(1):55–9.
134. Huppert JS, Gerber MA, Deitch HR, Mortensen JE, Staat MA, Adams Hillard PJ. Vulvar ulcers in young females: a manifestation of aphthosis. J Pediatr Adolesc Gynecol. 2006;19(3):195–204.
135. Slebioda Z, Szponar E, Kowalska A. Etiopathogenesis of recurrent aphthous stomatitis and the role of immunologic aspects: literature review. Arch Immunol Ther Exp (Warsz). 2014;62(3):205–15.
136. Martín JM, Godoy R, Calduch L, Villalon G, Jordá E. Lipschütz acute vulval ulcers associated with primary cytomegalovirus infection. Pediatr Dermatol. 2008;25(1):113–5.
137. Pelletier F, Aubin F, Puzenat E, Deprez P, Blanc D, Estavoyer JM, et al. Lipschütz genital ulceration: a rare manifestation of paratyphoid fever. Eur J Dermatol. 2003;13(3):297–8.
138. Dixit S, Bradford J, Fischer G. Management of non-sexually acquired genital ulceration using oral and topical corticosteroids followed by doxycycline prophylaxis. J Am Acad Dermatol. 2013;68(5):797–802.
139. Farhi D, Wendling J, Molinari E, Raynal J, Carcelain G, Morand P, et al. Non-sexually related acute genital ulcers in 13 pubertal girls: a clinical and microbiological study. Arch Dermatol. 2009;145(1):38–45.
140. Halvorsen JA, Brevig T, Aas T, Skar AG, Slevolden EM, Moi H. Genital ulcers as initial manifestation of Epstein-Barr virus infection: two new cases and a review of the literature. Acta Derm Venereol. 2006;86(5):439–42.
141. Slebioda Z, Szponar E, Kowalska A. Recurrent aphthous stomatitis: genetic aspects of etiology. Postepy Dermatol Alergol. 2013;30(2):96–102.
142. Lai K, Lambert E, Mercurio MG. Aphthous vulvar ulcers in adolescent girls: case report and review of the literature. J Cutan Med Surg. 2010;14(1):33–7.
143. Behçet H. Über rezidivierende, aphthöse, durch ein Virus verursachte Geschwüre am Auge, und an den Genitalien. Dermatol Wochenschr. 1937;105:1152–7.
144. Sung Bin Cho, Suhyun Cho, Dongsik Bang. New insights in the clinical understanding of Behçet's disease. Yonsei Med J. 2012;53(1):35–42.
145. Alli N, Gur G, Yalcin B, Hayran M. Patient characteristics in Behçet disease: a retrospective analysis of 213 Turkish patients during 2001–4. Am J Clin Dermatol. 2009;10(6):411–8.
146. Mat C, Yurdakul S, Sevim A, Özyazgan Y, Tüzün Y. Behçet's syndrome: facts and controversies. Clin Dermatol. 2013;31(4):352–61.
147. Yurdakul S, Yazici H. Behçet's syndrome. Best Pract Res Clin Rheumatol. 2008;22(5):793–809.
148. Zouhoulis CC, Kotter I, Djawari D, et al. Current epidemiological data from the German registry of Adamantiades-Behcet's disease. Adv Exp Med Biol. 2003;528:43–8.
149. Kotter I, Vonthein R, Müller CA, et al. Behcet's disease in patients of German and Turkish origin living in Germany: a comparative analysis. J Rheumatol. 2004;31(1):133–9.
150. Kitaichi N, Miyazaki A, Iwata D, Ohno S, Stanford MR, Chams H, Hirohata S. Ocular features of Behcet's disease: an international collaborative study. Br J Ophthalmol. 2007;91(12):1579–82.
151. Hirohata S. Central nervous system involvement in Behçet's disease. Rinsho Shinkeigaku. 2001;41(12):1147–9.
152. Hatemi G, Seyahi E, Fresko I, Hamuryudan V. Behçet's syndrome: a critical digest of the 2012–2013 literature. Clin Exp Rheumatol. 2013;31(3 Suppl 77):108–17.
153. Maldini C, Lavalley MP, Cheminant M, de Menthon M, Mahr A. Relationships of HLA-B51 or B5 genotype with Behcet's disease clinical characteristics: systematic review and meta-analyses of observational studies. Rheumatology (Oxford). 2012;51(5):887–900.
154. Xavier JM, Shahram F, Sousa I, Davatchi F, Matos M, Abdollahi BS, et al. FUT2: filling the gap between genes and environment in Behcet's disease? Ann Rheum Dis. 2013. doi:10.1136/annrheumdis-2013-204475 [Epub ahead of print].
155. Yazici H, Tuzun Y, Pazarli H, Yurdakul S, Ozyazgan Y, Ozdoğan H, et al. Influence of age of onset and patient's sex on the prevalence and severity of manifestations of Behçet's syndrome. Ann Rheum Dis. 1984;43(6):783–9.
156. Yazici H, Esen F. Mortality in Behçet's syndrome. Clin Exp Rheumatol. 2008;26(5 Suppl 51):S138–40.
157. Gürler A, Boyvat A, Türsen U. Clinical manifestations of Behçet's disease: an analysis of 2147 patients. Yonsei Med J. 1997;38(6):423–7.
158. Alpsoy E, Zouboulis CC, Ehrlich GE. Mucocutaneous lesions of Behçet's disease. Yonsei Med J. 1997;38(6):423–7.
159. Grigg EL, Kane S, Katz S. Mimicry and deception in inflammatory bowel disease and intestinal Behçet disease. Gastroenterol Hepatol (N Y). 2012;8(2):103–12.
160. International Study Group for Behçet's Disease. Criteria for diagnosis of Behçet's disease. Lancet. 1990;335(8697):1078–80.
161. Harlow BL, Stewart EG. A population-based assessment of chronic unexplained vulvar pain: have we underestimated the prevalence of vulvodynia? J Am Med Womens Assoc. 2003;58(2):82–8.
162. Harlow BL, Kunitz CG, Nguyen RH, Rydell SA, Turner RM, Maclehose RF. Prevalence of symptoms consistent with a diagnosis of vulvodynia: population-based estimates from 2 geographic regions. Am J Obstet Gynecol. 2014;210(1):40.e1–8.
163. Clare CA, Yeh J. Vulvodynia in adolescence: childhood vulvar pain syndromes. J Pediatr Adolesc Gynecol. 2011;24(3):110–5.
164. Ventolini G. Vulvar pain: Anatomic and recent pathophysiologic considerations. Clin Anat. 2013;26(1):130–3.

165. Reed B, Harlow S, Sen A, Legocki LJ, Edwards RM, Arato N, et al. Prevalence and demographic characteristics of vulvodynia in population-based sample. Am J Obstet Gynecol. 2012;206(2):170.e1–9.
166. Metts JF. Vulvodynia and vulvar vestibulitis: challenges in diagnosis and management. Am Fam Physician. 1999;59(6):1547–56, 1561–2.
167. Sadownik LA. Clinical profile of vulvodynia patients. A prospective study of 300 patients. J Reprod Med. 2000;45(8):679–84.
168. Giesecke J, Reed BD, Haefner HK, Giesecke T, Clauw DJ, Gracely RH. Quantitative sensory testing in vulvodynia patients and increased peripheral pressure pain sensitivity. Obstet Gynecol. 2004;104(1): 126–33.
169. Hampson JP, Reed BD, Clauw DJ, Bhavsar R, Gracely RH, Haefner HK, et al. Augmented central pain processing in vulvodynia. J Pain. 2013;14(6):579–89.
170. de Ramirez Knott HM, McCormick TS, Do SO, Goodman W, Ghannoum MA, Cooper KD, et al. Cutaneous hypersensitivity to Candida albicans in idiopathic vulvodynia. Cont Dermatitis. 2005; 53(4):214–8.
171. Ventolini G, Gygax SE, Adelson ME, Cool DR. Vulvodynia and fungal association: a preliminary report. Med Hypotheses. 2013;81(2):228–30.
172. Ventolini G. Vulvar pain: Anatomic and recent pathophysiologic considerations. Clin Anat. 2013;81(2):228–30.
173. Farmer MA, Taylor AM, Bailey AL, Tuttle AH, MacIntyre LC, Milagrosa ZE, et al. Repeated vulvovaginal fungal infections cause persistent pain in a mouse model of vulvodynia. Sci Transl Med. 2011;3(101):101ra91.
174. Bowen AR, Vester A, Marsden L, Florell SR, Sharp H, Summers P. The role of vulvar skin biopsy in the evaluation of chronic vulvar pain. Am J Obstet Gynecol. 2008;199(5):467.e1–6.
175. Harlow BL, He W, Nguyen RH. Allergic reactions and risk of vulvodynia. Ann Epidemiol. 2009; 19(11):771–7.
176. Mascherpa F, Bogliatto F, Lynch PJ, Micheletti L, Benedetto C. Vulvodynia as a possible somatization disorder. More than just an opinion. J Reprod Med. 2009;19(11):771–7.
177. Bullones Rodríguez MÁ, Afari N, Buchwald DS, National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Urological Chronic Pelvic Pain. Evidence for overlap between urological and nonurological unexplained clinical conditions. J Urol. 2013;189(1 Suppl):S66–74.
178. Kalra B, Kalra S, Bajaj S. Vulvodynia: an unrecognized diabetic neuropathic syndrome. Indian J Endocrinol Metab. 2013;17(5):787–9.
179. Nyirjesy P, Sobel JD. Genital mycotic infections in patients with diabetes. Postgrad Med. 2013;125(3): 33–46.
180. Lin MT, Rohwedder A, Mysliborski J, Leopold K, Wilson VL, Carlson JA. 'HPV vulvitis' revisited: frequent and persistent detection of novel epidermodysplasia verruciformis-associated HPV genotypes. J Cutan Pathol. 2008;35(3):259–72.
181. Perrigouard C, Dreval A, Cribier B, Lipsker D. Vulvar vestibulitis syndrome: a clinicopathological study of 14 cases. Ann Dermatol Venereol. 2008; 35(3):259–72.
182. Donders G, Bellen G. Characteristics of the pain observed in the focal vulvodynia syndrome (VVS). Med Hypotheses. 2012;78(1):11–4.
183. Bornstein J, Cohen Y, Zarfati D, Sela S, Ophir E. Involvement of heparanase in the pathogenesis of localized vulvodynia. Int J Gynecol Pathol. 2008;27(1):136–41.
184. Bornstein J, Goldschmid N, Sabo E. Hyperinnervation and mast cell activation may be used as histopathologic diagnostic criteria for vulvar vestibulitis. Gynecol Obstet Invest. 2004;58(3):171–8.
185. de Belilovsky C. 2013 vulvodynia update. Gynecol Obstet Fertil. 2013;41(9):505–10.
186. Foster DC, Sazenski TM, Stodgell CJ. Impact of genetic variation in interleukin-1 receptor antagonist and melanocortin-1 receptor genes on vulvar vestibulitis syndrome. J Reprod Med. 2004;49: 503–9.
187. Gupta MA, Gupta AK. Cutaneous sensory disorder. Semin Cutan Med Surg. 2004;49(7):503–9.
188. Davis SN, Bergeron S, Binik YM, Lambert B. Women with provoked vestibulodynia experience clinically significant reductions in pain regardless of treatment: results from a 2-year follow-up study. J Sex Med. 2013;10(12):3080–7.
189. Tommola P, Unkila-Kallio L, Paavonen J. Long-term well-being after surgical or conservative treatment of severe vulvar vestibulitis. Acta Obstet Gynecol Scand. 2012;91(9):1086–93.
190. Leclair CM, Goetsch MF, Korcheva VB, Anderson R, Peters D, Morgan TK. Differences in primary compared with secondary vestibulodynia by immunohistochemistry. Obstet Gynecol. 2011;117(6): 1307–13.
191. Halperin R, Zehavi S, Vaknin Z, Ben-Ami I, Pansky M, Schneider D. The major histopathologic characteristics in the vulvar vestibulitis syndrome. Gynecol Obstet Invest. 2005;59(2):75–9.
192. Perrigouard C, Dreval A, Cribier B, Lipsker D. Vulvar vestibulitis syndrome: a clinicopathological study of 14 cases. Ann Dermatol Venereol. 2008; 135(5):367–72.
193. Leclair CM, Goetsch MF, Li H, Morgan TK. Histopathologic characteristics of menopausal vestibulodynia. Obstet Gynecol. 2013;122(4):787–93.
194. Danby CS, Margesson LJ. Approach to the diagnosis and treatment of vulvar pain. Dermatol Ther. 2010;23(5):485–504.

Infectious Diseases and Infestations of the Vulva

6

Maria Angelica Selim, Viviana Parra, Omar P. Sangueza, Luis Requena, and Martin A. Sangueza

Introduction

The most prevalent infectious diseases of the vulva in industrialized countries include: condyloma acuminata induced by the human papilloma virus (HPV), herpes genitalis, syphilis, and molluscum contagiosum. Herpes simplex virus infection and syphilis are currently the most common causes of genital ulcers in the United States [1]. Other infectious diseases that are less prevalent but can also affect the vulva include: lymphogranuloma venereum, chancroid, granuloma inguinale, and several bacterial diseases caused by both Staphylococci and Streptococci. This chapter will discuss the wide range of clinicopathologic presentation of these diseases and highlights practical clues to reach a definitive diagnosis.

M.A. Selim, M.D. (✉)
Department of Pathology, Duke University Medical Center, Durham, NC, USA
e-mail: Angelica.selim@duke.edu

Viral Infections

Herpes Virus Infection

Herpes simplex virus (HSV) types 1 and 2 are double-stranded enveloped deoxyribonucleic acid (DNA) viruses that are ubiquitous and produce primary infection, latency, and recurrent orolabial and genital disease. HSV type 2 infects more than 500 million people worldwide with an estimate of 23 million new infections annually [2]. The incidence is higher with HSV type 1, with seroprevalence of >90 % in many nations [3]. In the United States, seroprevalence is around 54 % for HSV type 1 and 15.7 % for HSV type 2 [4]. These values have shown to vary over time and depend on factors like age, sex, race, and risk behavior profile [5]. Genital herpes is a lifelong infection representing the most frequent sexually transmitted disease worldwide. Until recently, the traditional thinking consisted of HSV type 2 as the major culprit for genital herpes [6], with a minor component attributed to type 1 virus. However, studies are demonstrating a shift in the epidemiology of genital herpes infection with an increasing role of HSV type 1 as the etiology at a certain age group [5]. Its incidence has been increasing through time in industrialized countries, especially affecting women of 25 years or younger [6, 7]. Among US college students, the

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_6,

percentage of genital herpes attributed to HSV type 1 went from 31 % in 1993 to 78 % in 2001 [8]. This trend is also seen in the recent HSV vaccine trial in the United States with nearly 60 % of genital herpes infections attributed to HSV type 1 [9, 10]. A postulated explanation to this increase in genital HSV type 1 in young women is the decline of childhood acquired orolabial HSV-1 infection and therefore the lack of HSV type 1 antibodies previous to become sexually active. Even more, the absence of HSV type 1 antibodies predispose to develop symptomatic disease if infected with HSV type 2 [11]. The seroprevalence of HSV type 2, estimated by the National Health and Nutrition Examination Survey data, has a plateau since 1999 in the United States [4]. Previous infection with HSV type 1 (e.g., cold sore) gives some protection from infection with HSV type 2, either reducing the severity of the primary attack or preventing it altogether.

The virus replicates at the site of infection, then travels to the dorsal root ganglia through retrograde axonal flow, and remains in a latent phase, with recurrent reactivations occurring spontaneously or following stimuli such as fever, stress, ultraviolet radiation, or immunosuppression. Fewer recurrences of lesions and less viral shedding are detected with HSV type 1 over type 2 [4].

Clinical Features

The clinical manifestations depend on the immune status of the patient and whether it is a primary versus a recurrent infection. HSV type 1 has characteristically transmitted nonsexually during childhood, while HSV type 2 is nearly always a sexually transmitted disease. Primary infection usually occurs 3–7 days after exposure, with a prodrome of general malaise associated with nonspecific genital findings. The systemic manifestations include fever, myalgias, headache, and back pain coexisting with painful erythema and vulvar swelling. In time, the classic genital lesions for HSV type 1 or 2 develop, which are vesicles on an erythematous base arranged in clusters that evolve to pustules and/or erosions (Fig. 6.1). These lesions are extremely painful and accompanied by regional lymphadenopathy that may last for more than a week. The vesicles heal without scarring unless there is secondary infection. Unusual clinical presentations include Well's syndrome in the form of vulvar edema [12, 13]. Patients suffering from human immunodeficiency virus (HIV) infection may present with severe clinical presentations like increased number and size of lesions, remarkable pain, slow resolution of lesions, and hypertrophic or verrucous lesions [14]. Vaginal discharge can occur. Extension of the lesions, systemic complaints, and complications are more frequent in women than in men with genital herpes. Lesions can spread to involve the cervix, buttock, and perineum. Severe local manifestations, marked regional lymphadenopathy, and systemic manifestations separate primary infection from recurrent disease. Recurrences are more frequent with HSV type 2 infection than type 1. HSV type 1 and type 2 can be transmitted perinatal from mother to child causing severe neurologic damage and in some cases death. Highest risk of neonatal herpes is seen with vaginal delivery and prolonged ruptured membranes before cesarean section or cervical infection. Other serious adverse events in herpetic adult infection include: blindness, encephalitis, and aseptic meningitis. Of concern is the interaction of HSV type 2 with HIV. A person infected with HSV type 2 has a two to threefold increased risk of acquiring HIV infection [15] and fourfold of transmitting HIV infection [16].

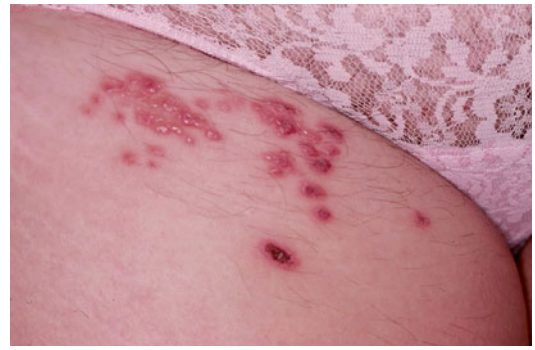


Fig. 6.1 *Herpes viral simplex infection*. Clinically, it presents as multiple grouped vesicles with an erythematous base

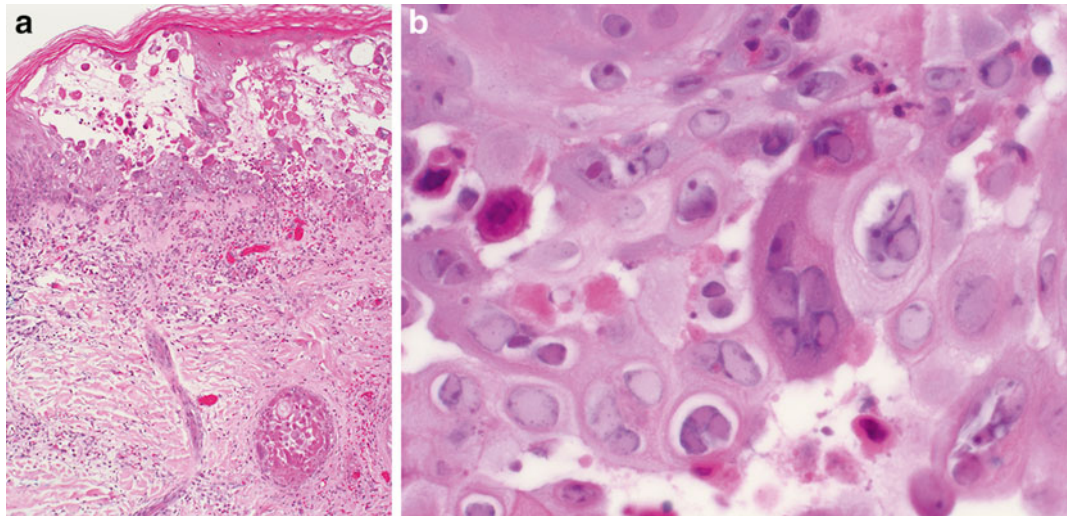


Fig. 6.2 (**a**, **b**) *Herpes viral simplex infection*. Intraepidermal vesicle with keratinocytic ballooning and reticular degeneration (**a**). Infected keratinocytes demonstrate multinucleation with molding and blue-gray nuclei with margination of the chromatin (**b**)

Originally, HSV shedding was thought to be infrequent given the low rate of symptomatic recurrences. Numerous studies are refuting this concept; daily samples study with PCR amplification have documented asymptomatic shedding in the genital tract of 80–90 % of HSV type 2 seropositive individuals around 10 % of days [17]. The same occurs with infected patients with HSV type 1 [18]. The viral shedding are typically brief and can be simultaneous or subsequent to reactivations and continues to occur over time [19]. These silent reactivations with frequent shedding episodes have a tremendous public health impact. Even more, studies indicate that most of HSV transmission occurs during these asymptomatic viral shedding episodes. Although the risk is higher with active lesions as the viral load is greater.

Histopathology

An early intact herpes simplex vesicle usually involves the entire epidermis with ballooning and reticular degeneration (Fig. 6.2a). Ballooning degeneration presents in the form of intense cytoplasmic eosinophilia with loss of intercellular cohesion leading to acantholysis. On the other hand, reticular degeneration typically shows hydropic swelling and rupture of the epidermal cells. Classically, infected keratinocytes demonstrate multinucleation with molding blue-gray nuclei with margination of the chromatin ("ground glass") (Fig. 6.2b). Cowdry A inclusions are eosinophilic intranuclear inclusions of 3–8 μm in diameter that are surrounded by a halo. With time, the cells undergo karyorrhexis and lysis, that clinically translate in erosions and ulcerations. At this stage, many times it is difficult to identify the characteristic features of HSV infection in the epidermis. Occasionally, sebaceous gland and follicular involvement can still harbor the diagnostic viral cytopathic changes (Fig. 6.3a, b). Herpetic syringitis, viral cytopathic changes in eccrine epithelium with necrosis and syringosquamous metaplasia, has been reported especially in immunocompromised patients [20]. The nerves are also affected by the infection in the form of neuronal atrophy and Schwann cell hypertrophy. Biopsy of nodular lesions demonstrates pseudoepitheliomatous hyperplasia of the epidermis and a dense inflammatory dermal process simulating a lymphoma. Cytological evalua-

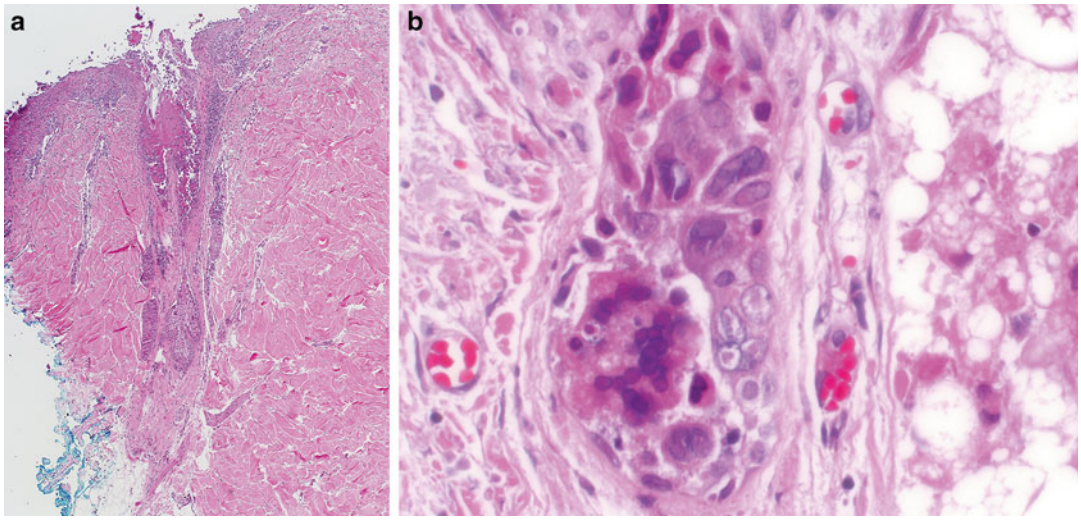


Fig. 6.3 (**a**, **b**) *Herpes simplex viral infection*. Follicular involvement is noted (**a**) and confirmed by viral cytopathic changes in high power (**b**)

tion of the scraping of the base and edges of a fresh ulcer, or freshly opened vesicle, usually will show the multinucleated cells with viral cytopathic effects, characteristic of HSV infection. Moistening the ulcer with a saline-soaked sponge and then scraping it with a wooden spatula may improve the diagnostic yield from ulcerative lesions. Morphologic changes seen with HSV infection are not reliable in separating from secondary infection or in distinguishing HSV type 1 from type 2 infection. Furthermore, herpes zoster can involve the vulva and may have similar cytological findings.

In difficult cases, HSV-specific fluorescein-conjugated antiserum may be placed on smears of ulcers or vesicles to identify HSV antigens. Immunoperoxidase techniques, utilizing HSV-specific antibodies, may be of value if the histopathological findings are nonspecific and can be employed on paraffin-embedded tissue to separate the different types of the herpes virus family.

Isolation of HSV types 1 or 2 can be achieved by the inoculation of tissue culture monolayers, such as WI-38 human embryonic lung fibroblasts or monkey kidney cells. Culture sensitivity increases if the sample lesion is a blister than an ulcer, from 10 % to 33 %. Both types of HSV produce characteristic cytopathic changes on these cell lines, which are confirmed by direct immunofluorescence employing monoclonal antibodies to HSV. Virus isolation can be achieved within 4 days. Rapid viral culture over 24 h, followed by a search for HSV antigen using immunoperoxidase technique, can give a prompt result (Fig. 6.4a, b). In situ hybridization and polymerase chain reaction (PCR) techniques, employing HSV-specific primers, are another approach to the positive identification of HSV infections. In considering diagnostic techniques for genital herpes infection, culture and direct fluorescent antibody staining of infected cells are labor-intensive techniques that require skilled laboratories and are impacted by collection technique, transport conditions, and subjective interpretation [21, 22]. On the other hand, PCR assays are more sensitive and are less time consuming that result in a fast turnaround time [22].

Differential Diagnosis

The identification of viral cytopathic changes confirms the diagnosis. However, the reactive changes associated with the infection and unusual clinical presentation can be misleading. Verrucous herpes infection, seen in HIV-infected

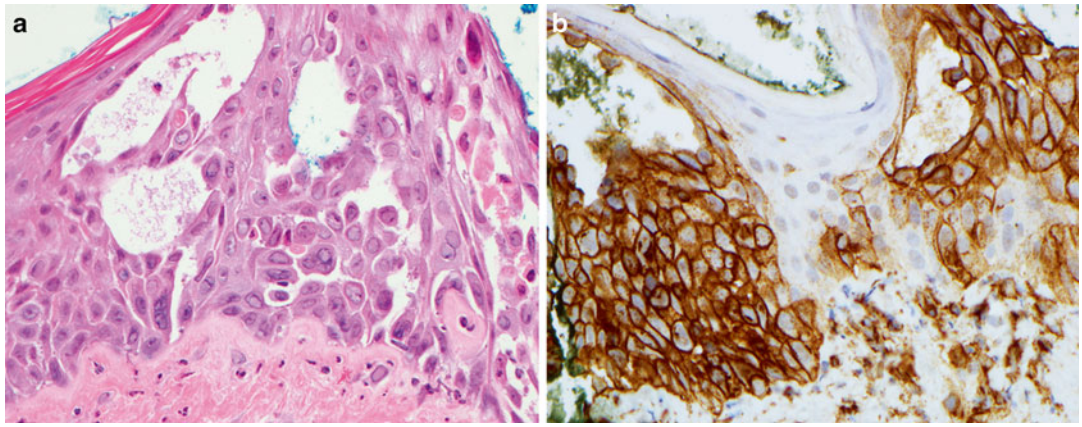


Fig. 6.4 (**a**, **b**) *Herpes simplex viral infection*. The infected keratinocytes (**a**) react with immunohistochemical antibody against the virus (**b**)

patients, raises the differential diagnosis of squamous cell carcinoma. The infiltrative irregular nests of severely atypical keratinocytes associated with desmoplastic stromal reaction as well as the presence of atypical mitoses support the interpretation of squamous cell carcinoma. Furthermore, no viral cytopathic changes will be noted. A dense lymphoid infiltrate can be associated with herpes viral infection. This may raise the consideration of a T-cell process like lymphomatoid papulosis due to the presence of CD30-positive reactive lymphocytes. The detection of T-cell clonality in the lymphoid infiltrate secondary to the viral infection can be even more confusing. The clinical history of a vesicular eruption and acute inflammation associated with CD4- and CD8-positive T-cell lymphocytes will support the diagnosis of herpes infection over lymphoproliferative disorders. Serial sections can be a useful tool to detect the viral changes. Lastly, reparative changes near an ulcer can produce multinucleated cells; the absence of "ground-glass" changes in the nuclei or the presence of Cowdry A cytopathic effect supports secondary reactive changes over viral infection. If in doubt, immunohistochemical stain can be of help.

Summary

Clinical Presentation

- Three stages: primary infection (initial manifestations in women without preexisting antibodies), latency (time after initial infection before reactivation periods), and recurrent episodes (reactivation of HSV after latency).
- Primary infection: 3–7 days after exposure, with a prodrome of general malaise.
- Classic clinical presentation consists of vesicles on an erythematous base arranged in clusters that evolve to pustules and/or erosions.
- Women suffering from HIV present with unusual clinical presentations.

Histologic Features

- Epidermis with ballooning and reticular degeneration.
- Epidermal blister +/– ulceration.
- Viral cytopathic changes:
  - Common: cells with multinucleated, molding nuclei with margination of chromatin (ground glass)

  - Less common: Cowdry A cytopathic effect (eosinophilic nuclear inclusion with clear halo)
- Involvement of folliculosebaceous units and eccrine gland occurs.
- Florid mixed inflammatory reaction.

Differential Diagnosis

- Squamous cell carcinoma
- CD30-positive lymphoproliferative disorder
- Reparative changes

Takeaway Essentials

Clinical Relevant Pearls

- There is a shift in the epidemiologic landscape of genital herpes infection.
- Increase risk associated with increased number of sexual partners, lower education, and poverty.
- Infected individuals experience frequent, asymptomatic brief shedding episodes.
- Prior infection likely reduces the burden of disease when infected by a second HSV type.
- Atypical presentation can be seen in HIV-infected patients with genital herpes.
- When considering cultures, selection of a blister increases the sensitivity.

Pathology Interpretation Pearls

- When the lesion is old and only an ulcer is present, adnexal structures like folliculosebaceous units and eccrine glands may demonstrate the characteristic viral cytopathic changes.
- Sometimes multiple levels are needed to detect the viral cytopathic changes.

Immunohistochemical Findings

- Immunohistochemical stains in paraffin-embedded tissue separate the different types of herpes virus implicated in the infection.

Varicella Zoster

Varicella zoster virus (VZV) is a ubiquitous, neurotropic alpha herpes virus. Primary infection results in varicella (chickenpox). This is followed by a latent infection of neurons of cranial nerve, dorsal root, and autonomic ganglia along the neuraxis. Twenty percent of immunocompetent hosts and half of immunocompromised infected people will undergo reactivation of the virus [23].

Clinical Features

Clinical reactivation is frequently seen in women around the fifth decade. An episode of herpes zoster usually starts with a prodrome of pain, pruritus, or tingling in a dermatomal distribution. In a short period of time, clusters of papules appear and change into vesicles with an erythematous base (Fig. 6.5). In the vulva, the vesicles have a short life span ending in erosions. Postherpetic neuralgia can be a debilitating sequelae of this infection [24]. Anogenital VZV infection in the pediatric population can be confused with child sexual abuse [25].

Histopathology

The histologic features of VZV infection overlap with those seen in HSV infection (Fig. 6.6a, b). VZV infection is less inflammatory than HSV but with a more prominent vasculitic component

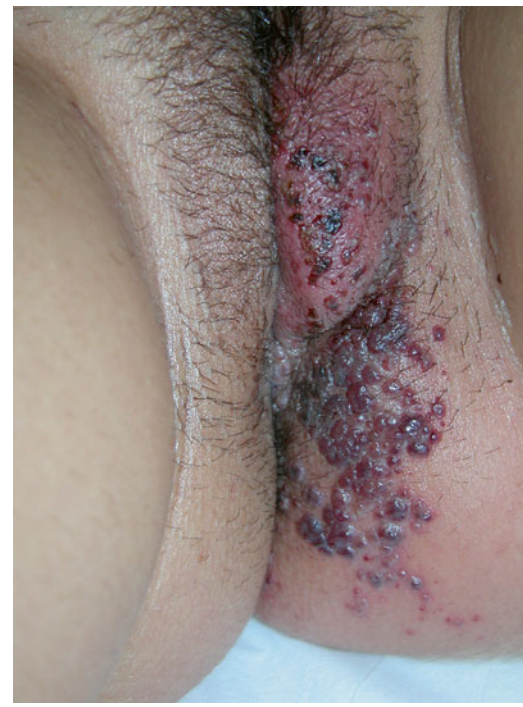


Fig. 6.5 *Varicella zoster*. Cluster of papules appear and change into vesicles with an erythematous base

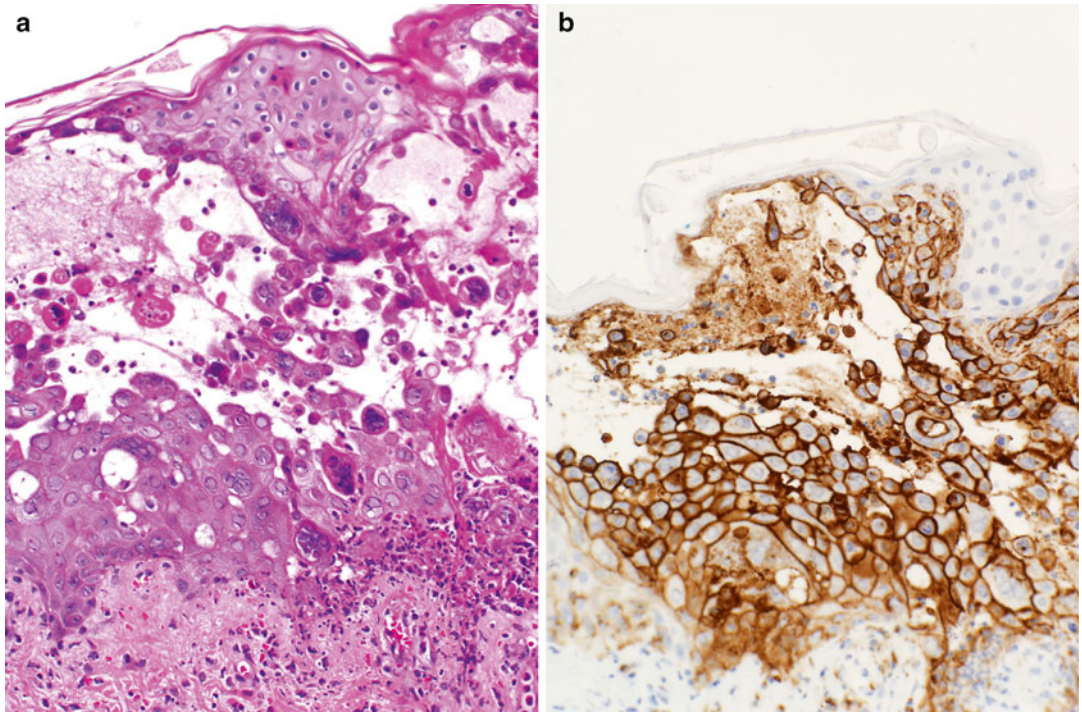


Fig. 6.6 (**a**, **b**) *Varicella zoster*. Its histologic features overlap with those of herpes viral simplex infection (**a**). An immunohistochemical stain can separate these viruses (**b**)

(Fig. 6.7a, b). When the vasculitis is severe, necrotizing lesions elicit. Confirmation of the infection can be reached using immunohistochemistry, direct florescence antibodies, or PCR test [26].

Differential Diagnosis

The identification of viral cytopathic changes confirms the diagnosis. Due to the overlap in histologic features between VZV and HSV, please refer to HSV section for discussion on differential diagnosis.

Summary

Clinical Presentation

- Dermatomal distribution
- Prodrome of burning or tingling
- Papules and vesicles rapidly turning into erosions

Histologic Features

- Similar to HSV

Differential Diagnosis

- Squamous cell carcinoma
- CD30-positive lymphoproliferative disorder
- Reparative epithelial changes

Takeaway Essentials

Clinical Relevant Pearls

- The distribution of the rash is a strong clue to reach the diagnosis.
- Atypical forms can be seen in immunosuppressed patients.
- Postherpetic neuralgia should be considered in women with unexplained dysesthesia.
- Anogenital *Varicell*a zoster in the pediatric population can be confused with sexual abuse.

Pathology Interpretation Pearls

- Histology of VZV is similar to HSV infection.

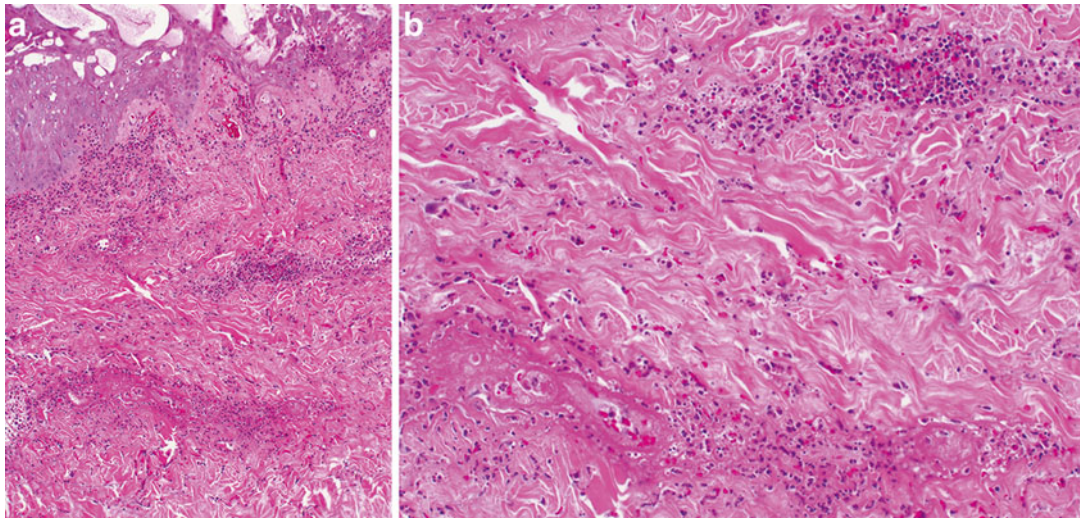


Fig. 6.7 (**a**, **b**) *Varicella zost*er. This viral infection is less inflammatory (**a**) than herpes simplex virus infection, but with a more prominent vasculitic component (**b**)

Immunohistochemical Findings

- Immunohistochemical stains are simple, reliable, and time-efficient diagnostic tools to separate HSV from VZV infection.

Cytomegalovirus

Cytomegalovirus (CMV) is part of the subgroup of beta herpesviruses. As other members of the *Herpesviridae* family, they have a primary infection, latent phase, and reinfection. CMV is one of the most fatal infection in immunocompromised individuals [27].

Clinical Features

Female genital CMV is a rare event. Routes of transmission are sexual, close contact, blood/tissue exposure, and perinatal. Clinical presentation exhibits a wide range of nonspecific cutaneous manifestations including generalized maculopapular rash, ulcers, hyperpigmented nodules, vesicles, and petechias, among others [27]. Single or multiple ulcers can be seen in the vulva, perineal, and perianal region [28]. In immunosuppressed patients, polymicrobial anogenital ulcers are the most frequent cutaneous presentation of CMV [29]. The detection of CMV in the lesion can be pathogenic, but in some cases, it is believed to be an epiphenomenon with colonization of endothelial cells during CMV viremia [30]. The latter has been argued in polymicrobial lesions in HIV-positive patients, especially in the presence of HSV and VZV virus infection. Intraneural location in the vulva and perianal skin is considered to be potential latency sites for this virus as seen in shingles [28].

Histopathology

The pathognomonic change in CMV consists of vascular dilatation with endothelial cells exhibiting large, eosinophilic intranuclear inclusions of 10 μm in size surrounded by a clear halo (owl's eye inclusions) (Fig. 6.8a, b). These inclusions can be seen not only in endothelial cells but also in keratinocytes, eccrine epithelium, macrophages, and fibroblasts [30]. Although the owl's eye intranuclear inclusions are the more characteristic viral cytopathic change; it is rare, with the most frequent form of viral change presenting as irregular-shaped cytomegaly and bubbly cytoplasm. Furthermore, the morphology of the viral inclusion varies through the different stages of the infection with an initial phase of nuclear enlargement, followed by cytoplasmic enlargement and viral inclusions in the cytoplasm and nucleus, and ends with nuclear inclusion fragmentation and disappearance of cytoplasmic inclusions [31]. Leukocytoclastic

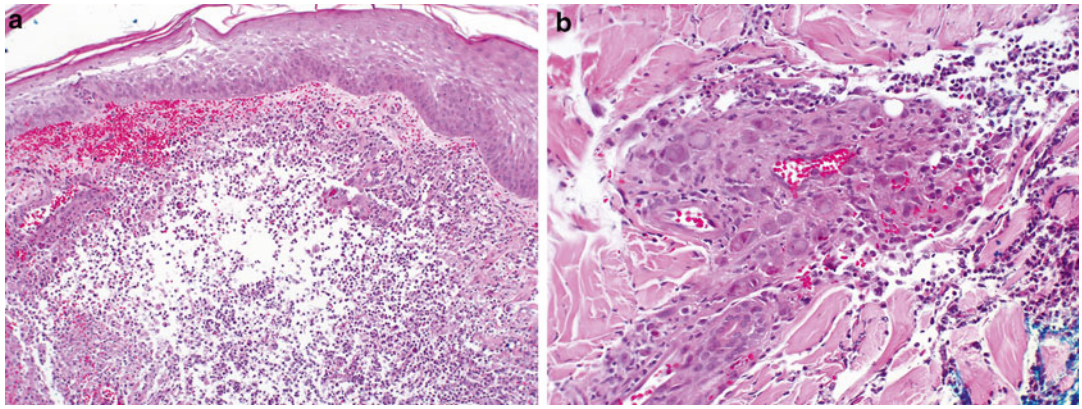


Fig. 6.8 (**a**, **b**) *Cytomegalovirus*. The dermal abscess (**a**) shows around the vessels the characteristic large, eosinophilic intranuclear inclusions surrounded by a clear halo (owl's eye inclusions) (**b**)

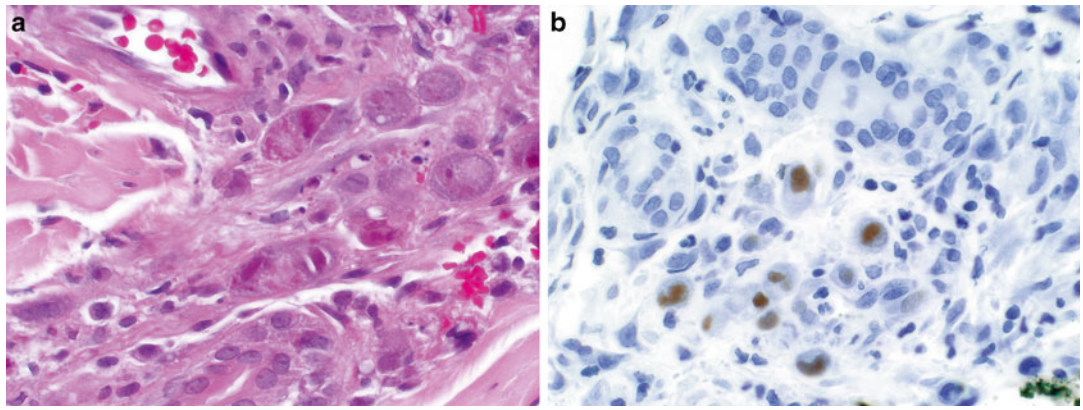


Fig. 6.9 (**a**, **b**) *Cytomegalovirus*. The infected cells (**a**) react with immunohistochemical antibody against the virus (**b**)

vasculitis, pauci-inflammatory thrombogenic vasculopathy [32], and eccrine squamous syringometaplasia [33] are reactive phenomenon associated with CMV infection.

Although light microscopy is a sensitive tool to detect CMV, immunohistochemistry (Fig. 6.9a, b), in situ hybridization, and molecular investigations can also be used to confirm the diagnosis in challenging cases.

Differential Diagnosis

The presence of the intranuclear inclusion in CMV is quite specific and definitive for a diagnosis. However, these inclusions are not present in all biopsies, and the differential diagnosis would include an anogenital ulcer due to a variety of etiologies including syphilis and other sexually transmitted diseases. For relevant information in the differential diagnosis, refer to Table 6.1.

Summary

Clinical Presentation

- Single or multiple ulcers can be seen in the vulva, perineal, and perianal region.

Histologic Features

- CMV inclusions ("owl's eye inclusions): large, eosinophilic intranuclear inclusions of 10 μm in size surrounded by a clear halo.
- Inclusions can be seen in endothelial cells but also in keratinocytes, eccrine epithelium, macrophages, and fibroblasts.
- Frequently the viral inclusions present as irregular-shaped cytomegaly and bubbly cytoplasm.

Differential Diagnosis

- Ulceration of other causes such as sexually transmitted diseases like syphilis.

Table 6.1 Sexually transmitted diseases

	Chancroid	Lymphogranuloma venereum	Granuloma inguinale (donovanosis)	Syphilis
Etiology	*Haemophilus ducreyi* (Gram-negative anaerobic coccobacillus)	*Chlamydia trachomatis* (obligatory intracellular Gram-negative coccoid or rod, serotype L1, L2, and L3)	*Klebsiella granulomatis* (obligatory intracellular Gram-negative rod)	*Treponema pallidum* (spirochete)
Location	Fourchette and labia minora	No specific location	Vulvar labia	Fourchette, labia majora
Incubation	1–14 days	3–42 days	2 weeks to 6 months after exposure	3 weeks
Clinical presentation	Chancroid: tender non-indurated ulcer with irregular undermined edges and purulent base	Three phases: (1) ulcerated skin lesion, (2) adenitis, and (3) fibrosis (genitoanorectal syndrome with perirectal abscesses, fistulas, and stenosis)	Ulcerated painless papules that merged to form a velvety beefy-erythematous granulation tissue surrounded by rolled edges	Primary: painless indurated ulceration with clean base
	Usually multiple	Primary lesion: inconspicuous ulcerated nontender papule healing without scar	Late manifestation: esthiomene	Secondary: maculopapular rash, pustular syphilis, lues maligna, and condyloma lata
		Late manifestation: esthiomene	If not treated is mutilating	Tertiary: nodules or ulcerated masses
Adenopathy	Inguinal adenopathy with flocculent nodes and drainage by fistula	Painful enlarged inguinal and/or femoral adenitis with bubo formation and rupture with drainage through sinus tract	Usually absent	Mildly enlarged
Histopathology	Lesion with three zones (1) ulcer, (2) granulation tissue and thrombosed vessels, and (3) chronic inflammation	Primary cutaneous lesion: nonspecific findings	Ulceration and granulation tissue. Donovan bodies: histiocytes with rod-shaped oval organisms with the form of a safety pin decorated by Warthin–Starry or Giemsa stain	Primary: ulcer with pseudoepitheliomatous hyperplasia
	Organisms: Gram-negative arranged as school of fish in the superficial aspect of lesion	Lymph nodes: serpiginous or stellate abscesses with granulomatous reaction		Endarteritis obliterans
				Secondary: psoriasiform pattern and lichenoid pattern with plasma cells component Condylomata lata: epidermal acanthosis with lymphoplasmacytic infiltrate and neutrophils in the surface
				Pustular syphilis: folliculocentric neutrophilic inflammation
				Lues maligna: thrombotic endarteritis
				Tertiary: granulomatous inflammation and endarteritis obliterans
Diagnostic tests	Cultures with selective agar medium or smears (better to show coccobacilli than histopathology)	Cultures with yolk sac of eggs, complement fixation antibodies, and PCR	Detection of Donovan bodies from smear of ulcer, culture, and PCR	Immunohistochemical stains, serologic tests (RPR, VDRRL, and PTHA), and molecular tests
	Immunofluorescent test and PCR			

Takeaway Essentials

Clinical Relevant Pearls

- Protean clinical presentation, anogenital region frequently present in the form of ulcers.
- The dermis is believed to be an inhospitable location for CMV.
- In immunosuppressed patient, the lesions are usually polymicrobial (HSV, VZV, and CMV).

Pathology Interpretation Pearls

- The "owl eye inclusion" however, it is not always present.
- Morphology of the inclusion will vary depending on the phase of the viral cycle.
- Serial sections may help to detect the viral cytopathic changes.

Immunohistochemical Findings

- Immunohistochemical stains are a useful tool to detect this virus in densely inflamed cases.

Molluscum Contagiosum

Molluscum contagiosum (MC) is a moderately contagious viral disease that, in adults, is often related to intimate and/or sexual contact. This poxvirus infection can present at any age, but it is frequently seen in children, sexually active adolescents, and immunocompromised individuals [34].

Clinical Features

Lesions of MC are usually asymptomatic; however, perianal lesions can become pruritic and undergo secondary infection. The lesions present as small, smooth papules, 3–6 mm in diameter with a central punctum or umbilication (Fig. 6.10). The most frequent affected anatomic locations include the labia majora, labia minora, and mons pubis [35, 36]. They are usually multiple and separated lesions, an unusual form of presentation is a plaque, which is made up of 50–100 individual clustered lesions. The incubation period varies between 14 and 50 days. The characteristic clinical presentation usually does not require biopsy. Cytological identification of the typical intracytoplasmic inclusion bodies (molluscum bodies or Henderson–Patterson bodies) within scrapings from the interior of molluscum papule is sufficient to confirm the diagnosis. Molluscum dermatitis (eczematous reaction to the viral infection) and Gianotti–Crosti-like reactions (erythematous papules or papulovesicles) can be seen in these patients [37]. Classically, lesions of molluscum contagiosum commonly regress within 6–12 months.

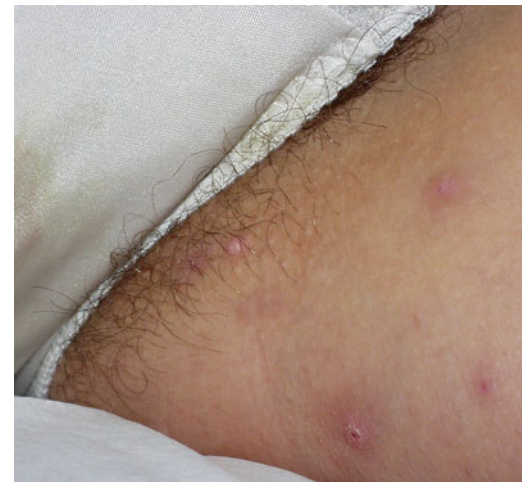


Fig. 6.10 *Molluscum contagiosum*. Clinically, the lesions consist of small, smooth papules, 3–6 mm in diameter with a central punctum or umbilication

Histopathology

MC presents as a cup-shaped keratinocytic endophytic growth containing the characteristic intracytoplasmic viral inclusions, which impart an eosinophilic appearance to the keratinocytic cytoplasm (molluscum body or Henderson–Patterson body) (Fig. 6.11a, b). The viral particles increase in size with progression toward the surface of the invagination causing the peripheral displacement of the nuclei. In older lesions, the cytoplasmic bodies take on a more basophilic appearance preceding lysis of the cell. The central dimple of the lesion is seen histologically if the lesion is carefully bisected. In the dermis, there is often an inflammatory infiltrate, which sometimes can be very prominent and simulate a lymphoma or a pseudolymphoma. Most lesions regress spontaneously; however, untreated lesions

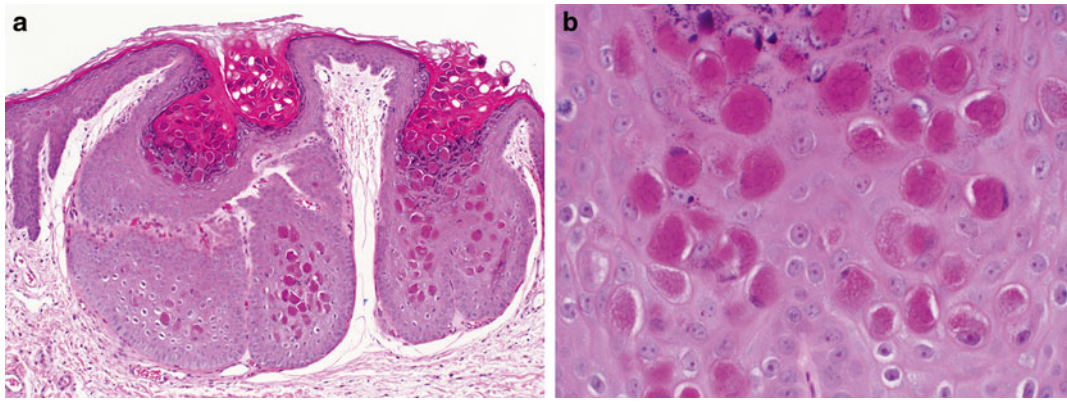


Fig. 6.11 (**a**, **b**) *Molluscum contagiosum*. Cup-shaped keratinocytic endophytic growth (**a**) containing the characteristic intracytoplasmic viral inclusions (Henderson–Patterson body) (**b**)

may persist for years, during which time they may be spread by close contact. Recently, a fluorescence resonance energy transfer-based real-time PCR is available for a sensitive and specific diagnosis of this infection [38].

Differential Diagnosis

The clinical and histologic appearance of MC is pathognomonic. The differential diagnosis in large lesions may include condyloma acuminatum. The presence of Henderson–Patterson bodies and the absence of koilocytes support the interpretation of MC.

Summary

Clinical Presentation

- Affect labia majora, labia minora, and mons pubis.
- The incubation period varies between 14 and 50 days.
- The lesions present as small, smooth papules, 3–6 mm in diameter with a central punctum or umbilication.

Histologic Features

- Cup-shaped endophytic epidermal growth
- Henderson–Patterson bodies

Differential Diagnosis

- Condyloma accuminata

Takeaway Essentials

Clinical Relevant Pearls

- Classic clinical presentation would not need a biopsy to confirm this infection.

Pathology Interpretation Pearls

- Exuberant lymphocytic reaction may mimic a lymphoproliferative disease.
- Serially sections through the block may be necessary to identify the virus.

Human Papillomavirus Infection (Condyloma Cuminata: Genital Warts)

Human papilloma virus (HPV) is a family of non-enveloped, circular, double-stranded DNA viruses. HPV is responsible for benign and malignant processes that affect the vulva, including condyloma acuminata and squamous cell carcinomas. Genital human papillomavirus is a common sexually transmitted infection (STI) with a transmission rate of approximately 65 % [39]. There are more than 40 types of HPV that can infect the genital areas of males and females, and they can infect the mouth and throat as well.

Condyloma acuminata (genital warts) are sexually transmitted benign neoplasms that may involve the vulva, vagina, cervix, urethra, anal canal, and perianal skin. Recurrent anogenital warts is a frequent cause of medical vulvitis with a reported 360,000 occurrences

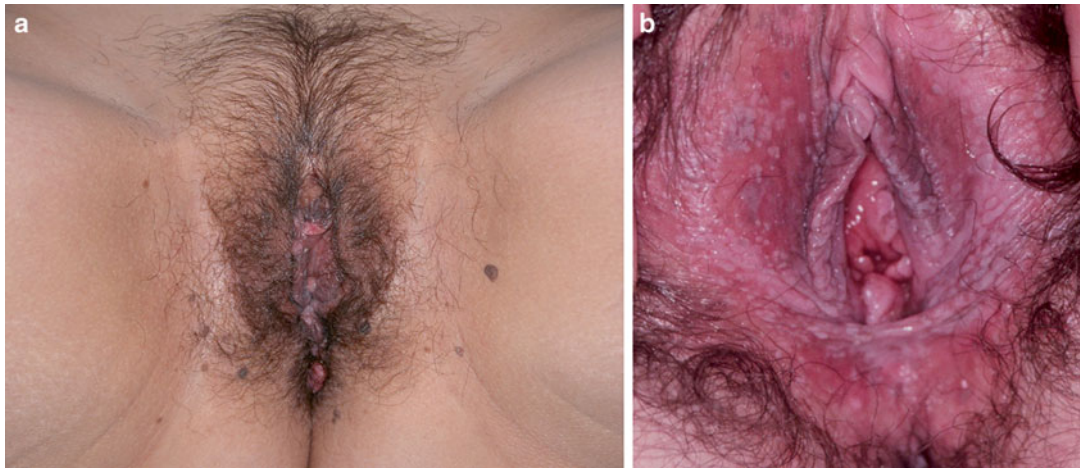


Fig. 6.12 (**a**, **b**) *Condyloma acuminata*. Clinically, condylomas present as flesh color or hyperpigmented multiple (**a**), confluent papillary, verrucous, filiform, flat (**b**), or papular lesions

in 2008 in the United States [39]. However, in recent years, the appearance of a quadrivalent (HPV genotypes 6, 11, 16, and 18) or bivalent (HPV types 6 and 11) vaccine is changing the epidemiologic landscape of genital warts. Future studies will reveal the impact of this vaccination in the patterns of origin and distribution of this infection. The prevalence of HPV infection varies greatly, depending on the analyzed population; in most studies, clinically evident vulvar involvement is less common than cervical HPV infection.

Molecular biology studies utilizing in situ hybridization have identified HPV-6 as the most prevalent HPV type in genital condylomata acuminata. HPV-11 is present in 25 % of genital warts [40]. These are low-risk HPV types in opposition to the high-risk (HPV16/16) types seen in vulva intraepithelial lesions and squamous cell carcinoma (see Chaps. 9 and 10).

Clinical Features

Clinically, condylomas present as flesh color or hyperpigmented, confluent papillary, verrucous, filiform, flat, or papular lesions of the skin and mucous membrane of the vulva that may extend to perineum and perianal region (Fig. 6.12a, b). Affects 1 % of sexually active adults with the highest incidence in the second and third decade [39]. The incubation period can range from 3 weeks to 8 months with a clinically apparent lesion within 2–3 months after infection [41]. Most lesions are asymptomatic unless secondarily infected. Small lesions are best appreciated with the application of 3–5 % acetic acid for 3–5 min, followed by colposcopic examination. Lesions that are only detected by colposcopic examination after performing HPV testing on the tissue in question are considered subclinical. Autoinoculation explains the high recurrence rates of condyloma, approximately 20–50 % [42]. Frequently, large proportion of genital warts may spontaneously regress within 1–2 years. Genotype-specific immunity may develop and protect against reinfection in the patient [43]. In immunocompromised patients, these lesions are frequently overgrown, large masses with a prologue history of recurrences [44, 45]. These large irregular and heavily keratinized lesions are frequently clinically confused with squamous cell carcinoma.

Condyloma acuminata are commonly associated with vaginitis, pregnancy, diabetes mellitus, oral contraceptive use, poor perineal hygiene, immunosuppression, and sexual activity with multiple partners. Approximately 30–50 % of women with vulvar condyloma acuminatum have associated cervical HPV infection. The presence of vulvar condyloma acuminatum in children is usually related to sexual abuse. A potential pitfall is the presence of HPV2 verruca vulgaris in young girls; their clinical presentation is similar

to the nongenital counterpart with more hyperkeratosis and papillomatosis than condylomas accuminatum [46]. When in doubt, DNA studies may help to distinguish these two clinical scenarios. To take into consideration is the fact that HPV-DNA has been detected on the fingers of children suffering from genital wart; therefore, it has been proposed that sexual contact is not always the route of transmission [47]. Other potential explanation for the presence of condylomas in children is the transmission from the mother to the infant during birth with a period of latency until clinical visible condylomas. Thorough clinical and social history and molecular tests are essential tools in the investigation of cases of potential sexual abuse.

Histopathology

Histologically, condylomas present as dome-shaped lesions with areas of acanthosis, papillomatosis, and hyperkeratosis. There is also a prominent granular layer with large and irregular keratohyaline granules and a few dyskeratotic keratinocytes. In some cases, the so-called koilocytes are seen in the superficial epidermis (Figs. 6.13a–c and 6.14a–d). These cells are characterized by the presence of cytoplasmic halos and enlarged irregular nuclei with slight hyperchromasia. Binucleated and multinucleated squamous cells often are found. The typical regular maturation upward from the basal layer is present, and the mitotic activity is restricted to the basal layer. A superficial chronic inflammatory infiltrate often is present in the dermis.

It is well recognized that treatment of genital warts with podophyllin leads to the presence of apoptosis, nuclear enlargement, and hyperchromasia. It is the clinical history and absence of atypia in the basal layer that can clarify the nature of these changes. Lately, the changes associated with podophyllin treatment were described in condylomas of young women in the absence of such treatment under the name of pseudobowenoid changes [48]. Here again, it is the absence of basal layer atypia that is a clue to separate these changes from high-grade vulva intraepithelial lesions.

On tissue specimen, tests available for detection of HPV include PCR, in situ hybridization, and

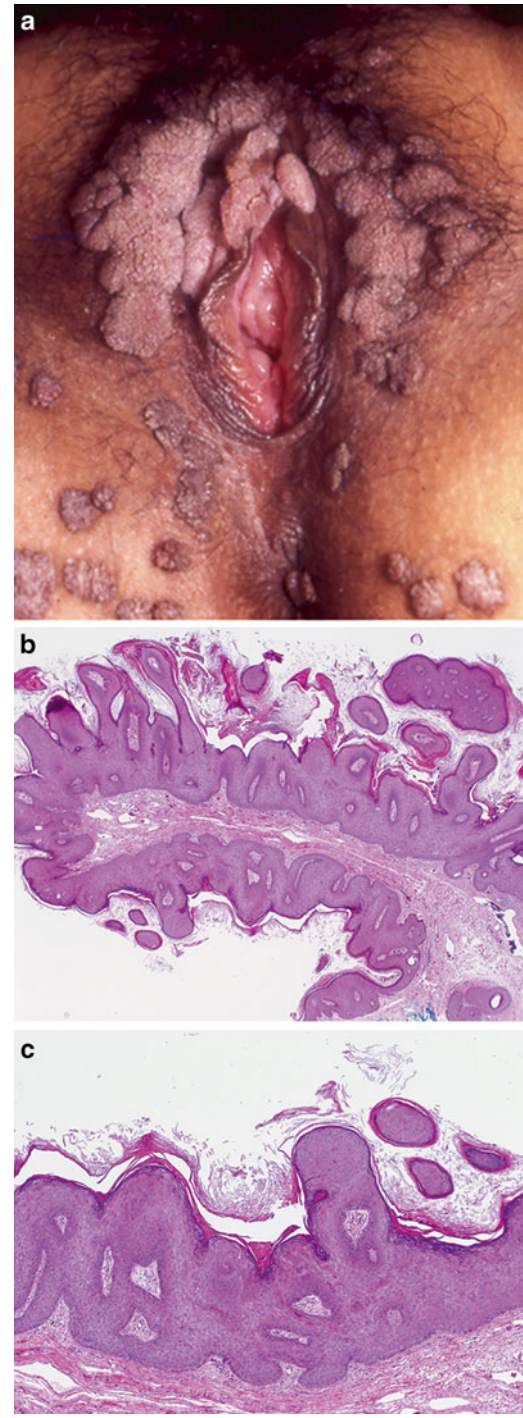


Fig. 6.13 (**a**–**c**) *Condyloma acuminata*. Verrucous filiform lesions (**a**) histologically present as a papillomatous acanthosis (**b**) with classic koilocytes (**c**)

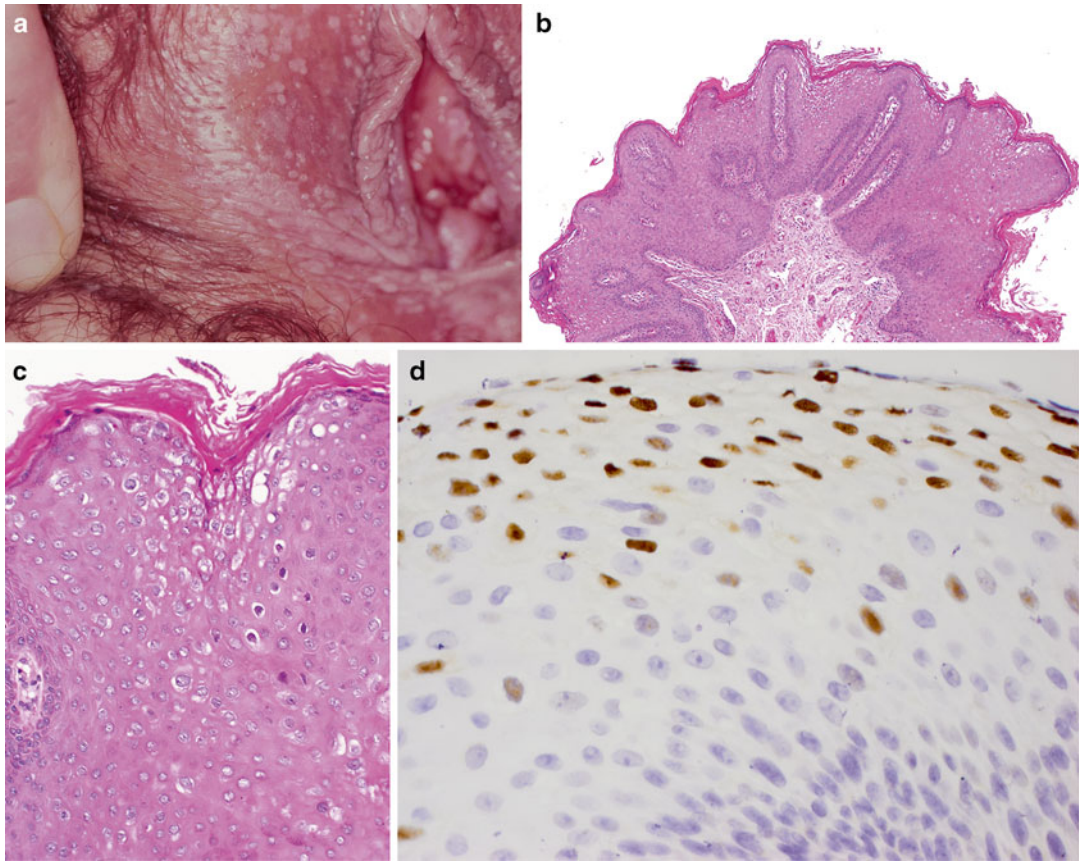


Fig. 6.14 (**a**–**d**) *Condyloma acuminata*. Clinically flat lesions (**a**) present as dome shaped (**b**) with koilocytes (**c**). Chromogenic in situ hybridization for HPV6/11 demonstrates strong nuclear staining of infected keratinocytes (**d**)

ISH. PCR is considered the most effective tests for HPV-DNA detection, but limitations include the absence of architecture preservation due to DNA extraction and the need of high expertise and rigorous laboratory conditions to avoid contaminations. ISH has the advantage of easy-implemented technique with preservation of morphology; however, it lacks in sensitivity. A solution to this limitation is the tyramide-based signal amplification kit, based on HPV chromogenic in situ (CISH) technology. This technology would help to distinguish episomal from integrated HPV [49].

Differential Diagnosis

The main differential diagnosis for condyloma acuminata is with high-grade vulva intraepithelial lesion. Condylomata are typically verrucous or papillary and have normal mitoses located in the basal layer, koilocytosis, parabasal hyperplasia, accentuated intracellular bridges, dyskeratosis, hypergranulosis, and compact hyperkeratosis. In contrast, high-grade vulva intraepithelial lesions present as flat macules with enlarged keratinocytes and prominent nuclei and nucleoli. However, in many cases, high-grade vulva intraepithelial lesions may develop in the setting of a condyloma. In those cases, the lesions at low power demonstrate the typical appearance of condylomas, but they usually show abnormal cytology with numerous atypical mitotic figures in the upper third of the epithelium.

Bowenoid papulosis that histologically presents with identical histologic features to squamous cell carcinoma in situ needs to be distinguished from condyloma acuminatum. Clinically, bowenoid papulosis usually presents with multiple small papules and histologically shows multiple mitotic figures and cellular atypia.

Small lesions of lichen simplex chronicus may resemble regressing or early flat condylomata

acuminatum. In those cases, the presence of koilocytes may help in the differential diagnosis. If this cannot be resolved by histopathological examination, molecular biologic methods such as a polymerase chain reaction (PCR) or in situ hybridization to detect the presence of HPV may help to make a more definitive diagnosis.

Vulvar vestibular papillomatosis also enters in the differential diagnosis of condylomata. In the former, the epithelium lacks hyperkeratosis and other typical microscopic features of condyloma, and the lesions are confined to the vulva vestibule [50] (see Chap. 12). Fibroepithelial polyps may have the shape of large condylomas; however, the epithelium also lacks evidence of HPV infection in the form of koilocytes. Seborrheic keratosis in this area can also create a diagnostic conundrum. The absence of koilocytes and thick keratohyaline granules are clues for seborrheic keratosis. Condylomata lata may resemble condylomata acuminata clinically; however, on biopsy, the presence of prominent inflammatory infiltrates composed mainly of plasma cells and the presence of spirochetes distinguishes these lesions. Warty surface of acquired lymphangiectasis can also mimic a genital wart; the presence of thin-walled lymphatics vessels in the superficial stroma confirms the diagnosis of lymphatic ectasias. Lastly, in systemic amyloidosis, vulva deposits can be associated with squamous proliferations like condylomas [51, 52]. Congo red stain can confirm the nature of the deposit for a final diagnosis of amyloidosis.

In large condylomas, the main differential diagnosis includes verrucous carcinoma. The latter has minimal cytologic atypia, no koilocytes, and a characteristic pushing broad-based rete ridges. HPV is commonly negative.

Summary

Clinical Presentation

- HPV-6 is the most prevalent HPV type in condylomata acuminata
- Affect the vulva, vagina, cervix, urethra, anal canal, and perianal skin.
- Present as multiple flesh color or hyperpigmented, confluent papillary, verrucous, filiform, flat, or papular lesions of the skin and mucous membrane.
- Immunosuppressed women have large hyperkeratotic lesions.

Histologic Features

- Dome-shaped lesions with areas of acanthosis, papillomatosis, and hyperkeratosis
- Koilocytes and prominent keratohyaline granules
- Presence of keratinocytic maturation upward with absence of basal layer atypia and superficial mitoses

Differential Diagnosis

- High-grade vulva intraepithelial lesion
- Bowenoid papulosis
- Small lesions of lichen simplex chronicus
- Vulvar vestibular papillomatosis
- Fibroepithelial polyp
- Condylomata lata
- Warty surface of acquired lymphangiectasis
- Verrucous carcinoma

Takeaway Essentials

- *Clinical Relevant Pearls*
- The different HPV subtypes can cause a wide spectrum of diseases in humans with varied morbidity and mortality.
- Most lesions are asymptomatic unless secondarily infected.
- Lesions that are only detected by colposcopic examination after performing HPV testing on the tissue in question are considered subclinical.
- In immunosuppressed women, condyloma accuminatum is frequently confused with squamous cell carcinoma due to the large size and hyperkeratosis.

Pathology Interpretation Pearls

- Changes associated with podophyllin treatment were described in condylomas of young women in the absence of such treatment under the name of pseudobowenoid changes.

Immunohistochemical Findings

- Immunohistochemical stains and CISH are tools to detect and subtype this virus in paraffin-embedded tissue.

Epstein–Barr Virus Infection

Nearly four decades ago, the first documented case of Epstein–Barr virus (EBV)-associated genital ulcers was published in the literature. It is believed that some of the cases described earlier under the name of "Lipschutz syndrome" in reality represent patients affected by EBV genital infection.

Clinical Features

Clinical presentation can be seen in the form of a single large (>1 cm in diameter) or multiple small ulcers. Characteristic ulcers are deep and necrotic with irregular borders and covered by a yellow adherent membrane. The mean age of these patients is 14.5 years (range 2–51 years old) [53]. Pain is the main complaint and usually proceeds to a prodrome of fatigue, fever, and headache. Symptoms similar to mononucleosis like tonsillitis and adenopathy can also be seen in these patients [54]. Detection of IgM in the absence of antibody to EBV nuclear antigen and later on in the disease identification of IgG antibody against EBV viral capsid confirms a primary EBV infection. PCR using a sample of the ulcer can also be helpful for the diagnosis.

Histopathology

Sections of the ulcer demonstrate nonspecific features like granulation tissue with acute and chronic inflammation and debris. Vessels with reactive endothelial swelling and thrombosis can be seen as secondary changes to the ulcer (Fig. 6.15a, b).

Differential Diagnosis

The two most frequent etiologies of genital ulcers are herpes viral infection and syphilis. Primary syphilis is painless in contrary to EBV ulcers. Serologies and immunohistochemical stains against the spirochete can be useful diagnostic tools. Multinucleated infected keratinocytes with molding nuclei exhibiting margination of the chromatin support the diagnosis of genital herpes infection. Aphthous ulcer can be excluded based on the serologic findings. Localized papulonecrotic tuberculid is also a consideration. The wedge-shaped dermal necrosis associated with leukocytoclastic vasculitis and granulomas will separate this hypersensitivity reaction to mycobacteria from EBV infection. Long-lasting history of the lesions and a distant focus of tuberculosis will confirm the diagnosis.

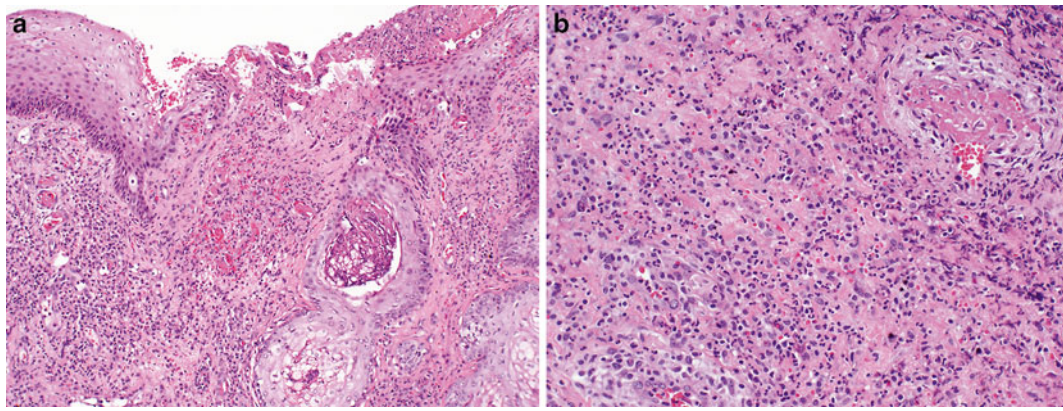


Fig. 6.15 (**a**, **b**) *Epstein–Barr virus infection*. Histology demonstrates ulcer with nonspecific features like acute and chronic inflammation, debris (**a**), and vascular thrombosis (**b**)

Summary

Clinical Presentation

- Single large ulcer (>1 cm in diameter) or multiple small ulcers.
- Ulcers are deep and necrotic with irregular borders and covered by a yellow adherent membrane.
- Prodrome of general malaise.
- Mononucleosis-like presentation can also be seen.

Histologic Features

- Sections of the ulcer demonstrate nonspecific features like granulation tissue with acute and chronic inflammation and debris.
- Vessels with reactive endothelial swelling and thrombosis are also present.

Differential Diagnosis

- Syphilis
- Herpes virus infection
- Aphthous ulcer
- Tuberculosis

Takeaway Essentials

Clinical Relevant Pearls

- Detection of IgM in the absence of antibody to EBV nuclear antigen and later on in the disease identification of IgG antibody against EBV viral capsid confirms a primary EBV infection.

Pathology Interpretation Pearls

- Vessels with marked reactive endothelialitis should not be confused with vascular changes seen in syphilis. When in doubt, immunohistochemical stain can be a rapid tool to reach the correct diagnosis.

Bacterial Infections

Bacterial vaginal and cervical infections often cause vulvar symptoms, partly by the production of an irritant discharge.

Bacterial Vaginosis

Bacterial vaginosis results from an overgrowth of commensal bacterial (*Gardnerella vaginalis*) and anaerobic organism (e.g., *Mobiluncus* or *Bacteroides*) producing an increased volume of vaginal secretions [55, 56]. The characteristic unpleasant fishy odor is the product of bacterial amines released after exposure to an alkaline substance such as a potassium hydroxide or semen.

Clinical Features

Although bacterial vaginosis lacks symptomatic manifestations (e.g., burning, itchiness, or erythema) and vulvitis is uncommon, it carries an increased risk of preterm labor in pregnant women.

Histopathology

Rarely, a biopsy is performed. The diagnosis is made with the presence of profuse milky vaginal discharge, with fishy odor when exposed to 10–20 % potassium hydroxide (positive whiff test) [57], and the presence of clue cells (squamous epithelial cells covered by coccobacilli producing a ground-glass appearance of the cytoplasm and obscuring the cell borders) on vaginal smear (Fig. 6.16).

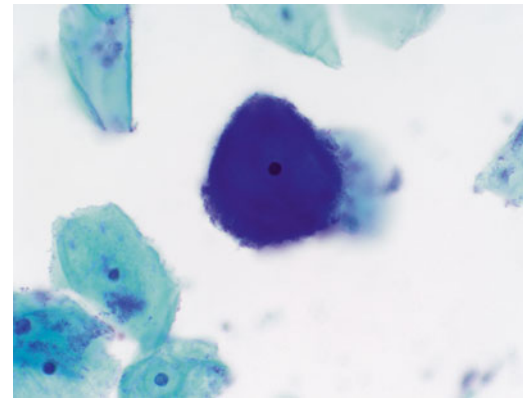


Fig. 6.16 *Bacterial vaginosis*. Clue cells: squamous epithelial cells covered by coccobacilli

Summary

Clinical Presentation

- Vaginal discharge can cause vulvitis by irritation.

Histologic Features

- Clue cells (squamous epithelial cells covered by coccobacilli producing a ground-glass appearance of the cytoplasm and obscuring the cell borders) on vaginal smear

Takeaway Essentials

Clinical Relevant Pearls

- Rarely biopsy due to the presence of milky vaginal discharge

Pathology Interpretation Pearls

- Unspecific inflammatory changes

Staphylococcal Infections

Like any hair-bearing skin, the labia or mons pubis may be the site of a superficial staphylococcal folliculitis and deep folliculitis producing furunculosis (boils). Occlusion, intertrigo, and depilation of pubic hair (particularly by shaving), all predispose to clinical infection. Other organisms producing similar changes include *Pseudomonas*, Malassezia furfur, and dermatophyte fungi.

Clinical Features

The warmth and moisture of the vulva provide the ideal environment for this infection. Clinical inspection reveals a variety of manifestations, from folliculitis presenting as perifollicular erythema with suppuration and follicular destruction to bullous impetigo showing yellow crust covering residual superficial erosions immediately after superficial fragile blisters rupture. Deep-seated abscess may arise upon infection of Bartholin gland, Skene ducts, Gartner ducts, and urethra diverticula. Although cultures identified in most of these settings are *S. aureus*, other bacteria like *E. coli*, *N. gonorrhea*, *K. pneumonia*, and *Bacteroides* have also been detected [58–61]. In recurrent abscesses, the possibility of a methicillin-resistant *S. aureus* infection should be suspected [62–64].

Microscopic Features

Histologically, areas of suppuration affecting the epidermis and epithelium of hair follicles are identified. The hair follicles are surrounded and infiltrated by neutrophils associated with edema (Fig. 6.17a–c). In advance stages of the infection, destruction of the follicle leads to the formation of granulomas and presence of naked hair shafts frequently surrounded by multinucleated giant cells. Bullous impetigo presents as a subcorneal bullae filled with neutrophils associated with a superficial perivascular lymphocytic infiltrate admixed with neutrophils. Brown and Brenn stain can be used to highlight the presence of bacteria in the bullae. The exfoliative toxin produced by *S. aureus* can induce flaccid blisters or pustules in the genitalia and thighs. In those cases, a subcorneal blister is formed with numerous neutrophils in the stratum corneum with areas of acantholysis (see Chap. 4). The latter is accompanied by a dermal sparse perivascular mixed inflammatory cell infiltrate and absence of identifiable bacteria. This is in contrast to bullous impetigo in which the dermal inflammatory component is heavier and bacteria colonies can be seen.

Differential Diagnosis

Neutrophilic folliculitis can be seen associated with a wide range of infections, from bacteria to virus to fungus. Herpes virus can affect the hair follicle and sebaceous glands; the presence of multinucleated keratinocytes with molding nuclei and margination of the chromatin will support a viral etiology. If in doubt, immunohistochemical stains can help to detect the virus. Dermatophytes can also produce a neutrophilic suppurative folliculitis; periodic acid–Schiff (PAS) and Gomori methenamine silver (GMS) stains would highlight the fungal forms. A subcorneal collection of neutrophils raises the differential diagnosis of infections (e.g., candida) and blistering disorders (e.g., IgA pemphigus and pemphigus foliaceus). The presence of budding yeast and pseudohyphae will point toward the diagnosis of candida, while immunofluorescent tests will be able to identify the blistering disorder.

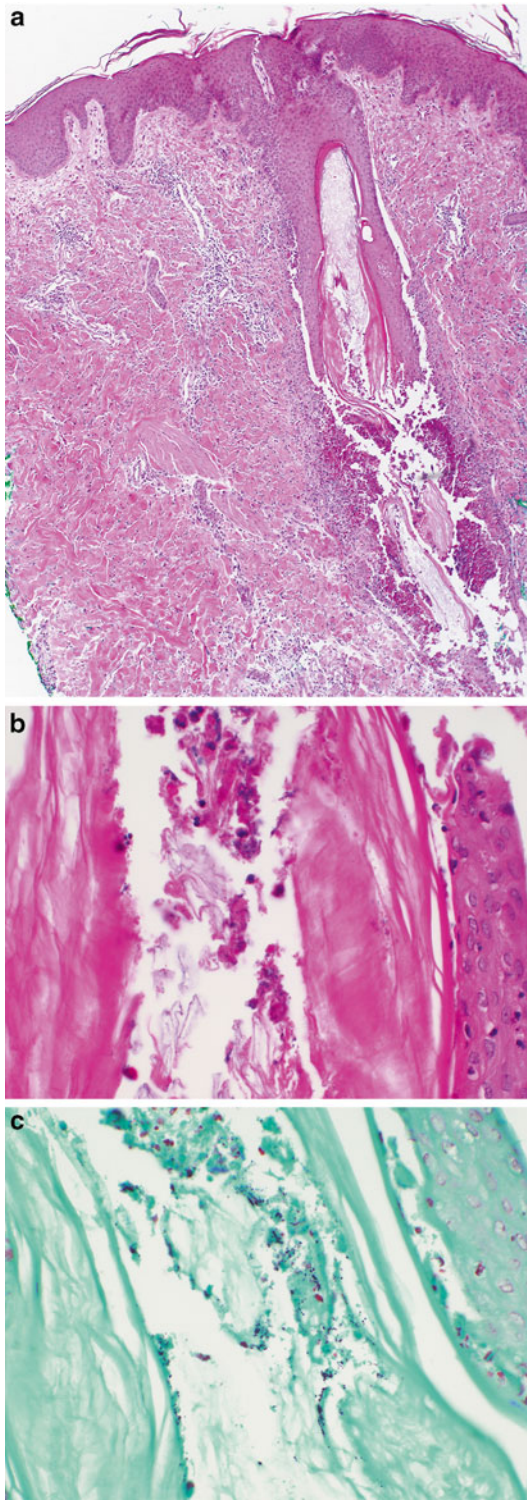


Fig. 6.17 (**a**–**c**) *Staphylococcal infection*. (**a**) Follicle with acute inflammation and (**b**, **c**) presence of Gram-positive cocci

Summary

Clinical Presentation

- Affect predominantly hair-bearing areas: labia majora and pubis
- Suppurative folliculitis
- Furunculosis (boils)
- Flaccid blisters or pustules

Histologic Features

- Similar to superficial or deep folliculitis elsewhere in the body.
- Subcorneal blister with numerous neutrophils.
- Brown and Brenn stain will highlight the bacterial forms in the folliculitis or bullae.

Differential Diagnosis

- Folliculitis caused by dermatophytes
- Herpetic folliculitis
- IgA pemphigus
- Pemphigus foliaceus

Takeaway Essentials

Clinical Relevant Pearls

- Location of the abscess may hint to the diagnosis (e.g., abscesses located at 4:00 and 8:00 o'clock positions in the vulva should raise concern of Bartholin gland abscess).
- Diagnosis is made by cultures, especially in recurrent infection in search of methicillin-resistant species.
- The epidermolytic toxin by staphylococci is responsible of bullous impetigo and staphylococcal scalded skin syndrome.

Pathology Interpretation Pearls

- In bullous impetigo, a Brown–Brenn stain would highlight the presence of bacteria producing the toxin in situ, while in staphylococcal scalded skin syndrome, the flaccid bullae is formed by a toxin that originated at a distant site.
- Serial histologic section may be necessary to identify the residual component of the infected glands (e.g., Bartholin gland abscess) or follicle.

- Histochemical stains to identify bacteria and fungus should be considered as part of the study of these cases (e.g., PAS, GMS, and B&B).
- A skin biopsy for immunofluorescent tests should be consider to workup patient with flaccid bullae.

Streptococcal Infections

Streptococcal infections can cause several alterations in the genital area, ranging from superficial cutaneous infection to necrotizing fasciitis, which causes a rapidly progressive necrosis of the skin and subcutaneous tissue. Superficial bacterial infections are common in children between 3 and 5 years and have a predominant perianal distribution that can extend to the vulva and vagina. These lesions commonly yield group A or C hemolytic streptococci. In adults, streptococcal infections are uncommon and can occur alone or in conjunction with another skin disorder. Sometimes they are associated with diseases such as lichen sclerosus or other dermatoses.

The most severe presentation of this bacterial infection is necrotizing fasciitis caused by group A streptococci, which has increased in frequency over the past decade. However, polymicrobial infection with anaerobic and aerobic bacteria like *Streptococcus*, *Staphylococcus aureus*, *Escherichia coli*, *Bacteroides*, and *Clostridium* spp. is very common. This rare and distinct form of rapidly progressive necrosis of subcutaneous tissue and fascia can be fatal if not recognized early and if aggressive therapeutic intervention is not used. Vulvar lesions can extend to the perineum and abdominal wall. It usually occurs in diabetics, particularly when complicated by obesity, hypertension, and peripheral vascular disease, or in immunocompromised host [65, 66]. The infection's origin can be a surgical incision (e.g., episiotomy) or a local abscess of skin or Bartholin gland. Mortality is up to 40 % if the disease is not recognized and debridement is delay or group A streptococcus is involved.

Clinical Features

Streptococcal infection clinical features are heterogeneous. Streptococcal vulvitis and perianal dermatitis produce tender, well-demarcated red skin with or without scale. The vagina shows erythematous mucosa and purulent discharge. Occasionally, anal fistulas with mucoid discharge and vulvar fissures develop. Lesions show excoriations and lichenification secondary to scratching and rubbing.

The patient suffering from necrotizing fasciitis presents with a hot, very tender, erythematous, swollen areas resistant to antibiotic treatment. Very rapidly, the skin changes from red to dusky-blue with bulla formation and necrosis. The soft tissue frequently feels indurated and wooden to palpation. Patients usually show signs or toxicity including fever, chills, malaise, shock, and tachycardia.

Microscopic Features

The characteristic features of necrotizing fasciitis are the presence of extensive necrosis extending from the epidermis to the subcutaneous tissue and sometimes to the muscle. Numerous polymorphonuclear leukocytes and mononuclear cells infiltrate the tissue and secondary vasculitis with trombi is present as large number bacterial colonies in the upper dermis [65]. Necrotizing fasciitis has been postulated as a form of septic vasculitis.

Differential Diagnosis

The differential diagnosis would include pyoderma gangrenosum and necrotizing Sweet syndrome. Pyodermal gangrenosum will be characterized by a folliculocentric neutrophilic dermal infiltrate with central necrotizing suppurative inflammation and peripheral lymphocytic vascular reaction. These histologic features, the absence of myonecrosis, and the clinical history of worsening after surgical procedures support the diagnosis of pyodermal gangrenosum. Necrotizing Sweet syndrome needs to be considered in patients with risk factors for this syndrome presenting with clinicopathologic features mimicking necrotizing fasciitis. Although histologic overlap preclude separation of these two diseases, multiple negative cultures, absence of significant myonecrosis, and improvement after steroid treatment will support the diagnosis of necrotizing Sweet syndrome [67].

Summary

Clinical Presentation

- Two main clinical forms: one as a limited superficial infection and the other as a fulminant life-threatening deep infection.
- In children, it presents as tender well-demarcated erythematous macules of the vulva with perianal involvement.
- Necrotizing fasciitis shows rapid progression from red tender swollen areas to dusky red-blue bullae leading to extensive necrosis up to the muscle.

Histologic Features

- Necrotizing fasciitis: extensive tissue necrosis affecting dermis up to the fascia and muscle with obliterative endarteritis and thrombosis

Differential Diagnosis

- Pyoderma gangrenosum
- Necrotizing Sweet syndrome

Takeaway Essentials

Clinical Relevant Pearls

- Necrotizing fasciitis of the external genitalia is an emergency due to the high rate of lethality if not recognized early, and aggressive therapeutic intervention is pursued.
- Superficial streptococcal infections in children should be considered in the differential diagnosis of sexual abuse.
- Superficial streptococcal infection can coexist with other inflammatory disorders of the vulva like lichen sclerosus.

Pathology Interpretation Pearls

- Cultures are useful to identify the infectious agent.

Syphilis

Syphilis is a worldwide highly contagious sexually transmitted disease that develops in four stages: primary, secondary, latent, and tertiary [68, 69]. The spirochete *Treponema pallidum* is the etiologic factor, and like other sexually transmitted diseases, coinfection with human immunodeficiency virus (HIV) is well recognized. Congenital and acquired syphilis present with cutaneous lesions including vulvar manifestation [70].

Clinical Features

Primary lesions (chancre) present as a painless, indurated, shallow, clean appearing ulcers with raised edges. The chancre usually presents within 3 weeks after initial contact at fourchette or labia majora; the range, however, is 7–90 days [69]. Spirochetes, Gram-negative slender and spiral-shaped bacteria, can be detected in the lesion. If secondarily infected, the chancre may become soft and painful and show an ulcerated surface. Most of the time, chancres are solitary lesions, but they can be multiple especially in immunocompromised women. Chancres may occur on inconspicuous surfaces, such as the vulva, perineum, cervix, anal mucosa, or oropharynx. In approximately 50 % of women and 30 % of men, the primary lesion cannot be identified. Lymphadenopathy presents 3–4 days after the chancre. The nodes are nontender, freely moveable, and rubbery. The exudate from the chancre is rich in spirochetes that can be detected using dark-field microscopy allowing a prompt diagnosis. Left untreated, the chancre will heal within 2–6 weeks and typically does not leave a scar; but the occurrence of vascular invasion will facilitate the progression to the secondary stage [69].

The secondary stage of the disease will become evident within 6 weeks to 6 months. At this point, the patient may present with a skin rash that often involves the trunk, genital area, flexor aspect of limbs, as well as the palms of the hands and soles of the feet. The cutaneous clinical phenotype is heterogeneous including maculopapular, annular, psoriasiform, pustular, lichenoid, and ulcerative lesions [71]. On occasion, the secondary lesions are papular, especially about the vulva, presenting as elevated flat, fleshy, moist plaques up to 3 cm in diameter (Fig. 6.18). These are known as condyloma lata and clinically may mimic condylomata acuminata. Condyloma lata are among the most contagious

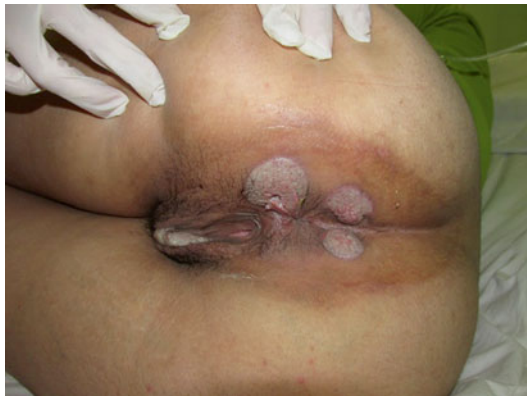


Fig. 6.18 *Secondary syphilis*. Clinically it presents as flat, fleshy, moist papules, and plaques. These lesions are known as Condyloma lata

lesions in syphilis. Such lesions also may occur on other mucocutaneous areas. After 3–8 weeks, they disappear spontaneously. Pregnant women may infect the fetus via transplacental passage of the spirochete. In secondary stage, diagnostic tools include the serologic tests that detect the presence of antibodies to cardiolipin by rapid plasma reagin (RPR) and venereal disease research laboratory (VDRL) assay [72, 73]. Interactions between HIV and *T. pallidum* can accelerate the disease with atypical clinical presentation (e.g., present with lues maligna and severe ulcerations and pseudolymphomatous lesion) [74, 75], change laboratory presentation, as well as increase the risk for complications [76, 77]. In women infected with HIV or immunosuppressed, a false-negative RPR results or the prozone effect can be encountered [78]. This effect results from extremely high antibody titers in the infected women that the visualization of the reaction is compromised. Other diagnostic methods include microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP) or detection of antibodies to surface proteins of *T. pallidum* by T. pallidum hemadsorption test (PTHA).

Latency is the period of time between healing of the clinical lesions and appearance of late manifestations and may last for years. The tertiary gumma of syphilis, an exuberant systemic immune response to a low border spirochete, is rarely seen on the vulva.

Microscopic Findings

If syphilis was not considered in the clinical differential, diagnosis may be quite difficult from histologic findings alone. A biopsy is necessary when the chancre has an atypical clinical presentation or it is located in an unusual anatomic site. Microscopically, the diagnosis of syphilis may be difficult, especially in the genital area, because the presence of plasma cells, which is the hallmark for the diagnosis of syphilis in other areas of the body, is not very reliable in genital areas. Plasma cells are usually present in many inflammatory processes. Other features commonly present in primary lesions of syphilis are areas of ulceration covered by cellular debris and mixed inflammatory infiltrates including neutrophils and plasma cells. Acanthosis of the epidermis is common at the edges of the ulcer, and blood vessels show prominent endothelial swelling and hyperplasia leading to endarteritis obliterans. If the diagnosis of primary syphilis is suspected clinically, immunoperoxidase stains for the identification of *T. pallidum* should be performed. Serologic tests to confirm the diagnosis are essential. The Warthin–Starry stain or other silver impregnation techniques were used in the past to demonstrate the spirochetes; nowadays, however, more sensitive immunohistochemical stains have replaced this capricious method [79]. Immunohistochemical stains while extremely useful, they are hampered by false positivity for other spirochetal infections and borreliosis [72]. In primary syphilis, spirochetes are usually identified at the dermoepidermal junction and within and around superficial dermal blood vessels.

Secondary syphilis exhibits lichenoid infiltrates composed of lymphocytes and plasma cells (Fig. 6.19a, b). The epidermis shows vacuolar changes and a few dyskeratotic keratinocytes. Areas of spongiosis may be also seen with neutrophils in the upper parts of the epidermis. Plasma cells are less prominent in macular lesions, but when present they can be seen around deep vessels. A second pattern of secondary syphilis can be seen in the form of epidermal psoriasiform hyperplasia with club-shaped elongation of rete ridges and areas of parakeratosis (Fig. 6.20a–d). The lymphocytic infiltrate may be

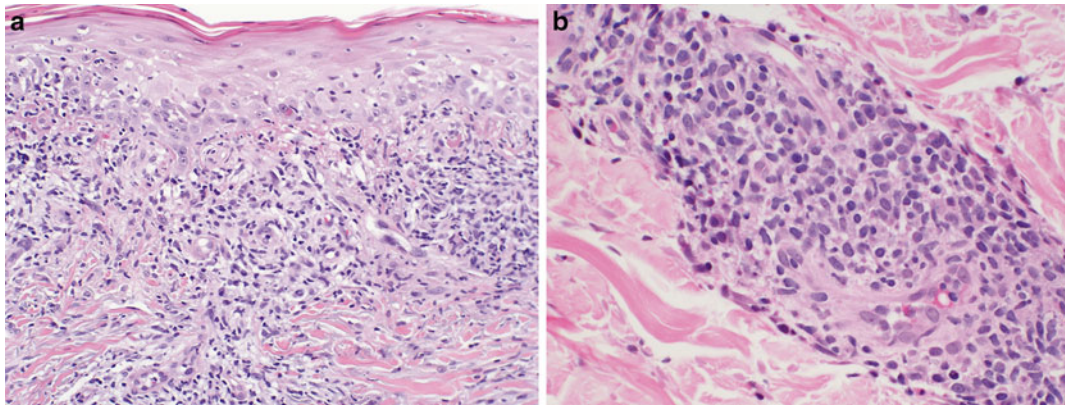


Fig. 6.19 (**a**, **b**) *Secondary syphilis*. One of the patterns of this disease is lichenoid infiltrate composed of lymphocytes (**a**) and plasma cells (**b**)

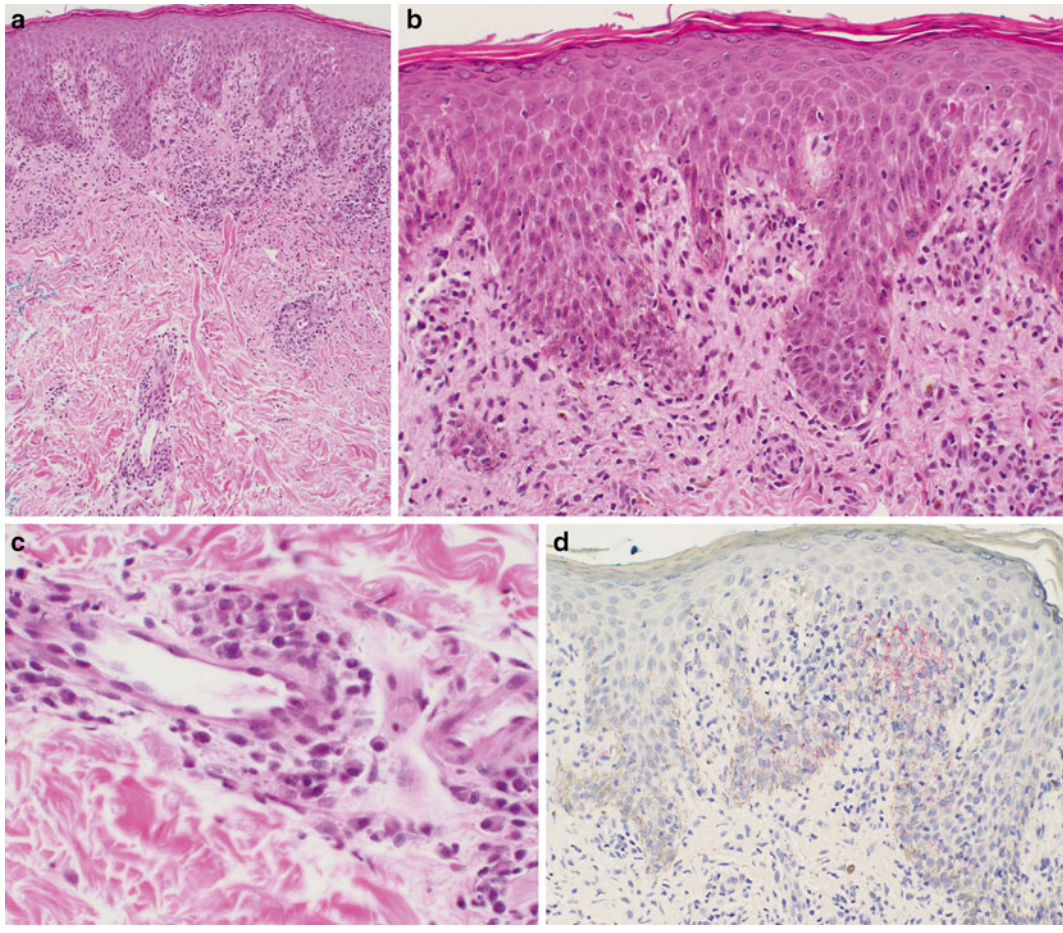


Fig. 6.20 (**a**–**d**) *Secondary syphilis*. A second pattern of secondary syphilis can be seen in the form of epidermal psoriasiform hyperplasia (**a**), club-shaped elongation of rete ridges, and areas of parakeratosis (**b**). Perivascular plasma cells are noted (**c**). Immunohistochemical stain reactivity for spirochete confirms the diagnosis (**d**)

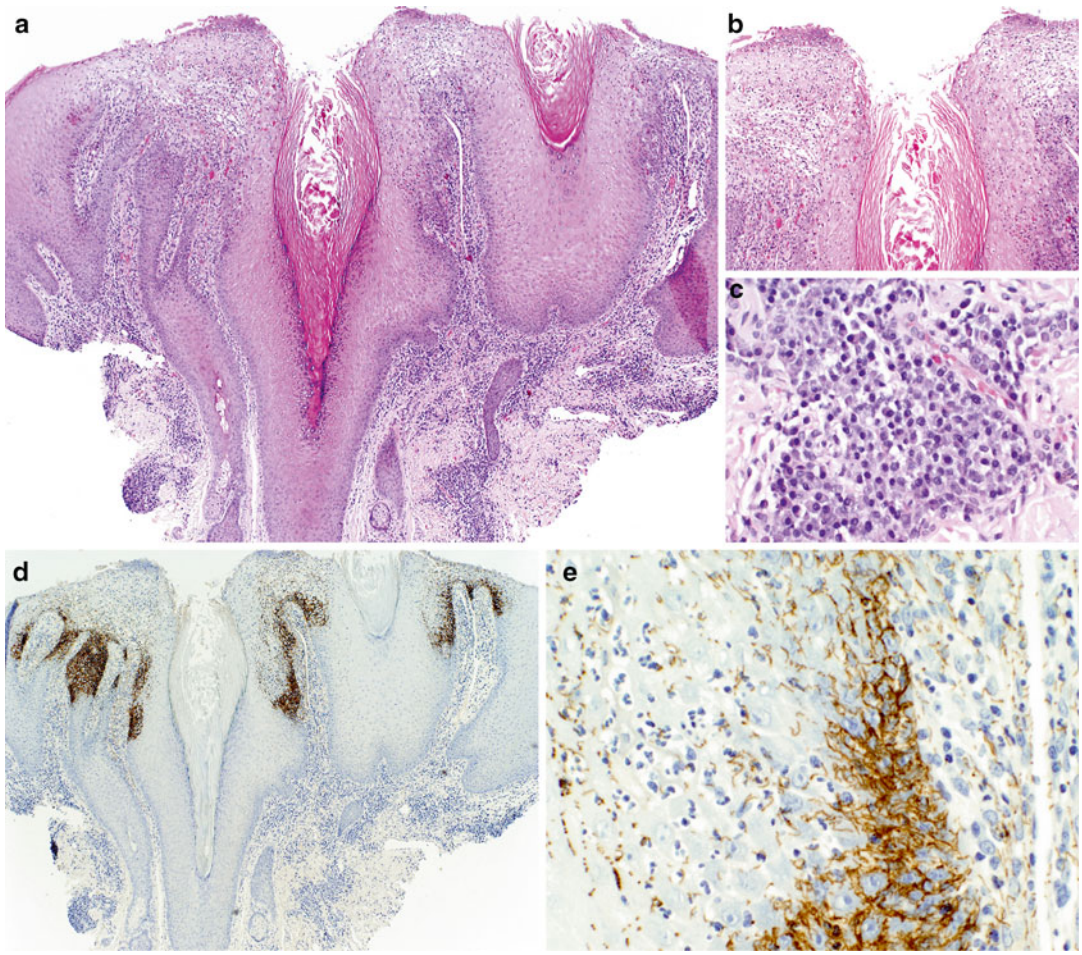


Fig. 6.21 (**a**–**e**) *Secondary syphilis*. Condyloma lata shows epidermal hyperplasia (**a**) with hyperkeratosis, patchy parakeratosis, and superficial intraepidermal microabscesses (**b**). Superficial and mid-dermal plasmacytic infiltrate is noted (**c**). The high density of spirochetes is demonstrated by immunohistochemical stain (**d**, **e**)

so exuberant as to simulate lymphoma, but its mixed nature would be rare in such neoplasm. Sometimes, early lesions of the secondary stage of syphilis exhibit prominent neutrophilic infiltrates that can resemble Sweet's disease (acute febrile neutrophilic dermatosis). Neutrophilic microabscesses in the outer root sheath of the hair follicle or even follicular pustules can be identified. Older lesions show granulomatous infiltrates, which may simulate granuloma annulare or sarcoidosis.

Condyloma lata have epidermal hyperplasia with hyperkeratosis, patchy parakeratosis, and superficial intraepidermal microabscesses [80] (Fig. 6.21a–e). The latter is associated with a superficial and mid-dermal perivascular lymphoplasmacytic infiltrate. Although secondary syphilis can mimic other inflammatory disorders, the predominance of plasma cells in the infiltrate is a valuable diagnostic clue. Classic cases of lues maligna show a thrombotic endarteritis obliterans, especially at the dermal–subcutaneous junction, causing ischemia, ulceration, and infarction.

In secondary syphilis, immunohistochemical stains will show an epitheliotropic pattern with abundant spirochetes in the lower portion of the epidermis [81]. Molecular detection of *T. pallidum* with PCR test is more sensitive [82–84]. Amplification products specific to the *TP47* gene are another useful diagnostic technique that lacks homology with other bacteria [72].

Tertiary syphilis presents in two forms: nodular form and gummas. In the nodular form, the granulomas are small with focal necrosis and limited to the dermis. On the other hand, gummas show superficial and deep perivascular plasmacytic infiltrate, granulomatous inflammation with prominent central necrosis, and endarteritis obliterans. Spirochetes can be detected to a lesser degree in this stage of the disease.

Differential Diagnosis

Syphilis is the greatest mimicker not only clinically but also histologically. In primary syphilis, chancroid, donovanosis, and lymphogranuloma venereum are among the differential diagnosis (Table 6.1). All these sexually transmitted diseases lack endarteritis and fail to show reactivity by immunohistochemical stain for spirochete. The lichenoid pattern of secondary syphilis mimics lichen planus, lichenoid drug eruptions, and pityriasis lichenoides. Lichen planus in mucosal or modified mucosal surface can present plasma cells; however, no endarteritis is noted or detection of spirochete by immunohistochemical techniques is observed. Lichenoid drug eruption and pityriasis lichenoides frequently affect the genitalia as part of a more diffuse eruption. The presence of numerous eosinophils and peripheral eosinophilia are evidence of a drug as a culprit. The psoriasiform pattern of secondary syphilis needs to be differentiated from psoriasis and psoriasiform drug reactions. The presence of deeper extends of the lymphoplasmacytic infiltrate and the presence of endarteritis can be a useful clue to favor a spirochetal infection. The latter can be confirmed by using immunohistochemical or molecular techniques to search for the microorganism. Perifollicular pustular syphilis can simulate bacteria or fungal folliculitis. Histochemical stains for bacterial and fungal forms can help to separate these infections. Condyloma lata can be differentiated from condyloma accuminatum by the presence of neutrophilic collections in the epithelium, dermal perivascular lymphoplasmacytic infiltrate, and exuberant reaction to immunohistochemical stain for spirochete. The granulomatous reactions of tertiary syphilis need to be distinguished from tuberculosis, Crohn disease, and sarcoidosis. In gummatous syphilis, the granuloma has a central necrotic zone in which ghost outlines of cells are seen without the typical caseative nature of tuberculosis [85]. Sarcoidosis fails to show the degree of necrosis and rich plasma cell infiltrate seen in syphilis. In Crohn disease, the granulomas are ill defined, and there is a lack of significant plasma cell infiltrate.

Summary

Clinical Presentation

- Primary syphilis (inoculation site): self resolving painless indurated ulceration with clean base and defined borders frequently seen in fourchette and labia majora
- Secondary syphilis (infiltration of skin by spirochetes): heterogeneous appearances including maculopapular rash and condyloma lata
- Tertiary syphilis (florid systemic immune response): nodules or ulcerated masses

Histologic Features

- Primary syphilis (chancre):
  - Ulcer with variable pseudoepitheliomatous hyperplasia
  - Endothelial swelling leading to hyperplasia and occlusion resulting in endarteritis obliterans
  - Dense mononuclear inflammatory infiltrate
- Secondary syphilis:
  - Psoriasiform pattern: epidermal acanthosis with club-shaped rete ridges and parakeratosis. Mixed infiltrate with plasma cells around vessels and adnexal structures
  - Lichenoid pattern: band-like inflammatory infiltrate with plasma cells
  - Condylomata lata: epidermal acanthosis and intraepidermal neutrophilic microabscesses. Perivascular lymphoplasmacytic infiltrate
  - Pustular syphilis: folliculocentric suppurative inflammation surrounded

by perivascular lymphoplasmacytic infiltrate
  - Lues maligna: thrombotic endarteritis obliterans, especially at the dermal subcutaneous junction leading to infarction and ulceration with rich lymphoplasmacytic infiltrate
- Tertiary syphilis:
  - Granulomatous inflammation
  - Endarteritis obliterans
  - Dermal and subcutaneous lymphoplasmacytic infiltrate

Differential Diagnosis

- Primary syphilis:
  - Chancroid
  - Donovanosis
  - Lymphogranuloma venereum
- Secondary syphilis:
  - Lichenoid pattern: lichen planus, lichenoid drug reaction, and pityriasis lichenoides
  - Psoriasiform pattern: psoriasis and psoriasiform drug reaction
  - Condyloma lata: condyloma accuminatum
- Tertiary syphilis:
- Tuberculosis
- Sarcoidosis
- Crohn disease

Takeaway Essentials

Clinical Relevant Pearls

- In primary stage, dark-field microscopy and serologies are more helpful than a biopsy. The latter play a role in atypical presentation of chancre.

Pathology Interpretation Pearls

- Consider syphilis when confronted to cases with unusual combination of inflammatory patterns (e.g., psoriasiform, lichenoid, and spongiotic patterns).
- Due to the reduced number of spirochetes in advanced stages of the disease, serologies may be more helpful.

Immunohistochemical Findings

- Immunohistochemical stains for spirochete should be requested in vulvar lichenoid or psoriasiform dermatitis with abundant plasma cells.
- When interpreting immunohistochemical stains, consider its cross reactivity with other spirochete infections and borreliosis.

Gonorrhea

Clinical Features

Gonorrhea, caused by *Neisseria gonorrhoeae*, a Gram-negative diplococcus, is the second most common notifiable communicable disease [86]. The urethra is the primary affected organ with potential spread to the vagina and cervix. Involvement of the paraurethral glands and Bartholin glands also occur. *Neisseria gonorrhoeae* vulvitis is unusual in adult women and can sporadically occur in prepuberal girls. Clinically, the lesions present as asymptomatic erythematous nodules that can ulcerate (Fig. 6.22).

Histopathology

Ulcerated epidermis frequently is seen overlying a dermal collection of neutrophils. The presence of Gram-negative intracellular diplococcic will confirm the diagnosis. If the cocci cannot be seen in histologic sections, microbiology culture is a useful diagnostic tool.

Differential Diagnosis

The presentation of an abscess raises the differential diagnosis of bacterial, fungal, and mycobacterial infections. Histochemical stains like PAS, GMS, and Brown–Brenn can help to recognize the etiologic agent. mmunohistochemical stains play an important role in the search of spirochetes. Cultures and PCR can be used to further explore the presence of these microorganisms.

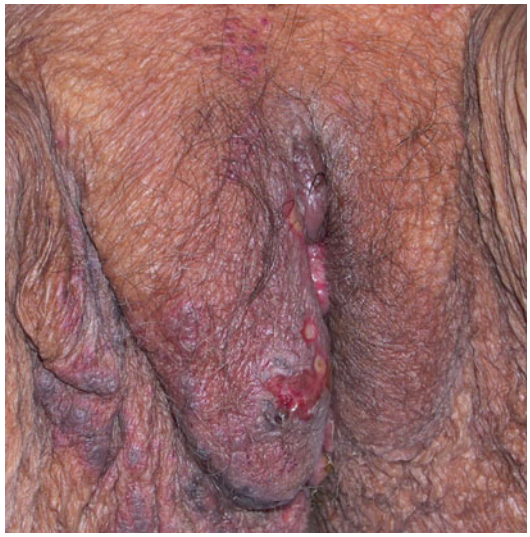


Fig. 6.22 *Gonorrhea.* Lesions present as asymptomatic erythematous nodules that can ulcerate

Summary
Clinical Presentation
- Erythematous nodules +/– ulcer
- Involve paraurethral and Bartholin glands

Histologic Features
- Dermal abscess +/– ulcer

Differential Diagnosis
- Other etiologies for dermal abscess: bacterial, fungal, or spirochetal

Takeaway Essentials
Clinical Relevant Pearls
- Gonorrhea should be considered in the workup of a Bartholin gland and paraurethral glands abscess.
- Cultures for *Neisseria gonorrhea* should be part of the routine bacterial cultures for vulvitis and vaginal discharge on a prepuberal girl.

Pathology Interpretation Pearls
- Consider Gram stain in dermal abscess to highlight the intracellular diplococci.

Chancroid

Chancroid is caused by *Haemophilus ducreyi* , a Gram-negative, nonmotile anaerobic coccobacillus, which in culture grows in pairs and parallel chains ("school of fish") [87, 88]. The infection is found mainly in developing and third-world countries [87–89]. Chancroid produces a 25-fold increased HIV acquisition and transmission rate [89]. Very few people are diagnosed in the United States each year with this infection. Most people in the United States who are diagnosed with chancroid have traveled outside the country to areas where the infection is more prevalent. Women likely act as reservoir. After entering the vulva through a cutaneous microabrasion, the organism elicits in 3–5 days the formation of papules that undergo necrosis and form an ulcer.

Clinical Features

Chancroid is relatively rare and presents with a genital ulcer that usually is tender, not indurated, and has irregular undermined edges and a friable purulent erythematous base (Fig. 6.23). Primary lesions may be single or multiple and tend to be small, measuring approximately 1–2 mm in diameter. In the vulva, the sites most frequently involved are the fourchette and labia minora and to a lesser extent urethra and vagina. Coalescence of the lesions leads to ulcers approaching up to 3 cm in diameter. Tender inguinal adenopathy with flocculent nodes may be present. Drainage through sinuses can occur in ipsilateral lymph nodes. The incubation period may be as short as 10 days. The clinical differential diagnosis includes herpes virus infection and primary syphilis.

Skin tests and biopsies may not be diagnostic. Identification of the organism by culture is necessary for accurate diagnosis. Selective agar medium has been developed for the organism, which has improved culture isolation.

Microscopic Features

Histologically, the inflammatory reaction is organized in three zones: a superficial ulcer with acute inflammation (e.g., neutrophils, erythrocytes, and fibrin), the second zone of granulation

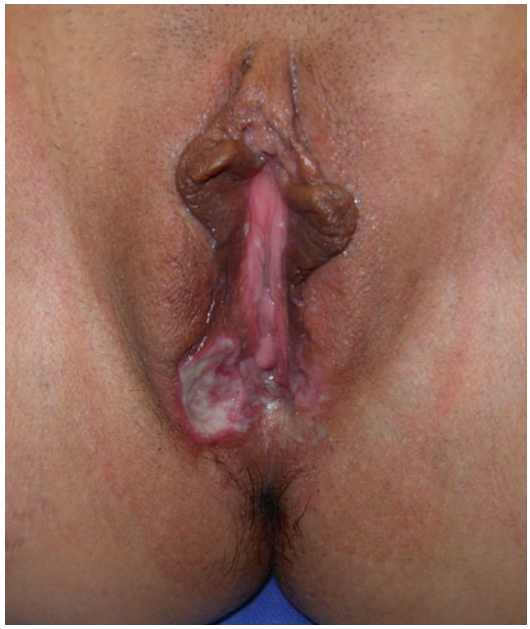


Fig. 6.23 *Chancroid.* Genital ulcer that usually is tender, not indurated, and has irregular undermined edges and a friable purulent erythematous base

tissue underneath the ulcer with thrombosed vessels, and the third or deepest zone represented by lymphocytes and plasma cells [90]. It is characterized by the presence of the Gram-negative organisms, which may be present in large numbers and in parallel chains, referred as "school of fish," "railroad," or "chaining," mainly in the superficial aspect of the lesion [90, 91]. The histochemical stains for bacteria such as Gram and Giemsa can decorate the microorganisms. Immunofluorescence antibodies and PCR analyses are very reliable diagnostic tests. 16S rDNA sequencing techniques may definitively confirm the diagnosis [82, 87].

Differential Diagnosis

Chancroid needs to be differentiated from other sexually transmitted diseases like syphilis, granuloma inguinale, and lymphogranuloma venereum (Table 6.1). Syphilis presents as painless ulcers with endothelialitis not seen in chancroid. Immunohistochemistry and serologies can be used to confirm the diagnosis of spirochetal infection. The search of large histiocytes engulfing bipolar organisms simulating safety pins will favor granuloma inguinale, while histiocytes with Gamna–Favre bodies will support the diagnosis of lymphogranuloma venereum.

Summary

Clinical Presentation

- Tender nonindurated ulcer with friable purulent erythematous base and serpiginous borders.
- Affect fourchette and labia minora.
- One quarter to half of the patients develop lymphadenopathy that can evolve to buboes (abscess).

Histologic Features

- Ulcer with adjacent variable epidermal acanthosis
- "Trilayer" from surface to deep aspect of the lesion: ulcer, granulation tissue, and chronic inflammation in the form of lymphocytes and plasma cells

Differential Diagnosis

- Syphilis
- Granuloma inguinale
- Lymphogranuloma venereum

Takeaway Essentials

Clinical Relevant Pearls

- It is the leading cause of genital ulcerations in developing countries, although is presently declining its incidence.
- Uncommon in the United States.
- Cultures and smear are more reliable to demonstrate the coccobacilli than biopsy.

Pathology Interpretation Pearls

- Trilayer (ulcer, granulation tissue, and lymphoplasmacytic infiltrate).

- When searching for the organism, focus your attention mainly to the superficial portion of the lesion.
- Organisms arranged in the characteristic "school of fish" best seen in smears of the ulcer stained with Gram stain.

Immunohistochemical Findings

- Fluorescent antibody test is very reliable.
- PCR analysis is the diagnostic test of choice.

Chlamydial Infection (Lymphogranuloma Venereum)

Lymphogranuloma venereum (LGV) is an unusual sexually transmitted disease (STD) caused by *Chlamydia trachomatis* occurring approximately three times more frequently in men than in women. LGV is endemic in certain areas of Africa, Southeast Asia, India, the Caribbean, and South America. It is rare in industrialized countries, but in the last 10 years has been increasingly recognized in North America, Europe, and the United Kingdom as causing outbreaks of proctitis among men who have sex with men (MSM). *Chlamydia trachomatis* is the organism responsible for LGV. This organism is an obligatory intracellular, and the serotypes L1, L2, and L3 are implicated in the transmission of this disease [92, 93]. The life cycle of *C. Trachomatis* has three stages. In stage 1, the elementary or infective particles penetrate the host cell; it is followed by stage 2 when the elementary particle transform into a metabolic active body that divides by binary fission and form reticulated body that exist only as an intracellular organism, and finally in stage 3, the reticulate bodies transform into elementary bodies that leave the host cell by exocytosis.

Clinical Features

The disease has three phases: [1] erosion of the skin, [2] adenitis, and [3] fibrosis and destruction. LGV spreads primarily via the lymphatics. The initial lesion is a small, painless papule, or vesicle that frequently erodes, healing within several days without residual scar. The initial ulcers are not tender or painful and form after an incubation period of 3–30 days after inoculation [93, 94]. Subsequently, the infection disseminates and manifests as enlarged painful inguinal and/or femoral lymph nodes. The lymph nodes can evolve to bubo formation, leading to spontaneous rupture and sinus tract formation (Fig. 6.24). The groove sign reflects matted lymph nodes that have formed a mass above and below the inguinal

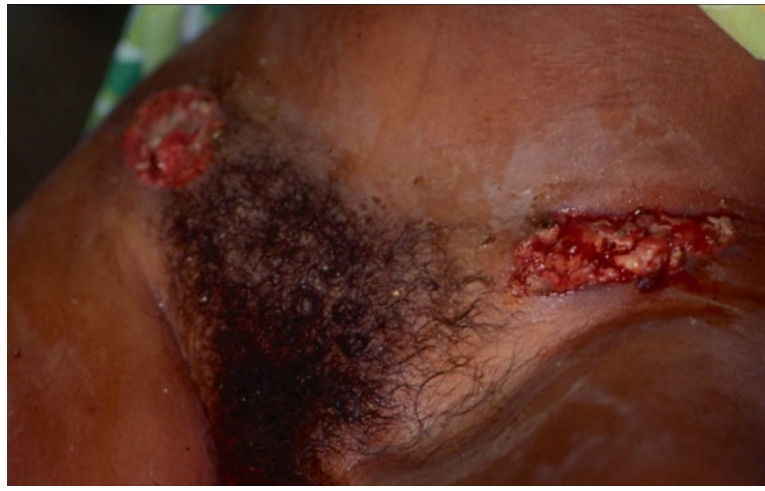


Fig. 6.24 *Lymphogranuloma venereum*. The lymph nodes can evolve to bubo formation, leading to spontaneous rupture and sinus tract formation

ligament. Without treatment, extensive necrosis and scarring can result in lymphedema. Esthiomene, a rare late manifestation of LGV, is a primary infection affecting the lymphatics of the vulva and can evolve to elephantiasis of the genitalia [95]. Progressive enlargement of the inguinal nodes follows.

Microscopic Features

The primary vulvar lesions have no specific histologic features and heal rapidly, often being no longer evident when the inguinal lymphadenopathy becomes marked. Smears and biopsy specimens should be evaluated for organisms (spirochetes, Donovan bodies, etc.) to rule out other diseases with a similar presentation. Histologically, multinucleated histiocytes may be seen along with lymphocytes and plasma cells. Older lesions may exhibit extensive fibrosis of the dermis and sinus tracts. The diagnosis is based on the typical clinical presentation, along with positive complement fixation tests. Culture, as well as other specific immunohistochemical tests, can assist in the diagnosis of LGV. Lymph nodes are occasionally excised for histologic diagnosis and show serpiginous or stellate abscesses with necrotic tissue and neutrophils in the center, surrounded by a macrophage and giant cell granulomatous reaction ("suppurating granulomas"). Culture in yolk sac of eggs, complement fixation antibody titers, molecular confirmation using PCR techniques, and DNA sequencing of the variable segments of the OMP1 [96] to determine serotype are all diagnostic modalities to confirm the suspicious of LGV.

Differential Diagnosis

The ulcer of LGV raises the differential diagnosis of syphilis, herpetic, and aphthous ulcers (Table 6.1). The presence of endotheliali tis can assist to the diagnosis of syphilis and confirmed by immunohistochemical stains. The detection of multinucleated cells with molding nuclei and margination of the chromatin will point to herpes virus infection. Aphthous ulcers can be differentiated from LGV by the marked lymphadenopathy and cultures and immunohistochemical tests available to confirm the *C. trachomatis* infection.

Summary

Clinical Presentation

- Three clinical stages:
  - Stage one: small painless papules or vesicle that frequently ulcerate after 3–10 days of inoculation, without residual scar.
  - Stage two: inguinal, femoral, and deep pelvic painful lymphadenopathy with necrosis, fistula formation, and ulceration that ends in sclerosing fibrosis.
  - Stage three: also known as genitoanorectal syndrome with perirectal abscesses, fistulae, and stenosis of rectum.
  - Severe lymph stasis results in massive vulvar edema or esthiomene.
- *Histologic Features*The ulcer in stage one has nonspecific findings.
- Lymph nodes demonstrate hyperplasia and foci of stellate necrosis surrounded by palisaded histiocytes and giant cells.

Differential Diagnosis

- Syphilis
- Herpetic ulcer
- Aphthous ulcers

Takeaway Essentials

Clinical Relevant Pearls

- Consider LGV as one of the causes of severe vulva edema.
- Lymph nodes involvement can extend up to deep pelvic nodes.

Pathology Interpretation Pearls

- When intrahistiocytic organism are seen, *C. tricomatis* should be considered in the differential diagnosis.
- The changes in the site of inoculation and lymph node are not specific.
- Only one-third of cultures are positive; therefore, serologic and molecular tests are better diagnostic tools.

Granuloma Inguinale

Granuloma inguinale (donovanosis, granuloma venereum) is caused by *Calymmatobacterium granulomatous*, an obligatory intracellular Gram-negative rod. The name of the organism reflects its pseudoencapsulated appearance in tissue sections ("Kalymma": Greek for hood or veil). It has been proposed that the etiologic agent been reclassified as *Klebsiella granulomatis* due to the 99 % phylogenetic similarity with *K. pneumoniae* [97]. This sexually transmitted disease is seen in New Guinea, the Caribbean, South Africa, Australia, Brazil, and parts of India [98]. Granuloma inguinale affects with approximately equal frequency in men and women.

Clinical Features

Primary lesions present as painless papules that ulcerate with rolled border affecting the labia in the vulva, vagina, or cervix. The ulcerated papules merged to form a hypertrophic, velvety, beefy-erythematous granulation tissue. An alternative clinical presentation takes the form of nodules as the results of confluent papules. Inguinal adenopathy usually is absent. The incubation period is from 2 week to 6 month after exposure [99]. Anal coitus and fecal contamination of the vulva or vagina have been incriminated as modes of transmission. The disease extends by local infiltration, although lymphatic permeation may occur during later stages of the disease. Chronic lymphatic infiltration and fibrosis frequently result in a massive edema of the external genitalia.

The clinical diagnosis of granuloma inguinale depends on the identification of the Donovan bodies within the tissue. This is best accomplished by preparing smears or imprinting of a biopsy from the edge of the ulcer after pressing the tissue between two slides. The tissue imprints are air dried, fixed in methanol, and stained with Giemsa stain. Any antibiotic treatment may obscure the diagnosis, necessitating a biopsy at a later date to identify the organisms. Alternatively, PCR test can be used in this setting [99].

Microscopic Features

Histologically, lesions of granuloma inguinale present as areas of ulceration and granulation tissue. The epithelium next to the areas of ulceration may show prominent pseudoepitheliomatous hyperplasia. In the epidermis, there are areas of necrosis and collections of neutrophils. Plasma cells are a prominent component of the granulation tissue. Admixed with the plasma cells, there are lymphocytes and histiocytes, some of them with large vacuoles containing the characteristic encapsulated 1–2 μm rod with the form of a safety pin (Donovan bodies). The organisms can be highlighted with a Warthin–Starry stain or Giemsa stain. The Donovan bodies may be found extracellular, as well as intracellular.

Differential Diagnosis

Donovan bodies need to be differentiated from other intracellular organisms: fungal (histoplasmosis, cryptococcosis, and pneumocystosis) and protozoal (toxoplasmosis and leishmania). Histochemical stains like PAS and GMS can be used to identify the fungal forms. In addition, mucin stains can also be considered for cryptococcosis. The presence of the kinetoplast can be used to recognize leishmania, while immunohistochemical stain are available to diagnosed toxoplasmosis.

Summary

Clinical Presentation

- At the sites of inoculation, ulcerated papules and nodules evolve into large areas of granulation tissue that bleed easily.

Histologic Features

- Granulation tissue response at the site of inoculation with dense inflammation composed by aggregates of neutrophils, plasma cells, and macrophages.
- Donovan bodies, 1–2 μm rod, are seen inside the macrophages.
- Organisms are better identified with Giemsa or silver-based Warthin–Starry stains.

Differential Diagnosis

- Intracellular microorganisms: fungal (histoplasmosis, cryptococcosis, and pneumocystosis) and protozoal (toxoplasmosis and leishmania)

Takeaway Essentials

Clinical Relevant Pearls

- Slowly progressive and destructive disease that leads to disfiguring fibrosis and extensive mutilation.
- Diagnostic delay may lead to significant morbidity.
- More destructive clinical presentation needs to raise concern for coexistence of HIV infection.
- When the patient is partially treated, PCR testing and nucleotide sequencing investigations are the selected diagnostic tools.

Pathology Interpretation Pearls

- Smear and histologic assessment of the lesion is necessary to confirm the diagnostic presence of Donovan bodies.
- If the patient has been already treated, PCR studies may be the best diagnostic tool to assist with the diagnosis.

Bacillary Angiomatosis

Thirty-one years ago, Stoler and colleagues described for the first time bacillary angiomatosis as a disease presenting with cutaneous lesions simulating Kaposi's sarcoma [100]. This systemic disease is caused by the Gram-negative coccobacilli *Bartonella henselae* and *Bartonella quintana. B. henselae* also causes cat scratch disease and peliosis transmitted by cat fleas, while *B. quintana* is responsible for trench fever transmitted by cat fleas, ticks, and body lice. Bacillary angiomatosis affects primary HIV-infected women or suffering from other immunosuppression conditions like leukemia or transplanted patients. Apparently, immunocompetent patients and even children can also be affected. Up to two-thirds of these cases are associated with cat bites or scratches [101].

Clinical Features

Cutaneous lesions are the most frequent manifestation of this systemic disease. The clinical presentation is heterogeneous and range from vulvar, nodules [102], warty lesions [102], and the more characteristic pyogenic granuloma-like presentation [103]. Pyogenic granuloma-like lesions are dusky red pedunculated lesions that commonly bleed.

Histopathology

Bacillary angiomatosis presents as a lobular proliferation of vessels lined by epithelioid endothelial cells. Varying degree of neutrophils and karyorrhectic debris can be seen in the interstitium of the lesion. The organism can be identified in the form of clumps of amphophilic or argyrophilic material in close relationship to neutrophils. Brown–Brenn stain can also be used to highlight the *Bartonella* species. Neutrophils are very prominent in the nodular/mass form described in the vulva to the point that it can be confused with an abscess. The verrucous vulvar form is due to associated pseudoepitheliomatous hyperplasia [102]. Immunohistochemical stains and molecular PCR-based methods have been used to confirm the presence of the microorganism.

Differential Diagnosis

The main differential diagnosis includes pyogenic granuloma. The presence of neutrophils in the absence of ulcer in conjunction with the identification of amorphous basophilic aggregates should alert the pathologist of this infection. To assist with the diagnosis of bacillary angiomatosis, silver-based stains like Warthin–Starry can be used to highlight the argyrophilic organisms. Other vascular lesions with prominent endothelial cells like epithelioid hemangioendothelioma and cutaneous epithelioid angiomatous nodule would also need to be differentiated from bacillary angiomatosis. Bacillary angiomatosis fails to show the intracytoplasmic vacuoles and present well-formed vascular channels not seen in epithelioid hemangioendothelioma. The detection of significant amount of neutrophils and associated *Bartonella* spp. would support the diagnosis of bacillary angiomatosis over epithelioid angiomatous nodule. Opposite to Kaposi's sarcoma, bacillary angiomatosis have vessels lined by epithelioid endothelial cells.

Summary

Clinical Presentation

- Classically present as a pyogenic granuloma-like lesion
- Other clinical presentations: erythematous papules, nodules, and warty-like lesions

Histologic Features

- Lobular proliferation of vessels lined by epithelioid endothelial cells
- Neutrophils and karyorrhectic debris
- Amphophilic aggregates of microorganisms close to neutrophils

Differential Diagnosis

- Pyogenic granuloma
- Epithelioid hemangioendothelioma
- Epithelioid angiomatous nodule
- Kaposi's sarcoma

Takeaway Essentials

Clinical Relevant Pearls

- Bacillary angiomatosis should be in the differential diagnosis of vascular-like lesions in immunocompromised women.
- Failure to recognized this infection may lead to overwhelming dissemination and death.

Pathology Interpretation Pearls

- A lobular vascular proliferation with the presence of neutrophils and karyorrhectic debris in the absence of ulceration should raise the suspicious of bacillary angiomatosis.
- The pseudoepitheliomatous hyperplasia of the epithelium in vulvar lesions of bacillary angiomatosis needs to be differentiated from squamous cell carcinoma or genital wart.

Tuberculosis

Tuberculosis of the vulva is usually the result of spreading from other genital sites, including the vagina, fallopian tubes, and endometrium [104, 105]. Genital involvement is frequently associated with pulmonary tuberculosis. Occasionally, primary inoculation or sexual transmission of tuberculosis can occur, but it is most uncommon [106]. Immunosuppression may play a role in susceptibility. Vulvar tuberculosis has been described in a renal transplant woman. The usual organism is *Mycobacterium tuberculosis*; however, atypical mycobacteria also have been incriminated. Diagnosis usually can be made by biopsy of the involved tissues. Cultures for identification and drug sensitivity testing and molecular testing with PCR methods are available diagnostic tools [107].

Clinical Features

Vulvar tuberculosis can present in the form of ulcers [108] with sinuses [109], hypertrophic lesions, or in rare occasions as esthiomene [109–112]. Vulvar tuberculids have been reported, lichen scrofulosorum and papulonecrotic tuberculids being the forms most frequently seen in the vulva [113, 114]. Vulvar cutaneous lymphangiectasias and Bartholin gland abscess can also be presentations of tuberculosis [105, 111, 115].

Histopathology

Caseating granulomas with multinucleated histiocytes are found, and acid-fast stains usually reveal the mycobacteria (Fig. 6.25a–c). The epithelium usually shows areas of pseudoepitheliomatous hyperplasia with acanthosis, papillomatosis, and hyperkeratosis. The papulonecrotic tuberculid shows a wedge-shaped inflammatory reaction with different components that from top to bottom include: crust, acute inflammation, coagulative necrosis, granulomas, and vasculitis. The folliculocentric vulvar papules of lichen scrofulosorum consist of granulomas situated along the folliculosebaceous units. Ziehl–Neelsen and AFB or Fite stains are essentially negative in tuberculids, supporting the concept of hypersensitivity to mycobacteria rather than direct inoculation.

Differential Diagnosis

The ulcerative presentation of tuberculosis needs to be differentiated from sexually transmitted

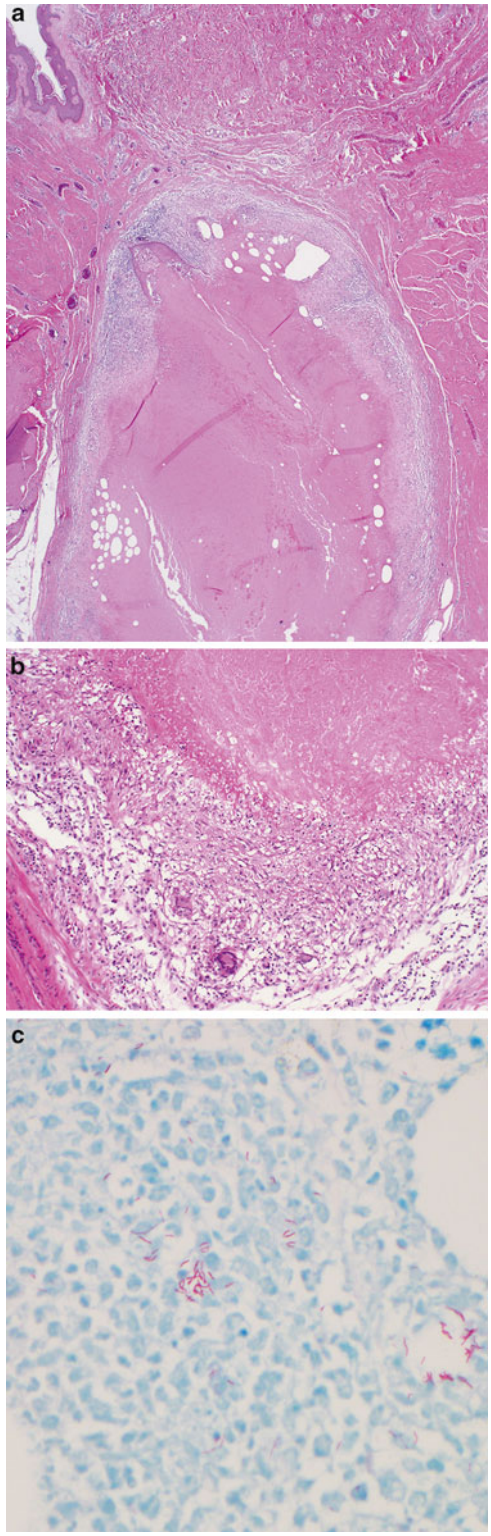


Fig. 6.25 (**a**–**c**) Tuberculosis. Caseating granuloma (**a**) with multinucleated histiocytes (**b**). AFB stain highlights the acid-fast bacilli (**c**) (Courtesy of Dr Mai Hoang, department of Pathology, Massachusetts General Hospital, Boston, MA)

diseases like chancroid, syphilis, and lymphogranuloma venereum. Chancroid presents a triple zonation with histiocytes showing the coccobacilli in "school-of-fish" arrangement. Granulomas in tertiary syphilis are associated with marked perivascular plasmacytic infiltrate and endarteritis obliterans. Lymphogranuloma venereum histologic features in the skin are not specific; however, the lymph node can help to confirm the diagnosis with the presence of necrotic palisading granulomas. Lesions with pseudoepitheliomatous hyperplasia may be confused with squamous cell carcinoma. However, tuberculosis lacks the keratinocytic pleomorphism, atypical mitosis, irregular infiltrative border, or desmoplasia seen in squamous cell carcinoma. While the granulomatous inflammation raises the differential diagnosis of sarcoidosis and Crohn disease, the presence of caseating necrosis in the granulomas points toward tuberculosis.

Summary

Clinical Presentation

- Large, painful ulcer(s), +\– sinuses
- Hypertrophic lesions
- Tuberculid
  - Papulonecrotic tuberculid: papules or vesicles with central necrosis
  - Lichen scrofulosorum: small firmed scaly perifollicular papules

Histologic Features

- Caseating granulomas
- Pseudoepitheliomatous hyperplasia
- Tuberculid
  - Papulonecrotic tuberculid showing wedge-shaped necrosis, vasculitis, and granulomas
  - Lichen scrofulosorum: granulomas along the hair follicles

Differential Diagnosis

- Granulomatous form: sarcoidosis and Crohn disease
- Ulceronecrotic form: Chancroid, syphilis, and lymphogranuloma venereum

Takeaway Essentials

Clinical Relevant Pearls

- Vulvar tuberculosis usually hints toward the presence of infection in other genital organs.
- Pulmonary disease is usually associated to vulva tuberculosis.
- Although rare, vulva lesions may be the main manifestation of tuberculosis.

Pathology Interpretation Pearls

- If treatment is not implemented, the infection will lead to scar and disfigurement.
- If considering a tuberculids, special stains are not helpful. Search of a distant focus of infection (e.g., lung or genitourinary system) will confirm the suspicious.
- PCR rarely has been described positive in tuberculids.

Fungal Infections

Candidiasis

Candidiasis is frequent in the genital and anal region. *Candida albicans*, the most common *Candida* species involved in human infection, is a normal dimorphic yeast inhabitant of the gastrointestinal tract and is found in the mouth of approximately half of normal individuals [116, 117]. In recent years, scientific advances suggests that vulvovaginal candidiasis is a hypersensitivity response to a commensal organism in potential genetically predisposed woman [118]. Pregnancy, immunologic and endocrine dysfunction, immunosuppression, high-dose estrogen, antibiotic or systemic corticosteroid therapy, and debilitating states all predispose to clinical infection [119, 120]. In the genital area, moisture, abrasion of epithelium, and heat also play a role in the development of this yeast infection. Candida can also cause intertrigo in the groins or in folds of a pendulous abdomen. Three-quarter of women in reproductive age will experience vulvovaginal candidiasis at least once in their lifetime, with half suffering > 1 recurrence and nearly 8 % multiple annual episodic infections [116, 121]. If four or more acute episodes occur annually, the women is considered to have recurring vulvovaginal candidiasis [122].

Clinical Features

Sore red, eroded patches occur in the flexures with small satellite pustules beyond their margins. Between 15 % and 30 % of asymptomatic women are carriers of Candida. Vulvovaginal candidiasis presents with vaginal and vulvar itching and burning, accompanied by vaginal discharge with a premenstrual exacerbation. Although only a fourth of all vaginitides are caused by Candida, it has the most prominent vulvar involvement. Therefore, a vaginitis that presents with vulvitis should initially be suspected as candidal in origin. Women of childbearing years are predominantly affected. A characteristic white curd (Candida comes from the LATIN candidus or dazzling white) appears on the vaginal walls. Erythema, edema, and fissuring of the vulva may occur, especially in mucosa and modified mucous membranes (e.g., inner labia and vestibule). Vulvar candidiasis, although less common than vaginal counterpart, increased in premenarchal girls, diabetic, obese, and patients with incontinence. Diagnosis is confirmed on culture of a high vaginal swab or by finding hyphae and spores on a Gram-stained film from the vaginal discharge. *C. albicans* is the culprit in most cases, but other *Candida* species such as *C. glabrata* and *C. tropicalis* are responsible in a minority and can be more resistant to conventional treatments. Non-*Candida albicans* species have been isolated in patients with recurrent vulvovaginal candidiasis, HIV-infected women, after menopause, and with uncontrolled diabetes [116]. Detection can be done in cultures using Sabouraud dextrose agar (standard media) with rapid growth in 3–5 days. Calcofluor-white fluorescent stain reacts with the chitin in the fungal wall leading to an apple green color under fluorescent scope. In rare and difficult diagnostic cases, PCR and hybridization can be helpful but not essential.

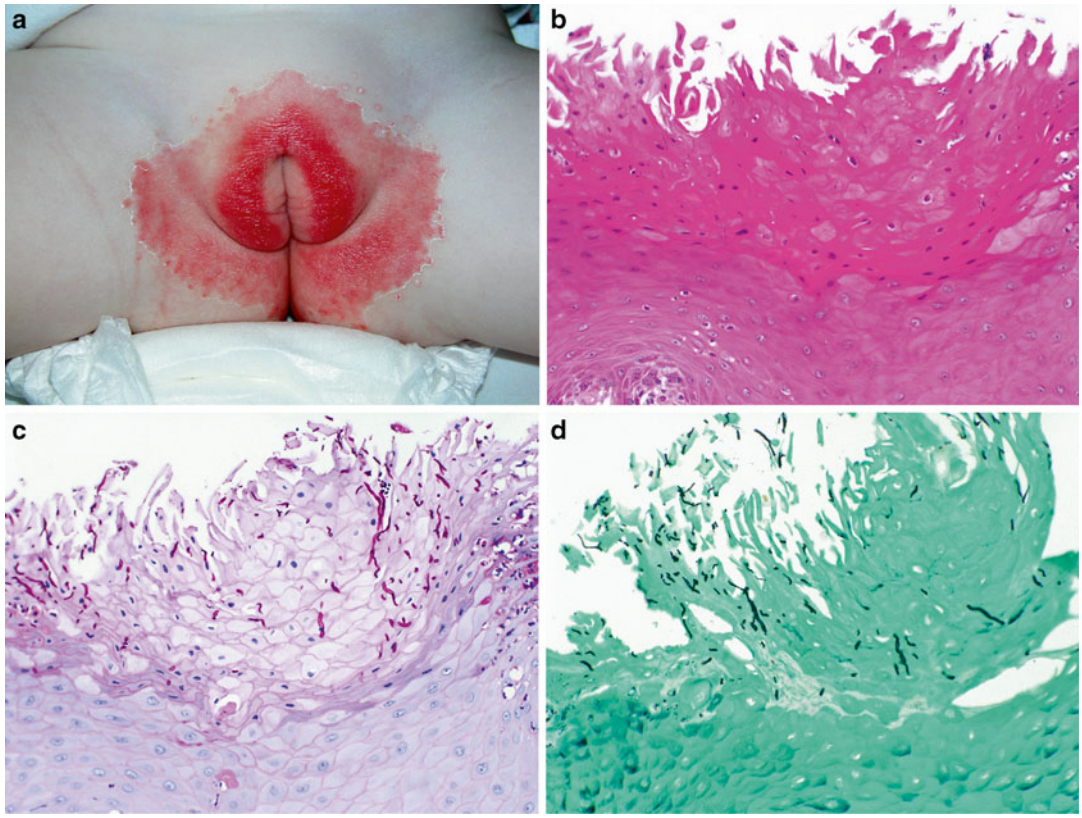


Fig. 6.26 (**a**–**d**) *Candidiasis*. It presents with an erythematous scaly area with pustules (**a**). In the stratum corneum, there are pseudohyphae and oval budding yeasts (**b**). PAS (**c**) and GMS (**d**) can be used to highlight the microorganisms

Microscopic Features

Histologically, the features of candidiasis are not specific. There is usually spongiosis with parakeratosis and collections of neutrophils in the stratum corneum. Candida can be seen as 3–6 μm in diameter round to oval budding yeasts associated with pseudohyphae of 2–4 μm in length. PAS and GMS stains are usually confirmatory (Fig. 6.26a–d).

Differential Diagnosis

In view of the epidermal changes associated with candidiasis, the infection can be confused with psoriasis. Although both share the presence of psoriasiform epidermal acanthosis, psoriasis will fail to show organisms in histochemical special stains (PAS and GMS). Dermatophytosis is also in the differential diagnosis. Clinically dermatophytosis is more annular and scaly than Candida and fails to show pustular presentation. In addition, no tendency to affect mucosal surface is noted in dermatophytosis. On histology, the presence of septated hyphae can help in separating dermatophytosis from candidiasis.

Summary

Clinical Presentation

- Erythematous and erosive patches
- Papular satellite lesions with pustules on top

Histologic Features

- Hyperkeratosis with variable parakeratosis
- Epidermal acanthosis with variable spongiosis
- Neutrophils migration through the epidermis and collecting in the stratum corneum

- Budding yeasts, pseudohyphae, and hyphae

Differential Diagnosis

- Psoriasis
- Dermatophytosis

Takeaway Essentials

Clinical Relevant Pearls

- Predominant vulvar involvement is rare and can be seen in premenarchal girls.
- Candida infection frequently affects mucosal and modified mucous membranes (e.g., vestibule and labia minora).

Pathology Interpretation Pearls

- Neutrophils in the stratum corneum should raise the suspicious of a fungal infection with the differential diagnosis of psoriasis.
- Due to the overlap with inflammatory dermatosis affecting the vulva, special histochemical stains like PAS are often needed to reach the correct diagnosis.

Pityriasis Versicolor

Pityriasis versicolor is a frequent benign fungal infection involving the stratum corneum. It is caused by lipophilic *Malassezia* species in its mycelial form. Species implicated in dermatologic diseases includes *Malassezia globosa*, *Malassezia sympodialis*, *Malassezia sloofiae*, and *Malassezia furfur*. Warm, humid environment, hyperhidrosis, application of oily substances, malnutrition, and immunosuppression are some of the factors that increase the predisposition to an infection. Although we know that Malassezia colonize genitalia in nearly half of healthy men, we do not know the colonization frequency in females [123].

Clinical Features

Vulvar infection by Malassezia will present in the form of pityriasis versicolor [124] and folliculitis [125]. It usually affects the labia majora as reddish-brown to whitish scaly macules. Areas of hyperpigmentation may be caused by large melanosomes, vascular ectasia, or orthokeratosis. Hypopigmentation can occur especially in darker pigmented women. The latter results from azelaic acid, a tyrosinase inhibitor that the organisms produce. Commonly, not significant pruritus is noted. Examination with Wood's lamp produces a dull green fluorescence and underscores the border of the lesion.

Histopathology

On histologic sections, clusters of round budding yeasts (blastoconidia), and short septate hyphae (pseudomycelia) can be seen in the stratum corneum (Fig. 6.27a, b). "Spaghetti and meatballs" is the term that has been used to describe the presen-

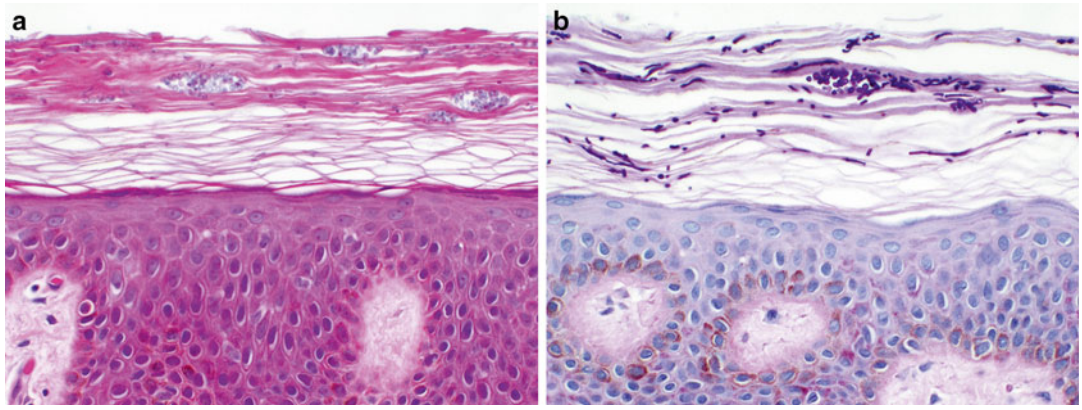


Fig. 6.27 (**a**, **b**) *Pityriasis versicolor*. Clusters of round budding yeasts (blastoconidia) and short septate hyphae (pseudomycelia) can be seen in the stratum corneum ("Spaghetti and meatballs") (**a**). PAS decorates the fungal forms in the stratum corneum (**b**)

tation of yeast and hyphae in skin biopsy and skin scrapings analyzed in 10 % potassium hydroxide (KOH). No significant inflammatory reaction is elicited in this infection.

Differential Diagnosis

Candida and tinea cruris should be differentiated from pityriasis versicolor. The presence of inflammatory reaction in the setting of infection would point toward candida and tinea cruris.

Summary

Clinical Presentation

- Usually affect labia majora
- Reddish-brown to whitish scaly macules

Histologic Features

- Organism is located in the stratum corneum
- "Spaghetti and meatballs" appearance

Differential Diagnosis

- Candida
- Tinea cruris

Takeaway Essentials

Clinical Relevant Pearls

- The patient should be reassured that pityriasis versicolor is not a sexually transmitted disease.

Pathology Interpretation Pearls

- Histochemical stains (e.g., PAS and GMS) may help to identify the organism.

Tinea Cruris

Clinical Features

Tinea cruris is a superficial dermatophyte infection, which usually involves the inner upper thighs and crural folds. *Trichophyton rubrum* is the most frequent causative agent, produces a chronic infection with frequent extension to the buttocks and waist. The lesions are annular, sharply demarcated, itchy, red patches with erythematous, scaly, advancing borders. Pustules or vesicles may occur at the edge. Secondary vulvar involvement may occur. Heat, sweating, and friction predispose to infection. When topical steroids are inadvertently applied, the appearance can change dramatically and become nondiagnostic, creating the so-called tinea incognito. Although some of the erythema and scaling is initially masked by steroid use, the infection ultimately becomes more extensive and inflammatory, with papules and pustules. The annular configuration may be a clue in these cases. Majocchi granuloma, another commonly presentation of infection by *T. rubrum*, presents with perifollicular erythema in immunocompetent women and deep plaque/nodule in immunosuppressed patients [126].

Microscopic Features

The epidermis can show areas of spongiosis with parakeratosis containing neutrophils. The superficial fungal infection can be seen between normal basket-weaved stratum corneum and underlying stratum corneum with hyperkeratosis or parakeratosis. This histologic feature is call "sandwich sign." Majocchi granuloma shows perifollicular granulomatous inflammation associated with neutrophils and lymphocytes. In many cases, the dermatophytes in the stratum corneum or hair follicle can be seen on H&E-stained slides, if not, PAS and GMS stains usually highlight the presence of organisms (Fig. 6.28a–d).

Differential Diagnosis

Erythrasma is a superficial infection caused by *Corynebacterium minutissimum* presenting as large, slowly enlarging, and confluent reddish-brown macules with variable central clearing and poorly defined borders. Its clinical presentation can be confused with tinea cruris, as both involve intertriginous spaces. In erythrasma, the causal organism *Corynebacterium minutissimum* is an aerobic diphtheroid rod highlighted with Gram stain. Using the Wood's lamp, erythrasma acquires a bright coral red fluorescence due to porphyrins produced by the bacteria. Candida has a predilection of mucosal surface involvement. The presence of yeasts and pseudohyphae will favor candida over dermatophytosis.

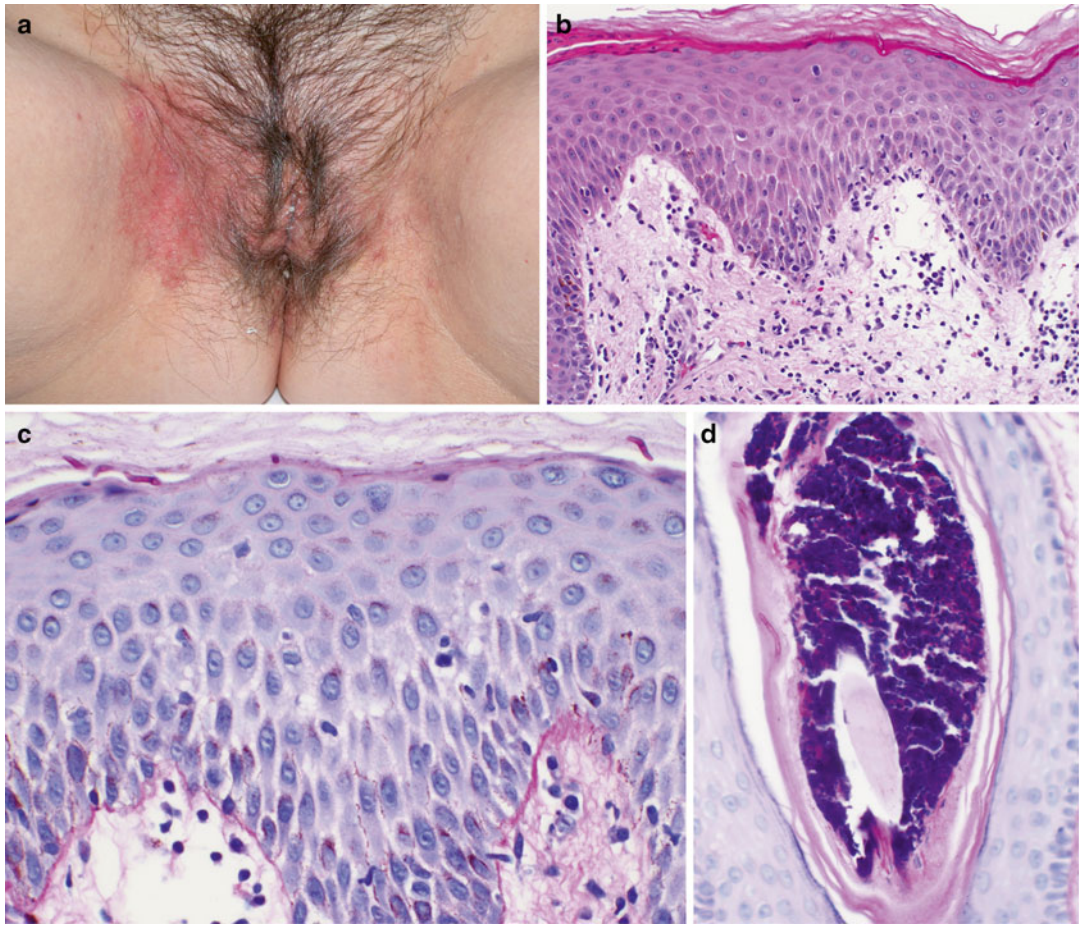


Fig. 6.28 (**a**–**d**) *Tinea cruris*. Erythematous slightly scaly area (**a**). In a background of epidermal spongiosis, dermatophytes can be seen in the stratum corneum (**b**) and or hair follicles. PAS highlights the fungal forms in the epidermal surface and hairs (**c**, **d**)

Summary

Clinical Presentation

- Erythematous annular plaques with scale and active advancing border

Histologic Features

- Different degrees of epidermal spongiosis and perivascular inflammation
- Neutrophils migration into the stratum corneum
- "Sandwich sign": fungal forms entrapped between hyper and/or parakeratosis and normal stratum corneum
- Fungal forms better demonstrated by PAS and GMS stains

Differential Diagnosis

- Erythrasma
- Candida

Takeaway Essentials

Clinical Relevant Pearls

- Wood's lamp is a convenient and practical tool to separate tinea cruris from erythrasma.
- Sample of the scale from the leading edge of the rash is the best place to identify the organism.

Pathology Interpretation Pearls

- Multiple sections through the block may be necessary to find the fungal forms in Majocchi granuloma.
- Subtle changes may be noted, therefore, in "normal-appearing skin," histochemical stains like PAS and GMS stains may help to highlight the infection.

Protozoal Infections

Trichomoniasis

Trichomoniasis is a sexually transmitted infection caused by the flagellated protozoan *T. vaginalis* that usually parasites the vagina and urethra. It is believed to represent a risk factor for HIV transmission [127].

Clinical Features

Approximately 70 % of the patients are asymptomatic. When this parasitic infection does cause symptoms, frequently it is in the form of an acute vaginitis, especially symptomatic when coexisting with bacterial vaginitis. The patient experiences a profuse vaginal discharge associated with dysuria, dyspareunia, and vaginal soreness. The vulva is acutely inflamed with marked erythema and edema, similar to the appearance of the vagina described as strawberry. Trichomoniasis is also associated with adverse pregnancy outcomes and infertility. Diagnosis is reached by examination of wet mount, culture, papanicolaou smears, serologic tests, and PCR investigation [128].

Histopathology

The examination of the foul smell, yellow-green discharge on a wet saline preparation demonstrates the presence of the motile, flagellate protozoan. The parasite measures 23–39 μm in length by 5–8 μm wide (Fig. 6.29). Four flagella and a fifth flagellum are present within the undulating membrane [129].

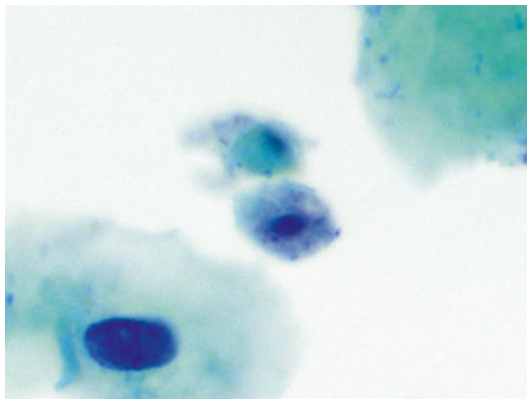


Fig. 6.29 *Trichomoniasis*. The Pap smear shows the flagellated protozoan

Differential Diagnosis

Bacterial vaginosis will have the classic clue cells in the papanicolaou-stained sections. Candida infection will show the yeast, pseudohyphae, and hyphae.

Summary

Clinical Presentation

- Asymptomatic
- Vaginal discharge and vulva irritation

Histologic Features

- Identification of the flagellated parasite

Differential Diagnosis

- Bacterial vaginosis
- Candida

Takeaway Essentials

Clinical Relevant Pearls

- The high prevalence worldwide of this parasite makes it a public health concern.
- Increase the risk of transmission of HIV.
- Thorough examination of other sexually transmitted diseases is recommended.
- Need to be considered in all women with vaginal discharge.

Pathology Interpretation Pearls

- Due to the poor reliability of history and physical findings, trichomoniasis depends on laboratory testing.

Infestations

Scabies

Scabies is a pruritic cutaneous eruption caused by the infestation of the female eight-legged mite *Sarcoptes scabiei var. hominis*. The dissemination of the disease occurs by intimate contact or fomite transmission, affecting especially areas

with low density of folliculosebaceous units and thin stratum corneum like web spaces, flexor surfaces of wrists, waistline, and genitalia. The female ectoparasite lives and reproduces in spaces created between the stratum corneum and granular layer called burrows where she lays eggs as part of the 30-day cycle of life. The population most affected includes young people in endemic areas, elderly, immunocompromised, nursing home residents, and resource poor, overcrowded populations. In developing countries, the average of prevalence is 2, 5:1,000 with an estimate of 300 million cases worldwide, although this data is difficult to confirm [130]. Immunosuppressed patients may exhibit an exuberant infestation called Norwegian or crusted scabies [14].

Clinical Features

The classic clinical presentation is fine tortuous superficial threadlike lesions or burrows with a black dot at the near blind end representing the mites. Marked nocturnal pruritus starts approximately 1 month after infestation, when mite or byproducts can elicit sensitization in the patient. "Post-scabietic pruritus" and persistent nodular scabies can last for months even in the absence of the mites. Other clinical presentations include papules, erythematous nodules, and eczematous reaction. Commonly scabies is a nuisance; however, morbidity increases by secondary bacterial infections. KOH preparation from skin scrapings can demonstrate the mite with its classic eight legs measuring 0.4 mm in length, golden spherules or scybala, and transparent ovals or eggs (Fig. 6.30). For clinical atypical forms, PCR can detect the mite in skin scrapings.

Histopathology

Sections show the presence of mites underneath the stratum corneum associated with epidermal spongiosis and superficial and deep perivascular lymphoeosinophilic infiltrate (Fig. 6.31a). Mites or its part can be seen in 20 % of cases, even after serial sectioning. *S. Scabies* is characterized by oval, ventrally flattened body with four pairs of legs and numerous cuticular spines (Fig. 6.31b). Scabietic spines are circular on cross section, with polarize light they show dark center and peripheral birefringence [131]. Eggs can be detected in the burrows as small geometric round to oval structures with few organelles; after hatching, the remnants simulate "pigtails" (Fig. 6.31c). A female mite can lay between 60 and 90 eggs during her life span. Fecal pellets or "scybala" can be seen in the burrows as golden globules showing stippled birefringence under polarized light. In some cases, only indirect evidence of the mite in the form of fecal material or ova is identified (Fig. 6.31d). In Norwegian scabies, the epidermis acquires a psoriasiform hyperplasia with hyperkeratosis and parakeratosis infested by numerous mites [132]. However, allergic symptoms like pruritus are not so pronounced.

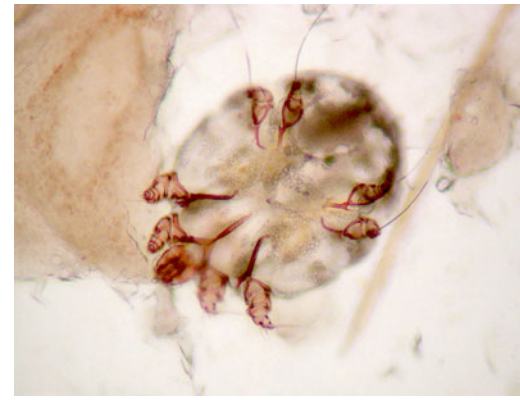


Fig. 6.30 *Scabies*. Skin scrapings show the mite with eight legs (Courtesy of Dr Mai Hoang, department of Pathology, Massachusetts General Hospital, Boston, MA)

Differential Diagnosis

Demodex mites are intrafollicular parasites commonly encountered in areas rich in follicular sebaceous units. It can be separated from *S. scabiei* because of their elongated bodies with prominent head and neck and intrafollicular habitat. In addition, they do not cause pruritus. Dermal hypersensitivity reactions can be elicited by multiple stimuli like scabies; the absence of burrows mites and eggs extends the potential triggers to drug or light, among others. As reaction to the ectoparasite, a rich CD30+ reactive population of lymphocytes can be seen and create confusion with CD30+ lymphoproliferative disorders. In contrast to scabies, lymphomatoid papulosis lacks significant noctur-

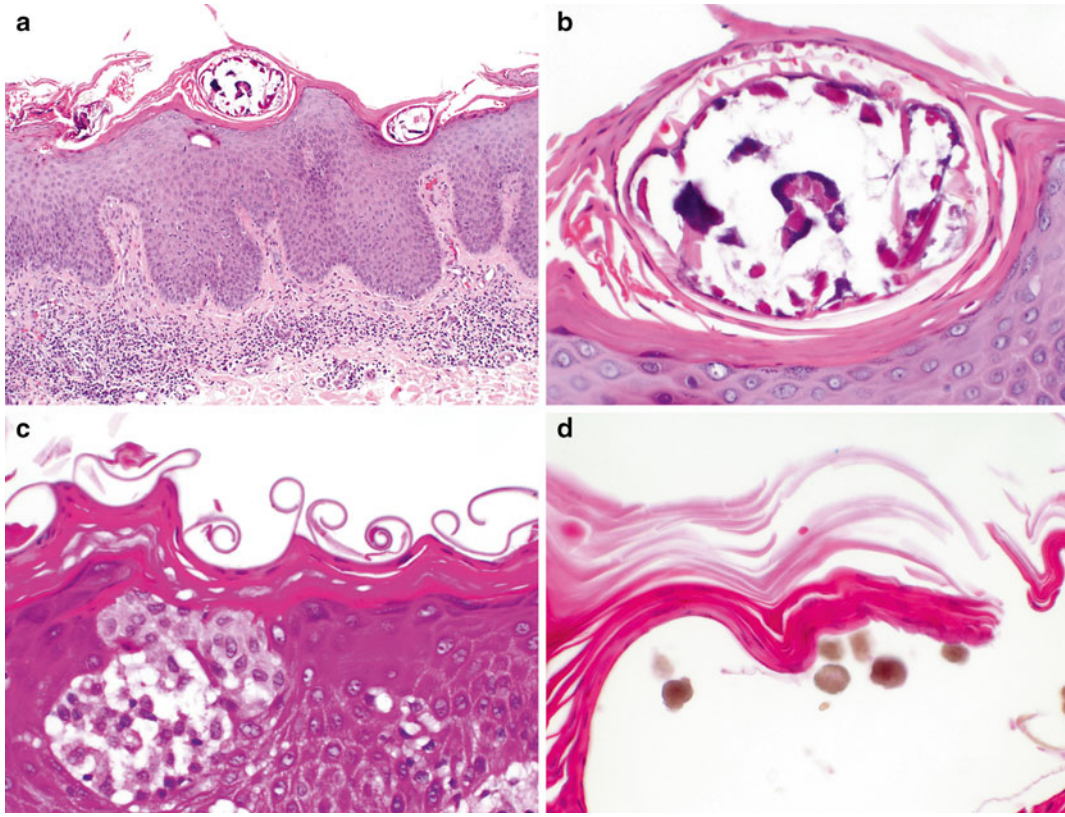


Fig. 6.31 (**a**–**d**) *Scabies*. The mite is located underneath the stratum corneum (**a**). The epidermis shows spongiosis and perivascular lymphoeosinophilic infiltrate. Note the mite with numerous cuticular spines that are refractile under polarized light (**b**). Other evidence of infestation can be seen in the form of "pigtails" or hatched eggs (**c**) and pellets (**d**)

nal pruritus and burrows containing mites: while exhibiting a characteristic presentation of crops of papules that spontaneously resolves [133]. Langerhans cells can also be increased in number as part of the reaction to the genital infestation infestation type: mimicking Langerhan cell histiocytosis.. Search for burrows and mites can avoid this pitfall and unnecessary treatment [134, 135].

Summary

Clinical Presentation

- Burrows, papules, and nodules
- Symptoms start usually after 1 month or months and half of the infestation.
- Marked pruritus, especially at night.

Histologic Features

- Mites in burrows (between stratum corneum and granular layer).
- Eggs remnants or "pigtails," transparent ovals.
- Fecal pellets or "scybala," golden-brown globules of 1–10 μm in diameter.
- Under polariscopic light, birefringence reaction is created by spines and scybala; mite bodies and egg cases are not polarizable.

Differential Diagnosis

- Demodex mites
- Dermal hypersensitivity reactions
- Demodex mites
- Lymphomatoid papulosis
- Langerhans cell histiocytosis

Takeaway Essentials

Clinical Relevant Pearls

- Significant public health problem.
- Pruritus can persist even in the absence of active infestation.
- Increased morbidity due to secondary bacterial infection.
- KOH preparation and PCR of skin scrapings can be useful diagnostic tools.

Pathology Interpretation Pearls

- Commonly, mites are not captured in the specimen planes of sections; serial sections and polarization can be useful diagnostic tools.
- Scabies is one of the pitfalls for CD30+ lymphoproliferative disorders.
- Before diagnosing Langerhans cell histiocytosis in the genital area, consider the possibility of scabietic infestation as trigger of increased number of Langerhans cells.

Pubic (Crab) Lice

Pediculosis pubis (pubic lice, crab lice) is caused by the blood-sucking *Phthirus pubis* and affects 10 % of the population worldwide [136]. This obligatory and highly contagious human parasite is transmitted by sexual contact, frequently in combination with other sexually transmitted diseases. Although it is considered a sexually transmitted disease, pubic lice infestation is not a reportable condition in the United States. Clinical confirmation can be reached by identification in skin scraping with a light microscope and mineral oil of adult parasites and/or viable eggs (nits) (Fig. 6.32) on hair shafts [136]. An adult female can lay 300 eggs; after 1 week, the eggs hatch and these nymphs will mature to adults in 10 days.

Fig. 6.32 *Pubic lice*. Egg (nit)

Clinical Features

The clinical presentation is dominated by severe pubic itch that worsen at night. It is believed that the latter is the result of a hypersensitivity reaction to feeding lice. Clinical findings include blue macules (maculae ceruleae) or feeding sites, erythema, and excoriations [137]. Identification of *Phthirus pubis* adult parasite and nits can be done with the naked eye if the clinician suspects this diagnosis.

Histopathology and Differential Diagnosis

In general, lice does not keep attached to their host after feeding; therefore, the histologic sections of the lesion of the bite site may show ulceration and a perivascular mixed inflammatory reaction with eosinophils. The adult parasite of 0.8–1.2 mm length has three pairs of legs; the first pair vestigial and the other two in the form of claws. The body is as wide as long, allowing grasping widely the spaced pubic hair. On histologic cut surface, the parasite shows a chitinous body containing straight muscle and red blood cells within the gut. The mouthparts can be pigmented. Its histomorphology has large overlap

with ticks like Amblyomma ticks and/or Ixodes ticks. Scraping of the skin for the adult parasite and search for nits in the hair shafts would clarify the diagnosis. Clinically, white piedra and trichomycosis pubis are also differential diagnosis. White piedra caused by *Trichosporon mucoides*, *Trichosporon asteroids*, *and Trichosporon cutaneum* can be diagnosed by direct observation of the hair shaft nodule with 10–15 % KOH preparation on a glass slide. The nodule will reveal hyphae, blastoconidia, and arthroconidia attached to the hair shaft. On the other hand, trichomycosis is a bacterial infection of hair shafts in sweat gland-bearing areas. Hair cultures showing the growth of the anaerobic diphtheroids will confirm the diagnosis.

Summary

Clinical Presentation

- Pruritus, especially at night
- Maculae ceruleae or feeding site and excoriations
- Secondary bacterial folliculitis

Histologic Features

- Phthirus pubis: 0.8–1.2 mm long adult parasite with three pair of legs
- Nits (eggs): white minute gritty projections attached to hair shaft

Differential Diagnosis

- White piedra
- Trichomycosis pubis

Takeaway Essentials

Clinical Relevant Pearls

- The presence of eggs attached to the hair shafts in the pubis is not an evidence of active disease as it can be empty eggs.
- These patients need to be thoroughly investigated for other sexually transmitted diseases.
- Digital epiluminescent dermatoscopy can help to identify the ectoparasite.

Pathology Interpretation Pearls

- Evaluation of the adult parasite and nits will confirm diagnosis.
- Advise the submission of hair shafts to work up the differential diagnosis of white nodules attached to the hair shaft.
- Direct inspection of hair shafts searching for fungal forms and culture for bacteria can rule out white piedra and trichomycosis pubis.

Case Vignettes

Vignette 1

Clinical history: 12-year-old girl with a painful deep and necrotic ulcer in the labia majora with yellow adherent membrane (Fig. 6.33). Tonsillitis and adenopathy are also noted. The patient denies to be sexually active.

Microscopic description: Sections show epidermal ulceration with necrosis of adnexal structures (Fig. 6.34a). Florid lymphoplasmacytic infiltrate with abundant neutrophils is noted (Fig. 6.34b). The vessels show thrombosis with presence of inflammation in the wall (Fig. 6.34c).

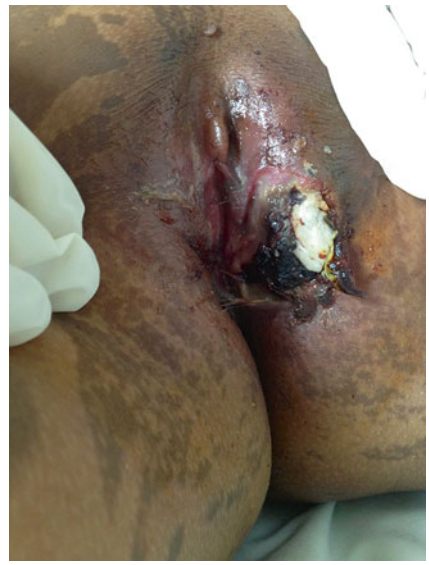


Fig. 6.33 Vignette 1. Deep necrotic ulcer in the labia majora

(continued)

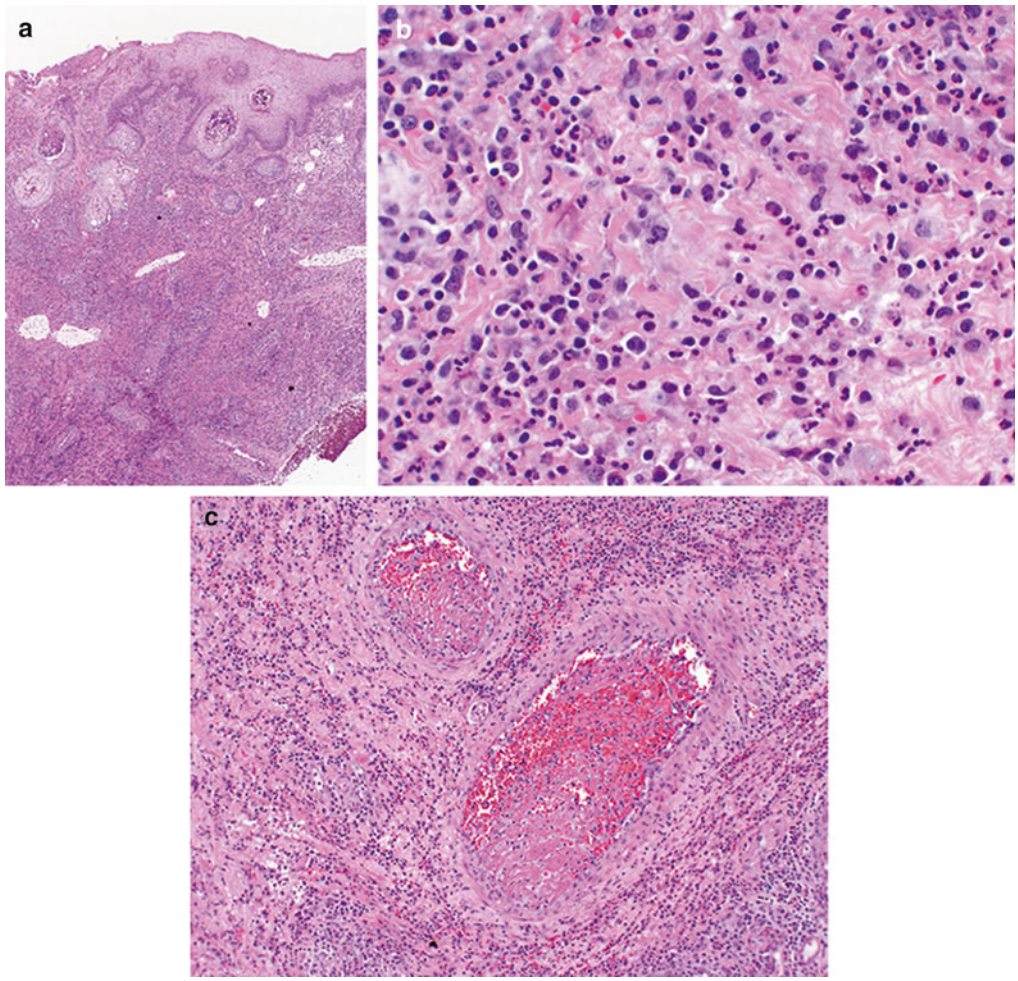


Fig. 6.34 (**a**–**c**) Vignette 1. Epidermal ulceration with necrosis of adnexal structures (**a**). Mixed acute and chronic inflammation is noted (**b**) as well as vascular thrombosis (**c**)

Diagnosis: Epstein–Barr virus vulvar ulcer

Discussion: The presence of a vulvar ulcer in prepuberal girls is of concern. Although aphthous ulcers can be seen in this population, the deep necrotic ulcer with irregular borders is somewhat unusual for that clinical diagnosis. The presence of ulcer with necrotic folliculosebaceous units raises the differential diagnosis of herpes virus infection; however, the classic viral cytopathic changes like multinucleation of keratinocytes with molding nuclei and margination of chromatin are not seen. The detection of tonsillitis and adenopathy or mononucleosis-like symptoms raises the concern for EBV infection. The latter is a known etiology of vulvar ulcers in nonsexually active prepuberal girls. Serologies demonstrating acute infection will confirm the diagnosis.

Vignette 2

Clinical history: 26-year-old HIV + pregnant woman with mass in the vulva (Fig. 6.35).

Microscopic description: Section shows pseudoepitheliomatous epidermal hyperplasia (Fig. 6.36a) overlying a massive lymphoplasmacytic infiltrate (Fig. 6.36b) with pockets of acute inflammation. Keratinocytes with multinucleated molding nuclei exhibiting margination of the chromatin are noted (Fig. 6.36c). Immunohistochemical stains for herpes virus simplex is positive (Fig. 6.36d).

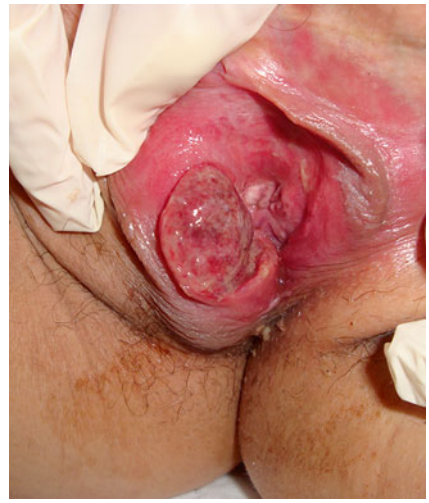


Fig. 6.35 Vignette 2. Vulvar ulcerated polypoid mass

(continued)

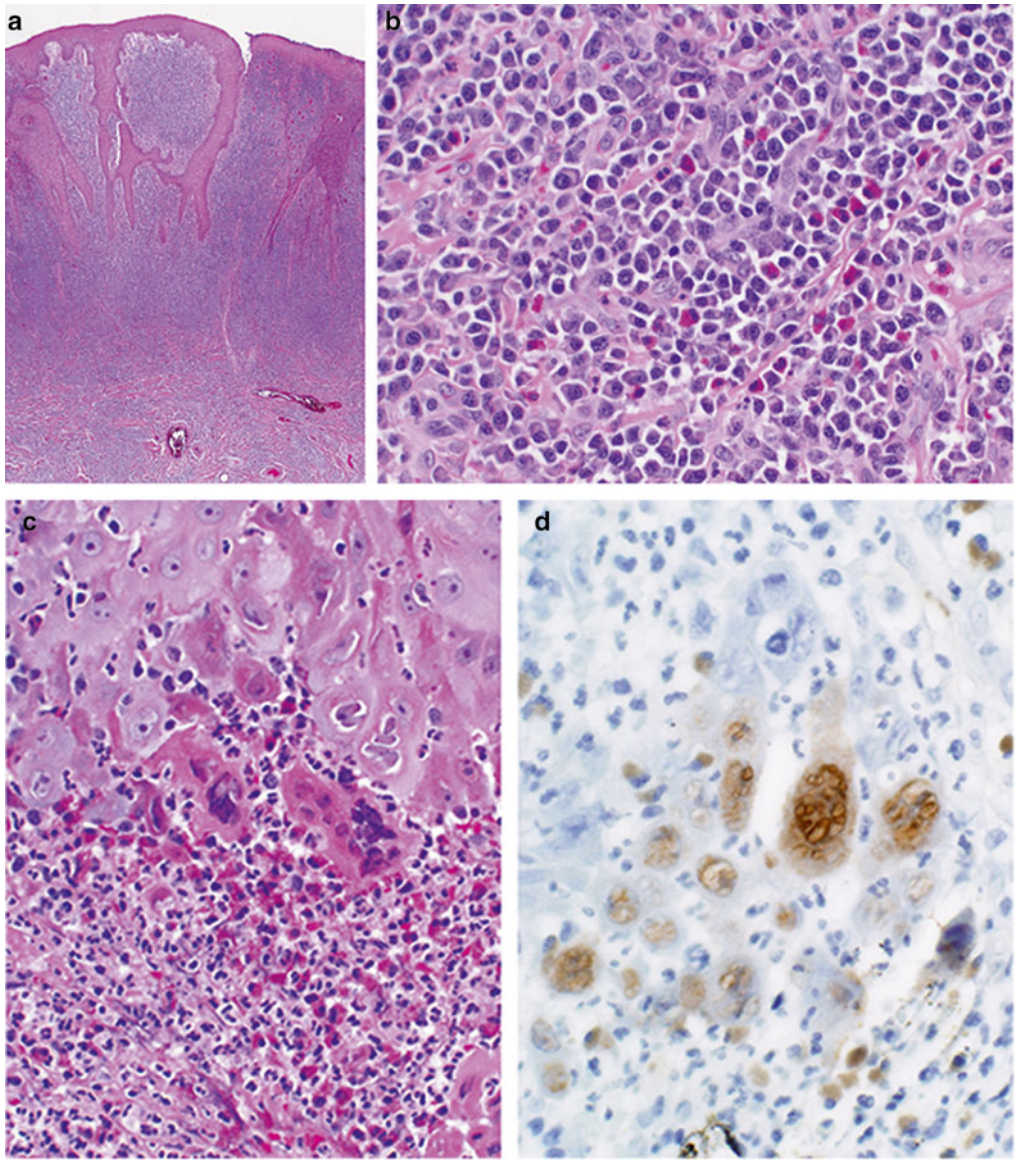


Fig. 6.36 (**a**–**d**) *Vignette 2*. Pseudoepitheliomatous hyperplasia (**a**) overlying a massive lymphoplasmacytic infiltrate (**b**). Keratinocytes with multinucleated molding nuclei are noted (**c**). Immunohistochemical for herpes viral simplex reacts with these cells (**d**)

Diagnosis: Chronic hypertrophic herpes infection

Discussion: Patients suffering from HIV infection may develop unusual and severe clinical presentation of herpes virus infection. Lesions in this clinical setting may be increasing in size and number, remarkably painful, and slow to heal. In addition, hypertrophic or verrucous presentation may be identified. Clinically, the chronic hypertrophic herpes needs to be differentiated from squamous cell carcinoma. The lack of significant cytologic atypia, atypical mitosis, and desmoplastic reaction coexisting with viral cytopathic changes supports the diagnosis of herpes infection. Immunohistochemical stains assist in diagnosis and subtyping of the virus.

Vignette 3

Clinical history: 41-year-old woman with clinical history of "wart nonresponsive to treatment," concerning for dysplasia.

Microscopic description: Well-circumscribed cup-shaped epidermal acanthosis with hyperkeratosis and degenerated keratinocytes with retained or loss nuclei (Fig. 6.37a, b). The keratinocytes of the granular and spinous layers show variable amount of vacuolar and reticular degeneration with coarse keratohyaline granules and eosinophilic material arranged in granules and strands (Fig. 6.37c). Mild perivascular chronic inflammation and dilated papillary vessels are identified.

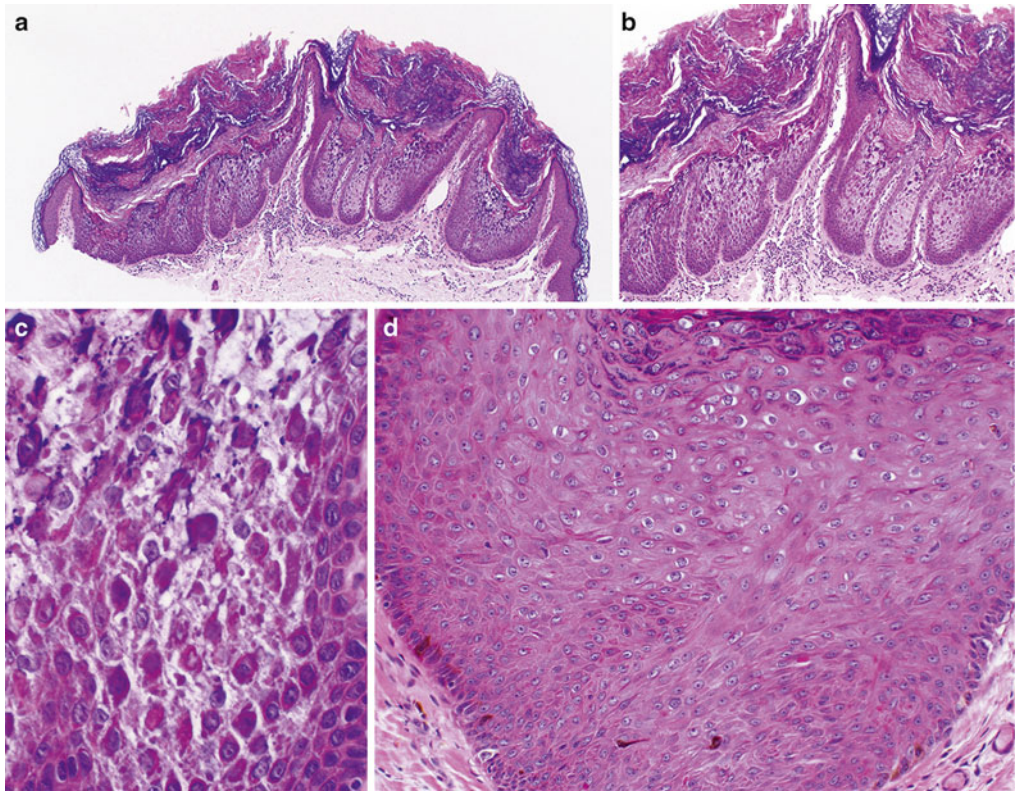


Fig. 6.37 (**a**–**d**) *Vignette 3*. Well-circumscribed cup-shaped epidermal acanthosis (**a**) with focal papillomatosis (**b**). Keratinocytes in the granular and spinous layers show vacuolar and reticulated degeneration, coarse keratohyaline granules, and eosinophilic material arranged in granules and strands (**c**). Although HPV lesion shows coarse keratohyaline granules, these lesions are characterized by koilocytes (**d**)

Diagnosis: Epidermolytic acanthoma

Discussion: Epidermolytic hyperkeratosis (EPH) is an abnormality of epidermal maturation characterized by hyperkeratosis associated with keratinocytes with clear spaces, eosinophilic perinuclear globules ("cell within a cell") or bands, reticular degeneration, and variably sized basophilic granules. All the previously enumerated changes affect predominantly the granular and spinous layers.

(continued)

EPH can be seen not only as part of ichthyosis but also in other keratinization disorders and even be a random event. The cutaneous manifestations of generalized or localized EPH are caused by mutations in the genes encoding keratins 1 and 10.

When EPH changes are noted in a benign localized proliferation of keratinocytes, it has been named epidermolytic acanthoma. Genital location is the most common location for solitary epidermolytic acanthomas [138]. Due to the clinical presentation as a small keratotic plaque, frequently they are clinically thought to be HPV lesions (e.g., verruca vulgaris or condyloma acuminatum), resulting in concern of sexually transmitted disease for the patient and potential unnecessary treatment. The thick keratohyaline basophilic granules and the occasionally papillomatous epidermis add to this confusion. However, HPV lesions will show koilocytes as evidence of the viral infection, not seen in epidermolytic hyperkeratosis [139] (Fig. 6.37d). When in doubt, immunohistochemical stains or CISH can confirm the absence of viral particles in epidermolytic hyperkeratosis.

Abbreviations

CMV	Cytomegalovirus
EBV	Epstein Barr virus
HPV	Human papilloma virus
HSV	Herpes simplex virus
LGV	Lymphogranuloma venereum
MC	Molluscum contagiosum
SCCIS	Squamous cell carcinoma in-situ
STD	Sexual transmitted diseases
VZV	Varicella-zoster virus

References

1. Roett MA, Mayor MT, Uduhiri KA. Diagnosis and management of genital ulcers. Am Fam Physician. 2012;85(3):254–62.
2. Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ. 2008;86(10):805–12.
3. Whitley RJ, Kimberlin DW, Roizman B. Herpes simplex viruses. Clin Infect Dis. 1998;26(3):541–53. quiz 554–5.
4. Bradley H, Markowitz LE, Gibson T, McQuillan GM. Seroprevalence of herpes simplex virus types 1 and 2–United States, 1999–2010. J Infect Dis. 2014; 209(3):325–33.
5. Hofstetter AM, Rosenthal SL, Stanberry LR. Current thinking on genital herpes. Curr Opin Infect Dis. 2014;27(1):75–83.
6. Kortekangas-Savolainen O, Orhanen E, Puodinketo T, Vuorinen T. Epidemiology of genital herpes simplex virus type 1 and 2 infections in southwestern Finland during a 10-year period (2003–2012). Sex Transm Dis. 2014;41(4):268–71.
7. Tuokko H, Bloigu R, Hukkanen V. Herpes simplex virus type 1 genital herpes in young women: current trend in Northern Finland. Sex Transm Infect. 2014; 90(2):160.
8. Roberts CM, Pfister JR, Spear SJ. Increasing proportion of herpes simplex virus type 1 as a cause of genital herpes infection in college students. Sex Transm Dis. 2003;30(10):797–800.
9. Belshe RB, Leone PA, Bernstein DI, Wald A, Levin MJ, Stapleton JT, et al. Efficacy results of a trial of a herpes simplex vaccine. N Engl J Med. 2012;366(1): 34–43.
10. Bernstein DI, Bellamy AR, Hook EW, Levin MJ, Wald A, Ewell MG, et al. Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. Clin Infect Dis. 2013;56(3): 344–51.
11. Langenberg AG, Corey L, Ashley RL, Leong WP, Straus SE. A prospective study of new infections with herpes simplex virus type 1 and type 2. Chiron HSV Vaccine Study Group. N Engl J Med. 1999; 341(19):1432–8.
12. Ludwig RJ, Grundmann-Kollmann M, Holtmeier W, Wolter M, Glas J, Podda M, et al. Herpes simplex virus type 2-associated eosinophilic cellulitis (Wells' syndrome). J Am Dermatol. 2003;48(5 Suppl): S60–1.
13. Griffith-Bauer K, O'Hearn M, Ehst BD. Chronic ulcerative herpes simplex virus infection of the vulva. Case Rep Dermatol. 2012;4(3):192–6.
14. Czelusta A, Yen-Moore A, Van der Straten M, Carrasco D, Tyring SK. An overview of sexually transmitted diseases. Part III. Sexually transmitted diseases in HIV-infected patients. J Am Dermatol. 2000;43(3):409–32; quiz 433–6.
15. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2

infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS. 2006;20(1):73–83.
16. Gray RH, Li X, Wawer MJ, Serwadda D, Sewankambo NK, Wabwire-Mangen F, et al. Determinants of HIV-1 load in subjects with early and later HIV infections, in a general-population cohort of Rakai, Uganda. J Infect Dis. 2004; 189(7):1209–15.
17. Tronstein E, Johnston C, Huang M-L, Selke S, Magaret A, Warren T, et al. Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. JAMA. 2011;305(14): 1441–9.
18. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. N Engl J Med. 1995;333(12):770–5.
19. Phipps W, Saracino M, Magaret A, Selke S, Remington M, Huang M-L, et al. Persistent genital herpes simplex virus-2 shedding years following the first clinical episode. J Infect Dis. 2011;203(2): 180–7.
20. Choi SW, Yang JE, Kang SJ, Lee HR, Kim CW. Herpetic infection on the vulva associated with eccrine squamous syringometaplasia in malignant lymphoma. Acta Derm Venereol. 1999;79(6): 500–1.
21. Rose L, Herra CM, Crowley B. Evaluation of real-time polymerase chain reaction assays for the detection of herpes simplex virus in swab specimens. Eur J Clin Microbiol Infect Dis. 2008;27(9):857–61.
22. Gitman MR, Ferguson D, Landry ML. Comparison of Simplexa HSV 1 & 2 PCR with culture, immunofluorescence, and laboratory-developed TaqMan PCR for detection of herpes simplex virus in swab specimens. J Clin Microbiol. 2013;51(11):3765–9.
23. Philip KEJ, Goodman A, Pallawela SNS, Sathia L, Webster DP. A not so simplex case of genital herpes. BMJ Case Rep. 2013. doi:10.1136/bcr-2013-009993.
24. Oaklander AL, Rissmiller JG. Postherpetic neuralgia after shingles: an under-recognized cause of chronic vulvar pain. Obstet Gynecol. 2002;99(4):625–8.
25. Simon HK, Steele DW. Varicella: pediatric genital/rectal vesicular lesions of unclear origin. Ann Emerg Med. 1995;25(1):111–4.
26. Cone RW, Hobson AC, Palmer J, Remington M, Corey L. Extended duration of herpes simplex virus DNA in genital lesions detected by the polymerase chain reaction. J Infect Dis. 1991;164(4): 757–60.
27. AbdullGaffar B, Raman LG, Muala AA. Cutaneous cytomegalovirus infection in a patient with acquired immunodeficiency syndrome. Int J Dermatol. 2008; 47(9):944–6.
28. Ramdial PK, Dlova NC, Sydney C. Cytomegalovirus neuritis in perineal ulcers. J Cutan Pathol. 2002; 29(7):439–44.
29. Choi Y-L, Kim J-A, Jang K-T, Kim D-S, Kim W-S, Lee J-H, et al. Characteristics of cutaneous cytomegalovirus infection in non-acquired immune deficiency syndrome, immunocompromised patients. Br J Dermatol. 2006;155(5):977–82.
30. Daudén E, Fernández-Buezo G, Fraga J, Cardeñoso L, García-Díez A. Mucocutaneous presence of cytomegalovirus associated with human immunodeficiency virus infection: discussion regarding its pathogenetic role. Arch Dermatol. 2001;137(4): 443–8.
31. Resnik KS, DiLeonardo M, Maillet M. Histopathologic findings in cutaneous cytomegalovirus infection. Am J Dermatopathol. 2000;22(5):397–407.
32. Ryan C, De Gascun CF, Powell C, Sheahan K, Mooney EE, McCormick A, et al. Cytomegalovirus-induced cutaneous vasculopathy and perianal ulceration. J Am Acad Dermatol. 2011;64(6):1216–8.
33. Chetty R, Bramdev A, Govender D. Cytomegalovirus-induced syringosquamous metaplasia. Am J Dermatopathol. 1999;21(5):487–90.
34. Lin H-Y, Linn G, Liu C-B, Chen C-J, Yu K-J. An immunocompromised woman with severe molluscum contagiosum that responded well to topical imiquimod: a case report and literature review. J Low Genit Tract Dis. 2010;14(2):134–5.
35. Palit A, Inamadar AC. Papulonodular genital growths in an HIV-infected woman. Clin Infect Dis. 2004;38(11):1585, 1633–4.
36. Fischer GO. Vulval disease in pre-pubertal girls. Australas J Dermatol. 2001;42(4):225–34; quiz, 235–6.
37. Berger EM, Orlow SJ, Patel RR, Schaffer JV. Experience with molluscum contagiosum and associated inflammatory reactions in a pediatric dermatology practice: the bump that rashes. Arch Dermatol. 2012;148(11):1257–64.
38. Hošnjak L, Kocjan BJ, Kušar B, Seme K, Poljak M. Rapid detection and typing of molluscum contagiosum virus by FRET-based real-time PCR. J Virol Methods. 2013;187(2):431–4.
39. Fathi R, Tsoukas MM. Genital warts and other HPV infections: established and novel therapies. Clin Dermatol. 2014;32(2):299–306.
40. Nuovo GJ, Friedman D, Richart RM. In situ hybridization analysis of human papillomavirus DNA segregation patterns in lesions of the female genital tract. Gynecol Oncol. 1990;36(2):256–62.
41. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010;117 (2 Suppl):S5–10.
42. Roden RB, Lowy DR, Schiller JT. Papillomavirus is resistant to desiccation. J Infect Dis. 1997;176(4): 1076–9.
43. de Koning MNC, ter Schegget J, Eekhof JAH, Kamp M, Kleter B, Gussekloo J, et al. Evaluation of a novel broad-spectrum PCR-multiplex genotyping assay for identification of cutaneous wart-associated human papillomavirus types. J Clin Microbiol. 2010; 48(5):1706–11.
44. Chiasson MA, Ellerbrock TV, Bush TJ, Sun XW, Wright TC. Increased prevalence of vulvovaginal

condyloma and vulvar intraepithelial neoplasia in women infected with the human immunodeficiency virus. Obstet Gynecol. 1997;89(5 Pt 1):690–4.
45. Marshburn PB, Trofatter KF. Recurrent condyloma acuminatum in women over age 40: association with immunosuppression and malignant disease. Am J Obstet Gynecol. 1988;159(2):429–33.
46. Aguilera-Barrantes I, Magro C, Nuovo GJ. Verruca vulgaris of the vulva in children and adults: a nonvenereal type of vulvar wart. Am J Surg Pathol. 2007; 31(4):529–35.
47. Sonnex C, Strauss S, Gray JJ. Detection of human papillomavirus DNA on the fingers of patients with genital warts. Sex Transm Infect. 1999;75(5): 317–9.
48. Nucci MR, Genest DR, Tate JE, Sparks CK, Crum CP. Pseudobowenoid change of the vulva: a histologic variant of untreated condylata acuminatum. Mod Pathol. 1996;9(4):375–9.
49. Zappacosta R, Colasante A, Viola P, D'Antuono T, Lattanzio G, Capanna S, et al. Chromogenic in situ hybridization and p16/Ki67 dual staining on formalin-fixed paraffin-embedded cervical specimens: correlation with HPV-DNA test, E6/E7 mRNA test, and potential clinical applications. Biomed Res Int. 2013;2013:453606.
50. Moyal-Barracco M, Leibowitch M, Orth G. Vestibular papillae of the vulva. Lack of evidence for human papillomavirus etiology. Arch Dermatol. 1990;126(12):1594–8.
51. Konig A, Wennemuth G, Soyer HP, Hoffmann R, Happle R, Krause W. Vulvar amyloidosis mimicking giant condylomata acuminata in a patient with multiple myeloma. Eur J Dermatol. 1999;9(1):29–31.
52. Schiera A, Pini M, Pioltelli P, Rossi E, Valente MG, Crippa D. Perianal condyloma-like lesions in multiple myeloma associated amyloidosis. Eur J Dermatol. 2004;14(3):193–5.
53. Halvorsen JA, Brevig T, Aas T, Skar AG, Slevolden EM, Moi H. Genital ulcers as initial manifestation of Epstein-Barr virus infection: two new cases and a review of the literature. Acta Derm Venereol. 2006; 86(5):439–42.
54. Barnes CJ, Alió AB, Cunningham BB, Friedlander SF. Epstein-Barr virus-associated genital ulcers: an under-recognized disorder. Pediatr Dermatol. 2007; 24(2):130–4.
55. Aroutcheva AA, Simoes JA, Behbakht K, Faro S. Gardnerella vaginalis isolated from patients with bacterial vaginosis and from patients with healthy vaginal ecosystems. Clin Infect Dis. 2001;33(7): 1022–7.
56. Udayalaxmi J, Bhat GK, Kotigadde S. Biotypes and virulence factors of Gardnerella vaginalis isolated from cases of bacterial vaginosis. Indian J Med Microbiol. 2011;29(2):165–8.
57. Modak T, Arora P, Agnes C, Ray R, Goswami S, Ghosh P, et al. Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: comparison of clinical and microbiological criteria. J Infect Dev Ctries. 2011;5(5):353–60.
58. Bleker OP, Smalbraak DJ, Schutte MF. Bartholin's abscess: the role of *Chlamydia trachomatis*. Genitourin Med. 1990;66(1):24–5.
59. Bora SA, Condous G. Bartholin's, vulval and perineal abscesses. Best Pract Res Clin Obstet Gynaecol. 2009;23(5):661–6.
60. Pinsky BA, Baron EJ, Janda JM, Banaei N. Bartholin's abscess caused by hypermucoviscous *Klebsiella pneumoniae*. J Med Microbiol. 2009;58 (Pt 5):671–3.
61. Rees E. Gonococcal bartholinitis. Br J Vener Dis. 1967;43(3):150–6.
62. Eady EA, Cove JH. Staphylococcal resistance revisited: community-acquired methicillin resistant *Staphylococcus aureus*–an emerging problem for the management of skin and soft tissue infections. Curr Opin Infect Dis. 2003;16(2):103–24.
63. Kilpatrick CC, Alagkiozidis I, Orejuela FJ, Chohan L, Hollier LM. Factors complicating surgical management of the vulvar abscess. J Reprod Med. 2010; 55(3–4):139–42.
64. Reichman O, Sobel JD. MRSA infection of buttocks, vulva, and genital tract in women. Curr Infect Dis Rep. 2009;11(6):465–70.
65. Cabrera H, Skoczdopole L, Marini M, Giovanna Della P, Saponaro A, Echeverría C. Necrotizing gangrene of the genitalia and perineum. Int J Dermatol. 2002;41(12):847–51.
66. Martinelli G, Alessandrino EP, Bernasconi P, Caldera D, Colombo A, Malcovati L, et al. Fournier's gangrene: a clinical presentation of necrotizing fasciitis after bone marrow transplantation. Bone Marrow Transpl. 1998;22(10):1023–6.
67. Kroshinsky D, Alloo A, Rothschild B, Cummins J, Tan J, Montecino R, et al. Necrotizing Sweet syndrome: a new variant of neutrophilic dermatosis mimicking necrotizing fasciitis. J Am Acad Dermatol. 2012;67(5):945–54.
68. Peeling RW, Hook EW. The pathogenesis of syphilis: the Great Mimicker, revisited. J Pathol. 2006; 208(2):224–32.
69. Avenel G, Goëb V, Abboud P, Ait-Abdesselam T, Vittecoq O. Atypical forms of syphilis: two cases. Joint Bone Spine. 2009;76(3):293–5.
70. Schnirring-Judge M, Gustaferro C, Terol C. Vesiculobullous syphilis: a case involving an unusual cutaneous manifestation of secondary syphilis. J Foot Ankle Surg. 2011;50(1):96–101.
71. Pournaras CC, Masouyé I, Piletta P, Piguet V, Saurat J-H, French LE. Extensive annular verrucous late secondary syphilis. Br J Dermatol. 2005;152(6):1343–5.
72. Buffet M, Grange PA, Gerhardt P, Carlotti A, Calvez V, Bianchi A, et al. Diagnosing *Treponema pallidum* in secondary syphilis by PCR and immunohistochemistry. J Invest Dermatol. 2007;127(10):2345–50.
73. Jethwa HS, Schmitz JL, Dallabetta G, Behets F, Hoffman I, Hamilton H, et al. Comparison of molecular and microscopic techniques for detection of *Treponema pallidum* in genital ulcers. J Clin Microbiol. 1995;33(1):180–3.

74. Don PC, Rubinstein R, Christie S. Malignant syphilis (lues maligna) and concurrent infection with HIV. Int J Dermatol. 1995;34(6):403–7.
75. Liotta EA, Turiansky GW, Berberian BJ, Sulica VI, Tomaszewski MM. Unusual presentation of secondary syphilis in 2 HIV-1 positive patients. Cutis. 2000;66(5):383–6, 389.
76. Carlson JA, Dabiri G, Cribier B, Sell S. The immunopathobiology of syphilis: the manifestations and course of syphilis are determined by the level of delayed-type hypersensitivity. Am J Dermatopathol. 2011;33(5):433–60.
77. Zinkernagel RM. Antiinfection immunity and autoimmunity. Ann N Y Acad Sci. 2002;958:3–6.
78. Smith G, Holman RP. The prozone phenomenon with syphilis and HIV-1 co-infection. South Med J. 2004;97(4):379–82.
79. Hoang MP, High WA, Molberg KH. Secondary syphilis: a histologic and immunohistochemical evaluation. J Cutan Pathol. 2004;31(9):595–9.
80. Jeerapaet P, Ackerman AB. Histologic patterns of secondary syphilis. Arch Dermatol. 1973;107(3): 373–7.
81. Martín-Ezquerra G, Fernandez-Casado A, Barco D, Jucglà A, Juanpere-Rodero N, Manresa JM, et al. Treponema pallidum distribution patterns in mucocutaneous lesions of primary and secondary syphilis: an immunohistochemical and ultrastructural study. Hum Pathol. 2009;40(5):624–30.
82. Gayet-Ageron A, Ninet B, Toutous-Trellu L, Lautenschlager S, Furrer H, Piguet V, et al. Assessment of a real-time PCR test to diagnose syphilis from diverse biological samples. Sex Transm Infect. 2009;85(4): 264–9.
83. Kouznetsov AV, Prinz JC. Molecular diagnosis of syphilis: the Schaudinn-Hoffmann lymph-node biopsy. Lancet. 2002;360(9330):388–9.
84. Wenhai L, Jianzhong Z, Cao Y. Detection of *Treponema pallidum* in skin lesions of secondary syphilis and characterization of the inflammatory infiltrate. Dermatology. 2004;208(2):94–7.
85. Chudomirova K, Chapkanov A, Abadjieva T, Popov S. Gummatous cutaneous syphilis. Sex Transm Dis. 2009;36(4):239–40.
86. Centers for Disease Control and Prevention. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*–2014. MMWR Recomm Rep. 2014;63 (RR-02):1–19.
87. Mohammed TT, Olumide YM. Chancroid and human immunodeficiency virus infection–a review. Int J Dermatol. 2008;47(1):1–8.
88. Trees DL, Morse SA. Chancroid and *Haemophilus ducreyi*: an update. Clin Microbiol Rev. 1995;8(3): 357–75.
89. Janowicz DM, Tenner-Racz K, Racz P, Humphreys TL, Schnizlein-Bick C, Fortney KR, et al. Experimental infection with *Haemophilus ducreyi* in persons who are infected with HIV does not cause local or augment systemic viral replication. J Infect Dis. 2007; 195(10):1443–51.
90. Freinkel AL. Histological aspects of sexually transmitted genital lesions. Histopathology. 1987;11(8): 819–31.
91. Abeck D, Freinkel AL, Korting HC, Szeimis RM, Ballard RC. Immunohistochemical investigations of genital ulcers caused by *Haemophilus ducreyi*. Int J STD AIDS. 1997;8(9):585–8.
92. Schachter J. Chlamydial infections (third of three parts). N Engl J Med. 1978;298(10):540–9.
93. Schachter J, Osoba AO. Lymphogranuloma venereum. Br Med Bull. 1983;39(2):151–4.
94. Wu JJ, Huang DB, Pang KR, Tyring SK. Selected sexually transmitted diseases and their relationship to HIV. Clin Dermatol. 2004;22(6):499–508.
95. Tchernev G, Salaro C, Costa MC, Patterson JW, Nenoff P. Lymphogranuloma venereum: "a clinical and histopathological chameleon?". An Bras Dermatol. 2010;85(4):525–30.
96. Joseph AK, Rosen T. Laboratory techniques used in the diagnosis of chancroid, granuloma inguinale, and lymphogranuloma venereum. Dermatol Clin. 1994;12(1):1–8.
97. O'Farrell N. Donovanosis. Sex Transm Infect. 2002;78(6):452–7.
98. Sardana K, Garg VK, Arora P, Khurana N. Malignant transformation of donovanosis (granuloma inguinale) in a HIV-positive patient. Dermatol Online J. 2008;14(9):8.
99. Carter JS, Bowden FJ, Bastian I, Myers GM, Sriprakash KS, Kemp DJ. Phylogenetic evidence for reclassification of *Calymmatobacterium granulomatis* as *Klebsiella granulomatis* comb. nov. Int J Syst Bacteriol. 1999;49 Pt 4:1695–700.
100. Stoler MH, Bonfiglio TA, Steigbigel RT, Pereira M. An atypical subcutaneous infection associated with acquired immune deficiency syndrome. Am J Clin Pathol. 1983;80(5):714–8.
101. Tappero JW, Mohle-Boetani J, Koehler JE, Swaminathan B, Berger TG, LeBoit PE, et al. The epidemiology of bacillary angiomatosis and bacillary peliosis. JAMA. 1993;269(6):770–5.
102. Ramdial PK, Sing Y, Ramburan A, Dlova NC, Bagratee JS, Calonje E. Bartonella quintana-induced vulval bacillary angiomatosis. Int J Gynecol Pathol. 2012;31(4):390–4.
103. Long SR, Whitfeld MJ, Eades C, Koehler JE, Korn AP, Zaloudek CJ. Bacillary angiomatosis of the cervix and vulva in a patient with AIDS. Obstet Gynecol. 1996;88(4 Pt 2):709–11.
104. Shen H-P, Chang W-C, Hsieh C-H, Yang T-C, Hung Y-C. Vulvar tuberculosis. Taiwan J Obstet Gynecol. 2011;50(1):106–8.
105. Dhall K, Das SS, Dey P. Tuberculosis of Bartholin's gland. Int J Gynaecol Obstet. 1995;48(2):223–4.
106. Brenner BN. Tuberculosis of the vulva: case reports. S Afr Med J. 1976;50(44):1798–800.
107. Amin I, Idrees M, Awan Z, Shahid M, Afzal S, Hussain A. PCR could be a method of choice for identification of both pulmonary and extrapulmonary tuberculosis. BMC Res Notes. 2011; 4:332.

108. Buppasiri P, Temtanakitpaisan T, Somboonporn W. Tuberculosis at vulva and vagina. J Med Assoc Thai. 2010;93(5):613–5.
109. Manoj K, Soma M, Ajay L, Ashish A, Rakesh S, Paliwal RV. Tubercular sinus of labia majora: rare case report. Infect Dis Obstet Gynecol. 2008;2008: 817515.
110. Naik RP, Srinivas CR, Balachandran C, Narayan PK, Ramnarayan K, Sahoo RC. Esthiomene resulting from cutaneous tuberculosis of external genitalia. Genitourin Med. 1987;63(2):133–4.
111. Mendiratta V, Harjai B, Sardana K. Tubercular lymphadenitis with lymphangiectases of the vulva. J Eur Acad Dermatol Venereol. 2005;19(2): 264–5.
112. Talwar A, Puri N, Sandhu HPS. Vulval lymphoedema following pulmonary tuberculosis. Int J STD AIDS. 2009;20(6):437–9.
113. Wong S, Rizvi H, Cerio R, O'Toole EA. An unusual case of vulval papulonecrotic tuberculid. Clin Exp Dermatol. 2010;36(3):277–80.
114. Pandhi D, Mehta S, Singal A. Genital tuberculid in a female child: a new entity (childhood vulval tuberculid). Pediatr Dermatol. 2007;24(5):573–5.
115. Bhat RM, Saldanha CS, Kambil SM, Dandakeri S. Cutaneous lymphangiectasia of the vulva secondary to tuberculosis. Indian J Sex Transm Dis. 2012; 33(1):35–7.
116. Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Microbiol Rev. 2010;23(2): 253–73.
117. Jacobsen MD, Duncan AD, Bain J, Johnson EM, Naglik JR, Shaw DJ, et al. Mixed *Candida albicans* strain populations in colonized and infected mucosal tissues. FEMS Yeast Res. 2008;8(8):1334–8.
118. Fischer G. Chronic vulvovaginal candidiasis: what we know and what we have yet to learn. Australas J Dermatol. 2012;53(4):247–54.
119. Goswami R, Dadhwal V, Tejaswi S, Datta K, Paul A, Haricharan RN, et al. Species-specific prevalence of vaginal candidiasis among patients with diabetes mellitus and its relation to their glycaemic status. J Infect. 2000;41(2):162–6.
120. Sobel JD. Vulvovaginal candidosis. Lancet. 2007; 369(9577):1961–71.
121. McClelland RS, Richardson BA, Hassan WM, Graham SM, Kiarie J, Baeten JM, et al. Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. J Infect Dis. 2009; 199(12):1883–90.
122. Foxman B, Muraglia R, Dietz J-P, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. J Low Genit Tract Dis. 2013;17(3):340–5.
123. Mayser P, Schütz M, Schuppe HC, Jung A, Schill WB. Frequency and spectrum of *Malassezia* yeasts in the area of the prepuce and glans penis. BJU Int. 2001;88(6):554–8.
124. Day T, Scurry J. Vulvar pityriasis versicolor in an immunocompetent woman. J Low Genit Tract Dis. 2014 Jul;18(3):e71–3.
125. Nyirjesy P, Nixon JM, Jordan CA, Buckley HR. Malassezia furfur folliculitis of the vulva: olive oil solves the mystery. Obstet Gynecol. 1994;84 (4 Pt 2):710–1.
126. Chang SE, Lee DK, Choi JH, Moon KC, Koh JK. Majocchi's granuloma of the vulva caused by Trichophyton mentagrophytes. Mycoses. 2005; 48(6):382–4.
127. Galvin SR, Cohen MS. The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol. 2004;2(1):33–42.
128. Vasoo S, Pritt BS. Molecular diagnostics and parasitic disease. Clin Lab Med. 2013;33(3):461–503.
129. Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin Microbiol Rev. 1998;11(2):300–17.
130. Mounsey KE, McCarthy JS, Walton SF. Scratching the itch: new tools to advance understanding of scabies. Trends Parasitol. 2013;29(1):35–42.
131. Foo CW, Florell SR, Bowen AR. Polarizable elements in scabies infestation: a clue to diagnosis. J Cutan Pathol. 2013;40(1):6–10.
132. Bakos L, Reusch MC, D'Elia P, Aquino V, Bakos RM. Crusted scabies of the vulva. J Eur Acad Dermatol Venereol. 2007;21(5):682–4.
133. Kempf W. CD30+ lymphoproliferative disorders: histopathology, differential diagnosis, new variants, and simulators. J Cutan Pathol. 2006;33(S1): 58–70.
134. Bhattacharjee P, Glusac EJ. Langerhans cell hyperplasia in scabies: a mimic of Langerhans cell histiocytosis. J Cutan Pathol. 2007;34(9):716–20.
135. Burch JM. Sarcoptes scabiei infestation misdiagnosed and treated as Langerhans cell histiocytosis. Pediatr Dermatol. 2004;21:58–62.
136. Anderson AL, Chaney E. Pubic lice (Pthirus pubis): history, biology and treatment vs. knowledge and beliefs of US college students. Int J Environ Res Public Health. 2009;6(2):592–600.
137. Leone PA. Scabies and pediculosis pubis: an update of treatment regimens and general review. Clin Infect Dis. 2007;44 Suppl 3:S153–9.
138. Kazlouskaya V, Lambe J, Elston D. Solitary epidermolytic acanthoma. J Cutan Pathol. 2013;40(8): 701–7.
139. Quinn TR, Young RH. Epidermolytic hyperkeratosis in the lower female genital tract: an uncommon simulant of mucocutaneous papillomavirus infection–a report of two cases. Int J Gynecol Pathol. 1997; 16(2):163–8.

Part III

Melanocytic and Squamous Proliferations of the Vulva

Pigmentary Alterations and Benign Melanocytic Lesions of the Vulva

7

Konstantinos Linos, Tien Anh Nguyen Tran, Martin A. Sangueza, and J. Andrew Carlson

Introduction

The number of melanocytes and melanin density is highest in genital skin relative to other anatomic regions. In addition, physiologic hormones and environmental factors affect melanocytes and melanin production [1, 2]. Not surprisingly, pigmentary alterations of the vulva are frequently encountered in clinical practice, in roughly 1 of 10 patients (mean, 7.6 %; range, 2.1–10.5 %) [3–5]. These lesions can be divided by whether the pigmentation is increased (hyperpigmentation) or decreased (hypopigmentation). Patients may present with concern about the areas of pigment alteration, but the majority is unaware of their lesions, particularly the hyperpigmented ones [4, 6]. Not uncommonly, the clinician first detects these pigmentary changes during a gynecologic examination. With respect to increased pigmentation, in a consecutive prospective series of 301 women, approximately 12 % of the patients had either a pigmented lesion or hyperpigmentation. Of the pigmented lesions (10.3 %), the majority were circumscribed lentigines (a.k.a. genital or vulvar melanosis) (7 %), followed by melanocytic nevi (2.3 %), seborrheic keratosis (0.3 %), and hemangioma (0.7 %). Included in the category of patchy or diffuse hyperpigmentation (2 %) were cases of post-inflammatory pigment changes (1.7 %) and pigmented vulvar intraepithelial neoplasia (VIN) (a.k.a. Bowen's disease) (0.3 %) [4]. The diversity of clinical and histologic characteristics of these pigmented disorders on mucous membranes and anogenital skin are less straightforward compared to other cutaneous surfaces. Large, irregular patches and plaques can mimic melanoma, whereas small, banal-appearing papules can represent melanoma. White patches can signify lichen sclerosus, vitiligo, or hyperkeratosis in the context of an eczematous dermatosis. Hence, biopsy is crucial in the accurate diagnosis and effective management of women presenting with vulvar pigmentation abnormalities.

Hyperpigmentation

Physiologic Hyperpigmentation

Clinical Features

Physiologic hyperpigmentation can be mistaken for a disorder of hyperpigmentation. It is characterized by symmetric, asymptomatic hyperpigmentation on the external genitalia in women

J.A. Carlson (✉)
Department of Pathology, Albany Medical College, Albany, NY, USA
e-mail: carlsoa@mail.amc.edu

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_7,

who are naturally dark complexioned and in women experiencing hormonal influences due to pregnancy and infertility therapy [7]. These pigmented patches are ill defined and accentuated on the tips of the labia minora, perianal skin, and, often, the posterior vestibule. Key to clinical diagnosis is the following findings: symmetric distribution, lack of symptoms, absence of surface change, and pigmentation that is accentuated on the labia minora [7]. Adrenal insufficiency (Addison disease) and hyperadrenocorticism (Cushing disease/syndrome) can also manifest as genital hyperpigmentation similar to physiologic hyperpigmentation as well as diffuse hyperpigmentation of sun-exposed areas, scars, and regions of friction [8, 9]. In these settings, high levels of alpha-melanocyte-stimulating hormone (α-MSH) are believed to be responsible for the hyperpigmentation [10].

Histopathology

Physiological hyperpigmentation is a clinical diagnosis. Skin biopsy is usually nondiagnostic or demonstrates an increase of melanin in the melanocytes and basal keratinocytes [11]. This histopathologic pattern is similar to cases of genital melanosis described below (e.g., presence of melanin incontinence, pattern of epidermal melanization, presence of dendritic melanocytes, and type of epidermal acanthosis). However, while the histologic findings overlap, the clinical pattern of pigmentation facilitates making the correct diagnosis.

Summary

Clinical presentation

- Symmetric, asymptomatic hyperpigmentation of the external genitalia
- Ill defined and accentuated on the labia minora

Histologic Features

- May demonstrate an increase of melanin in the melanocytes and basal keratinocytes

Differential Diagnosis

- Genital melanosis

Takeaway Essentials

Clinical Relevant Pearls

- Physiologic hyperpigmentation is a clinical diagnosis
- Adrenal insufficiency (Addison's disease) and hyperadrenocorticism (Cushing's disease/syndrome) can also manifest as genital hyperpigmentation similar to physiologic hyperpigmentation

Pathology Interpretation Pearls

- Histologic pattern similar to genital melanosis

Melanotic Macule (a.k.a. Vulvar Melanosis, Vulvar Lentiginosis)

Clinical Features

Various terms have been used to describe this entity with the most common ones including vulvar melanotic macule, vulvar melanosis, genital melanosis, and vulvar lentiginosis [12–16]. Vulvar melanosis is a fairly common, benign condition and comprises approximately 68 % of pigmented vulvar lesions [4]. Similar clinical and histopathologic features occur in penis, perianal region, lip, oral mucosa, conjunctiva, volar surfaces, and the nail apparatus [8]. Most of the time, vulvar melanotic macules go unnoticed by the patient, as they are asymptomatic and small. Rarely, they are accompanied by pruritus. Melanotic macules predominantly occur on the mucosal surfaces of the vulva rather than the keratinized skin. The labia minora and posterior vestibule are the sites most commonly involved [16]. They may, however, occur throughout the vulva including the medial labia majora, introitus, perineum, vagina, and uterine cervix [9, 17]. Vulvar melanosis is commonly encountered in premenopausal women, but when young girls are affected, several syndromes should be considered: Bannayan-Riley-Ruvalcaba syndrome, Peutz-Jeghers syndrome, Cockayne syndrome, NAME (nevi, atrial myxoma, myxoid neurofibromas, ephelides) syndrome, LEOPARD (lentigines,

electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retarded growth and deafness) syndrome, LAMB (lentigines, atrial myxomas, blue nevi) syndrome, Cushing disease, Carney syndrome, Laugier-Hunziker syndrome, and Albright syndrome [7–9]. Vulvar melanosis has been described in all races [8, 12, 14, 15].

Clinically, genital melanosis (vulvar melanotic macule/patch) presents as a single or multiple macules of brown to blue-black pigment, which may coalesce [7] and range from a few millimeters up to several centimeters in size [18]. These patches can appear uniform and well circumscribed but sometimes are asymmetric with very irregular contours meeting the "ABCD" (asymmetry, irregular borders, multiple colors, and diameter >5 mm) clinical criteria for melanoma (Fig. 7.1, left panel); therefore, a biopsy is required to establish the diagnosis. Melanotic macules and patches may have alternating areas of pigmented and normal mucosa resembling areas of regression. Unlike melanoma, vulvar melanosis is not associated with palpable nodules, induration, or ulceration [13]. Regarding terminology, some authors advocate the use of lentiginosis for clinically small, less than 5 mm, well-circumscribed tan-brown to blue-black hyperpigmented areas and reserve genital melanosis for large, ≥ 5 mm macules with irregular borders [4, 6]. However, we prefer the term genital melanosis over lentiginosis because melanosis specifically highlights the dominant histopathologic finding of pigmented/melanized keratinocytes.

The underlying cause and pathogenesis for vulvar melanosis is unknown, but it appears to be a reactive phenomenon [19] showing features that overlap with post-inflammatory hyperpigmentation, as it is known to occur in the setting of lichen sclerosus [20–22] and frequently associated with subepithelial fibrosis, melanophages, and sparse inflammatory infiltrates [23]. Some cases have been reported in patients with a clinical history of oral contraceptive usage [13] or psoralen ultraviolet A (PUVA) treatment [16]. Also, some consider the possibility that a defect in the normal transport of melanin to the suprabasal keratinocytes of the epidermis is responsible for the basal layer hyperpigmentation [24]. This theory is in line with the concept of "chromatic tendency" which determines the development of hyperpigmentation or hypopigmentation based on the robustness or lack thereof of melanocyte function and viability [25]. Lastly, no association has been shown between human papillomavirus (HPV) infection and vulvar melanotic macules [26]. However, reports in the literature of pigmented warts, frequent occurrence of pigmented high-grade vulvar intraepithelial neoplasia (VIN-3), and lentiginous melanocytic proliferations occurring in pigmented/nonpigmented warts and VIN as well as a possible association of HPV DNA with genital nevi and melanoma [27, 28] appear to provide indirect evidence of HPV infection inducing vulvar pigmentation in some instances. In a recent study of pigmented anogenital intraepithelial neoplasia [29], the main mechanisms of pigmented squamous intraepithelial neoplasia of the anogenital area include melanin incontinence and occurrence of melanin in dysplastic keratinocytes. Colonization of the dysplastic epithelium by dendritic melanocytes appeared to contribute but was rarely a prominent feature.

Vulvar melanosis tends to remain static, although new lesions may evolve over time [23]. Despite sometimes presenting with alarming features clinically indistinguishable from melanoma [13], genital melanosis is a benign entity as no reports of progression to vulvar melanoma exist. Nevertheless, cases of concomitant genital melanosis and melanoma of the urinary bladder, vulva, and/or vagina have been reported, suggesting a possible role of this pigmented lesion in the pathogenesis of melanoma [27, 28, 30]. In addition, vulvar melanosis is infrequently associated with lichen sclerosus, which is a known risk factor for squamous cell carcinoma [31] and has been reported in association with melanoma [27, 28]. Therefore, clinical follow-up of these patients would be a reasonable, prudent approach as a small, but increased association with melanoma exists. In syndrome-associated genital melanosis, the prognosis depends on the specific abnormalities of the syndrome.

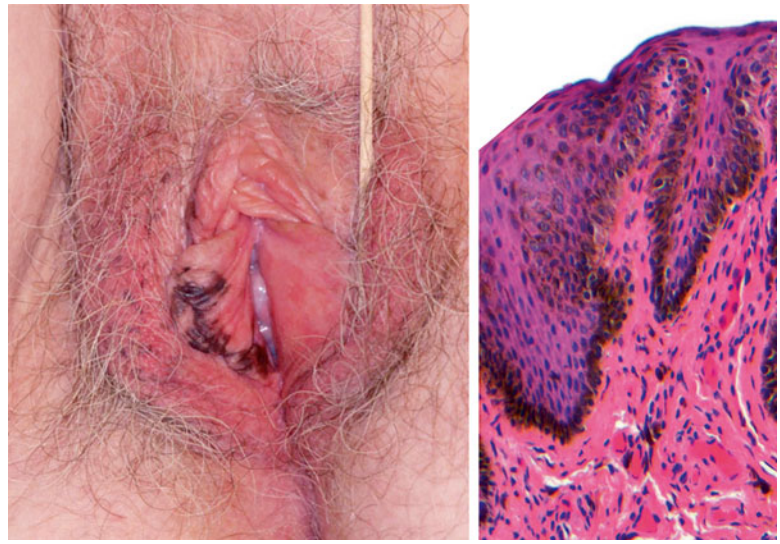


Fig. 7.1 Vulvar melanosis. An asymmetric, poorly circumscribed, variegated patch mimicking melanoma is evident (*left panel*). Histopathologically, vulvar (genital) melanosis exhibits, in most cases, increased melanization of the basal keratinocytes, subepithelial fibrosis, and free melanin and melanophages in the submucosa (*right panel*)

Histopathology

In general, there is increased melanin pigmentation within the keratinocytes, most prominent at the tips of the rete ridges, with normal or slightly increased number of melanocytes arranged as solitary units with no significant cytologic atypia or pagetoid upward migration [26] (Fig. 7.1, right panel). Because of the latter finding and the lack of nest formation, the term "genital lentiginosis" has been advocated by some [16]. In contrast to true melanocytic neoplasms, the lesional melanocytes are separated by keratinocytes and associated with an increased ratio of single melanocytes to keratinocytes along the basement membrane zone [26]. In some cases, the melanocytes have prominent dendritic processes containing melanin extending up into the upper epidermis [32]. Overall these descriptions match those described by Chapel et al. [24] for volar melanotic macules: (1) increased melanization of the basal keratinocytes only; (2) increased melanization of all keratinocytes (basal, spinous, and corneal); or (3) prominent dendritic melanocytes with scant pigmentation of adjacent keratinocytes [22].

Elongation of the rete ridges due to mild keratinocytic hyperplasia coupled with melanin incontinence (free melanin in the papillary dermis) and dermal melanophages can also be observed in vulvar melanotic macules. These findings overlap with post-inflammatory hyperpigmentation. An example of this phenomenon is genital melanosis coexisting with lichen sclerosus, where fibrosis of the papillary dermis is intermixed with melanophages, lymphocytes, and sclerotic collagen, in addition to the presence of hypermelanotic basal keratinocytes of a melanotic macule [20, 22, 33] (Fig. 7.2). While these changes mimic regression (e.g., regressed melanoma) [20], this constellation of histopathologic findings also fulfills minimal criteria for the diagnosis of lichen sclerosus [22] as well as melanotic macules [24]. Thus, it appears that an inflammatory milieu could induce melanotic macules; or conversely, genital melanotic macules can elicit a host immune response that progresses from a lichenoid host response to lichen sclerosus.

Differential Diagnosis

Vulvar melanosis needs to be differentiated from melanoma, benign melanocytic nevi, and post-inflammatory hyperpigmentation. The most important differential diagnosis is melanoma in situ. The larger the melanotic macule (e.g., >5 mm) and the more severe the irregularity

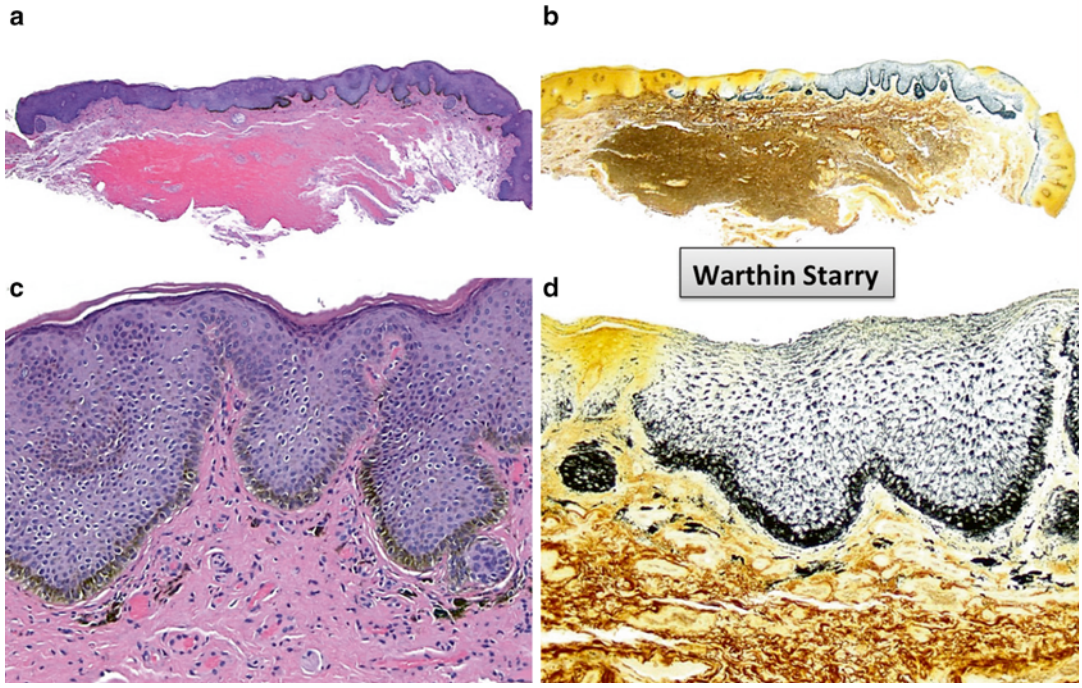


Fig. 7.2 Vulvar melanosis. Warthin-Starry stain is an effective histochemical stain for demonstrating the density and distribution of melanin within the epidermis and papillary dermis. Compared to routine hematoxylin and eosin staining (**a** and **c**), Warthin-Starry stain highlights the circumscription of mucosal pigmentation and demonstrates that most of the melanin is located in the basal keratinocytes with lesser melanin deposition in the spinous and corneal layers. In addition, free melanin and melanophages are readily seen in the submucosa (**b** and **d**). Subepithelial fibrosis commonly underlies these pigmentary changes, with or without lymphocytic infiltrates and histologic features highlighting the overlap of genital melanosis with post-inflammatory hyperpigmentation

of its borders, the greater the indication to biopsy to exclude the presence of melanoma in situ; patients with large or numerous macules of vulvar melanosis should be biopsied to confirm the diagnosis as the distinction from melanoma in situ cannot be made clinically with absolute certainty. In melanoma in situ, there is a significant increase of melanocytes along the basal layer of the epidermis that exhibits cytologic atypia and may form variably sized nests or extend into the spinous layer (pagetoid upward migration). Furthermore, mitoses may be present. Immunohistochemical studies are usually not necessary for the diagnosis of most vulvar melanotic macules because the presence of melanoma can be excluded by routine light microscopy [16]. However, in difficult cases, immunohistochemistry with Mart-1 and/or MiTF can be helpful in excluding melanoma in situ by demonstrating the absence of nests, lack of pagetoid upward migration, and/or no confluence of melanocytes along the basement membrane zone (similar to criteria applied in sun-damaged skin for lentigo maligna) [34]. In melanocytic nevi, the melanocytes form nests along the dermal-epidermal junction in a predictable and circumscribed fashion. In contrast, per definition, no melanocytic nests are seen in vulvar melanosis. In post-inflammatory hyperpigmentation, the basal layer of the epidermis is usually not hyperpigmented and the pigmentation is primarily due to the increase of melanophages and free melanin in the superficial dermis. See Vignette 1 at the end of this chapter.

Summary

Clinical Presentation

- Melanotic macules are asymptomatic, small (<0.5 cm), well-circumscribed, black macules.
- A minority are large macules/patches showing multiple macules with irregular borders, variegated pigmentation, and skip areas.

Histologic Features

- Hyperpigmentation of the basal keratinocytes with prominence, but not increase of mostly dendritic melanocytes
- Subjacent mucosal melanophages and fibrosis
- Absence of melanocytic nests, cytologic atypia, pagetoid scatter

Differential Diagnosis

- Post-inflammatory hyperpigmentation
- Lentiginous junctional melanocytic nevus
- Melanoma in situ

Takeaway Essentials

Clinical Relevant Pearls

- Can have variegated color and very irregular contours raising concern for melanoma

Pathology Interpretation Pearls

- Hyperpigmentation of the basal keratinocytes can be subtle
- Comparing the hyperpigmented areas with the adjacent normal, non-hyperpigmented epidermis may provide the only clue

Immunohistochemical/Molecular Findings

- In difficult cases immunohistochemistry for Mart-1 and/or MiTF-1 can be helpful in excluding melanoma in situ

Post-inflammatory Hyperpigmentation

Clinical Features

Post-inflammatory hyperpigmentation, as the name implies, is pigmentation that occurs in the setting of a genital eruption that has an inflammatory component, particularly one that is directed at the epithelium [7, 35]. Damage to the basement membrane zone, keratinocytes, and/or melanocytes allows melanin pigment to drop out of the epidermis into the dermis, most of which will be engulfed by macrophages (melanophages). Patients of all skin types can be affected, but it is more common and most pronounced in patients with darker skin. Notably, in dark-skin individuals, ongoing inflammation can appear more brown than red giving the impression of hyperpigmentation. Clinically, post-inflammatory hyperpigmentation presents as macules and patches with different shades of brown and gray (due to dermal melanin) and is usually asymmetric compared to the symmetry of physiologic hyperpigmentation (Fig. 7.3). Common causes include eczema (i.e., allergic or irritant contact dermatitis and seborrheic dermatitis) particularly those associated with chronicity such as lichen simplex chronicus, lichen sclerosus, lichen planus, lichenoid drug eruption, or fixed drug eruption [7, 32]. Typically, post-inflammatory hyperpigmentation is asymptomatic, as the primary process has resolved. Occasionally, symptoms related to the underlying causative skin disorder are present such as pruritus. Many times, the diagnosis can be made clinically based on the distribution and pattern of pigmentation as well as the history or clinical signs of an inflammatory process. However, there are some instances where clinical presentation is atypical, raising concern for melanoma; in those cases, the diagnosis should be confirmed with a biopsy. Lastly, the dark macules and patches of post-inflammatory hyperpigmentation are long lasting and diminish only slowly, if at all [7]. Control of the underlying causative inflammatory dermatosis is the most effective mode of management.

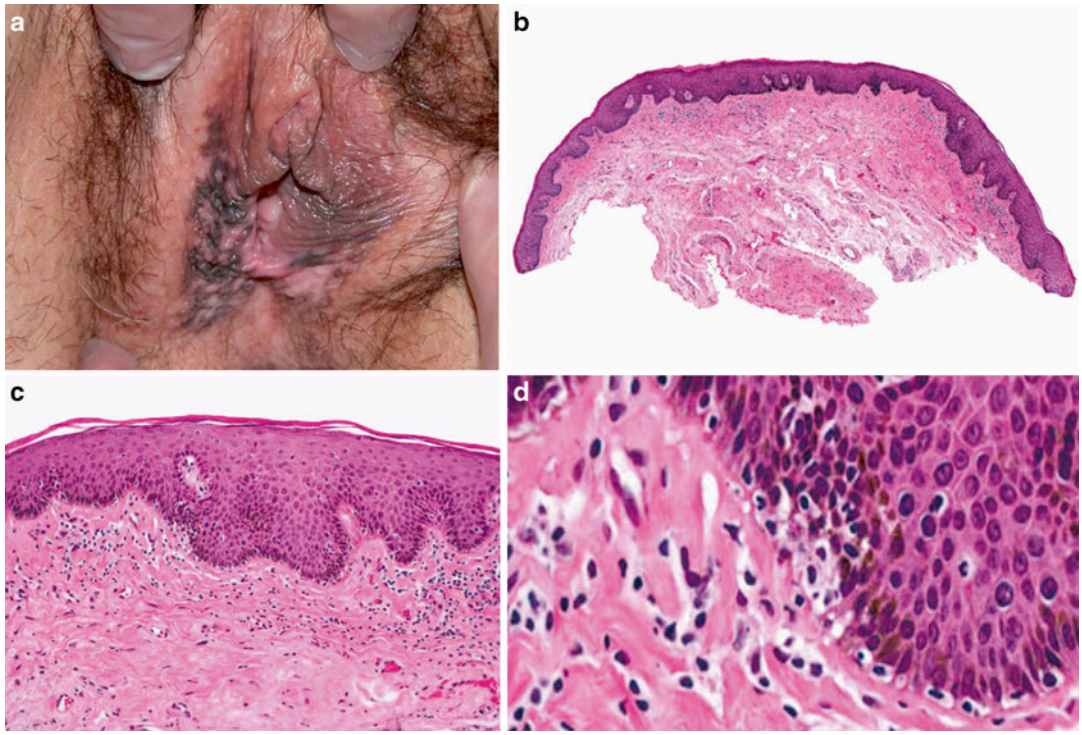


Fig. 7.3 Post-inflammatory hyperpigmentation due to lichen sclerosus. This is an example of an early lesion of lichen sclerosus presenting as an irregular, variegated brown and white patch, which occurs in 2 % of the cases [22]. The varying degrees of hyper- and hypopigmentation (**a**) are associated histopathologically with an irregularly melanized basal keratinocytic layer, fibrosis and sclerosis of the submucosa, lymphocytes infiltrating the fibrosis and basal layer, and rare melanophages (**b**, **c**). The (**c**) exhibits features that fulfill proposed minimal criteria for diagnosis of lichen sclerosus [22]

Histology

There is increased melanin and melanophages in the papillary dermis or submucosa and around blood vessels (Figs. 7.3 and 7.4). In addition, there may be increased amount of melanin within the basal layer of the epidermis although that is not usually the case. Occasionally, a superficial perivascular lymphocytic infiltrate and a mild increase in papillary dermal fibroblasts and collagen are present [32]. Usually, no evidence of the underlying causative dermatosis is identified by the time the patient presents for evaluation of vulvar pigmentation.

Differential Diagnosis

The differential diagnosis includes physiologic hyperpigmentation of the vulva and vulvar melanosis. Physiologic hyperpigmentation commonly occurs in dark-skin individuals and can change with different physiologic states such as pregnancy or the use of oral contraceptives. It is characterized by symmetric hyperpigmentation of the vulvar region, particularly in the perianal skin, posterior introitus, and the tips of the labia minora [7, 9]. The skin biopsy is usually nondiagnostic, although an increase of melanin and melanosomes in the melanocytes and basal keratinocytes has been reported [7]. Also, no dermal changes such as fibrosis or melanin incontinence would be present as these findings are expected for post-inflammatory hyperpigmentation. In general, if the pattern of hyperpigmentation is irregular, a biopsy to rule out a melanocytic lesion should be performed. Furthermore, since post-inflammatory

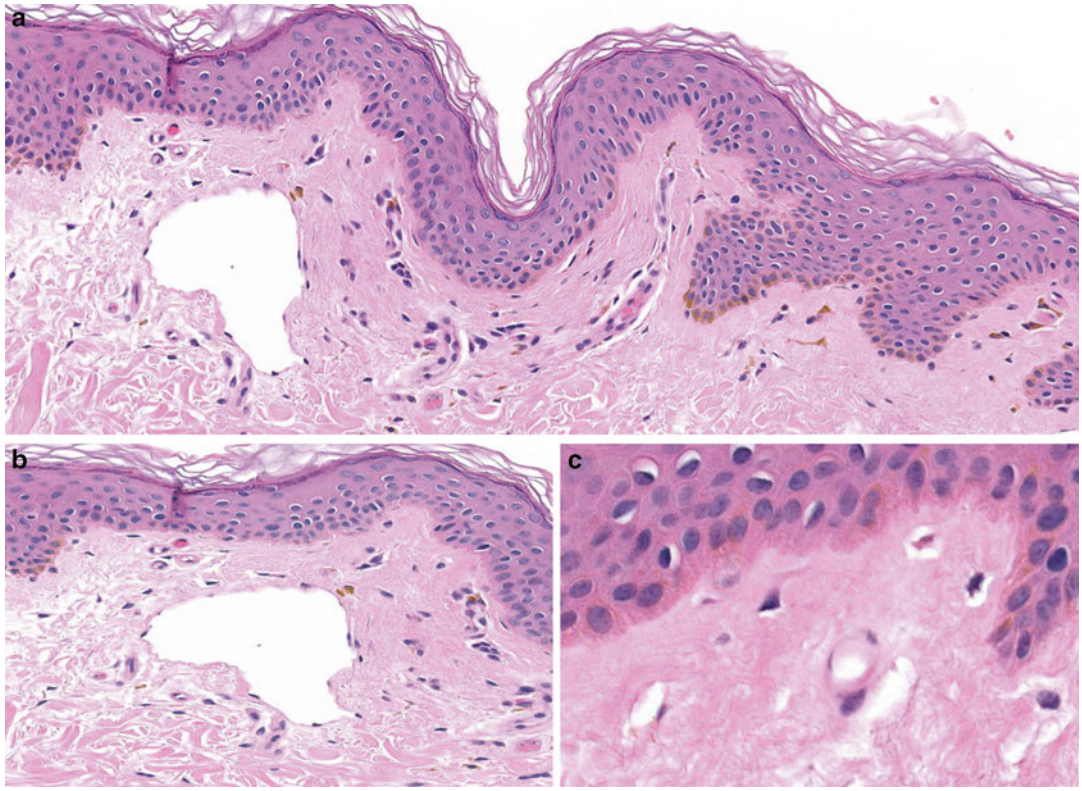


Fig. 7.4 Post-inflammatory hyperpigmentation due to fixed drug eruption. Fifty-eight-year-old woman presents complaining of recurring episodes of pruritus, swelling, and irregular vulvar pigmentation. Biopsy shows melanophages in the papillary dermis, irregular melanization of basal keratinocytes, and smudging of the basement membrane zone without significant inflammation consistent with a quiescent lesion of fixed drug eruption (**a**, **b**). Curiously, dermal sclerosis associated with dilated lymphatic spaces is also present (**c**). These later findings raise the possibility of early, evolving lichen sclerosus [36]

hyperpigmentation can occur in patients with lichen sclerosus, and the latter disease is a risk factor for squamous cell carcinoma or melanoma [27, 28, 31], skin biopsies are often required in these patients to accurately diagnose the nature of the hyperpigmentation. Due to hemosiderin deposition, it is possible that vulvar purpura and/or plasma cell vulvitis could mimic post-inflammatory hyperpigmentation [37], albeit these disorders are consequences of chronic inflammation and/or irritation. If there is doubt histologically regarding the nature of the pigment, the use of iron and Fontana-Masson stain can be helpful; the former will highlight hemosiderin whereas the latter melanin. Positive iron staining due to hemosiderin formation from chronic localized hemorrhage found in pigmented purpuras and plasma cell vulvitis would be confirmatory. See Vignette 2 at the end of this chapter.

Summary

Clinical Presentation

- Depends on the underlying inflammatory skin disease
- Usually asymptomatic

Histologic Features

- Melanin pigment and melanophages in the superficial dermis
- Subjacent mucosal melanophages and fibrosis
- A sparse perivascular lymphocytic infiltrate may be present

Differential Diagnosis
- Physiologic hyperpigmentation
- Genital melanosis
- Vulvar intraepithelial neoplasia, pigmented

Takeaway Essentials
Clinical Relevant Pearls
- Same distribution with the underlying inflammatory disease

Pathology Interpretation Pearls
- Increased melanin and melanophages in the papillary dermis

Acanthosis Nigricans

Clinical Features

Acanthosis nigricans is a dermatosis that exhibits a symmetrical pattern of hyperpigmentation with brown, poorly demarcated, thickened velvet-textured plaques with verrucous excrescences [7, 9]. The most commonly affected areas are the flexural areas of the body, especially the axillae, the hair-bearing surface of the vulva, crural creases, proximal medial thighs, umbilicus, and the neck and face; rarely it can be generalized [38, 39]. Because of the accentuation of the underlying papillomatous process, it is often associated with skin tags within the plaques. Acanthosis nigricans is not typically pruritic. Native Americans are most commonly affected, followed by African-Americans and Hispanics [40]. Acanthosis nigricans can be a cutaneous manifestation of a wide variety of less obvious internal conditions. Acanthosis nigricans is classified into five types based on its associations [41]. Type 1 is a hereditary, autosomal-dominant genodermatosis, which is exceedingly rare (Down syndrome, *FGFR3* mutations, and others) [32, 42]. Type 2 is a common asymptomatic manifestation of insulin resistance observed primarily in obese individuals [43, 44] associated with diabetes mellitus or other endocrinopathies (e.g., Cushing disease, Addison disease, or hypothyroidism). Type 3 is associated with obesity often in association with a metabolic syndrome. Type 4 is associated with various medications [45], especially niacin [46] and prednisone [9]. Type 5 is a paraneoplastic phenomenon, most commonly associated with adenocarcinoma but also seen in lymphoma, squamous cell carcinoma, and other malignancies [47]. The malignancy can precede, accompany, or follow the onset of acanthosis nigricans.

Biochemical mechanisms responsible for this hyperplastic lesion are unclear and depend on the associated disorder but likely involve local cutaneous growth factors [48] leading to the proliferation of keratinocytes and fibroblasts. Members of the tyrosine kinase receptor superfamily including epidermal growth factor receptor (EGFR), insulin growth factor receptor 1 (IGFR1), and fibroblast growth factor receptors (FGFRs) are some of the factors involved [49]. For example, in women with hyperandrogenism and insulin intolerance, anti-insulin receptor antibodies or loss of function mutation in the insulin receptor can be found [50]. In one study, 56 % of the women evaluated for hirsutism in a cohort of nondiabetic women with documented hyperandrogenism had acanthosis nigricans. Interestingly, although acanthosis nigricans was found in various body sites with different prevalences, vulvar acanthosis nigricans was always present in these women [51].

Acanthosis nigricans is benign albeit its course depends on the type. Type 1 becomes accentuated with time, but it can also regress in older patients. The course of type 2 depends on the underlying endocrinopathy while type 3 can regress after substantial weight loss. Type 4 resolves with withdrawal of the causative drug and type 5 with treatment of the malignancy. In thin individuals who develop acanthosis nigricans, particularly in atypical areas without evidence of insulin resistance, or of sudden onset, a thorough investigation for an internal malignancy should be performed [9, 52].

Histopathology

Acanthosis nigricans is a noninflammatory disorder characterized by marked papillomatosis with hyperkeratosis and mild acanthosis [53]. Fingerlike dermal papillae are covered by thin epidermis with overlying hyperkeratosis [54] (Fig. 7.5). A mild hyperpigmentation of the basal layer may be noted, but there is no increase of melanocytes. The thickened stratum corneum is largely responsible for the clinical pigmentation and hyperpigmentation found in acanthosis nigricans [41].

Differential Diagnosis

The differential diagnosis includes lichenified eczema and physiologic hyperpigmentation. Clinically, lichenified eczema can exhibit hyperpigmentation and mimic the velvety texture of acanthosis nigricans via accentuation of skin markings due to chronic rubbing or irritation [7]; in addition, eczema patients complain of pruritus whereas acanthosis nigricans is asymptomatic. By histopathology, lichenified eczema shows marked acanthosis and vertically oriented collagen bundles separating rete ridges and does not exhibit papillomatosis. Clinically, physiologic hyperpigmentation does not show the velvety, leathery texture of acanthosis nigricans and does not display papillomatosis and hyperkeratosis [9]. Some lesions of seborrheic keratosis show papillated epidermal hyperplasia indistinguishable from acanthosis nigricans; however, its circumscription, presence of horn pseudocysts, acanthotic growth pattern, and/or reticulate growth pattern distinguishes it from acanthosis nigricans in most instances.

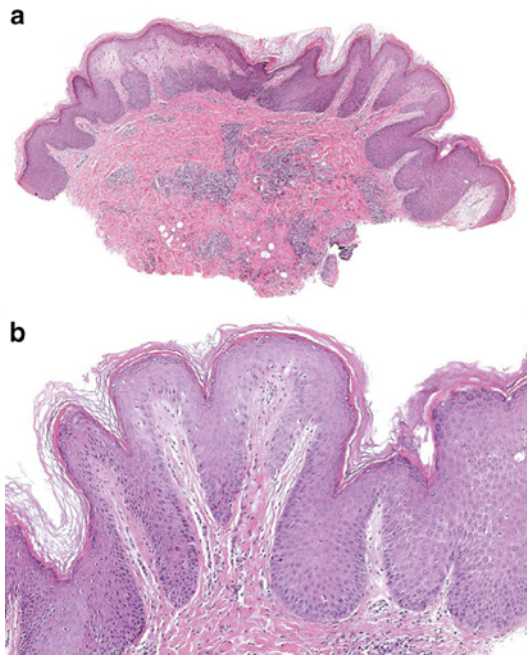


Fig. 7.5 Acanthosis nigricans manifests clinically as thickened-appearing, velvety, brown plaques in skinfolds of overweight and obese individuals [55]. Histopathologically, acanthosis nigricans is characterized by marked papillomatosis and orthokeratosis coupled with minimal acanthosis (**a**, **b**). This specimen shows a dermal nodular inflammatory infiltrate due to an adjacent suppurative folliculitis, which is another complication of obesity [55]. In this case, acanthosis nigricans was the secondary diagnosis, after confirmation of the folliculitis

Summary

Clinical Presentation

- Symmetrical, velvety, hyperpigmented plaques on the hair-bearing surface of the vulva and groins

Histologic Features

- Papillomatosis and hyperkeratosis but with very mild acanthosis
- No melanocytic proliferation and no dermal inflammation

Differential Diagnosis

- Lichenified eczema
- Physiologic hyperpigmentation

Takeaway Essentials

Clinical Relevant Pearls

- Symmetrical hyperpigmentation with brown, poorly demarcated, thickened velvet-textured plaques with verrucous excrescences

Pathology Interpretation Pearls

- Papillomatous hyperplasia without acanthosis

Hypopigmentation

Post-inflammatory Hypopigmentation

Clinical Features

Cutaneous infections (e.g., syphilis, leprosy, herpes) and many inflammatory conditions (atopic dermatitis, pityriasis lichenoides chronica (PLC), psoriasis, lichen striatus, and lichen sclerosus) can induce post-inflammatory hypopigmentation [25] (Figs. 7.6 and 7.7). Atopic dermatitis can show pigmentary changes, which can be particularly severe if high-potency corticosteroids are used [57]. In addition, cutaneous injuries from dermatologic procedures (such as laser therapy or cryotherapy), irritants (chemical peel), or burns can also lead to the same result [58]. The pathogenesis is not well understood as the melanocytes can react with normal, increased, or decreased melanin synthesis to the insult.

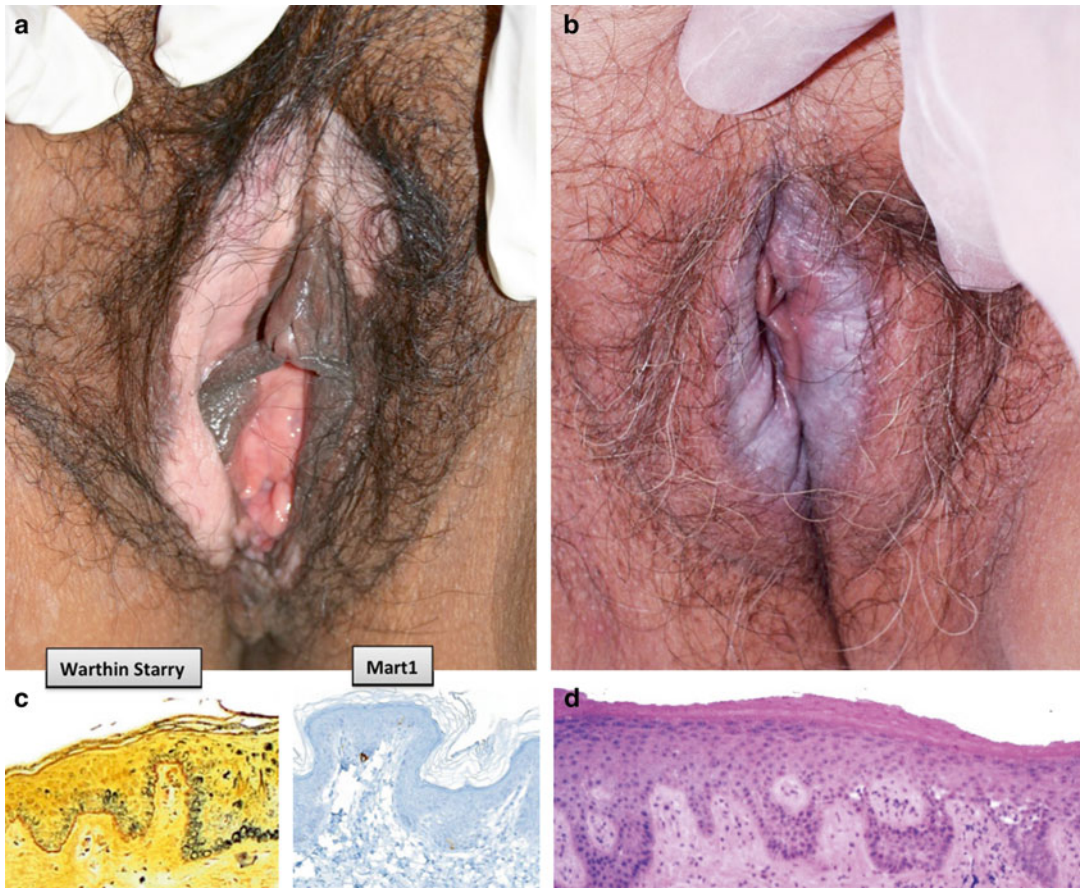


Fig. 7.6 Hypopigmentation: vitiligo and lichen sclerosus. Vitiligo manifests as loss of pigmentation without any other cutaneous or mucosal findings (**a**). Warthin-Starry or Fontana-Masson stain shows a loss of epidermal melanin and absence of dendritic melanocytes whereas the melanocyte-specific markers such as Mart-1 or MiTF highlight the loss of melanocytes (**c**). Post-inflammatory hypopigmentation is due to a combination of decrease of melanocytes, melanin incontinence due to basal keratinocyte or melanocyte damage, or blockade of transfer of melanin from melanocytes to keratinocytes, and from screening from sunlight of the epidermis by the hyperkeratosis. This example of lichen sclerosus (**b**) shows hypopigmentation caused by hyperkeratosis and hypergranulosis (**d**) from persistent rubbing of a pruritic vulva (lichen simplex chronicus)

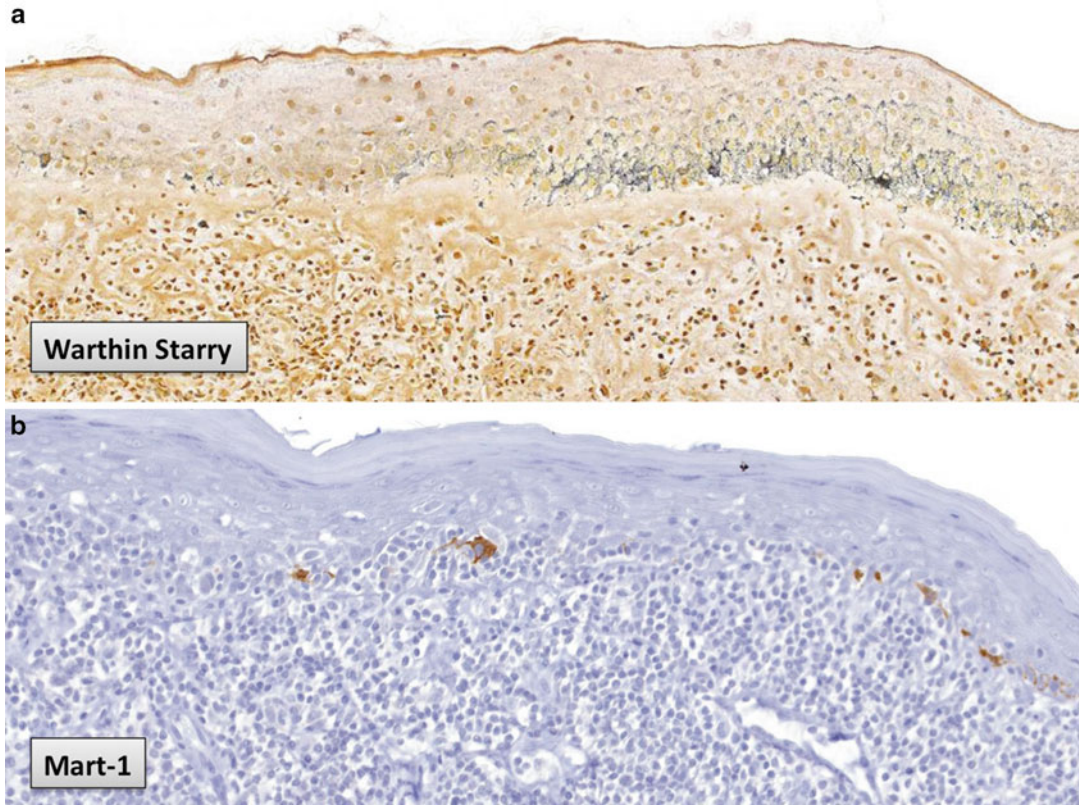


Fig. 7.7 Lichen sclerosus and hypopigmentation. Up to 98 % of vulvar lichen sclerosus cases show hypopigmentation that can mimic vitiligo [22]. Several mechanisms likely play a role in the pathogenesis of lichen sclerosus's inflammatory hypopigmentation: (1) decreased melanin production, (2) block in transfer of melanosomes to keratinocytes, and (3) melanocyte loss [56]. Illustrated herein is an early, evolving lesion of lichen sclerosus with areas of diminished or loss of melanin (**a**) in the basal keratinocytes and an almost complete loss of melanocytes (**b**)

Although post-inflammatory hypopigmentation depends on the duration, type, and severity of insult, there is definite predisposition of individuals to hypo- or hyperpigmentation, reflected by the concept of "individual chromatic tendency" where patients with "weak" melanocytes tend to develop hypopigmentation after inflammation whereas patients with "strong" melanocytes hyperpigmentation [25]; these outcomes are considered genetically determined and inherited in an autosomal-dominant pattern. Cutaneous inflammation may cause the release of multiple mediators, which can cause aberration of melanogenesis. It has been suggested that several factors play a role in the production of post-inflammatory hypopigmentation including defect in transfer of melanosomes to keratinocytes, melanocyte loss, and decreased/inhibited melanin production [25, 56, 58]. An example of the complexity of post-inflammatory hypopigmentation is found in lichen sclerosus, a disorder characterized clinically by cutaneous hypopigmentation of the anogenital area, particularly the vulva [22, 56] (Figs. 7.6 and 7.7). Several mechanisms, alone or in combination, likely play a role in lichen sclerosus's leukoderma: (1) decreased melanin production, (2) block in transfer of melanosomes to keratinocytes, and (3) melanocyte loss [56]. Absence of melanocytes also characterizes vitiligo, an autoimmune inflammatory disorder frequently associated with lichen sclerosus (LS). This association may be the pathogenic connection (lichenoid dermatitis triggering an autoimmune reaction to melanocytes) that

underlies the clinical association of LS with vitiligo [56].

Most cases with minimal hypopigmentation improve spontaneously within weeks or months if the primary cause ceases [58]. However, in patients with severe post-inflammatory hypopigmentation, the condition can be permanent if there is complete destruction of the melanocytes.

Histopathology

Histologic sections show decreased to absent melanin in the basal keratinocytes, melanophages in the upper dermis, and a sparse superficial lymphohistiocytic infiltrate. Sometimes the causative inflammatory condition such as lichen sclerosus may also be evident (Figs. 7.6 and 7.7). Fontana-Masson or Warthin-Starry histochemistry can help document the decreased melanin content of basal keratinocytes and none or only rare dendritic melanocytes within the epidermis. Similarly, immunohistochemical melanocytic markers (microphthalmia-associated transcription factor (MITF), HMB-45, and melanoma antigen recognized by T-cells 1 (Mart1) may show a decreased number or regional loss of melanocytes (Figs. 7.6 and 7.7).

Differential Diagnosis

The clinical differential diagnosis includes sarcoidosis [59], lichen sclerosus [56], hypopigmented mycosis fungoides, hypopigmented extramammary Paget's disease [60], hypopigmentation after high-potency topical corticosteroid usage, vitiligo, and chemical leukoderma [58]. Histologic examination and clinicopathologic correlation can easily differentiate these entities. In particular sarcoidosis shows noncaseating granulomas; mycosis fungoides, a lichenoid infiltrate of atypical lymphocytes with epidermotropism; extramammary Paget's disease exhibits large atypical epithelioid cells within the epidermis; vitiligo, absence of melanocytes; and chemical leukoderma, a reduction of melanocytes. Nevertheless, even if the biopsy is not conclusive, the exclusion of some of the entities in the differential diagnosis is useful for diagnostic classification and management.

Summary

Clinical Presentation

- More common in people with darker skin
- Correlates with the configuration and distribution of the causative inflammatory dermatosis
- Causes include inflammatory conditions, cutaneous injuries, irritants, or burns

Histologic Features

- Decrease melanin of the basal keratinocytes
- Upper dermal/submucosal melanophages
- Sparse superficial lymphohistiocytic inflammation
- Inflammatory cause may be coexisting

Differential Diagnosis

- Sarcoidosis
- Lichen sclerosus
- Hypopigmented mycosis fungoides
- Hypopigmented extramammary Paget's disease
- Hypopigmentation after medication
- Vitiligo
- Chemical leukoderma

Takeaway Essentials

Clinical Relevant Pearls

- Correlates with distribution of the causative inflammatory dermatosis

Pathology Interpretation Pearls

- Decreased melanin of the basal keratinocytes
- Upper dermal/submucosal melanophages

Immunohistochemical/Molecular Findings

- Fontana-Masson special stain may show decreased melanization of basal keratinocytes
- Immunohistochemical melanocytic markers (MITF, HMB45, etc.) may show a decreased number of melanocytes

Vitiligo

Clinical Features

Vitiligo, an acquired leukoderma, is one of the most frequent disorders of pigmentation of the skin and is characterized by distinct areas of skin and mucous membranes depigmentation. It affects up to 2 % of the population, tends to be progressive, and is more common between the ages of 10 and 30 years with no predilection for either sex or ethnic origin. The genitalia are one of the most commonly affected areas along with the face, neck, body creases, scalp, and axillae [61]. Vitiligo can be divided in four subtypes, referred to as generalized, segmental or localized, universal, and acrofacial. The generalized type tends to be symmetric, whereas the localized form, which can follow a mosaic distribution, is more common in children and more difficult to treat [62, 63]. The Koebner phenomenon, where trauma triggers the development of vitiligo, is a common phenomenon in the generalized type [64].

A family history exists in 30 % of the patients. Vitiligo is considered to have primarily an autoimmune etiology [65, 66] as it is commonly associated with other diseases of autoimmune etiology such as Hashimoto's thyroiditis, pernicious anemia, psoriasis, and lichen sclerosus [61]. The presence of anti-melanocyte antibodies supports the immune theory as an effector of melanocytic destruction. Patients with generalized form are more likely to have a concomitant autoimmune disease, whereas it is less frequent in patients with the localized, segmental (mosaic) form. Other theories implicate neural and cytotoxic factors, oxidant and antioxidant reactions, and alterations in melanocyte function in the pathogenesis of vitiligo; however, these mechanisms are not mutually exclusive and may exist in varied combinations [61].

On clinical examination, vitiliginous skin shows hypo- or depigmented macules and patches, sometimes with repigmentation that is frequently incomplete and ephemeral (Fig. 7.6). Ivory-white patches of skin with erythematous borders herald the initial stages and may indicate an ongoing inflammatory process. While hairs growing in patches of vitiligo are white, melanocytes sometimes remain in the hair follicles, resulting in tiny, annular rims of normally pigmented skin within the area of vitiligo. As vitiligo patients regain their pigmentation, the repigmentation process is believed to originate from these residual follicular (stem cell) melanocytes. Examination with the Wood's light can greatly assist in revealing areas of hypo- and depigmentation [67]. Depigmented areas are brightly white in comparison to the subdued reflections of the hypopigmented areas by Wood's light.

Histopathology

Vulvar skin affected by end-stage vitiligo is completely devoid of melanin within the basal keratinocytes, and melanocytes are entirely absent along the epidermal side of the basement membrane zone (Fig. 7.6). The ideal biopsy is taken through the edge of a lesion so that the vitiligo macule/patch can be compared with the adjacent normal skin. The edge of the vitiligo lesion may show a narrow zone of degenerating basal melanocytes and a lymphocytic infiltrate in the underlying papillary dermis and lower dermis hinting at the possibility that a local immune reaction is responsible for the melanocyte loss [65, 68]. Fontana-Masson or Warthin-Starry stain for melanin and immunohistochemical studies with various melanocytic markers such as S-100 protein, Mart1/MelanA, MITF-1, and HMB-45 are helpful in highlighting the differences in melanin content as well as the presence and density of melanocytes between normal and lesional skin samples (Fig. 7.6). In early vitiligo, some persistent melanocytes may be found, correlating with the clinically observed hypopigmentation instead of depigmentation. Since Langerhans cells are usually increased in vitiliginous skin, utilization of melanin/melanocyte-specific immunohistochemistry markers is recommended rather than the sensitive but nonspecific melanocyte marker S-100 protein, which could produce false-positive results [69].

Differential Diagnosis

In difficult cases, the differential diagnosis includes idiopathic guttate melanosis, tinea versicolor, post-inflammatory hypopigmentation, halo nevi, and regressing melanoma [61]. Histologically idiopathic guttate melanosis shows a decrease in

melanin pigment and the number of melanocytes but never a complete absence of them; tinea versicolor, a fungal infection caused by *Malassezia furfur*, will demonstrate spores and hyphae within the stratum corneum; in post-inflammatory hyperpigmentation, melanophages in the upper dermis and a sparse superficial lymphohistiocytic infiltrate will be evident; halo nevi will show nevus cells in a dense lymphocytic infiltrate; and in regressing melanoma, areas of loose fibrosis, dilated vessels, lymphocytes, and melanophages will be noted adjacent to a malignant melanoma. In general none of the above entities will show a complete absence of melanocytes as seen in vitiligo. Sometimes in cases of depigmentation, it may be difficult to differentiate vitiligo from lichen sclerosus (Fig. 7.7). Both are autoimmune disorders and sometimes coexist in the same patient. However, vitiligo shows macular loss of pigment, whereas lichen sclerosus shows indurated, elevated white papules and plaques due to its hallmark sclerotic alteration of the dermis.

Summary

Clinical Presentation

- Generalized, segmental/localized, universal, or acrofacial in distribution
- Amelanotic macules and patches
- Occasional repigmentation in vitiliginous skin

Histologic Features

- Absence of melanin in keratinocytes
- No melanocytes detected by light microscopy and immunohistochemistry
- Absent to scant melanin in subjacent dermis/submucosa
- Vacuolar interface alteration, but no significant inflammatory infiltrate

Differential Diagnosis

- Idiopathic guttate melanosis
- Tinea versicolor
- Post-inflammatory hypopigmentation
- Halo nevus
- Regressing melanoma
- Lichen sclerosus

Takeaway Essentials

Clinical Relevant Pearls

- Ideal biopsy is from the edge to allow for comparison of normal and vitiliginous skin compared with adjacent normal skin

Pathology Interpretation Pearls

- No melanocytes detected by light microscopy and immunohistochemistry

Immunohistochemical/Molecular Findings

- Fontana-Masson or Warthin-Starry for melanin and immunohistochemical studies with various melanocytic markers such as S-100 protein, Mart-1, MiTF-1, and HMB-45 show absence of melanin and melanocytes in well-developed lesion

Melanocytic Nevi

Common Acquired Melanocytic Nevus

Clinical Features

Vulvar nevi are relatively uncommon, occurring in up to 2.3 % of women presenting with pigmented vulvar lesions [3–5]. In children, the prevalence of genital nevi is 3.5 %, with a male to female ratio of 1.3:1 [70]. Acquired nevi appear in early childhood and continue to develop into early adulthood. They can occur in modified mucous membranes or keratinized skin and virtually all are acquired [4, 70]. Usually they are solitary but occasionally can be multiple. In a consecutive series of 59 vulvar nevi, 12 % were junctional, 36 % compound, and 52 % intradermal [71]. Typically, common vulvar nevi are small in size (<7 mm), symmetric with regular borders, and uniformly tan to brown in color (Fig. 7.8). Rarely, they can have a bluish appearance due to dermal pigment. Vulvar nevi can be flat-topped, dome-shaped, or macular [7]. Nevi that vary from these characterizations require biopsy [9]. These deviations may well represent atypical vulvar melanocytic nevi, but melanoma must be excluded.

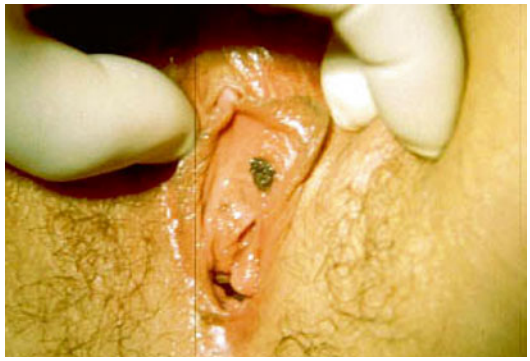


Fig. 7.8 Common vulvar junctional melanocytic nevus. Clinically, the pigmented macule could represent a genital melanotic macule, a junctional melanocytic nevus, or melanoma in situ. Twelve percent of vulvar nevi are junctional [71]

Histopathology

In general, on histologic examination, common vulvar melanocytic nevi display the same banal morphology without any cytologic atypia or unusual architectural patterns, indistinguishable from that of common nevi in other parts of the body [71] (Figs. 7.9, 7.10, and 7.11). Junctional melanocytic nevi are characterized by a proliferation of benign-appearing nevoid melanocytes arranged in solitary units and nests along the basement membrane zone of the epidermis with well-defined lateral margins and symmetric distribution. Although pure vulvar junctional melanocytic nevi are less frequently encountered under the microscope compared to compound and intradermal melanocytic nevi, the proportion of these three subtypes of common melanocytic

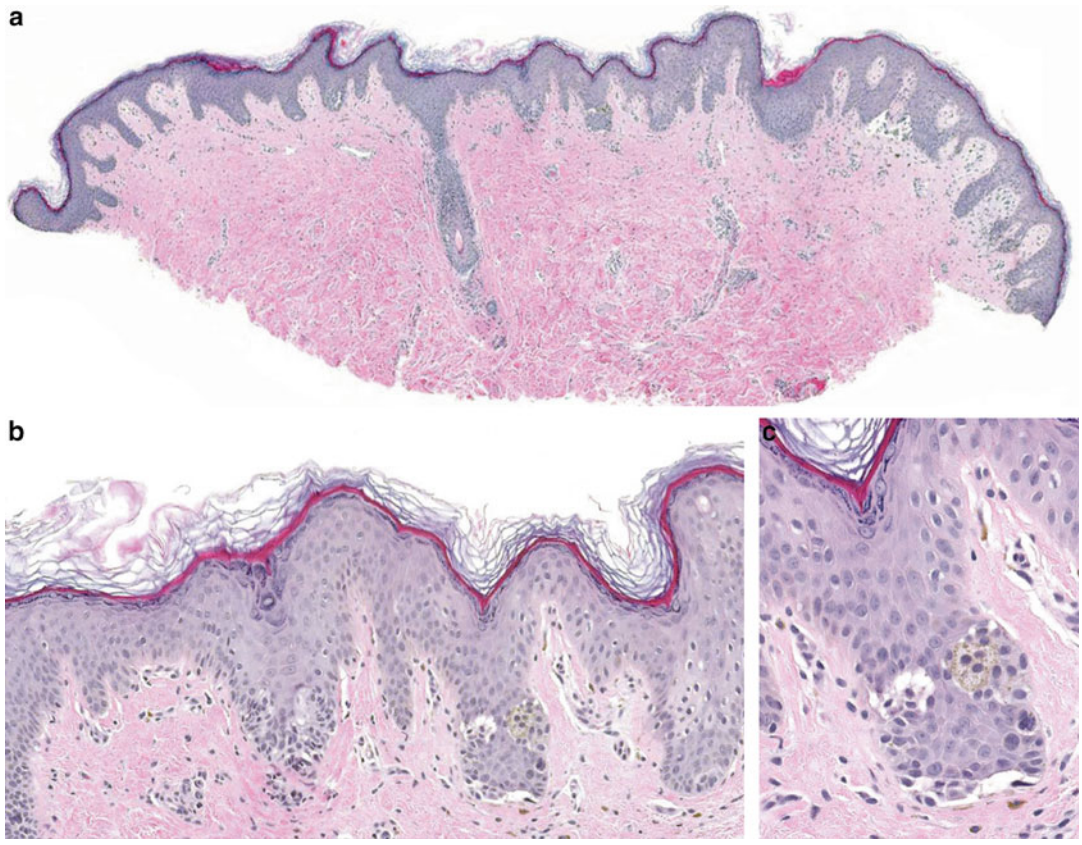


Fig. 7.9 Vulvar junctional melanocytic nevus. A symmetric, circumscribed proliferation of type A melanocytes arranged as nests along the sides and bases of elongated rete ridges (**a**, **b**). The melanocytes are enlarged but cytologically banal; nests or melanocytes outnumber solitary melanocytes along the basement membrane zone (**c**)

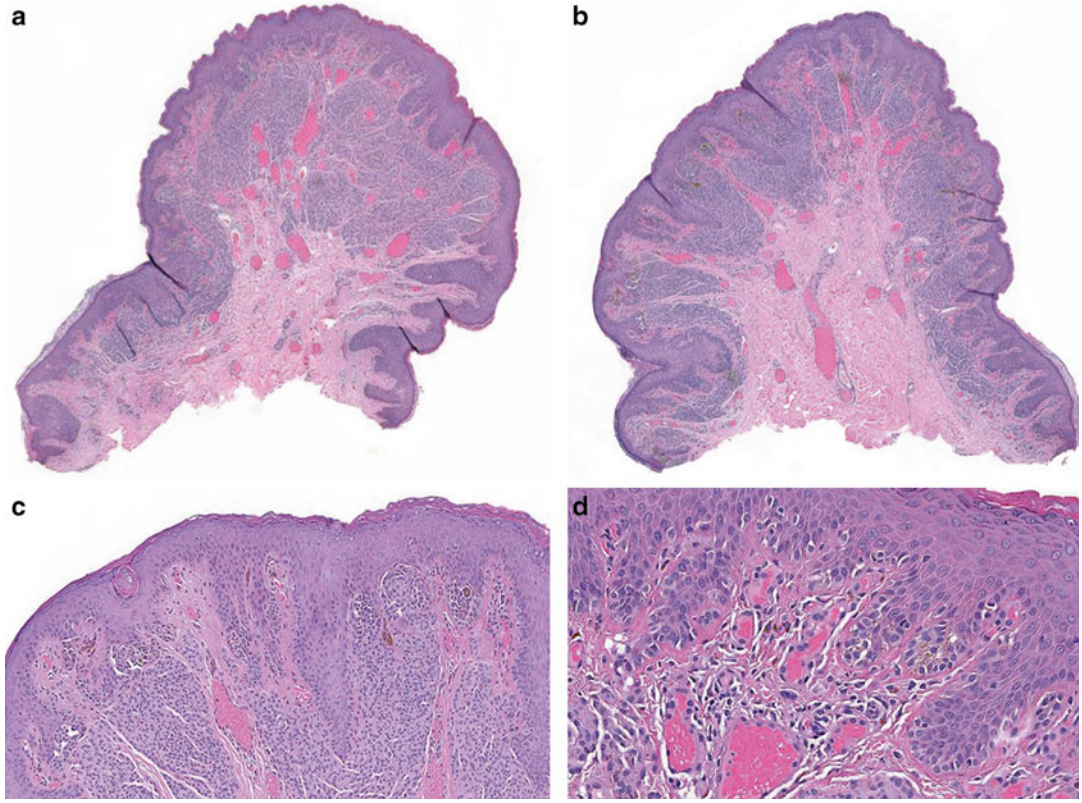


Fig. 7.10 Vulvar polypoid lentiginous compound melanocytic nevus. Polypoid melanocytic nevi are common in the inguinal region and vulva; they are either compound or more frequently predominately intradermal with congenital features (melanocytes forming single files in the dermis/submucosa and surrounding adnexal structures) (**a**, **b**). Within the epidermis, melanocytes are arranged as either solitary cells or nests along the sides and bases of rete ridges (**c**, **d**)

nevi in the vulva does not differ significantly from the torso [71]. In compound melanocytic nevi, nests of benign-appearing nevoid melanocytes are present in the dermis in conjunction with an epidermal component. The dermal melanocytes usually do not display discernible pigmentation, although in rare cases, heavy pigmentation may be present focally in some superficial dermal nests. In addition to the absence of cytologic atypia and conspicuous mitotic activity, the dermal component demonstrates zonal maturation as the melanocytes become smaller and more spindled (instead of epithelioid) and lose cytoplasmic pigment (in cases with superficial heavy pigmentation) with descent into the deep dermis/submucosa. In intradermal melanocytic nevi, the melanocytes, depending on their extent into the dermis or submucosa, exhibit zonal maturation varying from junctional type A (epithelioid) melanocytes to superficial dermal type B (nevoid) melanocytes and deep dermal type C (spindle/neurotized) melanocytes. In contrast to atypical genital melanocytic nevi or dysplastic nevi, common vulvar nevi do not exhibit any architectural distortion or cytologically atypia.

Differential Diagnosis

Clinically, vulvar melanocytic nevi can sometimes be difficult to differentiate from seborrheic keratosis, warts, pigmented vulvar intraepithelial neoplasia (VIN), or angiokeratomas; however, a biopsy can distinguish these entities from a vulvar melanocytic nevus without difficulty. Vulvar melanosis and lentigo simplex per definition do not show any melanocytic nests; the former shows an increase in dendritic melanocytes while

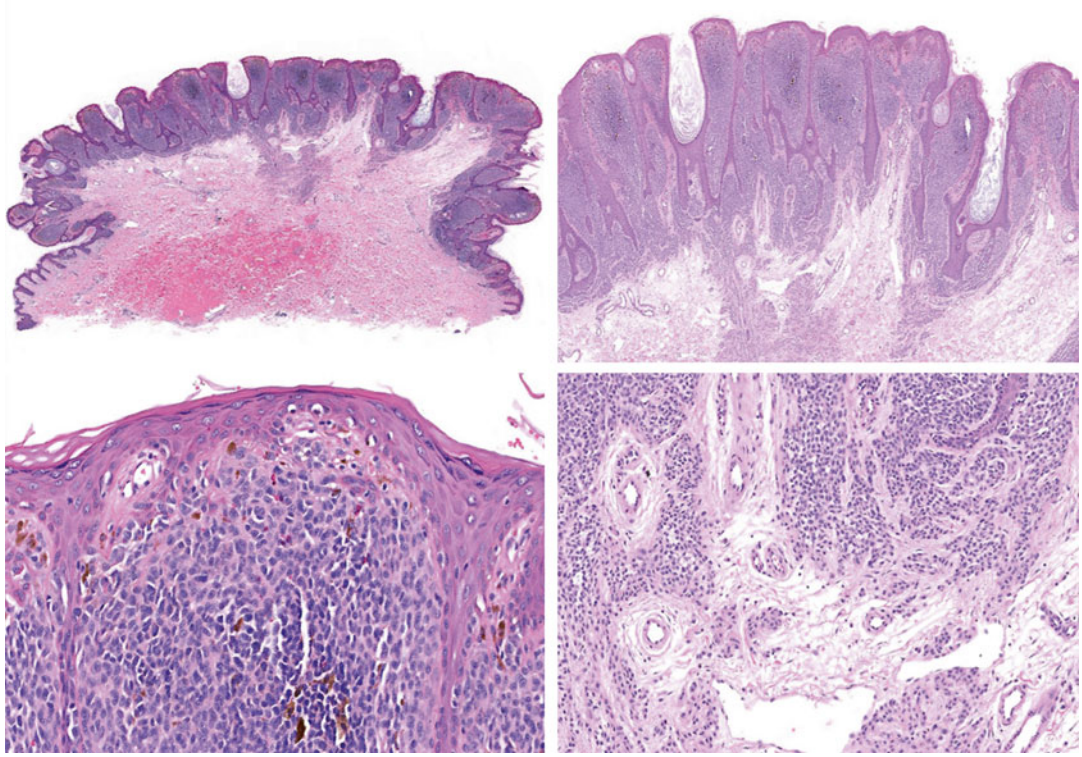


Fig. 7.11 Common vulvar melanocytic nevus—predominately intradermal congenital pattern. The majority, approximately 88 % of genital nevi, are either compound or intradermal [71]

the latter is associated with an increase in solitary, nevoid melanocytes along the sides of elongated, pigmented rete ridges. Nevoid melanoma of the anogenital region is rare but can be recognized by worrisome features such as deep dermal mitotic figures, absence of maturation, and subtle cytologic atypia [72, 73]. See Vignette 3 at the end of this chapter.

Summary

Clinical Presentation

- Small in size (<5 mm)
- Symmetric
- Regular borders
- Uniformly tan to brown in color

Histologic Features

- No cytologic atypia
- Maturation
- Decreased pigmentation with descent
- None to isolated mitotic figures

Differential Diagnosis

- Seborrheic keratosis, warts
- Pigmented VIN 3
- Nevoid melanoma
- Genital melanosis

Takeaway Essentials

Clinical Relevant Pearls

- Small, symmetric, and uniformly tan to brown in color

Pathology Interpretation Pearls

- Indistinguishable from common nevi in other parts of the body
- Benign-appearing nevoid melanocytes
- Well circumscribed with zonal maturation

Immunohistochemical/Molecular Findings

- Positive for melanocytic markers such as S100-protein, Mart-1, and HMB-45 showing maturation: minimal or absence of expression of the antibody in the deep aspect of the lesion

Blue Nevus

Clinical Features

Blue nevus, common or cellular subtype, is very rare in the vulva but has been reported in a handful of cases [74–77]. Vulvar blue nevi are believed to arise from Schwann cells of stromal nerves/perineural precursor cells or from latent dendritic melanocytes which have migrated from the neural crest [17, 78] or from mutated precursor stem cells [79]. Clinically, it is gray blue in color, which has been attributed to the scattering of light by dermal collagen and transmission of the blue wavelengths (Tyndall effect).

Histopathology

The common blue nevus is composed of varied populations of heavily pigmented bipolar dendritic melanocytes and nonpigmented spindle cells within the dermis. The hallmark cells of blue nevus are the dendritic melanocytes, which are characterized by elongated, slender dendrites with cytoplasm containing variable but usually quite prominent, fine melanin granules (Figs. 7.12 and 7.13). They do not exhibit any pleomorphism and are located between dermal collagen bundles, which may appear slightly thickened. Melanophages are usually conspicuous but mitoses are absent. Of note, in a typical case of blue nevus, no melanocytic proliferation is identified in the overlying epidermis or epithelium.

The cellular blue nevus is composed of spindle-type melanocytes that form a bulky nodule within the dermis, which commonly extends into the subcutaneous tissue in a characteristic dumbbell appearance. Typically, cellular blue nevus displays a biphasic pattern with clusters of clear, relatively light to nonpigmented spindle cells

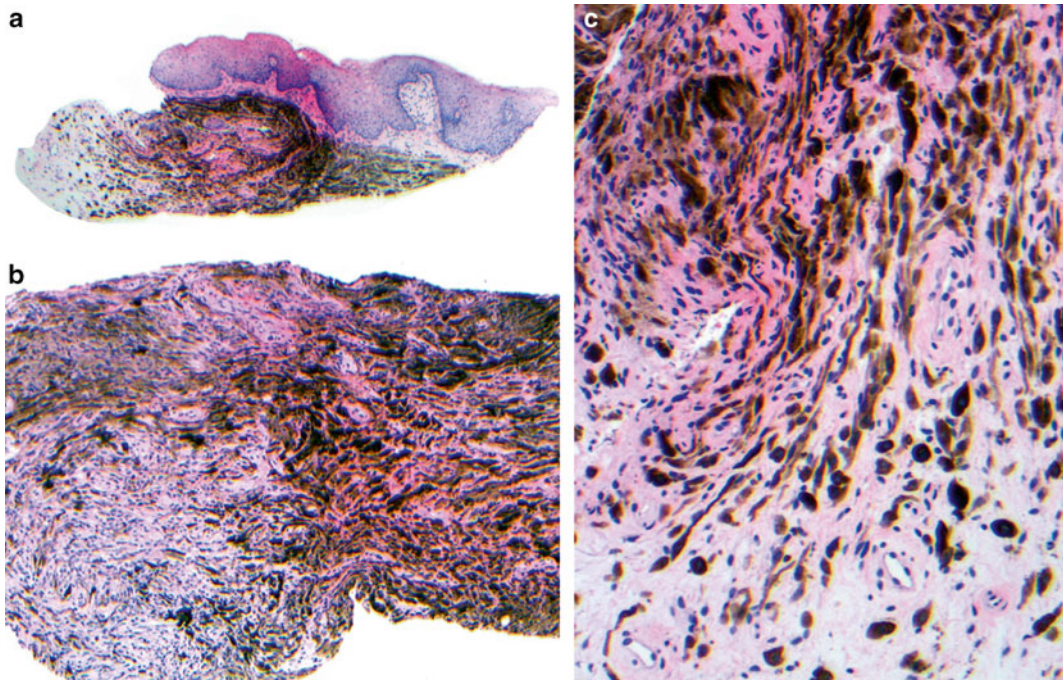


Fig. 7.12 Vulvar blue nevus, dendritic and spindle cell type. Vulvar blue nevus composed of heavily pigmented bipolar dendritic/spindle cells within the dermis (**a**, **b**). No pleomorphism or mitotic figures are identified (**c**)

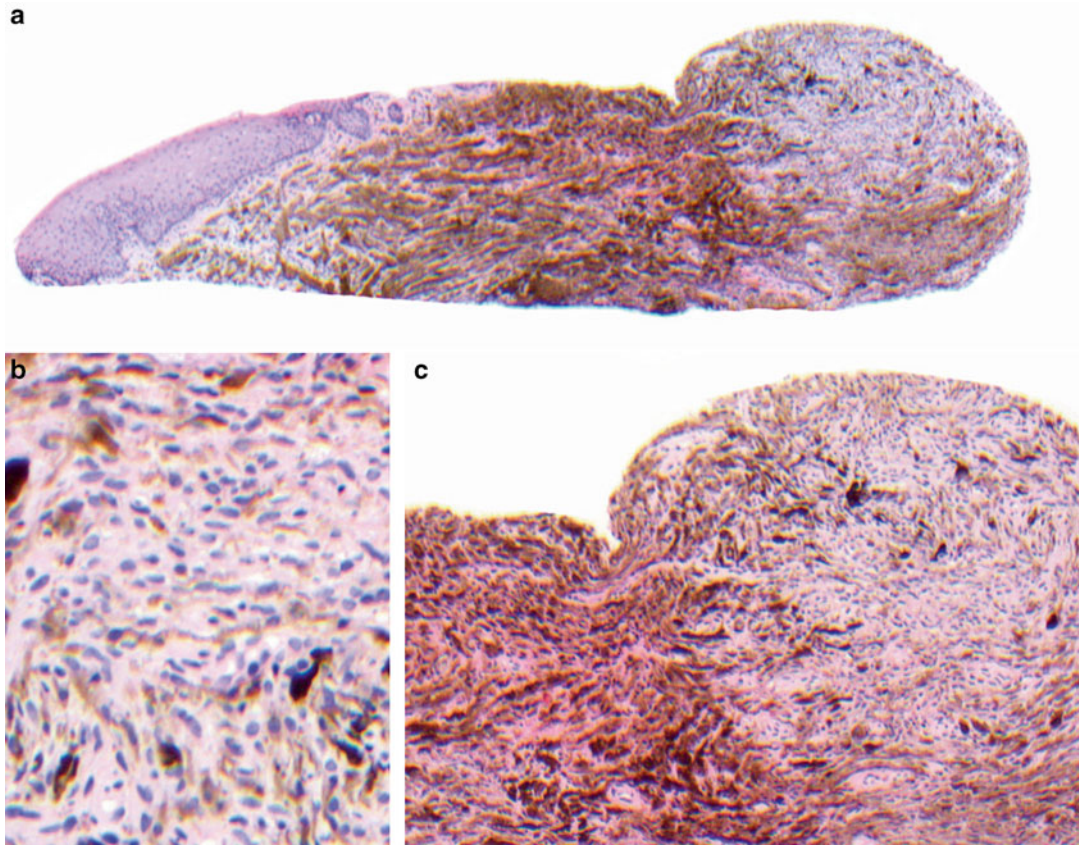


Fig. 7.13 Vulvar cellular blue nevi are cutaneous dermal melanocytic nevi that are rarely reported in the female genitalia. Marked cellularity and less pigmentation differentiate cellular from common blue nevus (**a**, **c**). Despite its cellularity, no worrisome findings are found such as mitotic figures, atypical cytology, or necrosis (**b**)

admixed with heavily pigmented spindle melanocytes. The tumor grows in an alveolar pattern with interspersed giant cells. Mitotic figures are sparse to absent. The spindle melanocytes may locally infiltrate superficial peripheral nerves and subcutaneous tissue, histologic features that should not be interpreted as malignancy [80]. A component of common blue nevus is frequently present at the periphery of the cellular nodule, whose recognition may facilitate the diagnosis of cellular blue nevus.

Another variant of blue nevus that can occur in genital mucosa is the epithelioid blue nevus [81]. This variant has been described mostly in patients with the Carney complex, but they may occur in isolation. Its pathognomonic features are polygonal epithelioid melanocytes situated within the dermis/submucosa that show no maturation with progressive depth of dermal infiltration. In contrast with the usual stromal changes in common blue nevi, epithelioid blue nevi exhibit no dermal fibrosis. In the genital region, no recurrence or metastasis has been reported and simple excision has been curative. However, epithelioid blue nevus is also known as pigmented epithelioid melanocytoma, which is a recently proposed term that encompasses melanocytic proliferations diagnosed as "animal-type melanoma" or "pigment-synthesizing melanoma." This variant of melanocytic tumors is considered a low-grade melanocytic proliferation that can give rise to locoregional lymph node metastases and rarely to distant metastases [82, 83].

Lastly, atypical cellular blue nevus is an exceedingly rare variant of cellular blue nevus, which exhibits a combination of architectural atypia (deeply infiltrative border and/or asymmetry) or cytologic atypia (focal nuclear pleomorphism and hyperchromatism, bizarre cells, and increased mitotic activity). Although the atypical histologic and cytologic features of atypical cellular blue nevus are worrisome for a malignant melanocytic lesion, there are subtle clinical and morphologic differences between atypical cellular blue nevus and malignant blue nevus, its malignant counterpart [80]. However, there is a significant disagreement between experienced dermatopathologists in the diagnosis of atypical cellular blue nevus due to a lack of strict clinical and morphologic criteria for this variant of cellular blue nevus [84]. Of note, atypical cellular blue nevus has never been reported in the vulva [80]; however, malignant blue nevus has occurred in vulva [76]. Mitotic figures and significant cytologic atypia differentiate malignant blue nevus form atypical blue nevus [80].

Differential Diagnosis

In rare instances, common blue nevus can be mistaken for post-inflammatory hyperpigmentation. The pigmented cells in post-inflammatory hyperpigmentation are melanophages and not dendritic melanocytes. Although dermal fibroplasia can be seen in both lesions, the dermal fibrosis in blue nevi is due to the thickened eosinophilic collagen bundles as opposed to the diffuse fibrosis in post-inflammatory hyperpigmentation. Lastly, the presence of a mild lymphocytic infiltrate, which can be observed in post-inflammatory hyperpigmentation, is not a histologic feature of common blue nevus.

The differential diagnosis of cellular blue nevus includes a malignant blue nevus or a blue nevus-like melanoma, which is extremely rare [85]. One case of malignant blue nevus has been reported in the vulva of a 28-year-old woman which resulted in a late ovarian metastasis [76]. A case of vaginal malignant blue nevus has also been published [86]. Malignant blue nevus usually has a clearly identifiable precursor lesion that is a dispersed blue nevus (e.g., nevus of Ito or nevus of Ota), a common dendritic blue nevus, and/or a cellular blue nevus. In a malignant blue nevus, a nodular or diffuse growth of cytologically malignant cells with hyperchromatic, pleomorphic nuclei, and prominent nucleoli is present, allowing for a confident diagnosis. Necrosis and high mitotic index including atypical mitotic figures may be observed.

The term blue nevus-like melanoma has been used to describe melanomas either with histologic features reminiscent of a blue nevus (de novo) or arising in association with a blue nevus (malignant blue nevi) [87]. Although the distinction between cellular blue nevus and blue-nevus-like melanoma can be achieved by conventional histopathologic examination in the majority of the cases as high mitotic activity, tumor necrosis, and nodular proliferation of cytologically atypical epithelioid cells are commonly seen in the latter, there is a subset of cases where the diagnosis can be extremely difficult. In problematic cases, multicolor fluorescent in situ hybridization (FISH) using four different melanoma probes has been shown to be very helpful, particularly in positive cases, as cellular blue nevus is reportedly negative for these probes [88]. Conversely, blue nevus and malignant blue nevus frequently harbor an activating mutation of *GNAQ* and/or *GNA11* in over 50 % of the cases [79].

For completeness, clear cell sarcoma can enter the histopathologic differential diagnosis of cellular blue nevus, albeit it has not been reported in the anogenital region [89, 90]. Clear cell sarcomas usually arise in the dermis or deep soft tissue of the distal extremities and can share the spindled cell morphology and multinucleated giant cells with cellular blue nevus. In contrast to the small, pinpoint nucleoli of cellular blue nevus, clear cell sarcoma usually displays prominent and basophilic nucleoli similar to those of melanomas. Since both tumors are positive for S100 protein and other melanocytic markers, in difficult cases, FISH study for the *EWSR1* gene rearrangement can discriminate the two, as clear cell sarcoma commonly shows a balanced translocation t(12;22)(q13;q12) resulting in the fusion of the gene *EWSR1* on chromosome 22 with *ATF1* on chromosome 12 [89].

Summary

Clinical Presentation

- Very rare
- Gray blue in color (Tyndall effect)

Histologic Features

- Heavily pigmented bipolar dendritic/spindle cells in the dermis
- No pleomorphism or mitoses

Differential Diagnosis

- Cellular blue nevus
- Blue nevus-like melanoma
- Dermal clear cell sarcoma

Takeaway Essentials

Clinical Relevant Pearls

- Gray blue in color (Tyndall effect)

Pathology Interpretation Pearls

- Heavily pigmented bipolar dendritic melanocytes

Immunohistochemical/Molecular Findings

- Approximately 50 % of cellular blue nevus and its malignant counterpart possess *GNAQ* and/or *GNA11* mutations

Spitz Nevus

First described by Sophie Spitz in 1948, Spitz nevi were originally believed to represent a special form of juvenile melanoma with an unusually good prognosis [91]. More than 60 years after her landmark study, there remains a certain degree of controversy surrounding the concept of Spitz nevus. However, except for a small percentage of problematic cases of so-called "spitzoid tumors," Spitz nevi are generally considered a benign melanocytic proliferation with a rather characteristic clinical manifestation and histologic appearance [92–94]. Clinically, a typical Spitz nevus commonly presents as a pink or pigmented dome-shaped papule with a smooth border, less than 6 mm in diameter, and located on the head and neck of a child or on the lower limbs of a young woman [95, 96]. The lesion commonly shows a rapid growth period followed by an unchanged, stable phase.

The defining histologic feature of a Spitz nevus is the cytologic appearance of their cell components, which are characterized by two particular cytologic features: the melanocytic cells are large and either epithelioid and/or spindled in shape [97]. The epithelioid melanocytes usually have abundant amphophilic to eosinophilic "glassy" cytoplasm and an enlarged nucleus with a central eosinophilic nucleolus (Fig. 7.14), whereas the spindle melanocytes display a benign nucleus and clear to pigmented cytoplasm [96]. Another cytologic feature commonly described in association with Spitz nevi are "Kamino" bodies which are eosinophilic structures frequently identified at the dermal-epidermal junction. Spitz nevi can be junctional, compound, or completely intradermal. On low-power magnification, Spitz nevi display a wedge-shaped appearance. Although the melanocytes of a typical Spitz nevus can exhibit various degrees of cytologic atypia, the melanocytic proliferation usually demonstrates the silhouette of a benign nevus including:

- Architectural symmetry: refers to the similarity of the melanocytes, the degree of pigmentation, and the extent of host response (inflammatory and epidermal) around a central axis on both sides of the lesion.
- Sharp lateral circumscription: no significant lateral extension of the junctional melanocytes beyond the dermal component. In particular, extensive lateral extension of the epidermal melanocytes beyond the dermal component as solitary units is not a feature of Spitz nevi.
- Maturation of the melanocytes with descent: the melanocytes in the deep aspect of the lesion are smaller compared to the melanocytic cells in the superficial dermis.

Although mitotic figures may be seen in a Spitz nevus, they are usually rare and observed in

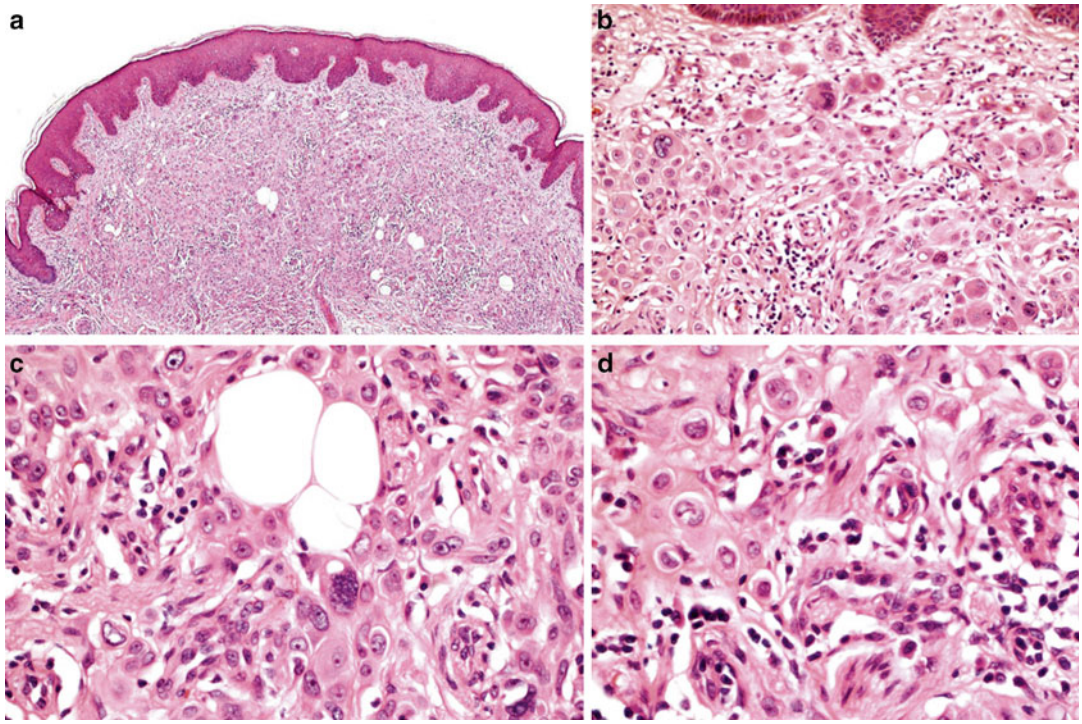


Fig. 7.14 Vulvar intradermal atypical Spitz nevus arising in a 61-year old. By scanning magnification, a relatively symmetric dome-shaped profile of epithelioid melanocytes is evident (**a**). Despite the symmetry, this intradermal Spitz nevus demonstrated worrisome histologic features such as severe cytologic atypia (**c**, **d**), lack of maturation (**a**, **b**), and an associated halo reaction/lymphocytic host response (**c**). No mitotic activity was observed and the patient was free of recurrence after 8 years of follow-up. (Images courtesy of Dr. J. A. Plaza, MD, Milwaukee, WI; in reference to study discussed in Ref. [98])

the superficial dermis. In contrast, the presence of mitotic activity in the base of the lesion should raise the possibility of a melanoma.

In addition to the classical histologic appearance, various histologic variants of Spitz nevus have been described in the literature such as angiomatoid Spitz nevus [99, 100], desmoplastic or sclerosing Spitz nevus [101, 102], plexiform Spitz nevus [103, 104], intradermal Spitz [98], and pagetoid Spitz nevus [105].

Histopathology

Reviewing the literature revealed that genital Spitz nevi are exceedingly rare with only nine reported cases of genital Spitz nevi including five penile and four vulvar cases, all in individuals less than 30 years old [70, 106–111], except for one case in a 61-year-old female [98]. Of the four vulvar Spitz nevi documented in the literature, three were observed in children (5, 9, and 10 years old) with two cases located on the labia minora and the remaining one on the labia majora. Interestingly, two of these vulvar Spitz nevi were not pure Spitz nevus but were described within a congenital compound melanocytic nevus [70, 107]. Notably, none of these three cases of juvenile vulvar Spitz nevus has well-described morphology [70, 107]. The single vulvar Spitz nevus in an adult (61 year old) was reported in a study of 74 cases of intradermal Spitz nevus with a prevalence of 1.3 % [98]. The Spitz nevus was polypoid and symmetrical architecturally. It

was composed predominantly of epithelioid melanocytic cells with severe cytologic atypia extending into the reticular dermis and associated with a halo reaction (Fig. 7.14). Of note, the melanocytic proliferation did not display any maturation of the epithelioid melanocytic cells with descent. Although this Spitz nevus demonstrated worrisome histologic features such as severe cytologic atypia, lack of maturation, and an associated halo reaction, mitotic activity was not observed and the patient was free of recurrence after 8 years of follow-up.

Differential Diagnosis

The differential diagnosis of Spitz nevi includes desmoplastic (sclerosing) nevi, Wiesner's nevus [112], and spitzoid melanomas. Desmoplastic nevus does not exhibit the characteristic epithelioid and spindle cytologic features of a Spitz nevus, but rather is predominantly composed of type B and C melanocytes set in a sclerotic, collagenous background. Wiesner's nevus is a recently described entity in BAP1-associated cancer susceptibility syndrome whose dermatologic hallmark is a dome-shaped nevus with distinct clinicopathologic features. It is composed of various nevomelanocytic populations all showing different degrees of atypia ranging from hyperchromatic nevus cell-like to large atypical epithelioid cells—features that can mimic Spitz nevi. By immunohistochemistry, Wiesner's nevus is BAP1 negative and VE1 (BRAF) positive [112]. Lastly, as the name implies, spitzoid melanoma resembles a Spitz nevus. However, in contrast to a typical Spitz nevus, spitzoid melanomas lack architectural symmetry, lateral circumscription, and maturation with descent. Cytologically, the melanocytic cells exhibit dusty cytoplasmic melanization, severe cytologic atypia, and high nuclear-to-cytoplasmic ratio. An expansile nodular dermal growth, the presence of deep dermal mitoses including atypical mitoses, and necrosis are additional worrisome features for a malignant melanoma [94]. However, it should be kept in mind that no single histologic feature is sufficiently specific, and a constellation of morphologic findings should be evaluated in conjunction with the clinical picture. In general immunohistochemical studies are not very helpful. Although diminished expression of HMB45 toward the base of the melanocytic lesion and low Ki-67 is more common in Spitz nevi, it is noteworthy that there are certainly melanomas expressing the same immunoprofile. Immunohistochemical studies with p-16 may add valuable information as the presence of areas with complete loss of p16 expression has been reportedly observed in atypical spitzoid tumors harboring homozygous deletion of 9p21, a genomic finding associated with an aggressive behavior in recent studies [113–115].

Significant progress has been made over the last few years in identifying cytogenetic/molecular alterations in spitzoid tumors, which have potential clinical implication in the distinction between Spitz nevi and melanomas. In this regard, ancillary genomic and/or genetic studies such as comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) have been increasingly used to improve diagnostic accuracy [115]. In the "melanoma FISH test," four probes are used [RREB1(6p25), CCND1(11q13), CEP6 (centromere 6), and MYB(6q23)] with a sensitivity of 86.7 % and specificity 95.4 % [117]. Recently, it has been shown that a variation of this probe set [PREB1(6p25), CCND1(11q13), C-MYC(8q24) and CDKN2A(9p21)] has an improved sensitivity and specificity of 94 % and 98 %, respectively [114, 115, 118, 119]. However, it should be kept in mind that there can be considerable interobserver variability in the interpretation of FISH results, most likely due to selection bias of nuclei enumerated [120–122]. Also, it cannot be overemphasized that a subset of bona fide melanomas are negative for these molecular tests [123]. CGH is considered more sensitive as it screens the entire genome for aberrations in contrast to the FISH study, which provides information for only the targeted loci. However, it is not widely available compared to FISH studies. Furthermore, it is conceivable that CGH can potentially miss cytogenetic/molecular aberrations detected by

FISH, when they are only present in a minority of the tumor cells [123].

Although histopathology remains the gold standard for the distinction of Spitz nevi from melanoma, some spitzoid melanocytic proliferations display atypical morphological characteristics and cannot be unequivocally separated into benign or malignant categories. In addition, the interobserver variability even among expert dermatopathologists can be significant. These tumors have been labeled as "atypical/borderline Spitz tumors" (tumors of uncertain malignant potential) and represent a controversial and incompletely defined diagnostic categorization [94]. In general, unequivocally benign Spitz nevi do not show chromosomal gains or losses, whereas more than 95 % of melanomas harbor multiple chromosomal aberrations [94]. However, the spectrum of chromosomal copy number aberrations reported in Spitz nevi and atypical Spitz tumors is rapidly expanding. The increasing identification of chromosomal aberrations in the category of "atypical spitzoid tumors" has led to the recognition of several subsets of spitzoid melanocytic lesions with distinct clinical, morphologic, and cytogenetic features. A subset of sclerosing Spitz nevi (approximately 20–25 %) can exhibit an isolated copy number increase of chromosome 11p and/or HRAS mutation [124, 125]. Patients with epithelioid and spitzoid tumors may carry a germline mutation and a neoplastic syndrome (cutaneous atypical melanocytic tumors/melanomas, uveal melanomas, and miscellaneous tumors such as mesotheliomas), particularly if the patients present with multiple lesions [126–128]. Another subset of atypical Spitz tumors displays a homozygous deletion of 9p21, where the p16 gene is located. These spitzoid tumors are reportedly associated with a more aggressive clinical course and therefore probably represent a low-grade spitzoid melanoma [115]. It is noteworthy that CGH plot does not allow discrimination of homozygous versus heterozygous loss of 9p21, and if this is the only chromosomal aberration, FISH study would be more sensitive. This cytogenetic finding may have clinical implication as recent studies have documented differences in biologic behavior of spitzoid tumors with heterozygous versus homozygous 9p21 loss [114]. Another variant of atypical spitzoid tumors that are associated with isolated 6q23 deletions frequently results in positive sentinel lymph node biopsy but very rarely progresses beyond this locoregional stage. Hence, some have advocated refraining from an unequivocal diagnosis of malignant melanoma for these spitzoid lesions although the data are still preliminary [115]. On the contrary, spitzoid tumors with 6p25 and 11q13 gains reportedly behave in a more aggressive fashion [115]. Very recently, it was shown that some of the Spitz lesions in the spectrum of spitzoid tumors (benign Spitz nevi, atypical Spitz tumors, and spitzoid melanomas) harbor gene fusions involving the tyrosine kinases ALK, ROS1, NTRK1, and RET or the serine-threonine kinase BRAF [129]. The fact that these fusion genes were identified along the entire spectrum of the spitzoid tumors suggests that the fusion occurs early in the pathogenesis and is not sufficient per se for malignant transformation. Notably, all kinase fusions appear to be mutually exclusive and occur only in tumors without HRAS mutations or loss of BAP1. Interestingly, among the spitzoid melanocytic lesions with gene fusions, the Spitz nevi and atypical spitzoid tumors with the ALK fusion are mostly amelanotic and exhibit a plexiform growth pattern of intersecting fascicles of fusiform melanocytes [130].

As a concluding remark, given their rarity and unknown natural history and prognosis, prophylactic excision and long-term follow-up of vulvar nevi that exhibit spitzoid features concomitant with large size and/or atypical, worrisome histopathology should be considered [70].

Summary

Clinical Presentation

- Very rare
- Only four cases reported in the vulva

Histologic Features

- Dome-shaped melanocytic proliferation composed of epithelioid and spindle melanocytes
- Architectural symmetry, well circumscription, and maturation with descent
- Mitotic activity if present is limited to the epidermis and superficial dermis
- The only case of vulvar Spitz nevus in the literature with well-documented morphology appears to be an "atypical Spitz nevus"

Differential Diagnosis

- Desmoplastic melanocytic nevus
- Wiesner's nevus
- Melanoma, particularly with spitzoid appearance

Takeaway Essentials

Clinical Relevant Pearls

- Vulvar Spitz nevi are exceedingly rare

Pathology Interpretation Pearls

- Exercise extreme caution in the diagnosis of a Spitz nevus in the vulva
- Since vulvar melanomas are significantly more common than Spitz nevi, particularly in adults, consultation with a dermatopathologist expert may be considered

Immunohistochemical/Molecular Findings

- Immunohistochemical studies with HMB-45, Ki-67, BAP-1, VE1(BRAF), and p-16 may be helpful
- New molecular studies such as comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) are promising

Nevus Associated with Lichen Sclerosus

Clinical Features

Melanocytic nevi with superimposed lichen sclerosus are a rare phenomenon that shows clinicopathologic features that most closely resemble those of persistent (recurrent) melanocytic nevi. They can clinically be extremely concerning simulating melanoma [131] (Fig. 7.15a–d). To date, no case of melanocytic proliferations with superimposed lichen sclerosus has progressed to advanced (metastatic) disease [20, 21, 70, 131–137]. Nevertheless, (adult) vulvar melanoma has been associated with background lichen sclerosus or changes suggestive of coexisting lichen sclerosus (e.g., ivory-white genital skin) in older women [27, 28, 131].

Lichen sclerosus, a chronic fibro-inflammatory dermatosis that presents as atrophic ivory-white small patches to large plaques, affects all ages and all sites but has a predilection for the vulva and prepubertal and postmenopausal women [22]. Clinical pigmentation of vulvar lichen sclerosus is infrequent (less than 2 %) and resembles genital melanosis [22]. In cases of melanocytic nevi with superimposed lichen sclerosus, the inflammatory and fibrotic changes of lichen sclerosus result in a lesion that can be large with irregular borders and black appearance admixed with white regions mimicking melanoma with regression [133]. Commonly, features of lichen sclerosus are evident at the time of the biopsy [20]; in other cases, its presence is appreciated retrospectively after learning the biopsy results and reexamining the patient. It is not clear if the inflammatory changes of lichen sclerosus promote the melanocytic proliferation or if the lichen sclerosus is an immune response tempered by the melanocytes [134]. Thirteen cases of vulvar melanocytic proliferations (labeled nevus or melanoma) associated with lichen sclerosis have been reported, all in females ≤14 years of age [70, 131–137] (two cases have also been described in males, 11 and 38 years old [20]). Because the risk for progression to melanoma is unknown, prophylactic excision is recommended [131, 134]. Given that chronic inflammation is a risk factor to cancer and

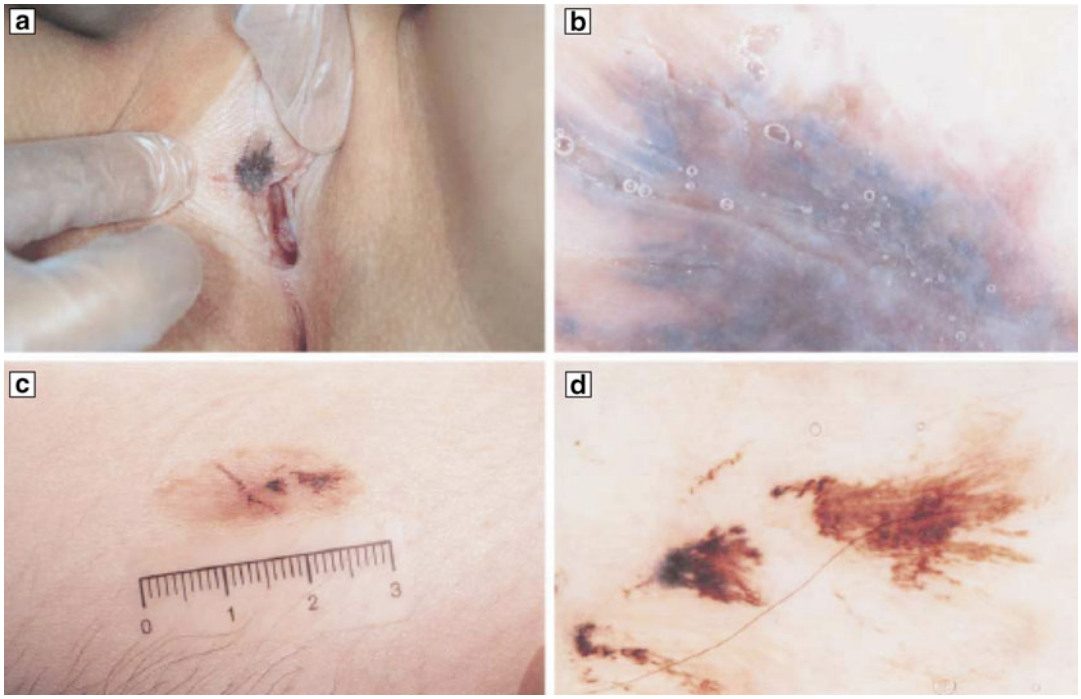


Fig. 7.15 Melanocytic nevi in lichen sclerosus show similarities with persistent (recurrent) melanocytic nevi. Clinical and dermoscopic views of melanocytic nevi arising in lichen sclerosus (**a** and **b**) and a surgical scar (**c** and **d**). Dermoscopy (10×) using immersion oil shows dark brown to black homogeneous pigmentation (**b**) and more irregular areas of pigmentation (**d**). In both cases, the band-like extensions of pigmentation indicate the presence of a melanocytic pigmentation. In addition, there is a pronounced whitish veil reflecting the presence of hyperkeratosis and perilesional inflammation in **b**. This persistent melanocytic nevus and the lichen sclerosus melanocytic nevus both meet the "ABCD" rule of dermoscopy for the identification of melanoma. The scale in part **c** is in centimeters (Used with permission from Carlson JA, Mu XC, Slominski A, Weismann K, Crowson AN, Malfetano J, et al. Melanocytic proliferations associated with lichen sclerosus. Arch Dermatol. 2002 Jan;138(1):77–87)

women with anogenital lichen sclerosus are at risk for squamous cell carcinoma, this course is prudent for melanocytic nevi with superimposed lichen sclerosus to prevent the possibility of progression to melanoma [31, 134].

Histopathology

The melanocytic nevi with superimposed lichen sclerosus are either junctional or compound types with symmetric architecture, well-circumscribed lateral margins, dermal maturation with descent, and no dermal mitoses. However, the melanocytes in this inflammatory milieu are enlarged, have an abundantly melanized cytoplasm, and are arranged in irregular, variably sized nests or confluent solitary melanocytes along the basement membrane zone (Figs. 7.16 and 7.17). These features overlap with melanoma but are also seen in halo nevi and persistent nevi. Melanocytes entrapped in the dermal sclerosis also have abundant dusky gray cytoplasm similar to the melanocytes within the epidermis; features found in persistent compound melanocytic nevi [131]. A lymphocytic host response either underlying the dermal sclerosis or infiltrating and disrupting the melanocytic nests is seen. The lichen sclerosus component extends beyond the borders of the melanocytic proliferation, which is a helpful, distinguishing feature from melanoma.

By immunohistochemistry, Ki-67 proliferation index is higher compared to common melanocytic nevi but is significantly lower than melanoma [131]. HMB-45 can be diffusely expressed by junctional and superficial dermal melanocytes,

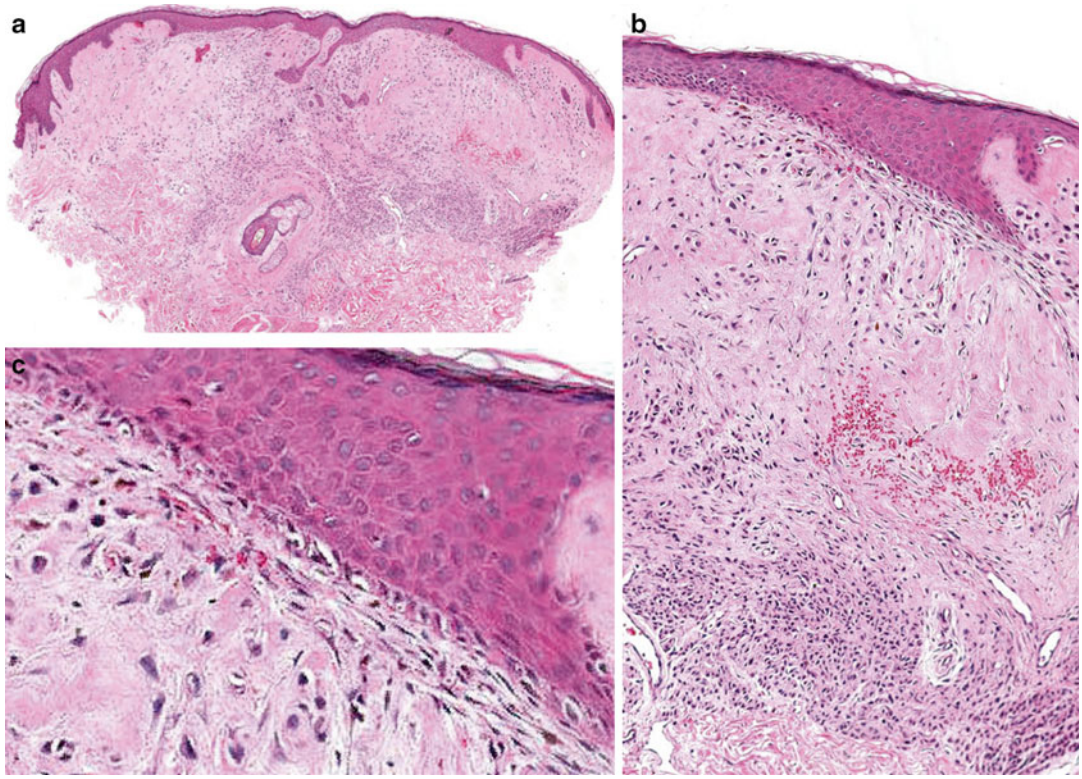


Fig. 7.16 Compound melanocytic nevus with superimposed scar. Stromal-melanocytic interactions secondary to a scar impart atypical features to this vulvar intradermal melanocytic nevus, congenital pattern (**a**) [131, 138]. These atypical changes consist of enlarged epithelioid melanocytes within the zone of dermal fibrosis (**b**) and an almost confluent array of melanocytes along the dermal-epithelial junction (**c**)

signifying an activated, melanogenic phenotype that is also observed in melanocytes overlying scars [131]. In one case report, the expression of soluble adenylyl cyclase antibody in a lichen sclerosis-associated melanocytic nevus was a dot-like Golgi pattern, resembling that found in benign melanocytic nevi as opposed to the pan-nuclear staining pattern reported in melanomas [134]. Lastly, in the same case report, multicolor FISH studies with probes to *MYB1*, *CCND1*, *RREB1*, and *CEP6* revealed a nevus genotype with no copy number chromosomal aberrations [134].

Differential Diagnosis

The differential diagnosis for melanocytic nevi with superimposed lichen sclerosus is melanoma, which necessitates an excisional biopsy to prevent a sampling error (Table 7.1). Compared to melanoma, melanocytic nevi with superimposed lichen sclerosus show: (1) symmetry and sharp circumscription, (2) the junctional melanocytic hyperplasia is limited in the region of lichen sclerosus, (3) minimal upward migration, (4) the majority of melanocytes are arranged in nests, (5) minimal nuclear atypia, (6) no dermal mitoses, and (7) gradient maturation of melanocytes with descent into the dermis [20, 131]. In contrast to melanoma regression, the dermal sclerosis is diffuse and homogenized and contains nests of melanocytes [134]. Ki-67 proliferation index is higher compared to common melanocytic nevi but significantly lower than melanoma. HMB-45 can be misleading as it simulates the diffuse expression seen in malignant melanomas.

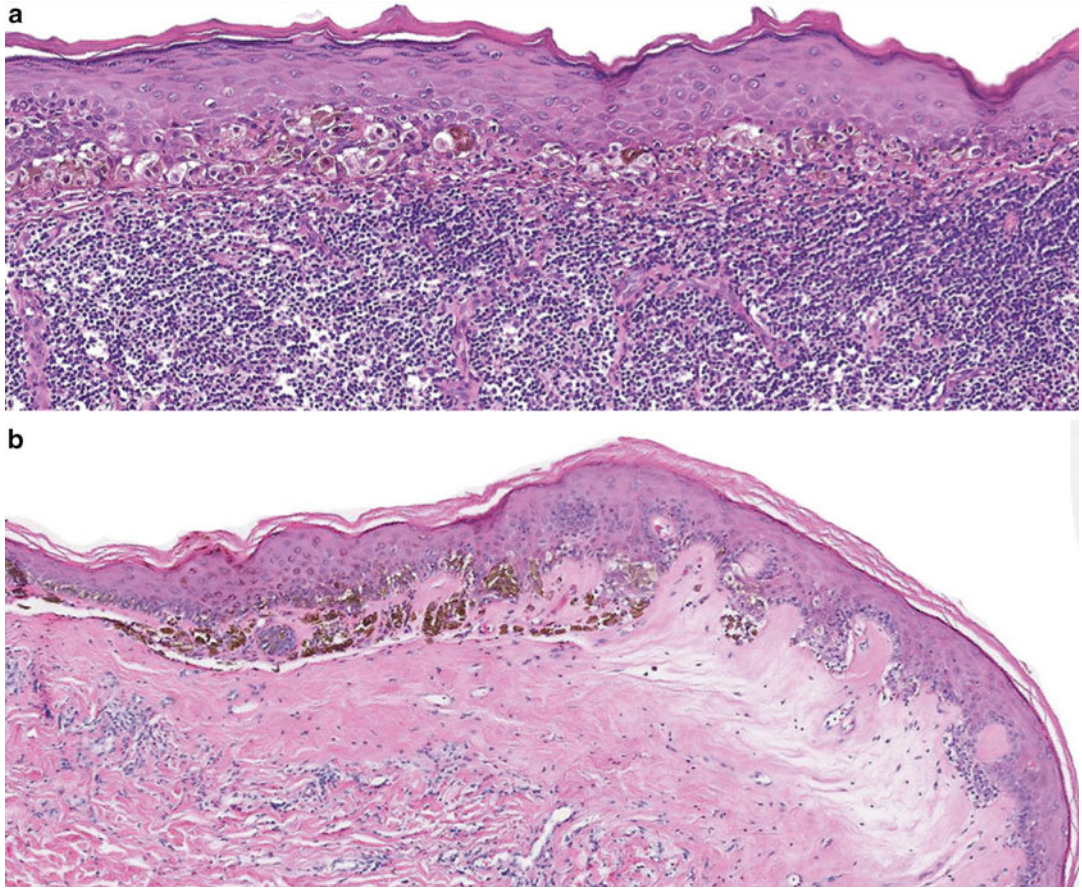


Fig. 7.17 Melanocytic nevi with superimposed lichen sclerosus, early (**a**) and late (**b**) lesions. Albeit cytologically atypical (confluent nests and enlarged melanocytes, respectively), features that argue against a diagnosis of melanoma for melanocytic proliferations arising in the setting of lichen sclerosus are the circumscribed borders without extension of the melanocytes past the region of lichen sclerosus, symmetry, as well as the absence of pagetoid upward migration and dermal mitotic figures

Table 7.1 Diagnostic features distinguishing atypical genital nevi, nevi associated with lichen sclerosus, and vulvar melanoma

Atypical genital nevi	Nevi associated with lichen sclerosus	Vulvar melanoma
Symmetrical	Sharply circumscribed borders	Extension of melanocytes past fibrosis
Focal and central pagetoid scatter	Minimal pagetoid scatter	Extensive pagetoid scatter
Can be junctional or compound	Concomitant dermal melanocytic nevus	No associated melanocytic nevus
Rare mitotic figures	No dermal mitoses	Dermal mitotic figures
Maturation with descent	Maturation with descend	No maturation with descent
Epidermal component does not extend beyond the dermal component	Junctional melanocytic hyperplasia limited to region of lichen sclerosus	Epidermal component can extend beyond the dermal component (radial growth phase)
Usually loss of HMB-45 with maturation	HMB-45 expression confined to dermal melanocytes within sclerosis	Deep dermal HMB-45 expression
Ki-67 index <10 %	Ki-67 index <10 %	Ki-67 index >10 %

Modified with permission from Carlson JA, Mu XC, Slominski A, Weismann K, Crowson AN, Malfetano J, et al. Melanocytic proliferations associated with lichen sclerosus. Arch Dermatol. 2002 Jan;138(1):77–87

Summary

Clinical Presentation

- Large in size, hyperchromatic with irregular borders
- Features of lichen sclerosus (atrophic, ivory patches to plaques) may be evident

Histologic Features

- Well circumscribed
- Single junctional melanocytes with large nuclei and abundant cytoplasm
- Focal upward migration
- Dermal melanocytes with abundant dusky gray cytoplasm, maturation, and absent mitoses
- Lichen sclerosus component beyond the borders of nevus

Differential Diagnosis

- Melanoma

Takeaway Essentials

Clinical Relevant Pearls

- Features of lichen sclerosus may be evident

Pathology Interpretation Pearls

- Criteria
  - Minimal upward migration
  - Minimal nuclear atypia
  - Symmetry and sharp circumscription
  - No dermal mitoses
  - Maturation with descent
- Majority of melanocytes arranged in nests
- Junctional melanocytic hyperplasia limited to region of lichen sclerosus

Immunohistochemical/Molecular Findings

- Ki-67 proliferation index is higher compared to common melanocytic nevi but significantly lower than melanoma
- HMB-45 can be diffusely expressed

Atypical Genital Melanocytic Nevi

Clinical Features

Atypical genital nevi, first described by Friedman and Ackerman [139], are benign melanocytic nevi, which fit within the category of "nevi of special sites" (axillae, breasts, periumbilical region, groin, flexures, acral sites, and the ears) [140]. These nevi have distinctive histologic features that overlap significantly with those of melanoma and dysplastic melanocytic nevi [71, 74, 138, 141, 142], leading to a misdiagnosis in up to one third of the cases according to a sentinel study [138]. They are uncommon, representing approximately 5–7 % of benign vulvar nevi [71, 143], and are seen predominantly in teenage girls, young premenopausal women, and occasionally children (≤10 years old) [70]. In addition, atypical vulvar melanocytic nevi are commonly detected during pregnancy, routine physical, or gynecologic examination or during skin surveillance because of history of melanoma or dysplastic nevi. These nevi show a strong predilection for the clitoris, labia majora, and labia minora with equal involvement of hair-bearing and glabrous surfaces [141]; they can also arise in the mons pubis, perineum, and male genitalia [74]. They are usually symmetric, uniformly hyperpigmented papular lesions, most often smaller than 1 cm in diameter with well-circumscribed borders.

Atypical vulvar melanocytic nevi are benign and rarely can recur after biopsy [141]. Thus, it is considered prudent to conservatively reexcise any incompletely excised lesion to allow a complete pathologic examination and prevent local recurrence; however, a wide excision in this sensitive area is not indicated. These patients should be followed in a similar fashion to those with regular dysplastic nevi [141].

Histologic Features

Atypical genital nevi are typically junctional or compound and display architectural symmetry with good lateral circumscription. They often have a florid junctional melanocytic proliferation

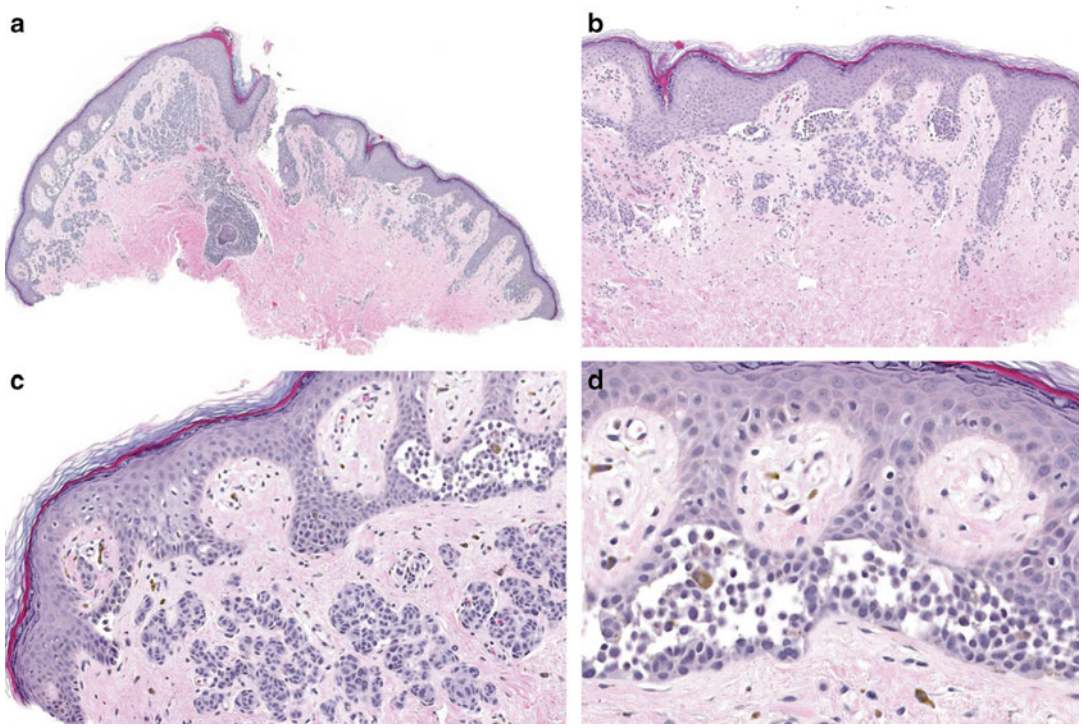


Fig. 7.18 Atypical genital melanocytic nevus—a nevus of special site [140]. Atypical vulvar (genital) melanocytic nevi are papular/nodular tumors formed by compound melanocytic nevi that show well-defined lateral circumscription, symmetric profiles (**a**, **b**), and prominent junctional melanocytes that form dyscohesive but confluent nests along the sides and bases of rete ridges with retraction artifact (**c**, **d**), similar but more extensive as what can be found in compound dysplastic melanocytic nevi. Unlike dysplastic melanocytic nevi, atypical genital nevi show minimal shouldering

composed of large variably sized nests with cellular dyscohesion and prominent retraction artifact [138]. The junctional nests can originate from the sides as well as the tips of the rete ridges and sometimes bridge the adjacent rete ridges to form elongated fusiform nests oriented parallel to the dermoepidermal junction [74] (Figs. 7.18 and 7.19). Concomitant with the unusual junctional changes, compound atypical vulvar melanocytic nevi exhibit a large, "mushroom-shaped" dermal nevus component. Unlike authentic dysplastic nevi, the epidermal component usually does not extend beyond its shoulder. The cytologic atypia can be variable from mild to severe with epithelioid cytomorphology, vesicular nuclei, and readily identified, occasionally eosinophilic nucleoli. Pagetoid upward migration is not uncommon, but is usually focal and centrally located overlying the large junctional nests [141]. Adnexal extension of single cells or nests can be seen in almost half of the cases, a feature shared with congenital nevi. The superficial part of the dermal component can show cytologic atypia, which can be occasionally severe, and rare mitoses can be seen. However, maturation with descent is always present [141]. Features classically associated with a dysplastic nevus are seen in a subset of cases including bridging of adjacent rete ridges, a "shoulder" component (extension of the epidermal melanocytes beyond the dermal component),

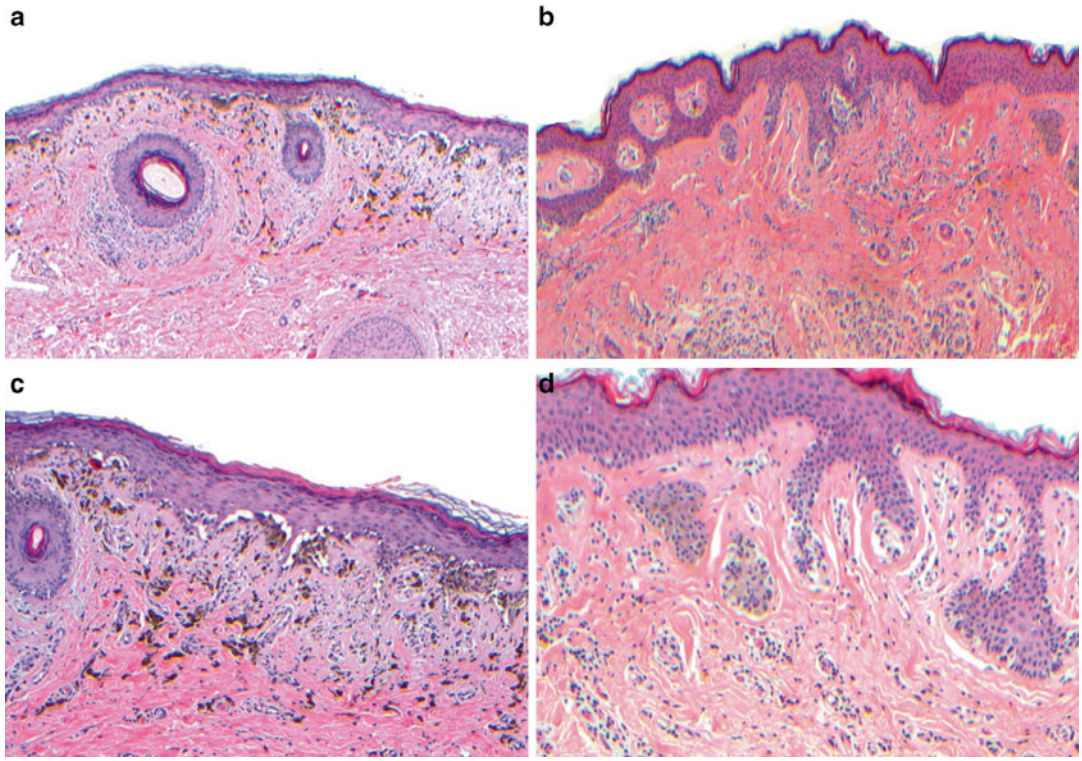


Fig. 7.19 Atypical genital melanocytic nevus—stromal alterations are common. Here are two more examples of atypical vulvar melanocytic nevi exhibiting features that overlap with melanoma (confluence of atypical melanocytes along the dermal-epidermal junction associated with fibrosis (**a**, **c**)) and dysplastic nevus (lamellar fibrosis and enlargement of melanocytes (**b**, **d**)). The dermal fibrosis is suspected to induce these pseudo-melanoma changes, similar to that of persistent melanocytic nevi and nevi associated with lichen sclerosus [131, 138]. Awareness of these features is important to avoid overdiagnosis of melanoma and unnecessary treatment [142]

or a lamellar pattern of fibroplasia in the papillary dermis [141]. Cytoplasmic pigmentation can be minimal, coarse, or so-called dusty. Some cases can exhibit dermal fibrosis of the papillary and superficial reticular dermis, which can entrap the dermal melanocytes in single cells or nests resembling regression. In a subset of cases, superficial dermal lymphohistiocytic infiltrate associated with ectatic vessels and melanophages can be seen [141].

Differential Diagnosis

The main differential diagnosis includes a dysplastic nevus and melanoma (Table 7.1). Clinically atypical genital nevi are more common on the labium minora and mucosal surfaces, whereas dysplastic nevi are more common on the labium majora, and may be associated with a dysplastic nevus syndrome. Dysplastic nevi are composed of a disorganized lentiginous proliferation of single cells and small nests of melanocytes along

elongated rete ridges, whereas large dyscohesive nests without elongation of rete ridges compose atypical genital nevi. Bridging is present in both lesions. Pagetoid spread is absent in dysplastic nevi whereas it can be focal and central in atypical vulvar melanocytic nevi. The lentiginous single-cell pattern is a minor component in atypical genital nevi and usually at the periphery. In dysplastic nevi, the cytologic atypia is random and focal and the pattern of dermal fibroplasia is described as concentric eosinophilic and lamellar, whereas in the atypical genital nevi, the cytologic atypia is uniformly mild to moderate (sometimes severe) and the fibroplasia is denser. Significant number of dermal mitoses is not present in dysplastic nevi. However, there is histologic overlap between these two entities in some lesions.

Most importantly though is the distinction from melanoma which sometimes can be particularly difficult. First of all, vulvar melanoma is primarily a disease of postmenopausal women as opposed to atypical genital nevi, which are common in women of reproductive age. Melanoma is often larger than 1 cm in diameter as opposed to atypical vulvar melanocytic nevi, which are 1 cm or less. Clinically, irregular pigmentation is commonly observed in melanoma. Histopathologically, melanoma demonstrates a predominance of single atypical hyperchromatic melanocytes compared to a nested junctional population, displays diffuse cytologic atypia throughout as opposed to regional atypia in the junctional nests, and lacks architectural symmetry. Furthermore, the dermal, invasive component of melanoma shows no maturation and mitoses are easily identifiable [141]. See Vignette 4 at the end of this chapter.

Summary

Clinical Presentation

- Predominantly in older girls and premenopausal women
- Symmetric, uniformly hyperpigmented papular lesions most often smaller than 1 cm in diameter with well-circumscribed borders

Histologic Features

- Symmetrical
- Large variably sized nests with cellular dyscohesion and prominent retraction artifact
- Elongated fusiform nests oriented parallel to the dermoepidermal junction
- Epidermal component does not extend beyond the dermal component
- Focal and central pagetoid upper migration

Differential Diagnosis

- Dysplastic nevus
- Melanoma

Takeaway Essentials

Clinical Relevant Pearls

- Rarely can recur
- Conservative reexcision of any incompletely excised lesion is prudent; however, a wide excision is not indicated

Pathology Interpretation Pearls

- Histologic overlap between atypical genital nevi and dysplastic nevi

Immunohistochemical/Molecular Findings

- Not usually applied in routine cases

Case Vignettes

Vignette 1

Clinical History: A 43-year-old female was referred to dermatology for chronic vulvar itching. On clinical exam, she was found to have multiple irregular, brown, and white papules (line arrow) and plaques (block arrow) affecting the vestibule and labia minora (Fig. 7.20). Biopsies were taken of the affected area.

Microscopic Description: Slight papillated epidermal hyperplasia and marked acanthosis is present and the spinous layer is replaced by pleomorphic keratinocytes, which have hyperchromatic nuclei and pigmented cytoplasm. The upper spinous layer shows koilocytes indicative of human papillomavirus infection (Fig. 7.21).

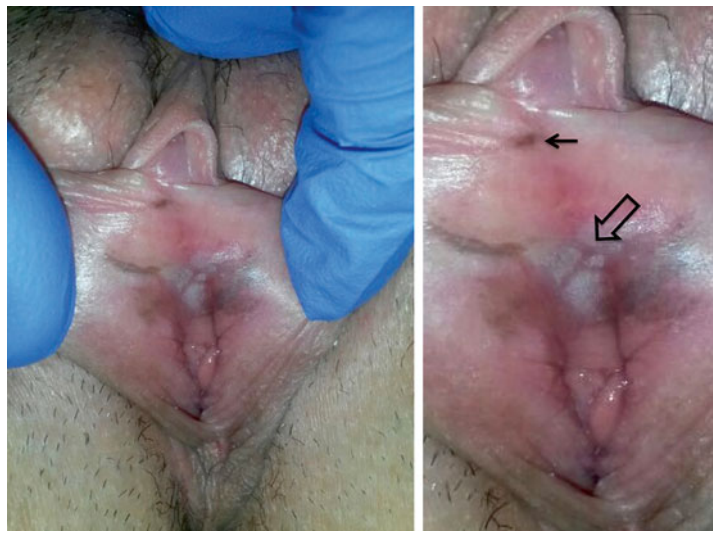


Fig. 7.20 Vignette 1. Multiple irregular, brown, and white papules (*line arrow*) and plaques (*block arrow*) affecting the vestibule and labia minora

(continued)

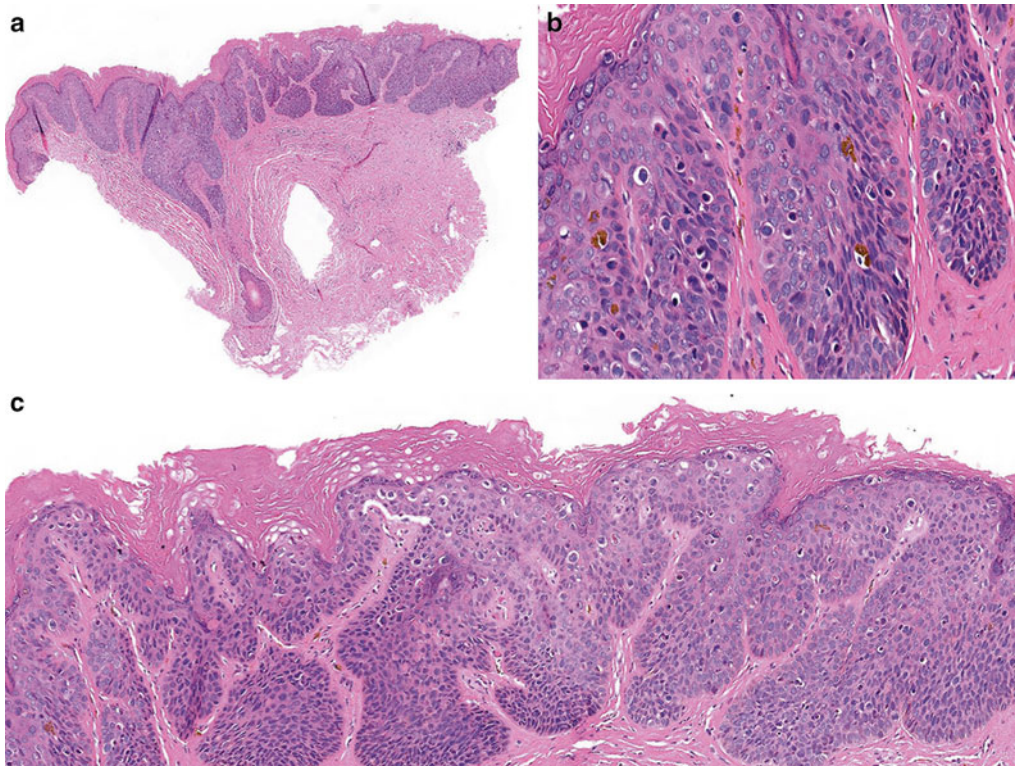


Fig. 7.21 Vignette 1. Slight papillated epidermal hyperplasia and marked acanthosis (**a**). The spinous layer is replaced by pleomorphic keratinocytes, which demonstrate hyperchromatic nuclei and pigmented cytoplasm (**b**). The upper spinous layer shows koilocytes indicative of human papillomavirus infection (**c**)

Diagnosis: Pigmented vulvar intraepithelial neoplasia (VIN 3), warty type

Discussion: High-grade (warty or basaloid) VIN3 frequently presents as pigmented, multifocal papules and plaques with relatively sharp margins. While the diagnosis is based clinically, histologic confirmation is necessary as seborrheic keratoses, genital warts, and melanocytic nevi can present with similar clinical morphologies. If left untreated, VIN3 has a low risk, approximately 9–16 %, of progressing to invasive squamous cell carcinoma [144].

Vignette 2

Clinical History: A 73-year-old female presented with chronic itching of the labia majora. Vulvar exam shows dusky red-brown pigmentation (a combination of erythema and post-inflammatory hyperpigmentation) of her vulva. Excoriations and marked accentuation of skin markings of the vulva and inguinal region are evident (Fig. 7.22). She also has erythema and scale affecting her scalp, face, and axilla.

Microscopic Description: Corresponding to the clinical findings, histopathologic evidence of lichen simplex chronicus in the form of psoriasiform hyperplasia, spongiosis, dermal fibrosis, hypergranulosis, and hyperkeratosis is seen (top panels) (Fig. 7.23). Also, foci of ulceration, erosion, and epidermal necrosis are present and are confirmatory signs of excoriation.

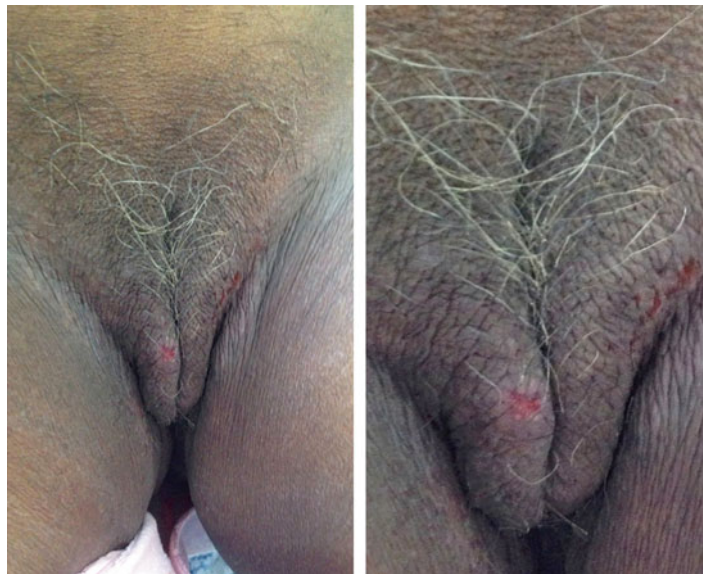


Fig. 7.22 Vignette 2. Dusky red-brown pigmentation with excoriations and marked accentuation of skin markings of the vulva and inguinal region

(continued)

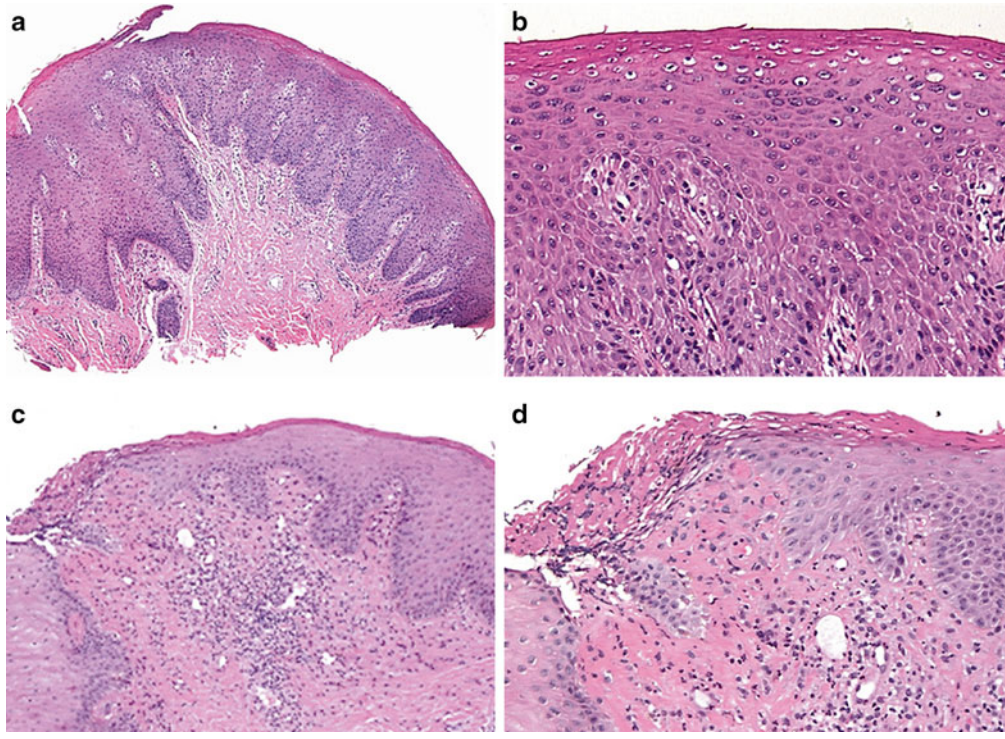


Fig. 7.23 Vignette 2. Psoriasiform hyperplasia, spongiosis, dermal fibrosis, hypergranulosis, and hyperkeratosis characterize the histopathologic changes (**a**, **b**). Also, foci of ulceration, erosion, and epidermal necrosis are noted consistent with excoriation (**c**, **d**)

Diagnosis: Hyperpigmented lichen simplex chronicus arising in chronic spongiotic dermatitis due to seborrheic dermatitis

Discussion: This elderly lady likely has seborrheic dermatitis. An underlying atopic diathesis and/or secondary allergic or irritant contact dermatitis may be exacerbating her vulvar symptoms producing hyperpigmented lichen simplex chronicus due to post-inflammatory hyperpigmentation.

Vignette 3

Clinical History: A young 32-year-old woman presented with an asymptomatic, black macule on the labia minora-vestibule, 7 × 5 mm (Fig. 7.24). An excisional biopsy was performed.

Microscopic Description: A circumscribed proliferation of melanocytes is present associated with marked basal keratinocyte melanization (top panel) (Fig. 7.25). Mostly solitary, cytologically banal melanocytes are present along the sides and bases of elongated and hyperpigmented rete ridges, similar to that of cutaneous simple lentigo (left bottom panel) (Fig. 7.25). Notably, a region of enlarged, solitary and rare, nested epithelioid melanocytes found along rete ridges is also present (right bottom panel) (Fig. 7.25). All of these changes are associated with underlying fibrosis and melanophages suggesting prior injury and/or inflammation.

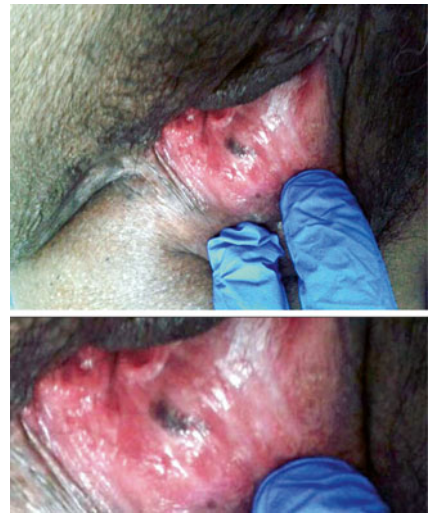


Fig. 7.24 Vignette 3. Asymptomatic, black macule on the labia minora-vestibule

(continued)

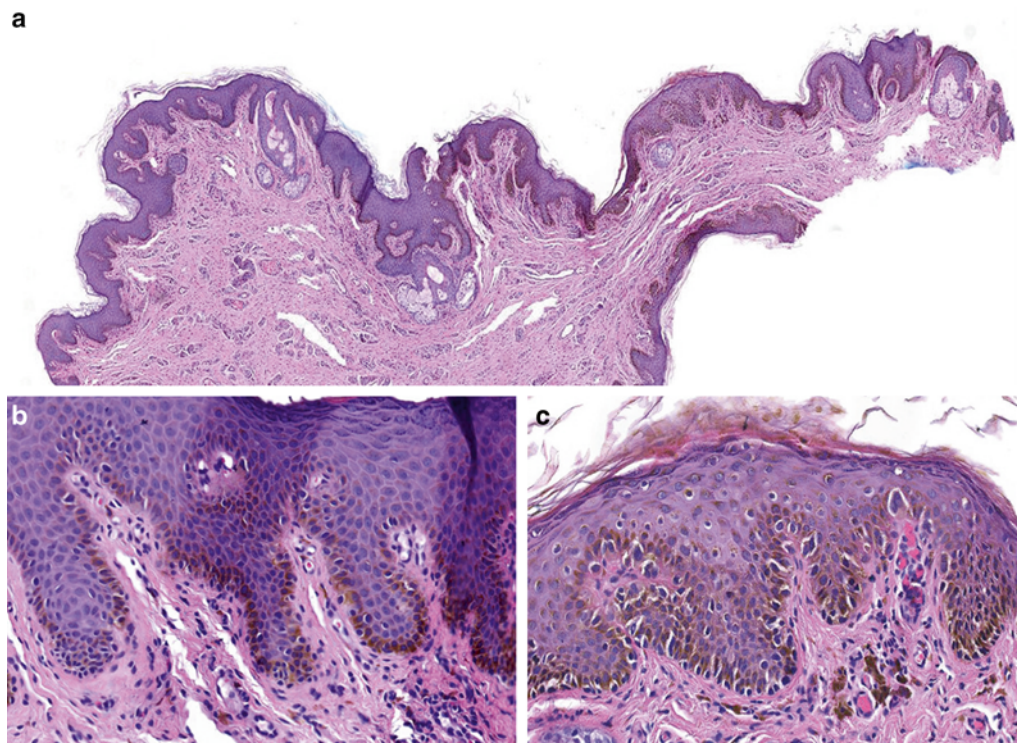


Fig. 7.25 Vignette 3. A circumscribed proliferation of melanocytes associated with marked basal keratinocyte melanization (**a**). Mostly solitary, cytologically banal melanocytes along the sides and bases of elongated and hyperpigmented rete ridges (**b**). A region of enlarged, solitary and rare, nested epithelioid melanocytes found along ridges is also present (**c**). Underlying fibrosis and melanophages suggesting prior injury and/or inflammation

Diagnosis: Lentiginous junctional melanocytic nevus

Discussion: The small size, circumscription, symmetry, and lack of pagetoid scatter of melanocytes indicate that this melanocytic proliferation is a melanocytic nevus and not melanoma in situ. The keratinocytic hyperpigmentation, submucosal fibrosis, and numerous melanophages are all features that overlap with vulvar melanosis (lentiginoses) and suggest that a continuum might exist between these entities (melanotic macule to lentigo simplex to lentiginous junctional melanocytic nevus). Large, irregular patches of genital melanosis should be well sampled to prevent a sampling error.

Vignette 4

Clinical History: A woman, 45 years old, presented with an ulcerated, variegated periclitoral nodule because of recent onset of symptoms of a palpable mass and bleeding (Fig. 7.26). She does not report any history of vulvar abnormalities before the onset of symptoms and signs. An excisional biopsy was performed.

Microscopic Description: A compound, asymmetric proliferation of pleomorphic epithelioid and spindle melanocytes is present that has extensively infiltrated the surrounding squamous mucosa and has formed a large nodule (Fig. 7.27).

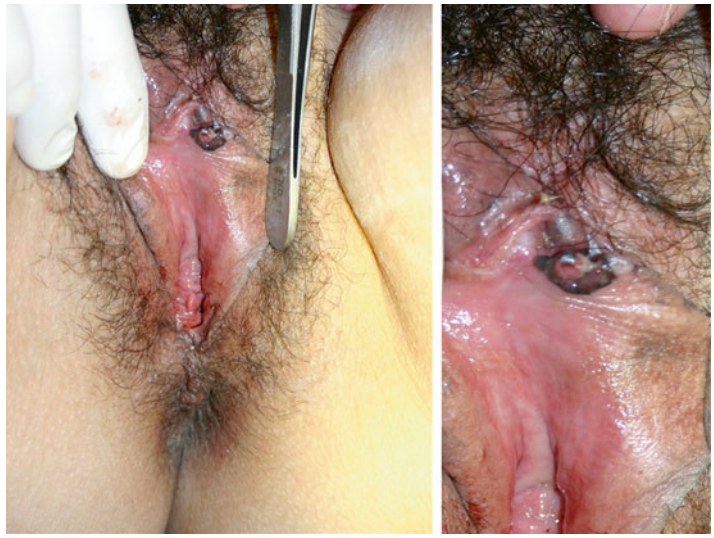


Fig. 7.26 Vignette 4. Ulcerated, variegated periclitoral nodule

(continued)

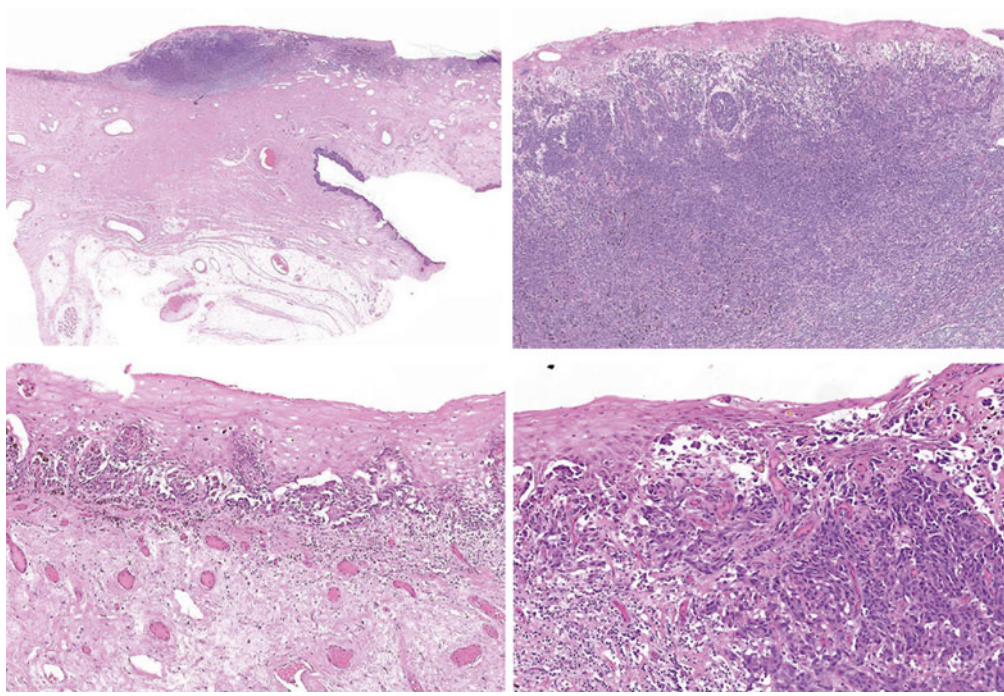


Fig. 7.27 Vignette 4. Compound, asymmetric proliferation of pleomorphic epithelioid and spindle melanocytes infiltrating the surrounding squamous mucosa

Diagnosis: Vulvar melanoma, lentiginous mucosal type

Discussion: This is unquestionably a melanoma, based on its large size and extensive but asymmetric proliferation of pleomorphic melanocytes. Most vulvar melanomas are detected when they have reached a large size/greater thickness compared to cutaneous, sun-associated melanomas. Vulvar melanomas are thought to arise de novo; however, studies examining for the prevalence of genital melanosis, lichen sclerosus, or HPV in the setting of vulvar melanoma are few. As vulvar melanoma is a disease of older woman, a lower threshold for vulvar biopsy of lesions in this age group is recommended.

References

1. Szabo G. Quantitative histological investigation on the melanocyte system in the human epidermis. In: Gordon M, editor. Pigment cell biology. New York: Academic; 1959. p. 99–124.
2. Lu S, Slominski A, Yang SE, Sheehan C, Ross J, Carlson JA. The correlation of TRPM1 (Melastatin) mRNA expression with microphthalmia-associated transcription factor (MITF) and other melanogenesis-related proteins in normal and pathological skin, hair follicles and melanocytic nevi. J Cutan Pathol. 2010;37 Suppl 1:26–40.
3. Friedrich Jr EG, Burch K, Bahr JP. The vulvar clinic: an eight-year appraisal. Am J Obstet Gynecol. 1979;135(8):1036–40.
4. Rock B, Hood AF, Rock JA. Prospective study of vulvar nevi. J Am Acad Dermatol. 1990;22(1):104–6.
5. Tovell HM, Young Jr AW. Diseases of vulva. Classification and incidence of 877 patients seen consecutively in vulva clinic. N Y State J Med. 1977;77(6):938–41.
6. Rock B. Pigmented lesions of the vulva. Dermatol Clin. 1992;10(2):361–70.
7. Edwards L. Pigmented vulvar lesions. Dermatol Ther. 2010;23(5):449–57.

8. Blossom J, Altmayer S, Jones DM, Slominski A, Carlson JA. Volar melanotic macules in a gardener: a case report and review of the literature. Am J Dermatopathol. 2008;30(6):612–9.
9. Venkatesan A. Pigmented lesions of the vulva. Dermatol Clin. 2010;28(4):795–805.
10. Shizume K. Thirty-five years of progress in the study of MSH. Yale J Biol Med. 1985;8(6):561–70.
11. Wisernan MCKE. Genital dermatology atlas. 1st ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
12. Jackson R. Melanosis of the vulva. J Dermatol Surg Oncol. 1984;10(2):119–21.
13. Sison-Torre EQ, Ackerman AB. Melanosis of the vulva. A clinical simulator of malignant melanoma. Am J Dermatopathol. 1985;7(Suppl):51–60.
14. Rudolph RI. Vulvar melanosis. J Am Acad Dermatol. 1990;23(5 Pt 2):982–4.
15. Estrada R, Kaufman R. Benign vulvar melanosis. J Reprod Med. 1993;38(1):5–8.
16. Barnhill RL, Albert LS, Shama SK, Goldenhersh MA, Rhodes AR, Sober AJ. Genital lentiginosis: a clinical and histopathologic study. J Am Acad Dermatol. 1990;22(3):453–60.
17. Tran TA, Niu G, Tomasello CA, Tran HV, Ross JS, Carlson JA. The spectrum of grossly visible pigmented lesions in the uterine cervix: a prospective study. Int J Gynecol Pathol. 2014;33(1):89–99.
18. Oliveira A, Lobo I, Selores M. Asymptomatic vulvar pigmentation. Clin Exp Dermatol. 2011;36(8): 921–2.
19. Veraldi S, Cavicchini S, Benelli C, Gasparini G. Laugier-Hunziker syndrome: a clinical, histopathologic, and ultrastructural study of four cases and review of the literature. J Am Acad Dermatol. 1991; 25(4):632–6.
20. El Shabrawi-Caelen L, Soyer HP, Schaeppi H, Cerroni L, Schirren CG, Rudolph C, et al. Genital lentigines and melanocytic nevi with superimposed lichen sclerosus: a diagnostic challenge. J Am Acad Dermatol. 2004;50(5):690–4.
21. Schaffer JV, Orlow SJ. Melanocytic proliferations in the setting of vulvar lichen sclerosus: diagnostic considerations. Pediatr Dermatol. 2005;22(3):276–8; author reply 8–9.
22. Carlson JA, Lamb P, Malfetano J, Ambros RA, Mihm Jr MC. Clinicopathologic comparison of vulvar and extragenital lichen sclerosus: histologic variants, evolving lesions, and etiology of 141 cases. Mod Pathol. 1998;11(9):844–54.
23. Lenane P, Keane CO, Connell BO, Loughlin SO, Powell FC. Genital melanotic macules: clinical, histologic, immunohistochemical, and ultrastructural features. J Am Acad Dermatol. 2000;42(4):640–4.
24. Chapel TA, Taylor RM, Pinkus H. Volar melanotic macules. Int J Dermatol. 1979;18(3):222–5.
25. Ruiz-Maldonado R, Orozco-Covarrubias ML. Postinflammatory hypopigmentation and hyperpigmentation. Semin Cutan Med Surg. 1997;16(1): 36–43.
26. Jih DM, Elder DE, Elenitsas R. A histopathologic evaluation of vulvar melanosis. Arch Dermatol. 1999;135(7):857–8.
27. Rohwedder A, Philips B, Malfetano J, Kredentser D, Carlson JA. Vulvar malignant melanoma associated with human papillomavirus DNA: report of two cases and review of literature. Am J Dermatopathol. 2002;24(3):230–40.
28. Rohwedder A, Slominski A, Wolff M, Kredentser D, Carlson JA. Epidermodysplasia verruciformis and cutaneous human papillomavirus DNA, but not genital human papillomavirus DNAs, are frequently detected in vulvar and vaginal melanoma. Am J Dermatopathol. 2007;29(1):13–7.
29. Kacerovska D, Requena L, Carlson JA, Santonja C, Michal M, Bouda J, et al. Pigmented squamous intraepithelial neoplasia of the anogenital area: a histopathological and immunohistochemical study of 64 specimens from 45 patients exploring the mechanisms of pigmentation. Am J Dermatopathol. 2014;36(6):471–7.
30. Kerley SW, Blute ML, Keeney GL. Multifocal malignant melanoma arising in vesicovaginal melanosis. Arch Pathol Lab Med. 1991;115(9):950–2.
31. Carlson JA, Ambros R, Malfetano J, Ross J, Grabowski R, Lamb P, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. Hum Pathol. 1998; 29(9):932–48.
32. Weedon D. Disorders of pigmentation. Weedon's skin pathology. 3rd ed. London: Churchill Livingstone; 2010. p. 281–99.
33. Reynolds N, Colins C, Kennedy C. Penile melanosis and lichen sclerosus et atrophicus. Eur J Dermatol. 1993;3:41–2.
34. Hendi A, Brodland DG, Zitelli JA. Melanocytes in long-standing sun-exposed skin: quantitative analysis using the MART-1 immunostain. Arch Dermatol. 2006;142(7):871–6.
35. Lacz NL, Vafaie J, Kihiczak NI, Schwartz RA. Postinflammatory hyperpigmentation: a common but troubling condition. Int J Dermatol. 2004;43(5): 362–5.
36. Carlson JA, Carlson GD, Murphy M, Rohwedder A. Lichen sclerosus exhibiting histologic signs of lymphedema: an essential factor in the pathogenesis of verruciform xanthoma. Arch Dermatol. 2012; 148(2):260–2; author reply 2.
37. Li Q, Leopold K, Carlson JA. Chronic vulvar purpura: persistent pigmented purpuric dermatitis (lichen aureus) of the vulva or plasma cell (Zoon's) vulvitis? J Cutan Pathol. 2003;30(9):572–6.
38. Denadai R, Souto RM, Auada-Souto MP. Generalized acanthosis nigricans without systemic disease associated. Dermatol Online J. 2013;19(9):19614.
39. Piccolo V, Russo T, Picciocchi R, Errico M, Ametrano O, Moscarella E. Generalized idiopathic

benign acanthosis nigricans in childhood. Ann Dermatol. 2013;25(3):375–7.
40. Grandhe NP, Bhansali A, Dogra S, Kumar B. Acanthosis nigricans: relation with type 2 diabetes mellitus, anthropometric variables, and body mass in Indians. Postgrad Med J. 2005;81(958):541–4.
41. Weedon D. Miscellaneous conditions Weedon's skin pathology. 3rd ed. London: Churchill Livingstone; 2010. p. 502–9.
42. Berk DR, Spector EB, Bayliss SJ. Familial acanthosis nigricans due to K650T FGFR3 mutation. Arch Dermatol. 2007;143(9):1153–6.
43. Brickman WJ, Binns HJ, Jovanovic BD, Kolesky S, Mancini AJ, Metzger BE. Acanthosis nigricans: a common finding in overweight youth. Pediatr Dermatol. 2007;24(6):601–6.
44. Brickman WJ, Huang J, Silverman BL, Metzger BE. Acanthosis nigricans identifies youth at high risk for metabolic abnormalities. J Pediatr. 2010;156(1): 87–92.
45. Pedro S. Letter: drug-induced acanthosis nigricans. N Engl J Med. 1974;291(8):422.
46. Tromovitch TA, Jacobs PH, Kern S. Acanthosis nigricans-like lesions from nicotinic acid. Arch Dermatol. 1964;89:222–3.
47. Talsania N, Harwood CA, Piras D, Cerio R. Paraneoplastic acanthosis nigricans: the importance of exhaustive and repeated malignancy screening. Dermatol Online J. 2010;16(8):8.
48. De Sanctis V, Soliman A, Arsciani A, Timoncini G, Reggiani L, Zucchini A, et al. Acanthosis nigricans in adolescents: a practical approach. Georgian Med News. 2013;222:73–8.
49. Torley D, Bellus GA, Munro CS. Genes, growth factors and acanthosis nigricans. Br J Dermatol. 2002;147(6):1096–101.
50. Grasso V, Colombo C, Favalli V, Galderisi A, Rabbone I, Gombos S, et al. Six cases with severe insulin resistance (SIR) associated with mutations of insulin receptor: is a Bartter-like syndrome a feature of congenital SIR? Acta Diabetol. 2013;50(6): 951–7.
51. Grasinger CC, Wild RA, Parker IJ. Vulvar acanthosis nigricans: a marker for insulin resistance in hirsute women. Fertil Steril. 1993;59(3):583–6.
52. Kurzrock R, Cohen PR. Mucocutaneous paraneoplastic manifestations of hematologic malignancies. Am J Med. 1995;99(2):207–16.
53. Rogers DL. Acanthosis nigricans. Semin Dermatol. 1991;10(3):160–3.
54. Brown J, Winkelmann RK. Acanthosis nigricans: a study of 90 cases. Medicine (Baltimore). 1968;47(1): 33–51.
55. Yosipovitch G, DeVore A, Dawn A. Obesity and the skin: skin physiology and skin manifestations of obesity. J Am Acad Dermatol. 2007;56(6):901–16; quiz 17-20.
56. Carlson JA, Grabowski R, Mu XC, Del Rosario A, Malfetano J, Slominski A. Possible mechanisms of hypopigmentation in lichen sclerosus. Am J Dermatopathol. 2002;24(2):97–107.
57. Larregue M, Martin J, Bressieux JM, Canuel C, De Giacomoni P, Ramdenee P, et al. Achromies vitiligoides et dermatite atopique grave. A propos de quatre cas. fre. [Vitiligoid achromias and severe atopic dermatitis. Apropos of 4 cases]. Ann Dermatol Venereol. 1985;112(8):589–600.
58. Vachiramon V, Thadanipon K. Postinflammatory hypopigmentation. Clin Exp Dermatol. 2011;36(7): 708–14.
59. Handa S, Handa U. Sarcoidosis presenting as cutaneous hypopigmentation [letter; comment]. Int J Dermatol. 1995;34(11):824.
60. Yang CC, Lee JY, Wong TW. Depigmented extramammary Paget's disease. Br J Dermatol. 2004;151(5): 1049–53.
61. Rose PT. Pigmentary disorders. Med Clin North Am. 2009;93(6):1225–39.
62. Njoo MD, Vitiligo WW. Pathogenesis and treatment. Am J Clin Dermatol. 2001;2(3):167–81.
63. van Geel N, Speeckaert R, Melsens E, Toelle SP, Speeckaert M, De Schepper S, et al. The distribution pattern of segmental vitiligo: clues for somatic mosaicism. Br J Dermatol. 2013;168(1):56–64.
64. Mollet I, Ongenae K, Naeyaert JM. Origin, clinical presentation, and diagnosis of hypomelanotic skin disorders. Dermatol Clin. 2007;25(3):363–71, ix.
65. Le Poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. Curr Dir Autoimmun. 2008; 10:227–43.
66. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. Pigment Cell Res. 2003;16(2):90–100.
67. Asawanonda P, Taylor CR. Wood's light in dermatology. Int J Dermatol. 1999;38(11):801–7.
68. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. Am J Pathol. 1996;148(4):1219–28.
69. Montes LF, Abulafia J, Wilborn WH, Hyde BM, Montes CM. Value of histopathology in vitiligo. Int J Dermatol. 2003;42(1):57–61.
70. Hunt RD, Orlow SJ, Schaffer JV. Genital melanocytic nevi in children: experience in a pediatric dermatology practice. J Am Acad Dermatol. 2014; 70(3):429–34.
71. Christensen WN, Friedman KJ, Woodruff JD, Hood AF. Histologic characteristics of vulvar nevocellular nevi. J Cutan Pathol. 1987;14(2):87–91.
72. Zembowicz A, McCusker M, Chiarelli C, Dei Tos AP, Granter SR, Calonje E, et al. Morphological analysis of nevoid melanoma: a study of 20 cases with a review of the literature. Am J Dermatopathol. 2001;23(3):167–75.
73. Fulciniti F, Ascierto PA, Simeone E, Bove P, Losito S, Russo S, et al. Nevoid melanoma of the vagina: report of one case diagnosed on thin layer cytological preparations. CytoJournal. 2007;4:14.

74. Ribe A. Melanocytic lesions of the genital area with attention given to atypical genital nevi. J Cutan Pathol. 2008;35 Suppl 2:24–7.
75. Yamazhan M, Ertas IE, Kandiloglu G, Ozeren M. Cellular blue nevus of the vulva mimicking Bartholin's gland abscess in a 15-year-old girl: a case report. Arch Gynecol Obstet. 2012;285(4): 1009–11.
76. Spatz A, Zimmermann U, Bachollet B, Pautier P, Michel G, Duvillard P. Malignant blue nevus of the vulva with late ovarian metastasis. Am J Dermatopathol. 1998;20(4):408–12.
77. Rodriguez HA, Ackerman LV. Cellular blue nevus. Clinicopathologic study of forty-five cases. Cancer. 1968;21(3):393–405.
78. Craddock KJ, Bandarchi B, Khalifa MA. Blue nevi of the Mullerian tract: case series and review of the literature. J Low Genit Tract Dis. 2007;11(4):284–9.
79. Zembowicz A, Phadke PA. Blue nevi and variants: an update. Arch Pathol Lab Med. 2011;135(3):327–36.
80. Tran TA, Carlson JA, Basaca PC, Mihm MC. Cellular blue nevus with atypia (atypical cellular blue nevus): a clinicopathologic study of nine cases. J Cutan Pathol. 1998;25(5):252–8.
81. Izquierdo MJ, Pastor MA, Carrasco L, Moreno C, Kutzner H, Sangueza OP, et al. Epithelioid blue naevus of the genital mucosa: report of four cases. Br J Dermatol. 2001;145(3):496–501.
82. Zembowicz A, Carney JA, Mihm MC. Pigmented epithelioid melanocytoma: a low-grade melanocytic tumor with metastatic potential indistinguishable from animal-type melanoma and epithelioid blue nevus. Am J Surg Pathol. 2004;28(1):31–40.
83. Gavriilidis P, Michalopoulou I, Chatzikakidou K, Nikolaidou A. Pigmented epithelioid melanocytoma: a new concept encompassing animal-type melanoma and epithelioid blue nevus. BMJ Case Rep. 2013; 22 Mar 2013. pii: bcr-2013-008865. doi: 10.1136/bcr-2013-008865
84. Barnhill RL, Argenyi Z, Berwick M, Duray PH, Erickson L, Guitart J, et al. Atypical cellular blue nevi (cellular blue nevi with atypical features): lack of consensus for diagnosis and distinction from cellular blue nevi and malignant melanoma ("malignant blue nevus"). Am J Surg Pathol. 2008;32(1): 36–44.
85. Requena L, Carlson J. Melanoma arising from blue naevus. In: LeBoit P, Burg G, Weedon D, Sarasin A, editors. WHO classification of tumors: pathology & genetics of skin tumors. Lyon: IARC; 2006. p. 79–82.
86. Cerez-Pham H, Bertrand G, Tigori J, Simard C. Association of a malignant vaginal melanoma with vaginal melanosis and a blue nevus of the cervix. Apropos of a case. Arch Anat Cytol Pathol. 1984;32(1):48–51.
87. Barnhill RL, Cerroni L, Cook M, Elder DE, Kerl H, LeBoit PE, et al. State of the art, nomenclature, and points of consensus and controversy concerning benign melanocytic lesions: outcome of an international workshop. Adv Anat Pathol. 2010;17(2):73–90.
88. Gammon B, Beilfuss B, Guitart J, Busam KJ, Gerami P. Fluorescence in situ hybridization for distinguishing cellular blue nevi from blue nevus-like melanoma. J Cutan Pathol. 2011;38(4):335–41.
89. Hantschke M, Mentzel T, Rutten A, Palmedo G, Calonje E, Lazar AJ, et al. Cutaneous clear cell sarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 12 cases emphasizing its distinction from dermal melanoma. Am J Surg Pathol. 2010;34(2):216–22.
90. Falconieri G, Bacchi CE, Luzar B. Cutaneous clear cell sarcoma: report of three cases of a potentially underestimated mimicker of spindle cell melanoma. Am J Dermatopathol. 2012;34(6):619–25.
91. Spitz S. Melanomas of childhood. Am J Pathol. 1948;24(3):591–609.
92. Shimek CM, Golitz LE. The golden anniversary of the Spitz nevus. Arch Dermatol. 1999;135(3):333–5.
93. Spatz A, Calonje E, Handfield-Jones S, Barnhill RL. Spitz tumors in children: a grading system for risk stratification. Arch Dermatol. 1999;135(3):282–5.
94. Zedek DC, McCalmont TH. Spitz nevi, atypical spitzoid neoplasms, and spitzoid melanoma. Clin Lab Med. 2011;31(2):311–20.
95. Elder D, Murphy G. Spindle and epithelioid cell melanocytic tumors. In: Elder D, Murphy G, editors. Melanocytic tumors of the skin. Silver Spring: ARP; 2010. p. 83–113.
96. Crowson AN, Magro CM, Mihm MC. Spitz nevus. In: Crowson AN, Magro CM, Mihm MC, editors. The melanocytic proliferations of the skin. New York: Wiley; 2001. p. 121–76.
97. Massi G, LeBoit PE. Spitz nevus. Histological diagnosis of nevi and melanoma. Darmstadt: Steinkopf Verlag; 2004. p. 169–234.
98. Plaza JA, De Stefano D, Suster S, Prieto VG, Kacerovska D, Michal M, et al. Intradermal spitz nevi: a rare subtype of spitz nevi analyzed in a clinicopathologic study of 74 cases. Am J Dermatopathol. 2014;36(4):283–97.
99. Diaz-Cascajo C, Borghi S, Weyers W. Angiomatoid Spitz nevus: a distinct variant of desmoplastic Spitz nevus with prominent vasculature. Am J Dermatopathol. 2000;22(2):135–9.
100. Tetzlaff MT, Xu X, Elder DE, Elenitsas R. Angiomatoid Spitz nevus: a clinicopathological study of six cases and a review of the literature. J Cutan Pathol. 2009;36(4):471–6.
101. Barr RJ, Morales RV, Graham JH. Desmoplastic nevus: a distinct histologic variant of mixed spindle cell and epithelioid cell nevus. Cancer. 1980;46(3): 557–64.
102. Mackie RM, Doherty VR. The desmoplastic melanocytic naevus: a distinct histological entity. Histopathology. 1992;20(3):207–11.
103. Clarke B, Essa A, Chetty R. Plexiform spitz nevus. Int J Surg Pathol. 2002;10(1):69–73.
104. Spatz A, Peterse S, Fletcher CD, Barnhill RL. Plexiform spitz nevus: an intradermal spitz nevus with plexiform growth pattern. Am J Dermatopathol. 1999;21(6):542–6.

105. Busam KJ, Barnhill RL. Pagetoid Spitz nevus. Intraepidermal Spitz tumor with prominent pagetoid spread. Am J Surg Pathol. 1995;19(9):1061–7.
106. Aoyagi S, Sato-Matsumura KC, Akiyama M, Tanimura S, Shibaki H, Shimizu H. Spitz naevus of the glans penis: an unusual location. Acta Derm Venereol. 2004;84(4):324–5.
107. Polat M, Topcuoglu MA, Tahtaci Y, Hapa A, Yilmaz F. Spitz nevus of the genital mucosa. Indian J Dermatol Venereol Leprol. 2009;75(2):167–9.
108. Filippov SV, Kniaz'kin IV, Anichkov NM, Zeziulin PN, Shinkarenko AV, Bykov NM. Nevus Spitz ("iuvenil'nyi" nevus) kozhi polovogo chlena. [Spitz nevus (juvenile nevus) of the penile skin]. Arkh Patol. 2002;64(1):46–8.
109. Monfrecola G, Ianniello S, Donofrio V, DeRosa G. Multiple agminated Spitz nevi of the penis. J Eur Acad Dermatol. 1994;3:189–93.
110. Dawe RS, Wainwright NJ, Evans AT, Lowe JG. Multiple widespread eruptive Spitz naevi. Br J Dermatol. 1998;138(5):872–4.
111. Weedon D, Little JH. Spindle and epithelioid cell nevi in children and adults. A review of 211 cases of the Spitz nevus. Cancer. 1977;40(1):217–25.
112. Llamas-Velasco M, Perez-Gonzalez YC, Requena L, Kutzner H. Histopathologic clues for the diagnosis of Wiesner nevus. J Am Acad Dermatol. 2014; 70(3):549–54.
113. Gerami P, Cooper C, Bajaj S, Wagner A, Fullen D, Busam K, et al. Outcomes of atypical spitz tumors with chromosomal copy number aberrations and conventional melanomas in children. Am J Surg Pathol. 2013;37(9):1387–94.
114. Yazdan P, Cooper C, Sholl LM, Busam K, Rademaker A, Weitner BB, et al. Comparative analysis of atypical Spitz tumors with heterozygous versus homozygous 9p21 deletions for clinical outcomes, histomorphology, BRAF mutation, and p16 expression. Am J Surg Pathol. 2014;38(5):638–45.
115. Gerami P, Scolyer RA, Xu X, Elder DE, Abraham RM, Fullen D, et al. Risk assessment for atypical spitzoid melanocytic neoplasms using FISH to identify chromosomal copy number aberrations. Am J Surg Pathol. 2013;37(5):676–84.
116. Yeh I, Mully TW, Wiesner T, Vemula SS, Mirza SA, Sparatta AJ, et al. Ambiguous melanocytic tumors with loss of 3p21. Am J Surg Pathol. 2014;8(8): 1088–95.
117. Gerami P, Jewell SS, Morrison LE, Blondin B, Schulz J, Ruffalo T, et al. Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. Am J Surg Pathol. 2009;33(8):1146–56.
118. Gammon B, Beilfuss B, Guitart J, Gerami P. Enhanced detection of spitzoid melanomas using fluorescence in situ hybridization with 9p21 as an adjunctive probe. Am J Surg Pathol. 2012;36(1): 81–8.
119. Gerami P, Li G, Pouryazdanparast P, Blondin B, Beilfuss B, Slenk C, et al. A highly specific and discriminatory FISH assay for distinguishing between benign and malignant melanocytic neoplasms. Am J Surg Pathol. 2012;36(6):808–17.
120. Gerami P, Mafee M, Lurtsbarapa T, Guitart J, Haghighat Z, Newman M. Sensitivity of fluorescence in situ hybridization for melanoma diagnosis using RREB1, MYB, Cep6, and 11q13 probes in melanoma subtypes. Arch Dermatol. 2010;146(3):273–8.
121. Gerami P, Busam K, Cochran A, Cook MG, Duncan LM, Elder DE, et al. Histomorphologic assessment and interobserver diagnostic reproducibility of atypical spitzoid melanocytic neoplasms with long-term follow-up. Am J Surg Pathol. 2014;38(7):934–40.
122. Barnhill RL, Argenyi ZB, From L, Glass LF, Maize JC, Mihm Jr MC, et al. Atypical Spitz nevi/tumors: lack of consensus for diagnosis, discrimination from melanoma, and prediction of outcome. Hum Pathol. 1999;30(5):513–20.
123. North JP, Garrido MC, Kolaitis NA, Leboit PE, McCalmont TH, Bastian BC. Fluorescence in situ hybridization as an ancillary tool in the diagnosis of ambiguous melanocytic neoplasms: a review of 804 cases. Am J Surg Pathol. 2014;38(6):824–31.
124. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features. Am J Pathol. 2000;157(3):967–72.
125. Bastian BC, Wesselmann U, Pinkel D, Leboit PE. Molecular cytogenetic analysis of Spitz nevi shows clear differences to melanoma. J Invest Dermatol. 1999;113(6):1065–9.
126. Busam KJ, Wanna M, Wiesner T. Multiple epithelioid Spitz nevi or tumors with loss of BAP1 expression: a clue to a hereditary tumor syndrome. JAMA dermatology. 2013;149(3):335–9.
127. Busam KJ, Sung J, Wiesner T, von Deimling A, Jungbluth A. Combined BRAF(V600E)-positive melanocytic lesions with large epithelioid cells lacking BAP1 expression and conventional nevomelanocytes. Am J Surg Pathol. 2013;37(2):193–9.
128. Wiesner T, Murali R, Fried I, Cerroni L, Busam K, Kutzner H, et al. A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression. Am J Surg Pathol. 2012; 36(6):818–30.
129. Wiesner T, He J, Yelensky R, Esteve-Puig R, Botton T, Yeh I, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun. 2014;5:3116.
130. Busam KJ, Kutzner H, Cerroni L, Wiesner T. Clinical and pathologic findings of Spitz nevi and atypical Spitz tumors with ALK fusions. Am J Surg Pathol. 2014;38(7):925–33.
131. Carlson JA, Mu XC, Slominski A, Weismann K, Crowson AN, Malfetano J, et al. Melanocytic proliferations associated with lichen sclerosus. Arch Dermatol. 2002;138(1):77–87.
132. Bussen SS. Melanocytic proliferations associated with lichen sclerosus in adolescence. Arch Gynecol Obstet. 2009;280(6):1039–40.
133. Hassanein AM, Mrstik ME, Hardt NS, Morgan LA, Wilkinson EJ. Malignant melanoma associated with

lichen sclerosus in the vulva of a 10-year-old. Pediatr Dermatol. 2004;21(4):473–6.
134. Pinto A, McLaren SH, Poppas DP, Magro CM. Genital melanocytic nevus arising in a background of lichen sclerosus in a 7-year-old female: the diagnostic pitfall with malignant melanoma. A literature review. Am J Dermatopathol. 2012;34(8):838–43.
135. Rosamilia LL, Schwartz JL, Lowe L, Gruber SB, Quint EH, Johnson TM, et al. Vulvar melanoma in a 10-year-old girl in association with lichen sclerosus. J Am Acad Dermatol. 2006;54 Suppl 2: S52–3.
136. Friedman RJ, Kopf AW, Jones WB. Malignant melanoma in association with lichen sclerosus on the vulva of a 14-year-old. Am J Dermatopathol. 1984;6(Suppl):253–6.
137. Egan CA, Bradley RR, Logsdon VK, Summers BK, Hunter GR, Vanderhooft SL. Vulvar melanoma in childhood. Arch Dermatol. 1997;133(3):345–8.
138. Clark Jr WH, Hood AF, Tucker MA, Jampel RM. Atypical melanocytic nevi of the genital type with a discussion of reciprocal parenchymal-stromal interactions in the biology of neoplasia. Hum Pathol. 1998;29(1 Suppl 1):S1–24.
139. Friedman RJ, Ackerman AB. Difficulties in the histologic diagnosis of melanocytic nevi on the vulvae of premenopausal women. In:Ackerman AB, ed. Pathology of Malignant Melanoma. New York, NY:Masson;1981:119:127.
140. Elder DE. Precursors to melanoma and their mimics: nevi of special sites. Mod Pathol. 2006;19 Suppl 2:S4–20.
141. Gleason BC, Hirsch MS, Nucci MR, Schmidt BA, Zembowicz A, Mihm Jr MC, et al. Atypical genital nevi. A clinicopathologic analysis of 56 cases. Am J Surg Pathol. 2008;32(1):51–7.
142. Brenn T. Atypical genital nevus. Arch Pathol Lab Med. 2011;135(3):317–20.
143. Blickstein I, Feldberg E, Dgani R, Ben-Hur H, Czernobilsky B. Dysplastic vulvar nevi. Obstet Gynecol. 1991;78(5 Pt 2):968–70.
144. McCluggage WG. Premalignant lesions of the lower female genital tract: cervix, vagina and vulva. Pathology. 2013;45(3):214–28.

Malignant Melanoma of the Vulva

8

Doina Ivan and Victor G. Prieto

Introduction

Pigmented lesions of the vulva are relatively common, affecting approximately 12 % of women during their lifetime [1, 2]. The majority of them are the result of inflammatory conditions that lead to hyperpigmentation, non-melanocytic neoplastic disorders, or mucosal lentigo/melanotic macule [3]. Although the vast majority of vulvar melanocytic lesions are benign (conventional, atypical, or blue nevi), a small proportion is melanoma. Vulvar melanoma is the second most common malignant neoplasm of the vulva and accounts for 8–10 % of all malignant vulvar tumors [4]. Approximately 3 % of all melanomas in women occur in the genital region, and the vulva is the most common location. The annual incidence of vulvar melanoma is reported as approximately 0.1 per 100,000 women per year in the United States [5].

Vulvar melanoma carries a poor prognosis, with a high recurrence rate and metastatic potential, likely due to its often delayed diagnosis. The treatment of choice of primary vulvar melanoma is complete surgical excision and, in selected cases, may benefit from examination of sentinel lymph nodes. Recently, great progress has been made in understanding the molecular pathways and mutations involved in various types of melanomas, including in vulvar melanoma, and potential therapeutic targets are recognized, especially for patients with metastatic disease.

D. Ivan (✉)
Departments of Pathology and Dermatology,
University of Texas MD Anderson Cancer Center,
Houston, TX, USA
e-mail: dsivan@mdanderson.org

Clinical Features

Clinical Presentation

Due to their location, vulvar melanomas are usually detected late, resulting in more advanced lesions at presentation. The most common symptom at presentation is a vulvar mass (approximately 40 % of cases) followed by pain, bleeding, and itching. Dysuria is rarely reported, possibly due to urethral involvement by melanoma. In addition, ulceration, local discoloration, foul odor, and discharge may also be presenting symptoms [4]. It has been reported that the mean interval between the onset of the symptoms and the moment that the patient seeks medical advice is on average 4 months [4]. The clinical differential diagnosis includes usually benign melanocytic lesions but also inflammatory and non-melanocytic conditions, such as extramammary Paget disease, squamous intraepithelial lesions, and vascular lesions, among others.

Melanomas are usually large (over 7 mm) lesions, with variegate pigmentation and irregular

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_8,

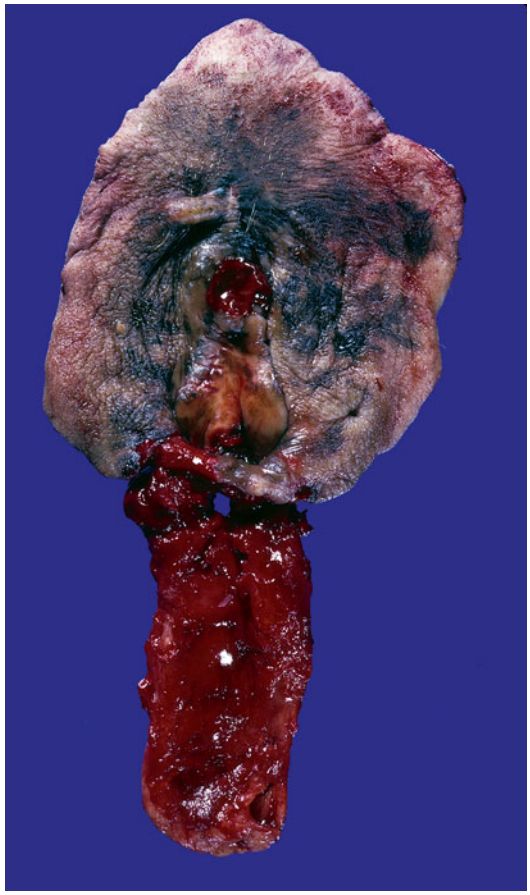


Fig. 8.1 Clinical vulvar melanomas present often as large lesions, with variegate pigmentation and irregular borders. Multifocality is not uncommon in vulvar melanomas

or poorly demarcated borders (Fig. 8.1). Blue, black, or gray colors in pigmented lesions are especially suggestive of aggressive behavior. This assumption does not apply to nodular melanomas that display less variegation of pigmentation and asymmetry. The most frequent types of melanoma affecting the vulva are mucosal/acral-lentiginous and superficial spreading types. Although amelanotic melanomas are uncommon on the skin, about 25 % of all vulvar melanomas are amelanotic [6]. Cutaneous melanomas are nearly always solitary, but vulvar melanomas are often multifocal. These particular clinical characteristics of vulvar melanoma, their multifocality and frequent amelanotic nature, may account for the delay in the diagnosis of these tumors and relatively high local recurrence rate. The clinicians should be aware of this clinical challenge and potential pitfall. Therefore, biopsies should be performed whenever a melanocytic lesion is suspected and also consider the possibility of performing multiple biopsies in a larger pigmented lesion.

Dermoscopy

Dermoscopy may be used as a noninvasive examination of vulvar melanocytic lesions and can play a role in selection of suspicious pigmented lesions in need of a biopsy. The dermoscopic criteria for benign and malignant lesions on the vulva are not very well established, mainly due to the lack of large case series of such lesions. A recent study revealed that in vulvar melanomas, 60 % of cases had a multicomponent pattern by dermoscopy [7]. The same study revealed that there may be an irregular dermoscopic pattern in vulvar melanomas, with white or white-blue veil, irregularly distributed dots and globules, and atypical vascular pattern [7].

Location

Melanoma can occur on the keratinized surfaces of the vulva, but more often affects the modified mucous membranes of the vulva and the true mucous membranes of the vestibule and the vagina. In one of the large retrospective studies, more than half of the patients presented with melanoma involving the vulvar mucosa (labia minora, urethra, clitoris, introitus, vagina), and in 21 % of the patients, the melanoma was on the epidermal site of the vulva (labia majora or mons pubis). Overall, the most common location is on the labia minora, followed by the labia majora. It is important to emphasize that 20 % of the patients may have multifocal disease at the diagnosis [8]. In rare instances, melanomas can occur on the distal aspect of the urethra and may be visible at the urethral meatus at clinical examination [9].

Epidemiology

The female genitalia and especially the vulvar region have one of the highest densities of melanocytes in the body. Approximately 3 % of all melanomas in women occur in the genital region. Among the female genital tract, the most common is vulvar melanoma (76.7 %), followed by vaginal (19.8 %), while cervical melanoma is least common [10]. Despite the sun-protected location and apparent rare incidence, vulvar melanomas are in fact more frequent than other types of cutaneous melanomas, when accounting for the region's surface in rapport with the entire body surface [11, 12]. The annual incidence of vulvar melanoma is approximately 0.1 per 100,000 per year in the United States. According to one of the largest studies to date using data from the Surveillance, Epidemiology, and End Results (SEER) database, vulvar melanomas occur most often in middle-aged to elderly (median age of 68), postmenopausal white women [13, 14]. Childhood vulvar melanoma is extremely rare [15].

Vulvar melanoma can occur in all skin types. In comparison with nongenital cutaneous-counterpart melanomas, 3.6 % of vulvar melanomas affect black patients, in contrast with only 0.6 % of cutaneous melanomas [14]. As in cutaneous melanomas, family history of melanoma correlates with an increased incidence in vulvar melanoma and may be identified in up to 15 % of cases [16].

Histopathology

When reporting the diagnosis of melanoma, certain prognostic factors and characteristics are usually noted. We will present those parameters with specific comments regarding vulvar melanoma.

Histological Subtypes

Considering that melanomas involve primary the mucosal aspect of the vulva, the majority of melanomas arising in this anatomic region are mucosal/acral-lentiginous type, followed by superficial spreading and nodular types. Desmoplastic melanomas, usually occurring in sun-damaged skin of elderly people, are rarely reported in the vulvar area [17]. Vulvar melanomas arise most commonly de novo, but in approximately 5 % of cases, especially in those affecting the cutaneous aspect of the vulva, they may be associated with a preexisting nevus [18].

Mucosal/acral-lentiginous melanoma is characterized by a poorly circumscribed, asymmetrical proliferation of cytologically atypical melanocytes (i.e., cellular pleomorphism, with high nuclear/cytoplasmic ratio, irregular nuclei, and frequently prominent nucleoli). The junctional component may be composed of nests of melanocytes, but more often there are numerous single cells with confluent, lentiginous growth pattern, and prominent pagetoid upward migration (Fig. 8.2a).

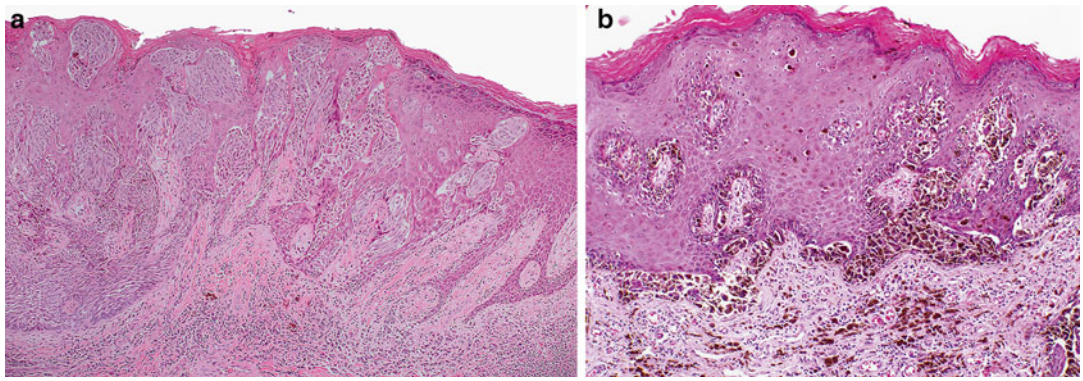


Fig. 8.2 (**a**) Vulvar melanoma is characterized by a proliferation of cytologically atypical melanocytes with numerous single cells with confluent, lentiginous growth pattern, and prominent pagetoid upward migration. (**b**) Vulvar superficial spreading melanomas occur on the cutaneous aspect of the vulva and are characterized by a predominately nested melanocytic proliferation, with a lesser degree of pagetoid upward migration

When invasive, the dermal melanocytes have similar histomorphology with the junctional ones and fail to demonstrate maturation growth pattern with depth. Mitotic figures may be identified within the dermal melanocytes. Lymphovascular and perineural invasion is more commonly seen in this type of melanoma in comparison with cutaneous melanomas.

Vulvar superficial spreading melanomas that occur on the cutaneous aspect of the vulva are characterized by a poorly circumscribed, asymmetrical proliferation of atypical melanocytes, mostly having a nested growth pattern. The superficial spreading-type melanoma has a lesser degree of pagetoid upward migration, when compared with the mucosal/acral-lentiginous type (Fig. 8.2b).

Nodular melanoma is defined by an invasive component of melanoma in which the melanoma in situ (intraepithelial) does not extend for three or more rete ridges from the invasive component. In deeply invasive lesions, the tumor cells may show a higher degree of heterogeneity, frequently displaying both epithelioid and spindle cell histology.

Clark Level

In vulvar melanomas, the Clark level assessment is only valid for melanomas arising on the cutaneous aspect of the vulva (similarly used as in nongenital cutaneous melanomas). Clark level is not applicable for the mucosal type of vulvar melanomas since the Clark levels are related to cutaneous and not mucosal structures. The anatomic levels of melanoma invasion are: level I, melanoma in situ; level II, invasion into the superficial papillary dermis; level III, invasive melanoma fills and expands the papillary dermis; level IV, invasion into the reticular dermis; and level V, infiltration into subcutaneous adipose tissue. It has been reported that the assessment of Clark levels II, III, and IV has a very high interobserver variability. Moreover, in melanomas with polypoid growth pattern, the tumor may be deeply invasive by Breslow thickness but still a Clark level III. In these cases, there is no significant prognostic value of the Clark level, and the thickness of the tumor dictates the clinical behavior. Currently, the Clark level is no longer recommended to be used as a staging criterion, since it is not an independent prognostic factor when mitotic rate is included in the analysis [19].

In the largest case series of vulvar melanoma, the median Clark level when reported was IV, highlighting that these tumors are usually diagnosed when they are relatively advanced [8, 18].

Tumor Thickness

Breslow proposed in 1970 that measuring the vertical thickness of cutaneous melanoma is a good tool to predict the metastatic potential of this tumor. Until today, this measurement is the single most important prognostic factor for melanoma clinical behavior. Moreover, the evaluation of this parameter has the least interobserver variability. The Breslow thickness is measured from the top of the epidermal granular layer to the deepest melanocyte of the invasive component. When there is tumor-induced ulceration, the measurement should be done from the base of the ulceration to the deepest aspect of the invasive component of melanoma.

The term of "Breslow thickness" should be reserved for vulvar melanomas occurring in the cutaneous aspect of the vulva; for mucosal melanomas, only the term "thickness" is considered appropriate since the term "Breslow" was not intended for mucosal sites [20].

Involvement of follicular or adnexal structures by melanoma in situ, even if they are situated deeper in the dermis, should not be considered for the measurement of melanoma thickness. On the other hand, the presence of perineural invasion by melanoma (relatively commonly seen in mucosal type of vulvar melanoma), in our opinion, should be measured and reported as part of the Breslow thickness.

Several large case series of vulvar melanoma report that the median Breslow thickness ranges from 3.2 to 4.4 mm [8, 21].

Radial and Vertical Growth Phase

The definition of radial and vertical growth phases is based on the concept that tumor progression implicates different evolutionary steps that can be identified on histological examination and helps predict the tumor metastatic potential. By definition, all in situ melanomas have only radial growth phase. When melanoma invades into the dermis or submucosa, it may still have only radial growth phase (single cells or small nests within the dermis, without mitotic figures).

Vertical growth phase has been defined as "tumorigenic" growth, when the dermal nests are larger than any nest in the junctional component of the lesion or when mitotic figures can be identified within the dermal melanocytes. It has been reported that the metastatic potential of a melanoma strongly correlates with the presence of vertical growth phase, but it seems that the predictive power of vertical growth phase is likely related to the presence of the dermal mitotic figures rather than the size of the dermal tumoral nests.

Mitotic Figures

The counting of mitotic figures should be done only in the invasive component of melanoma. Currently, the preferred method is to report the mitotic count per square millimeter (usually represents 4 and ½ consecutive high-power fields at a magnification of 40×, but it depends on the size of the field in that particular microscope). It has been reported that a high mitotic rate correlates with a poor survival rate. In one of the largest case series of vulvar melanoma, the average of mitotic figure per square millimeter was reported to be 6.6 [21].

Ulceration

The presence of ulceration is regarded as an independent prognostic factor for melanoma-associated survival. Survival rates of patients with an ulcerated melanoma are lower than those of patients with a non-ulcerated melanoma of similar thickness. Thus, the presence of ulceration results in upstaging the lesion. The presence of ulceration and possibly its width should be always reported. It is also important to recognize the difference between tumor-related ulceration (due to "epidermal consumption and attenuation") and ulceration due to trauma, since the latter is probably not associated with such an impaired prognosis (Fig. 8.3). Therefore, morphologists should be very cautious before reporting ulceration in re-excision specimens (in which the cause for ulceration may be surgical trauma).

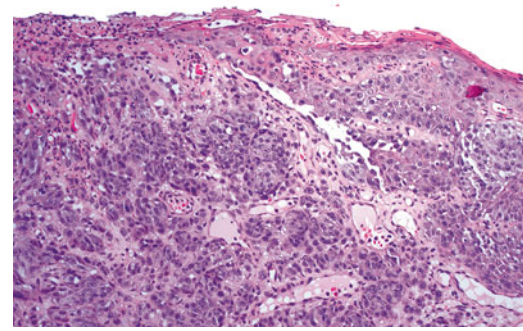


Fig. 8.3 Presence of ulceration is an independent prognostic factor for melanoma-associated survival and its presence should be reported. Histologically, it is characterized by the absence of the epidermis with fibrin deposition and neutrophilic aggregates

Lymphovascular Invasion

The presence of tumor cells in lymphovascular spaces is considered a major prerequisite for metastatic spread (Fig. 8.4a). Several authors have reported that vascular invasion in melanoma may be associated with an increased risk of relapse, lymph node metastasis, distant metastases, overall survival, and disease-free survival. However, other authors showed that this feature is not an independent factor in predicting prognosis in patients with melanoma. This apparent discrepancy may be due to the difficulty of identifying this feature on

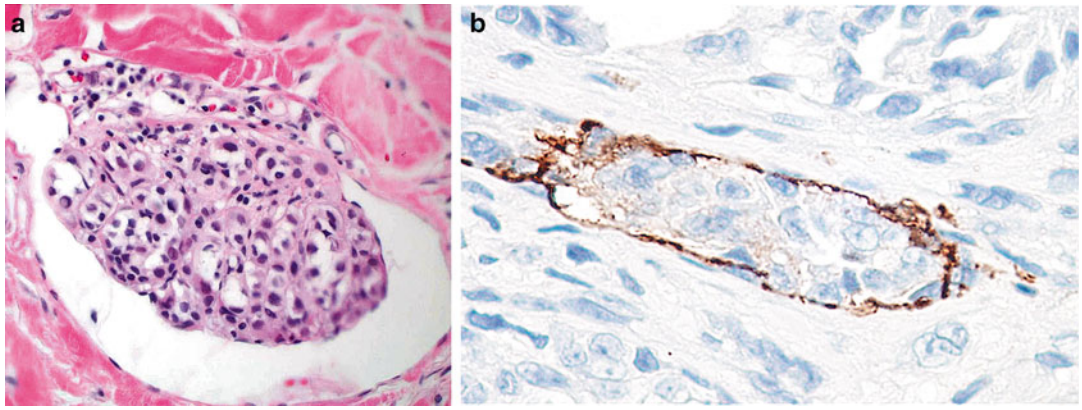


Fig. 8.4 (**a**) Lymphovascular invasion is characterized by the presence of tumor cells in lymphovascular spaces. (**b**). D2-40 immunohistochemical study can highlight the vascular spaces and can be used as an aid in identification of lymphovascular invasion

hematoxylin and eosin sections alone. Previous reports on immunohistochemical detection of lymphovascular invasion and correlation with metastatic rate have yielded conflicting results. More recently, studies have shown that immunohistochemical detection of lymphovascular invasion using anti-D2-40, a monoclonal antibody against podoplanin, in melanomas thicker than 1 mm, correlates with sentinel lymph node status and survival (Fig. 8.4b). Therefore, we suggest that such analysis might be incorporated in the routine work-up of primary cutaneous melanoma thicker than 1 mm. In one of the largest case series of vulvar melanomas, vascular invasion of tumor cells did not reach the significance level as a predictor of survival in the univariate analyses [18].

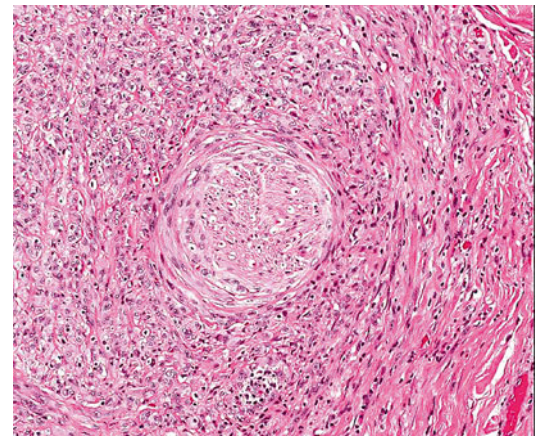


Fig. 8.5 Perineural invasion is relatively commonly seen in mucosal melanomas, such as vulvar melanoma

Perineural Invasion

Infiltration of nerves by tumor cells should also be recorded (Fig. 8.5). Some types of melanomas, such as desmoplastic or spindle cell melanomas, have a high propensity for perineural invasion. This feature is also relatively commonly seen in mucosal melanomas.

Microscopic Satellitosis

Microsatellites are defined as discrete tumor aggregates, designated as having the diameter larger or equal to 0.05 mm in largest dimension and are separated from the main tumor by normal (not fibrosis or inflammation) dermal collagen or subcutaneous fat. Only few studies have evaluated the role of microsatellites as prognostic factors in cutaneous melanomas and so far there is no definite published data in this regard in vulvar melanomas. It is controversial whether their presence is an independent prognostic factor, but they do appear to correlate with a higher risk of local recurrence and with an increased frequency of regional lymph node metastasis (from 12 % to 53 %) in tumors thicker than 1.5 mm.

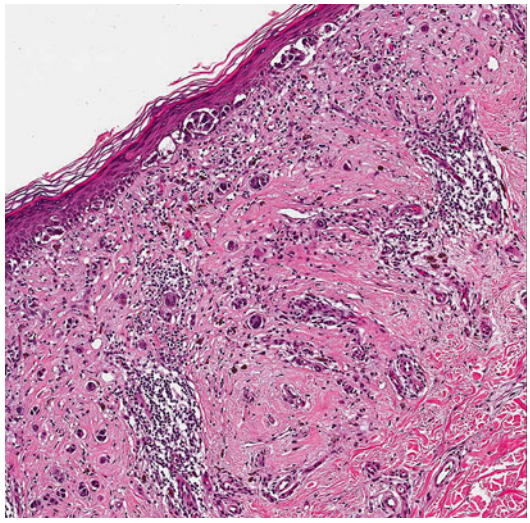


Fig. 8.6 Regression can be recognized by the presence of dermal fibrosis, vascular proliferation, inflammatory infiltrate, and presence of melanophages in association with complete or partial (as in this case) loss of melanocytes

Regression

Histologically, it can be recognized by the presence of dermal fibrosis, vascular proliferation, inflammatory infiltrate, and the presence of melanophages in association with complete or partial loss of melanocytes. There may be also thinning of the overlying epidermis with loss of rete ridges (Fig. 8.6). The regression changes can range from focal to extensive and can be partial or complete. The correlation of regression with prognosis is controversial. Although most studies have not found a significant role for regression in determining survival, others suggested that the metastatic rate is higher in thin melanomas with extensive regression. One of the possible explanations is that a regressed lesion was previously thicker, and the assessment of current thickness is an underestimate of the tumor's metastatic potential [22].

Tumor-Infiltrating Lymphocytes

The host immune response is recognized by the presence of a lymphocytic inflammatory infiltrate. It can be "brisk," "non-brisk," and "minimal" ("absent") and is usually measured by the extent of the lymphocytic infiltrate surrounding the invasive dermal component of melanoma. The presence of a brisk inflammatory infiltrate has been reported that correlates with improved survival. However, the assessment of this feature is observer dependent, mainly due to the lack of a uniform definition of host response in terms of type and location of the infiltrate.

"Multifocality" of Vulvar Melanoma

"Multifocality" of vulvar melanomas, especially those arising on the mucosal aspect, is a well-known characteristic with a significant impact in the capability to obtain tumor-free surgical margins. Multifocality is defined as multiple foci of melanoma separated by intact epithelium, or arising on follow-up, but not in the vicinity of the surgical scar. In one study, six of nine (66 %) vulvar melanomas had more than one focus of melanoma: one had multiple primary melanomas both at the time of diagnosis and during follow-up period, one had multiple foci of melanoma but had no local recurrences, and three had secondary melanomas during follow-up.

Interestingly, in a recent study, almost half of the patients had atypical melanocytic hyperplasia adjacent to primary melanoma [21]. Verschraegen et al., in their recently published series, raise the suggestion of a "field effect" in vulvar melanomas; some patients having atypical melanocytic hyperplasia at the surgical margins as well as in noninvolved mucosa, and local recurrences in these patients were described.

Prognostic Factors, 2009 AJCC Staging, and Its Applicability on Vulvar Melanomas

The understanding of prognostic factors and their clinical significance in cutaneous melanomas is continuously evolving. The American Joint Committee on Cancer (AJCC) adopted in 2002 a version of the melanoma staging system on the basis of an analysis of 17,600 patients in AJCC Melanoma Staging Database. Recently, in November 2009, a new version of AJCC

Melanoma Staging and classification has been released, based on an expanded sample size of the Melanoma Staging Database (over 30,000 patients) and multivariate analysis of different independent prognostic factors [19].

There is variability between different institutions and pathologists regarding the routine reports of histological parameters in melanomas. Some of the reports contain only minimal information (such as tumor thickness and presence or absence of ulceration) while others are very comprehensive. In such detailed reports, although some of the information provided might not be of immediate relevance to a given patient, its importance might become apparent at a later date, especially when used in conjunction with information gathered from large number of patients in prospective or retrospective studies. As an example, mitotic count is now included as an essential histopathological element in the newest version of AJCC Melanoma Staging and classification.

In contrast with the 2002 AJCC Melanoma Staging, the mitotic rate replaces the level of invasion (Clark) in defining T1 categories of cutaneous melanomas. The presence of one or more mitotic figures per square millimeter is now used as one of the two criteria (along with ulceration) for defining T1b-stage melanoma. A possible drawback of applicability of this criterion may be in cases in which there is a very small dermal component. However, it is not recommended by AJCC or College of American Pathologists (CAP) to perform multiple, serial sections with the intent to detect mitotic figures. The tumor thickness is the primary determinant for T staging in both 2002 and 2009 AJCC recommendations for melanoma staging. In the newer 2009 AJCC, recommendation immunohistochemical detection of nodal metastases is included and has to be performed in all the cases in which a metastasis is not obvious in the initial hematoxylin- and eosin-stained sections examined. In such cases, the markers that can be used for immunohistochemical studies may be MART1, Melan A, or HMB45. The number of nodal metastases is the primary determinant for N staging and there is no lower threshold of staging N+ disease (isolated tumor cells and very small tumor deposits should be scored N+). The TNM staging categories for cutaneous melanomas (including vulvar melanoma) that are currently recommended are included in Table 8.1.

Parameters that correlate strongly with the prognosis and should be always mentioned in the report include tumor thickness, number of mitoses per square millimeter, as well as presence or absence of ulceration, lymphovascular, perineural invasion, satellitosis, and regression. In addition, it is recommended to include Clark level (only for vulvar melanomas affecting the cutaneous aspect of the vulva). It is important to emphasize that according to literature, most of the vulvar melanomas at the time of diagnosis are Clark level IV, reach a Breslow thickness ranging between 3.2 and 4.4 mm, have an average of six mitoses per square mm, and a large proportion may be ulcerated [8, 21].

Therapy

The treatment of choice consists of surgical removal or vulvectomy (hemivulvectomy or radical vulvectomy). There is a trend toward less extensive resection, since there have not been found a difference of survival rates between patients treated with radical vulvectomy and conservative surgery [23] which yields results as good as radial vulvectomies. Obtaining tumor-free resection margins is critical to prevent local recurrence. However, due to the multifocality commonly seen in vulvar melanomas, their frequent amelanotic nature and highly subclinical peripheral extension, complete resection was proven to be a difficult task. Moreover, in up to 20 % of cases, there is a background of atypical junctional melanocytic hyperplasia that makes the assessment of resection margins even more challenging. Frozen section analysis is neither specific nor sensitive for histological evaluation of margins of resection and is not generally indicated. Chemotherapy and radiation therapy add little to overall survival.

The sentinel lymph node biopsy (SLNB) is a less invasive alternative to elective lymph node dissection. The SLNB has a lower associated

Table 8.1 2009 AJCC melanoma pathologic staging

Stage	T (tumor)	N (lymph nodes)	M (metastases)
0	**Tis** (in situ)	**N0**	**M0**
IA	**T1a** (thickness < 1 mm, without ulceration, <1 mitosis/square mm)	**N0**	**M0**
IB	**T1b** (thickness <1 mm, with ulceration and/or 1 or >1 mitoses/square mm) OR **T2a** (thickness 1–2 mm, without ulceration)	**N0**	**M0**
IIA	**T2b** (thickness 1–2 mm, with ulceration) OR **T3a** (thickness 2–4 mm, without ulceration)	**N0**	**M0**
IIB	**T3b** (thickness 2–4 mm, with ulceration) OR **T4a** (thickness >4 mm, without ulceration)	**N0**	**M0**
IIC	**T4b** (thickness >4 mm, with ulceration)	**N0**	**M0**
IIIA	**T1–T4a**	**N1a** (1 lymph node with micrometastasis) OR **N2a** (2–3 lymph nodes with micrometastases)	**M0**
IIIB	**T1–T4b**	**N1a** (1 lymph node with micrometastasis) OR **N2a** (2–3 lymph nodes with micrometastases)	**M0**
IIIB	**T1–T4a**	**N1b** (1 lymph node with macrometastasis) OR **N2b** or **N2c** (2–3 lymph nodes with macrometastases or in transit metastases/ satellites without lymph node involvement)	**M0**
IIIC	**T1–T4b**	**N1b** (1 lymph node with macrometastasis) OR **N2b** or **N2c** (2–3 lymph nodes with macrometastases or in transit metastases/ satellites without lymph node involvement)	**M0**
IIIC	**Any T**	**N3** (4 or more 4 lymph nodes with metastases or in transit metastases/ satellites with lymph node metastases)	**M0**
IV	**Any T**	**Any N**	**M1** (distant metastases)

Data from Ref. [19]
Micrometastases are diagnosed after sentinel lymph node biopsy
Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically
NA not applicable, *LDH* lactate dehydrogenase

morbidity and higher sensitivity in detecting lymph node metastases. According to 2009 AJCC recommendations for melanoma staging, immunohistochemical studies have to be performed if tumor is not identified in the routine H&E sections (Fig. 8.7a, b). Up to date the studies analyzing the potential role of SLNB in vulvar melanomas are relatively small but appears that the procedure is capable of identifying patients who have occult lymph node metastases and may benefit from local lymphadenectomy performed in order to prevent distant metastases [24, 25].

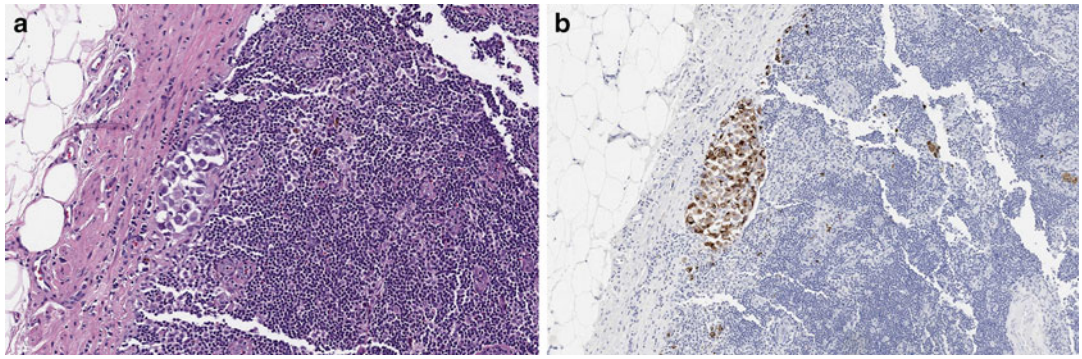


Fig. 8.7 The sentinel lymph node biopsy can identify micrometastases in lymph nodes (**a**) that can be also highlighted by immunohistochemical studies using MART1 (**b**)

Survival

According to a one of the largest studies of 644 patients with vulvar melanoma using SEER database, the 5-year disease-specific rates for those with localized, regional, and distant metastases were 75.5 %, 38.7 %, and respectively 22.1 % [13]. Women aged 68 years or younger had a better survival rate than older patients (72 % compared with 47.7 %). Overall, the prognosis of vulvar melanoma is poorer relative to cutaneous melanoma. The overall outcome of melanoma of the vulva and vagina is poor primarily because of the sociocultural barriers, which may delay the patient seeking timely medical attention; therefore, melanoma in these areas is usually diagnosed late. The median survival rate of the patients with vulvar melanoma was reported as 16 and 39 months in black and Caucasian patients. In contrast, the median survival rate of cutaneous melanomas is 124 months for black patients and 319 months for Caucasian patients [14]. Lymph node metastases were diagnosed in 9–17 % of the patients and the survival rate was also related with the number of positive lymph nodes [8, 13, 26]. In contrast with cutaneous melanomas, 8.5 % of patients with vulvar melanoma present with advanced disease, while only 2.7 % of patients with cutaneous melanoma present with regional or distant metastases [14]. In patients with positive lymph nodes 5-year disease-specific survival is 24 %, compared with 68.3 % for those with negative lymph nodes [13]. In a multivariable analysis of vulvar melanomas, younger age, localized disease, and negative lymph nodes were independent prognostic factors for improved survival [13]. Recent population-based studies demonstrate that the 5-year melanoma-specific survival rates are only 61 % for vulvar melanoma, lower than 91.3 % for cutaneous melanomas, and 80.3 % for acral melanomas [21].

Differential Diagnosis

The differential diagnosis of melanoma has to be made not only with other types of vulvar melanocytic lesions but also with other pigmented non-melanocytic vulvar lesions that clinically may mimic melanoma. For example, postinflammatory hyperpigmentation and vulvar lentigo/melanosis can be clinically very similar with melanoma. Considering the importance of early detection of melanoma and because, on the genital skin, significant and less significant lesions can appear grossly similar and less characteristic than on the extragenital skin, the clinicians are encouraged to perform biopsies in any instance in which melanoma is included in the differential diagnosis [3]. As a rule, any suspicious vulvar lesion, for which the clinical diagnosis of melanoma is considered, requires a biopsy (either punch or an excisional biopsy) of the most abnormal area(s). Shave biopsies are generally not recommended if melanoma is suspected, since it can compromise either the diagnosis or some prognostic parameters, such as thickness.

Differential Diagnosis of Vulvar Melanoma from Non-melanocytic Lesions That Appear Clinically Pigmented

- **Angiokeratomas**—small, vascular tumors often located on the labia majora (see Chap. 14). They present as shiny red, purple, or black papules and are often multiple. The histology reveals ectatic vessels with overlying epithelial acanthosis with hyperkeratosis.
- **Purpura or other vascular lesions**—such as Kaposi sarcoma, can be clinically considered in the differential diagnosis of vulvar melanoma (see Chap. 14). Histologically, these lesions differ by the presence of telangiectasia and extravasated red blood cells in purpura and identification of vascular spaces lined by spindle cells as seen in Kaposi sarcoma. In challenging cases, immunohistochemical studies may be performed, such as CD31 or CD34, that highlight vascular spaces of vascular lesions or antibodies against herpes virus 8 (HHV8) which labels endothelial cells in Kaposi sarcoma.
- **Lichen sclerosus**—may sometimes be considered clinically in the differential diagnosis of melanoma due to variegated pigmentation and association with telangiectatic superficial capillaries (see Chap. 5). Although hyperpigmentation and melanin incontinence can be seen in lichen sclerosus, the latter will also show dermal sclerosis and lack of melanocytic proliferation.
- **Lichen simplex chronicus and acanthosis nigricans**—may clinically be considered in the differential diagnosis of a pigmented melanocytic lesion due to epithelial acanthosis with hyperkeratosis (see Chap. 3). In some cases, the melanocytes are either producing more melanin or there is a mild increase in the density of melanocytes, but they lack cytologic atypia.
- **Seborrheic keratosis**—common benign lesions usually occurring with aging and are often reddish brown and have a "stuck on" gross appearance. When they occur on the vulva, they may raise concern of melanoma. Histologically, seborrheic keratoses show hyperkeratosis, acanthosis, and pseudohorn cysts. Occasional dendritic melanocytes may be seen in the epidermis.
- **Condyloma accuminatum**—may be pigmented, particularly in darker-skinned individuals. Histologically, they are characterized by hyperplastic squamous epithelium, with hyperkeratosis, parakeratosis, acanthosis, and papillomatosis. Koilocytes (the "hallmark" of human papilloma virus infection) are noted and they are characterized by enlarged irregularly shaped nucleus, with a perinuclear halo. Sometimes, prominent melanin granules may be seen in these lesions (see Chap. 6).
- **Vulvar intraepithelial neoplasia (VIN)**—clinically may appear brown, red, or white. Pigmented VIN may raise concern for melanoma and lead to biopsy. Histologically, epithelial keratinocytic atypia as well as evidence of human simplex virus (HPV) infection is noted (see Chap. 9). Sometimes pigment incontinence in the papillary dermis may be noted.
- **Basal cell carcinoma**—is less commonly seen in the vulvar area comparing with the sun-exposed skin but may present with pruritus (similar to melanoma), has a nodular/papular appearance, and occasionally can be pigmented (either due to dendritic melanocytes within the tumor nests or pigment incontinence) (see Chap. 11). The classic basaloid proliferation with peripheral palisading, retraction artifact, and associated myxoid stroma will support the diagnosis of basal cell carcinoma.
- **Extramammary Paget disease**—may have sometimes a pigmented appearance and mimic clinically melanoma. Moreover, sometimes even the histological differential diagnosis may be challenging. In cases of pigmented Paget disease, there is an intraepidermal proliferation of epithelioid cells, with marked pagetoid spread, focally containing mucin and melanin pigment (see Chap. 11). Intraepidermal reactive melanocytes with a dendritic morphology are usually also present. The pigment within the neoplastic cells is likely the result from the uptake of melanin by Paget cells from surrounding melanocytes within the epidermis. In cases in which only a superficial biopsy is performed, pigmented Paget disease can be confused easily with melanoma in situ [27] (Fig. 8.8a). Immunohistochemical studies can resolve the differential diagnosis: Paget

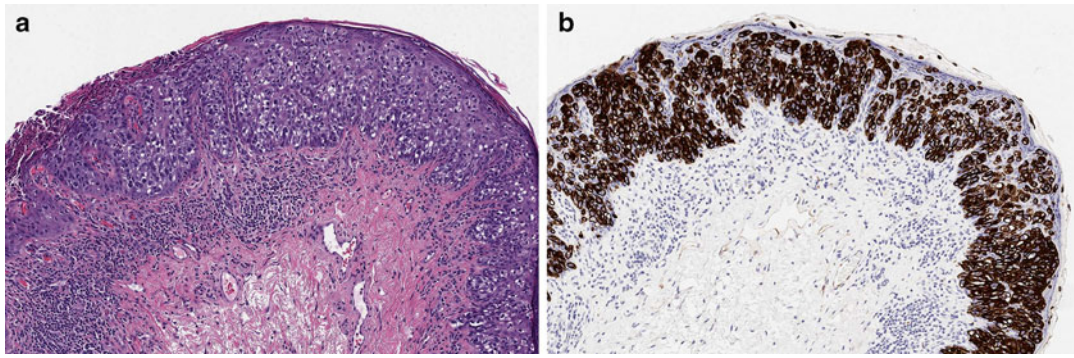


Fig. 8.8 Clinical and histological extramammary Paget disease involving the vulva can mimic vulvar melanoma (**a**), but the lesional cells are strongly and diffusely positive for cytokeratin 7 (**b**) and negative for melanocytic markers

cells label for CK7 (Fig. 8.8b), low-molecular-weight keratins (CAM 5.2), EMA, and CEA. There is variable expression of GCDFP-15 (BRST2) and mucicarmine. The pagetoid cells are negative for melanocytic markers such as S100 protein, HMB45 antigen, MART1, Sox10, and MITF. It is important to mention a potential pitfall in the interpretation of immunohistochemical studies with MART1: due to the presence of numerous background dendritic melanocytes, the stain can be easily over interpreted; we should base our evaluation/interpretation of the stain only on the number of labeled cellular bodies and not their dendritic processes.

Differential Diagnosis of Vulvar Melanoma from Melanin-Related Lesions That Appear Pigmented But Are Not Due to Melanocytic Proliferation

- **Mucosal lentigo/melanosis/lentigines—**they are usually incidental; lentigines are smaller while larger areas of hyperpigmentation may be seen in mucosal melanosis (see Chap. 7). Since melanosis may be extensive and occurs usually on the mucosal aspect of the vulva and introitus, they frequently raise the concern for melanoma due to their extent and sometimes variable pigmentation. Histologically, hyperpigmentation of basal keratinocytes and dendritic melanocytes are noted, without a definite increase in the number of melanocytes. Melanophages may be also seen in the superficial dermis/submucosa.
- **Postinflammatory hyperpigmentation—**numerous inflammatory conditions of the vulva, such as a variety of interface or lichenoid dermatoses, may lead to postinflammatory hyperpigmentation (see Chap. 7). This condition is due to secondary disruption if the basement membrane and the melanin from the basal keratinocytes are released in the dermis. Histologically, it is characterized by superficial dermal interstitial melanin or dermal melanophages.

Differential Diagnosis of Vulvar Melanoma from Vulvar Nevi

The clinical and/or histological differential diagnosis of these entities may be sometimes very challenging, including for experienced dermatopathologists. This is especially true for nevi with architectural disorder, as commonly seen in the vulvar area. In this region, these so called nevi of special sites have often histological features that in other sites could be interpreted as malignant melanoma [28] (see Chap. 7). They are characterized by a proliferation of melanocytes with variable degree of cytologic atypia, frequently arranged in large nests or single cells, sometimes with focal pagetoid upward migration (usually in the central portion of the lesion). Lentiginous growth, bridging of epithelial rete ridges, and adnexal involvement are often identified. Extension of junctional

melanocytic proliferation beyond the dermal component ("shoulder phenomenon") is commonly seen and associated with papillary dermal fibroplasia (Fig. 8.9). It is important to note that these histological features when identified in genital melanocytic nevi (as well as in nevi along the mammary line) do not carry the same associations of the cutaneous dysplastic nevus syndrome but is rather dependent on the site of biopsy.

Clinically, melanomas are larger, more irregular in comparison to nevi, and have variegated pigmentation, with irregular or poorly demarcated borders. Histologically, a melanocytic nevus is favored when the lesion is well circumscribed (ends with nests of melanocytes) and symmetrical; the majority of melanocytes are arranged in nests; there is only minimal and central pagetoid upward migration, mild to moderate cytologic atypia, and no evidence of deep dermal mitotic figures; and there is maturation of the dermal component (Table 8.2).

While in the great majority of cases the diagnosis is based only on the histopathological examination of the lesions, applying the described criteria in the appropriate clinical context, in

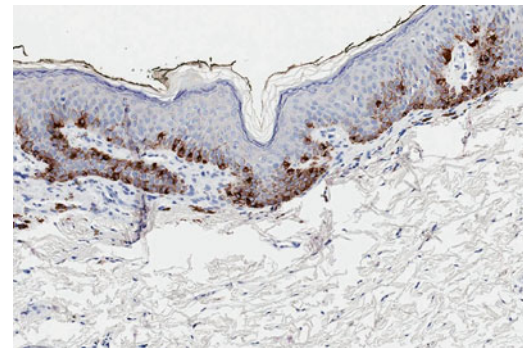


Fig. 8.9 Melanocytic hyperplasia in the vulva highlighted by HMB45 immunohistochemical studies is characterized by a relatively increased number of junctional melanocytes, but without confluent growth pattern or pagetoid upward migration as seen in melanomas

Table 8.2 Vulvar melanoma versus atypical vulvar nevi: clinical features, histological features, and immunohistochemical studies

	Vulvar melanoma	Atypical vulvar nevi
Clinical features		
Age	Postmenopausal	Premenopausal
Size	Large	Relatively small, less than 1 cm
Pigmentation	Variegated	Diffuse and homogenous
Clinical appearance	Irregular, poorly circumscribed	Usually well circumscribed
Histological features		
Circumscription	No	Yes
Symmetry	No	Yes
Junctional nests and single cells	Predominately single cells	Predominately nested, frequent large nests
Lentiginous growth	Yes, confluent growth pattern	Only focal
Junctional nests	Poorly cohesive or coalescent	Cohesive
Pagetoid upward migration	Present, frequently prominent	Usually absent. If present, it is focal and central
Cytology	Significant cytologic atypia	Mild cytologic atypia
Ulceration	Present	Absent
Dermal maturation	Absent	Present
Dermal mitoses	Present, including in the deep aspect	Rare and superficial
Associated dermal inflammatory host response	Present, extensive	Present
Dermal fibrosis	Regression type	Papillary dermal fibroplasia
Immunohistochemical studies		
HMB45	Patchy expression, in both superficial and deep aspect of the lesion	Expressed in the intraepidermal and superficial dermal melanocytes only (maturation pattern)
Ki67	Increased proliferative rate (>10 %), including in deeper melanocytes	Low proliferative rate (<10 %) and only in the superficial melanocytes

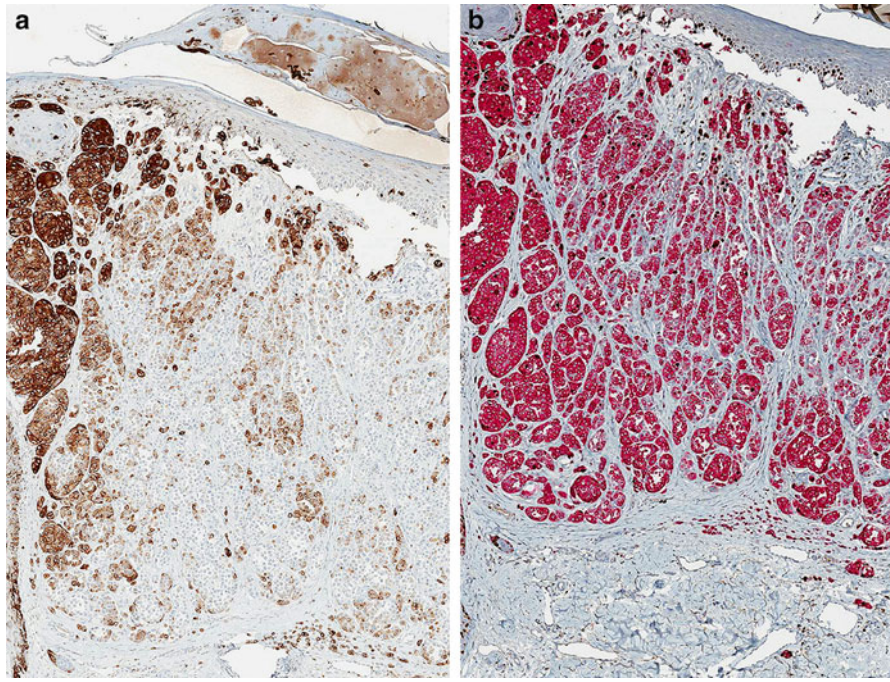


Fig. 8.10 An immunohistochemical study using HMB45 reveals a solid staining pattern in melanoma (**a**), and the cocktail-combining MART1-red chromogen, cytoplasmic staining, and Ki67 brown nuclear staining highlight a high proliferative rate of invasive melanocytes (**b**)

selected situations immunohistochemistry can be a useful aid in the differential diagnosis of these entities.

The immunohistochemical studies that may be used are a combination of HMB45 and a proliferation marker, such as Ki67/MIB1. In melanoma, HMB45 has a patchy to solid pattern of staining, labeling melanocytes in both superficial and deep portion of the lesion (Fig. 8.10a) [29–31]. In contrast to nevi, melanomas tend to have an increased proliferative rate (Fig. 8.10b). Moreover, the examination of the pattern of expression of Ki67 in the dermal melanocytes may be helpful: nevi have scattered positive intraepidermal, upper dermal, or periadnexal cells, while melanomas tend to reveal a more diffuse positivity for Ki67 or a higher proliferation rate within the deeper aspect of the lesion. One of the most common problems in interpreting the proliferation rate using Ki67 is the fact that sometimes the cells that label may be tumor-associated lymphocytes or proliferating background fibroblasts or endothelial cells that account for an overvaluation of tumor cells' proliferative rate. For this reason, it may be used as a cocktail that includes both anti-MART1 (a cytoplasmic marker) and anti-Ki67 (that labels the nucleus) that also uses two chromogens: aminoethylcarbazol (red) and diaminobencidine (brown). Therefore, when we evaluate this immunohistochemical stain we are able to reliably quantify the proliferating melanocytes having a red cytoplasmic and brown nuclear labeling.

One important aspect of using immunohistochemical studies in evaluation of vulvar melanocytic lesion is to use caution when interpreting stains such as MART1, considering the known association of vulvar melanomas with background melanocytic hyperplasia.

The diagnosis of melanoma in situ implies the detection of a confluent growth pattern of atypical intraepithelial melanocytes compared with the scattered atypical melanocytes, albeit increased in number, which can be seen in melanocytic hyperplasia. Some authors discourage the use of anti-MART1 because the labeling of the melanocytic dendrites may simulate the confluent pattern of growth of melanoma. However, using HMB45 or particularly MITF1 or Sox10 (which are nuclear

markers) will allow a better quantitative appreciation of the melanocytic proliferation.

Differential Diagnosis of Vulvar Melanoma from Vulvar Melanocytic Proliferations in Lichen Sclerosus

Melanocytic nevi arising in lichen sclerosus can mimic both clinical and histological melanoma, and therefore they are often referred to as "pseudomelanoma" (see Chap. 7). The overlapping morphologic features with melanoma are likely due to the stromal alterations that may induce a change in the melanocytes' phenotype. Histologically, melanomas are poorly circumscribed and have more confluent growth pattern, and dermal mitotic figures are often noted.

Among the histopathological features favoring lichen sclerosus are the presence of a homogenized papillary dermis rather than a thinned epidermis as seen in regressed melanoma. Usually a band-like lymphocytic infiltrate separates the dermis from the epidermis by a thin layer of sclerosis. Marked basal vacuolar alteration is noted; the epidermis contains numerous necrotic keratinocytes and the basal membrane may be thickened. In lichen sclerosus, melanocytes are not easily identified but finding melanocytic nevi within a field of lichen sclerosus is not an uncommon phenomenon; the concomitant secondary pigment alterations affecting the normally hyperpigmented vulvar skin, as well as a melanocytic nevus, can produce a lesion clinically suspicious for melanoma. Also the postinflammatory pigmentary alteration can explain the variegated pigmentation and irregular borders of the lichen sclerosus that mimic melanoma.

A melanocytic nevus associated with lichen sclerosus is usually well circumscribed, absent or minimal, and only central pagetoid upward migration of melanocytes has no mitotic figures identified within dermal melanocytes and has a low proliferative rate by Ki67, and HMB45 is expressed only in epidermal melanocytes or in the focal superficial dermis (maturation phenomenon) (Table 8.3).

Table 8.3 Vulvar melanoma versus nevi arising in a background of lichen sclerosus: histological features and immunohistochemical studies

Vulvar melanoma		Nevi arising in a background of lichen sclerosus
Histological features		
Circumscription	No	Yes
Symmetry	No	Yes
Junctional nests and single cells	Predominately single cells	Predominately nested
Lentiginous growth	Confluent growth pattern, extending beyond the area of fibrosis	Trizonal growth pattern
Junctional nests	Poorly cohesive or coalescent	Cohesive
Pagetoid upward migration	Present, frequently prominent	Usually absent
Cytology	Significant cytologic atypia	No or minimal cytologic atypia
Ulceration	Present	Absent
Dermal maturation	Absent	Present
Dermal mitoses	Present, including in the deep aspect of the lesion	Not easily identified
Dermal fibrosis	Regression type	Homogenized, sclerotic papillary dermis with underlying band-like lymphocytic infiltrate
Epidermal changes	Loss of rete ridges, especially in regressed lesions	Evidence of interface dermatitis with basal vacuolar changes and scattered colloid bodies (necrotic keratinocytes)
Immunohistochemical studies		
HMB45	Patchy expression, in both superficial and deep aspect of the lesion	Expressed in the intraepidermal and superficial dermal melanocytes only (maturation pattern)
Ki67	Increased proliferative rate (>10 %), including in deeper melanocytes	Low proliferative rate (<10 %) and only in the superficial melanocytes

However, it is also important to remember that lichen sclerosus may be also associated with vulvar melanoma and extreme caution may be used for the diagnosis [32].

Molecular Biology of Vulvar Melanoma and Its Therapeutic Implications

Despite new treatments attempted and developed, there is no definite evidence of improved melanoma survival, including in vulvar melanomas, in the last few decades. Patients with thinner melanomas may be managed using surgical resection and had a better prognosis than those with more extensive or metastatic disease.

In a need to develop new treatment options for these patients, over the last decade, significant progress has been made in identifying genetic alterations with the potential to revolutionize the treatment of melanoma. Distinctive chromosomal aberrations were identified in melanomas arising on chronically sun-exposed skin in comparison with melanomas arising in areas intermittently exposed to the sun, such as vulvar melanomas, leading to different therapeutic approaches. Moreover, it has been noted that melanomas arising at different mucosal sites differ also depending on their site of origin, having a significant heterogeneity in expression and different gene alterations. It has been also shown that vulvar melanomas located on the glabrous skin (mucosa) have different biological properties when compared with melanomas involving the hairy vulvar skin [12]. Similar to acral melanoma, mucosal melanomas show a high degree of genomic instability with frequent amplifications.

Activating V600E or V600K mutations in the *BRAF* kinase that are common in cutaneous melanomas arising in sun-exposed areas and for which *BRAF*-specific targeted therapies are available. They are extremely rare in melanomas occurring in non-sun-exposed areas or mucosal sites, such as vulvar melanoma [33]. Somatic *BRAF* and *NRAS* mutations are mutually exclusive; however, *NRAS* mutations are also relatively infrequent in vulvar melanomas (approximately 12 % of cases) in comparison with melanomas arising in chronically sun-damaged skin (approximately seen in 24 % of cases) [33]. Interestingly, in melanomas at other mucosal sites, such as esophagus, *NRAS* alterations are more frequent, so this discrepancy may not be due to a direct association to ultraviolet light irradiation [33]. Similarly, *ras* mutation, especially in codon 61 of N-ras oncogene, is common in cutaneous melanomas, but this is an exceptional occurrence for vulvar melanoma [34].

Recently, mutations and/or increased copy numbers of the gene encoding the receptor tyrosine kinase *KIT* have been described in mucosal and acral-lentiginous melanomas. The frequency of *KIT* mutations in mucosal melanomas varies significantly with anatomical site, but the highest *KIT* mutation rate (35–40 %) was detected in vulvar melanomas. The majority of the *KIT* mutations identified occur within exon 11, encoding the juxtamembrane domain of the *KIT* receptor. In addition, a minority of mutations was found in *KIT* exon 17, which encodes the tyrosine kinase-2 domain of *KIT*. Single amino acid substitutions were the most common type of alterations identified. It is interesting to note that many vulvar melanomas with *KIT* mutations had a mutation other than L576P, having a better clinical response to imatinib and sorafenib.

The correlation between *KIT* expression levels by immunohistochemistry and mutation status is still controversial. It has been shown that rare tumors for which *KIT* mutation was demonstrated were negative for c-kit immunohistochemical expression and cases of *KIT* wild-type melanomas demonstrated positivity for c-kit by immunohistochemistry. The immunohistochemical expression of c-kit is so far insufficient to predict the response to KIT-targeted therapy with imatinib mesylate [33]. The current recommendation is that immunohistochemistry should not replace mutation analysis in the identification of patients who may benefit from *KIT* inhibitors-targeted therapies. However, other studies have shown that immunohistochemical expression of *KIT* in less than 10 % of the cells of the invasive component of acral-lentiginous/mucosal melanomas appears to be a strong negative predictor of *KIT* mutation and therefore can potentially be used to triage cases for additional *KIT* genotyping [35].

Recent studies showed that the response rates for metastatic melanomas treated with imatinib mesylate were better in patients with *KIT* exon 11 mutations than in those with *KIT* exon 17 mutations or *KIT* amplifications [33].

It has been also reported that RAF/MEK/ERK and PI3K/AKT signaling pathways are activated in a significant proportion of mucosal melanomas, which occurs irrespective of the KIT/NRAS/BRAF mutation status of the tumors [36]. Both the RAF/MEK/ERK and PI3K/AKT pathways may represent promising alternative therapeutic targets in mucosal melanoma, especially in the subset of tumors lacking activating-*KIT* mutations.

Summary

Clinical Presentation

- Melanoma often presents as a vulvar mass (40 %) followed by pain, bleeding, and itching.
- Affect middle-aged or postmenopausal women (median age of 68).
- Usually large, with variegate pigmentation and irregular or poorly demarcated borders.

Histological Features

- Commonly involve the mucosal aspect of the vulva and are mucosal/acral-lentiginous type and may also be nodular or superficial spreading (on the cutaneous aspect of the vulva).
- Melanomas are poorly circumscribed, asymmetrical, composed of melanocytes with a significant degree of cytologic atypia, arranged in nests and numerous single cells with confluent growth pattern, and prominent pagetoid upward migration.
- Ulceration is also noted and mitotic figures within the dermal melanocytes are noted.

Differential Diagnosis

- Non-melanocytic pigmented lesions:
  - Angiokeratoma
  - Purpuric lesions
  - Lichen sclerosus
  - Lichen simplex chronicus
  - Acanthosis nigricans
  - Seborrheic keratosis
  - Condyloma accuminatum
  - Vulvar intraepithelial neoplasm (VIN)
  - Basal cell carcinoma
  - Pigmented extramammary Paget disease
- Melanocytic pigmented lesions:
  - Lentigo/melanosis/lentigines
  - Nevus of special site
  - Nevus in a background of lichen sclerosus

Takeaway Essentials

Clinical Relevant Pearls

- Because of their location, vulvar melanomas are usually detected late, resulting in more advanced lesions at presentation.
- Vulvar melanomas are often multifocal and amelanotic which may explain the relatively high rate of local recurrence.
- Can mimic both clinically and histologically a wide variety of non-melanocytic and melanocytic lesions. When in doubt, a biopsy should be performed!

Pathology Interpretation Pearls

- The most important prognostic factors in melanoma and which should be mandatory included in the pathology report are: tumor thickness, presence or absence of ulceration, and mitotic count per square mm.
- The most challenging differential diagnosis is with melanocytic nevi with dysplastic features and nevi occurring in association with lichen sclerosus.

Immunohistochemical/Molecular Findings

- Immunohistochemical studies with HMB45 and Ki67 (or cocktail-combining MART1 and Ki67) may be useful in the differential diagnosis of melanoma: melanoma has usually a patchy or solid labeling with HMB45, and Ki67 is increased, including in the deep aspect of the lesion.
- *KIT* mutations have been described in up to 40 % of vulvar melanomas and currently targeted therapies with tyrosine kinase inhibitors may be used.

Case Vignettes

Vignette 1

Clinical History: A 44-year-old female with irregular dark-brown plaque on labia majora.

Microscopic Description: The epidermis showed a proliferation of epithelioid cells with severe cytologic atypia arranged in nests and single cells. Upper migration was noted. The proliferative cells exhibited large amount of basophilic cytoplasm and brown pigment. Immunohistochemical studies demonstrated that the malignant cells were positive for CK7, CAM 5.2, EMA, and CEA while negative for S100 protein, HMB45, and MART1.

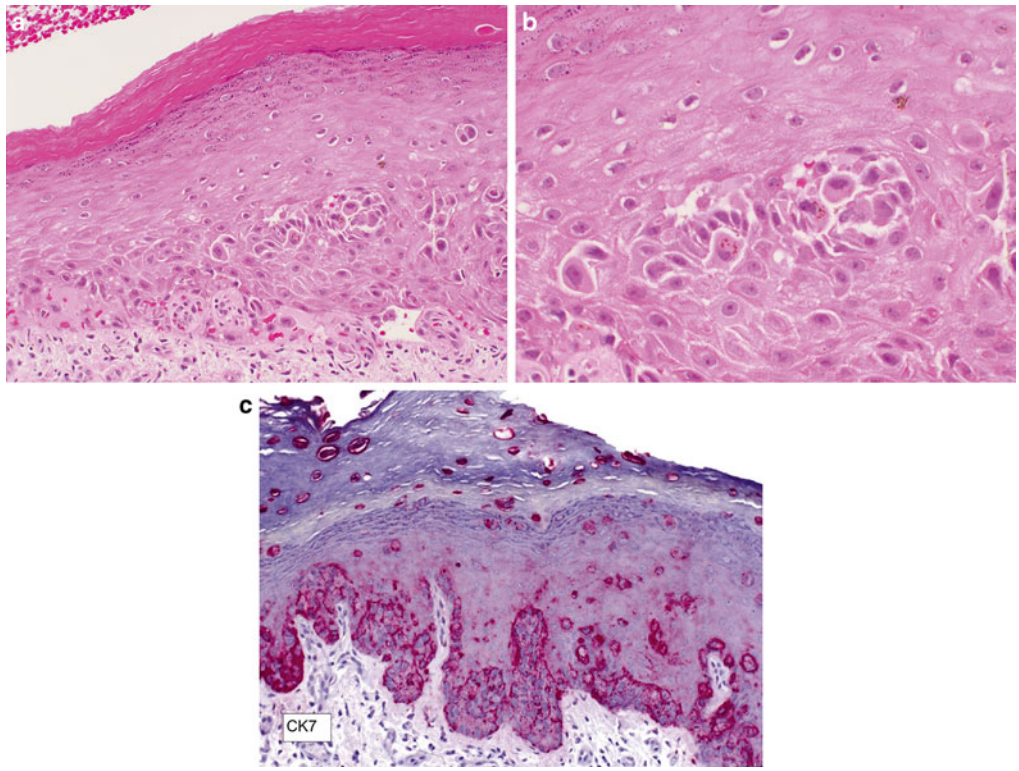


Fig. 8.11 Vignette 1, Proliferative malignant cells arranged as single cells and nests (**a**) with focal collection of pigment (**b**). CK7 decorates the malignant cells (**c**)

Diagnosis: Pigmented extramammary Paget's diseases.

Discussion: The arrangement of the malignant cells in extramammary Paget's disease in nests and single cells (Fig. 8.11a, b), as well as the presence of pagetoid spread, can create a diagnostic confusion with malignant melanoma. The difficulty is increased by the presence of melanin uptake from the surrounding melanocytes within the epidermis and the presence of intraepidermal reactive melanocytes with a dendritic morphology. In cases in which only a superficial biopsy is performed, pigmented extramammary Paget's disease can be confused easily with melanoma in situ. Immunohistochemical studies can resolve the differential diagnosis: Paget cells label for CK7 (Fig. 8.11c), low-molecular-weight keratins (CAM 5.2), EMA, and

(continued)

CEA. There is variable expression of GCDFP-15 (BRST2) and mucicarmine. The pagetoid cells are negative for melanocytic markers such as S100 protein, HMB45 antigen, MART1, Sox10, and MITF. It is important to mention a potential pitfall in the interpretation of immunohistochemical studies with MART1: due to the presence of numerous background dendritic melanocytes, the stain can be easily over interpreted; we should base our evaluation/interpretation of the stain only on the number of labeled cellular bodies and not their dendritic processes.

Vignette 2

Clinical History: A 12-year-old girl with a brown macule of 1 cm in greatest diameter surrounded by an ill-defined indurated white area.

Microscopic Description: The sections showed a compound proliferation of melanocytes with focal homogenous eosinophilic area in the dermis and epidermal atrophy (Fig. 8.12a). A proliferation of single and nested melanocytes arranged in a focal confluent growth pattern with pagetoid spread was noted (Fig. 8.12b). The dermal component exhibits maturation with presence of small melanocytes in the reticular dermis (Fig. 8.12d). No mitotic figures were noted. HMB45 decorates most of the lesion (Fig. 8.12c).

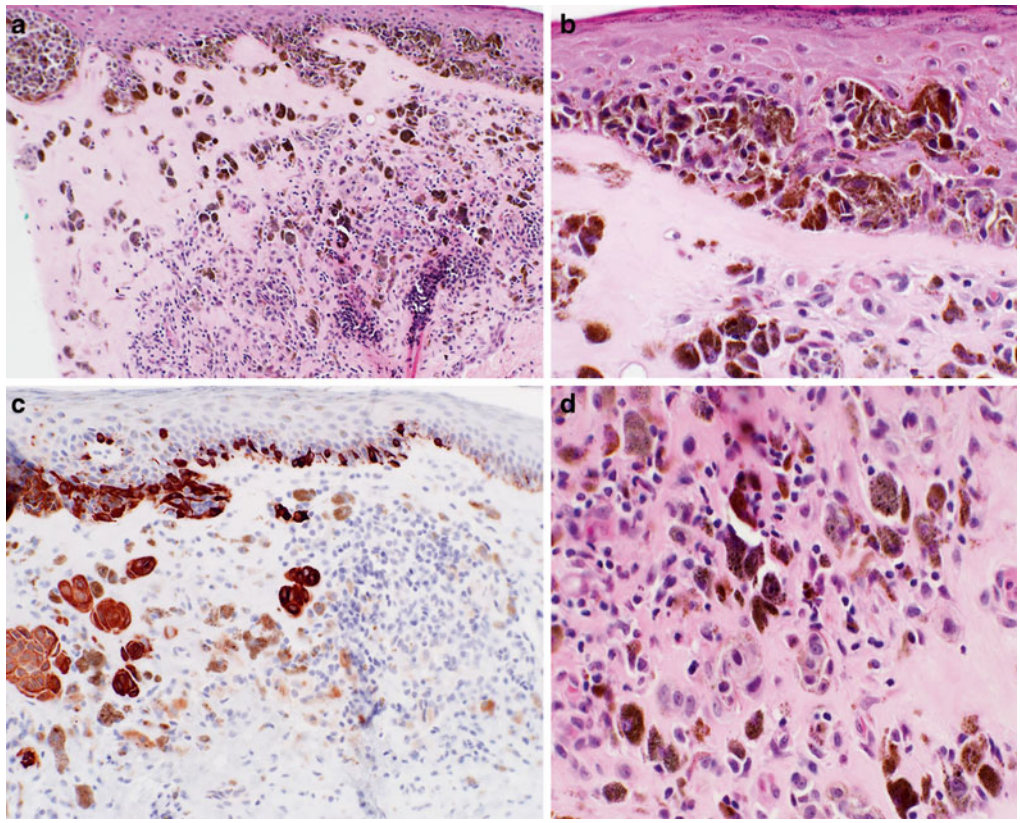


Fig. 8.12 Vignette 2, Low power demonstrated a compound proliferation of melanocytes with the dermal area of eosinophilic sclerosis (**a**). Junctional melanocytes showed confluent growth pattern (**b**). HMB-45 decorates the melanocytes in the epidermis and dermis (**c**). Dermal melanocytic maturation is noted (**d**)

(continued)

Diagnosis: Compound melanocytic nevus arising in lichen sclerosus.

Discussion: Melanocytic nevus growing over an area of lichen sclerosus can mimic melanoma.

The overlapping morphologic features with melanoma are likely due to the stromal alterations that may induce a change in the melanocytes phenotype. Histological features that favor a melanocytic nevus associated with lichen sclerosus are: a well-circumscribed proliferation, absent or minimal, and only central pagetoid upward migration of melanocytes, no mitotic figures identified within dermal melanocytes, and low proliferative rate by Ki67, and HMB45 is an expression only in epidermal melanocytes or in the focal superficial dermis (maturation phenomenon) (Table 8.3).

However, it is also important to remember that lichen sclerosus may be also associated with vulvar melanoma and extreme caution may be used for the diagnosis.

References

1. Rock B, Hood AF, Rock JA. Prospective study of vulvar nevi. J Am Acad Dermatol. 1990;22(1):104–6.
2. Rock B. Pigmented lesions of the vulva. Dermatol Clin. 1992;10(2):361–70.
3. Heller DS. Pigmented vulvar lesions-a pathology review of lesions that are not melanoma. J Low Genit Tract Dis. 2013;17(3):320–5.
4. De Simone P, Silipo V, Buccini P, Mariani G, Marenda S, Eibenschutz L, et al. Vulvar melanoma: a report of 10 cases and review of the literature. Melanoma Res. 2008;18(2):127–33.
5. Weinstock MA. Malignant melanoma of the vulva and vagina in the United States: patterns of incidence and population-based estimates of survival. Am J Obstet Gynecol. 1994;171(5):1225–30.
6. Moxley KM, Fader AN, Rose PG, Case AS, Mutch DG, Berry E, et al. Malignant melanoma of the vulva: an extension of cutaneous melanoma? Gynecol Oncol. 2011;122(3):612–7.
7. Ronger-Savle S, Julien V, Duru G, Raudrant D, Dalle S, Thomas L. Features of pigmented vulval lesions on dermoscopy. Br J Dermatol. 2011;164(1):54–61.
8. Verschraegen CF, Benjapibal M, Supakarapongkul W, Levy LB, Ross M, Atkinson EN, et al. Vulvar melanoma at the M. D. Anderson Cancer Center: 25 years later. Int J Gynecol Cancer. 2001;11(5):359–64.
9. Günther V, Alkatout I, Lez C, Altarac S, Fures R, Cupic H, et al. Malignant melanoma of the urethra: a rare histological subdivision of vulvar cancer with a poor prognosis. Case Rep Obstet Gynecol. 2012;2012:385175.
10. McLaughlin CC, Wu X-C, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. Cancer. 2005;103(5):1000–7.
11. Moan J, Porojnicu AC, Dahlback A, Grant WB, Juzeniene A. Where the sun does not shine: is sunshine protective against melanoma of the vulva? J Photochem Photobiol B Biol. 2010;101(2):179–83.
12. Ragnarsson-Olding BK. Primary malignant melanoma of the vulva–an aggressive tumor for modeling the genesis of non-UV light-associated melanomas. Acta Oncol. 2004;43(5):421–35.
13. Sugiyama VE, Chan JK, Shin JY, Berek JS, Osann K, Kapp DS. Vulvar melanoma. Obstet Gynecol. 2007;110(2, Part 1):296–301.
14. Mert I, Semaan A, Winer I, Morris RT, Ali-Fehmi R. Vulvar/vaginal melanoma: an updated surveillance epidemiology and end results database review, comparison with cutaneous melanoma and significance of racial disparities. Int J Gynecol Cancer. 2013;23(6):1118–25.
15. Egan CA. Vulvar melanoma in childhood. Arch Dermatol. 1997;133(3):345–8.
16. Wechter ME, Gruber SB, Haefner HK, Lowe L, Schwartz JL, Reynolds KR, et al. Vulvar melanoma: a report of 20 cases and review of the literature. J Am Acad Dermatol. 2004;50(4):554–62.
17. Collina G. "Combined" desmoplastic melanoma of the vulva with poor clinical outcome. Pathologica. 2011;103(6):337–9.
18. Ragnarsson-Olding BK, Nilsson BR, Kanter-Lewensohn LR, Lagerlöf B, Ringborg UK. Malignant melanoma of the vulva in a nationwide, 25-year study of 219 Swedish females: predictors of survival. Cancer. 1999;86(7):1285–93.
19. Balch CM, Gershenwald JE, Soong S-J, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199–206.
20. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg. 1970;172(5):902–8.
21. Tcheung WJ, Selim MA, Herndon JE, Abernethy AP, Nelson KC. Clinicopathologic study of 85 cases of melanoma of the female genitalia. J Am Acad Dermatol. 2012;67(4):598–605.
22. Guitart J, Lowe L, Piepkorn M, Prieto VG, Rabkin MS, Ronan SG, et al. Histological characteristics of

metastasizing thin melanomas: a case-control study of 43 cases. Arch Dermatol. 2002;138(5):603–8.
23. Piura B. Management of primary melanoma of the female urogenital tract. Lancet Oncol. 2008; 9(10):973–81.
24. De Hullu JA, Hollema H, Lolkema S, Boezen M, Boonstra H, Burger MPM, et al. Vulvar carcinoma. The price of less radical surgery. Cancer. 2002; 95(11):2331–8.
25. Abramova L, Parekh J, Irvin WP, Rice LW, Taylor PT, Anderson WA, et al. Sentinel node biopsy in vulvar and vaginal melanoma: presentation of six cases and a literature review. Ann Surg Oncol. 2002;9(9):840–6.
26. Raspagliesi F, Ditto A, Paladini D, Fontanelli R, Stefanon B, DiPalma S, et al. Prognostic indicators in melanoma of the vulva. Ann Surg Oncol. 2000;7(10):738–42.
27. la Garza Bravo De MM, Curry JL, Torres-Cabala CA, Ivan DS, Drucker C, Prieto VG, et al. Pigmented extramammary Paget disease of the thigh mimicking a melanocytic tumor: report of a case and review of the literature. J Cutan Pathol. 2014;41(6): 529–35.
28. Gleason BC, Hirsch MS, Nucci MR, Schmidt BA, Zembowicz A, Mihm MC, et al. Atypical genital nevi. A clinicopathologic analysis of 56 cases. Am J Surg Pathol. 2008;32(1):51–7.
29. Carlson JA, Mu XC, Slominski A, Weismann K, Crowson AN, Malfetano J, et al. Melanocytic proliferations associated with lichen sclerosus. Arch Dermatol. 2002;138(1):77–87.
30. Mulcahy M, Scurry J, Day T, Otton G. Genital melanocytic naevus and lichen sclerosus. Pathology. 2013;45(6):616–8.
31. Pinto A, McLaren SH, Poppas DP, Magro CM. Genital melanocytic nevus arising in a background of lichen sclerosus in a 7-year-old female: the diagnostic pitfall with malignant melanoma. A literature review. Am J Dermatopathol. 2012;12.
32. Heinzelmann-Schwarz VA, Nixdorf S, Valadan M, Diczbalis M, Olivier J, Otton G, et al. A clinicopathological review of 33 patients with vulvar melanoma identifies c-KIT as a prognostic marker. Int J Mol Med. 2014;33(4):784–94.
33. Aulmann S, Sinn HP, Penzel R, Gilks CB, Schott S, Hassel JC, et al. Comparison of molecular abnormalities in vulvar and vaginal melanomas. Mod Pathol. 2014
34. Jiveskog S, Ragnarsson-Olding B, Platz A, Ringborg U. N-ras mutations are common in melanomas from sun-exposed skin of humans but rare in mucosal membranes or unexposed skin. J Invest Dermatol. 1998;111(5):757–61.
35. Torres-Cabala CA, Wang W-L, Trent J, Yang D, Chen S, Galbincea J, et al. Correlation between KIT expression and KIT mutation in melanoma: a study of 173 cases with emphasis on the acral-lentiginous/mucosal type. Mod Pathol. 2009;22(11):1446–56.
36. Omholt K, Grafström E, Kanter-Lewensohn L, Hansson J, Ragnarsson-Olding BK. KIT pathway alterations in mucosal melanomas of the vulva and other sites. Clin Cancer Res. 2011;17(12):3933–42.

Part IV

Vulvar Intraepithelial Neoplasia and Squamous Cell Carcinoma

Squamous Intraepithelial Lesions of the Vulva

9

Demaretta S. Rush and Edward J. Wilkinson

Introduction

All squamous intraepithelial lesions of the vulva have in common the presence of nuclear atypia and abnormalities of proliferation and maturation. They may be distinguished from one another by the degree of such alterations and by determination of which layers of the epithelium are affected by them. The majority of these lesions are the result of infection with human papillomavirus (HPV). Such HPV-associated lesions demonstrate cellular and architectural changes analogous to HPV-induced lesions first characterized in the uterine cervix and subsequently observed throughout the squamous mucosa of the entire lower anogenital tract.

Because of the shared pathogenesis and morphology of HPV-associated squamous lesions throughout this anatomic region, it has recently been proposed by the *Lower Anogenital Squamous Terminology (LAST) Standardization Project for HPV-Associated Lesions* (referred to as the "LAST project") that a unified terminology be applied to them all [1]. In the vulva, this new terminology replaces the previous "Vulvar-Vaginal Intraepithelial Neoplasia (VIN)" nomenclature, introduced in the 1980s [2] and endorsed by the International Society for the Study of Vulvovaginal Disease (ISSVD) [3] in which lesions were classified as either condyloma acuminatum, a benign lesion caused by low-risk HPV types, or as premalignant VIN, which was initially further stratified into three grades with multiple histological subtypes (Table 9.1). Subsequently the ISSVD classified VIN lesions into two categories. This revision by the ISSVD [4, 5] abandoned the VIN 1 category and combined VIN 2 and VIN 3 into one category called simply "VIN" which was then divided into two types, the HPV-related or "usual" type (u-VIN) and the differentiated type (d-VIN) (Table 9.1). This classification offered an excellent integration of differentiated lesions into the overall scheme, but for precisely this reason it is not applicable to other anatomic sites affected by HPV and is therefore unsatisfactory if a unified terminology for HPV-associated lesions is to be desired. The new "LAST" terminology represents the culmination and synthesis of decades of work on HPV-induced lesions and allows for a simplified and more clinically oriented categorization of lesions into either low-grade or high-grade categories, retaining the option to include the VIN nomenclature in parentheses for purposes of clarification (Table 9.1).

Insofar as the majority of squamous intraepithelial lesions of the vulva are HPV-related and

D.S. Rush (✉)
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL, USA
e-mail: rushd@pathology.ufl.edu

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_9,

Table 9.1 Evolution of terminology for vulvar squamous intraepithelial lesions

1986 ISSVD		2004 ISSVD	LAST 2012	ISGYP/WHO 2013
Condyloma		Condyloma	LSIL (VIN 1)	LSIL (VIN 1)
VIN 1		–		
VIN 2	Warty	VIN Usual type (u-VIN)	HSIL (VIN 2-3)	HSIL (VIN 2-3)
	Basaloid			
	Mixed			
VIN 3	Warty			
	Basaloid			
	Mixed			
	Differentiated	Differentiated type (d-VIN)		HSIL, differentiated type (VIN-d)

ISSVD International Society for the Study of Vulvovaginal Disease, *LAST* Lower Anogenital Squamous Terminology (does not address differentiated lesions), *ISGYP/WHO* International Society of Gynecologic Pathologists/World Health Organization, *VIN* vulvar intraepithelial neoplasia, *LSIL* low-grade squamous intraepithelial lesions, *HSIL* high-grade squamous intraepithelial lesions

analogous to HPV-induced lesions of other sites, the LAST terminology achieves its goal of establishing a unified terminology. In the vulva, however, there exists a second, unique category of intraepithelial squamous lesion which has no counterpart in the rest of the lower female anogenital tract. This type of lesion, formerly termed "differentiated" or "simplex" VIN, is not HPV-related and demonstrates a unique morphology distinct from the changes caused by HPV. It has no low-grade counterpart and is high-grade by definition. These lesions were initially categorized as a subtype of VIN 3. The LAST standardization, since it is focused on HPV-related disease, fails to address differentiated lesions of the vulva altogether, making it inadequate for use in the classification of all vulvar intraepithelial disease. In 2013 the International Society of Gynecologic Pathologists (ISGyP) and the World Health Organization (WHO) addressed this problem and proposed something of a hybrid system for vulvar nomenclature [6], reintroducing the differentiated category into the terminology (Table 9.1). It is this terminology that will be used in this work.

Lesions categorized as low-grade intraepithelial lesion (LSIL) in the new classification comprise those formerly categorized as both flat condylomata and VIN 1, while lesions categorized as high-grade intraepithelial lesion (HSIL) in the new classification comprise those formerly categorized as VIN 2 and VIN 3. The histological criteria for determination as to whether a lesion belongs in the high-grade or low-grade category, as put forth by the LAST Project, are summarized in Table 9.2 and will be discussed in depth in the sections to follow.

Table 9.2 LAST Standardization Project criteria for two-tiered classification system

	LSIL	HSIL
Maturation	Little in the bottom third, begins in middle third, relatively normal in upper third	Little to none in the middle and upper thirds
Mitoses	Limited to lower third	May be found in middle and upper thirds
Nuclear Features	Increased nuclear size	Increased nuclear size
	Irregular nuclear membranes	Irregular nuclear membranes
	Increased nuclear: cytoplasmic ratios	Increased nuclear: cytoplasmic ratios
	And/or koilocytosis *without* features of high-grade lesion	

LAST Lower Anogenital Squamous Terminology, *LSIL* low-grade squamous intraepithelial lesions, *HSIL* high-grade squamous intraepithelial lesions

The decision to combine flat condyloma acuminatum with VIN 1 into a single category of lesions and to abandon the intermediate category of VIN 2 was justified by multiple factors.

For one thing, the diagnoses of VIN 1 and VIN 2 were found to be both uncommon and poorly reproducible [7, 8], and interobserver variability was found to be improved by including flat condyloma and VIN 1 together in one category and VIN 2 and VIN 3 in another [5, 7, 9–11]. On a practical level, this made sense as well, given that the treatment is the same for both flat condyloma and VIN 1 and likewise for VIN 2 and VIN 3.

Most importantly, however, current understanding of HPV infection does not support the existence of three categories of risk, but rather, only two. HPV infection is thought to result in either self-limited infection, in which viral particles actively replicate in the infected cells, corresponding to low-grade lesions with no potential to progress to carcinoma, or in persistent infection, in which the viral genome becomes integrated into the host DNA or persists in the host cell in episomal form, and which are truly premalignant lesions. To date there has been no convincing difference in biologic behavior demonstrated between lesions diagnosed as VIN 1 and those diagnosed as flat condyloma, suggesting they are merely variant morphologies of the same low-grade process [5, 12]. Likewise, there is no convincing evidence that VIN 2 is a distinct biologic or clinical entity. No morphologic criteria have been identified to distinguish a different biologic behavior for VIN 2 as opposed to VIN 3, and subsequent excisions performed for an initial diagnosis of VIN 2 on biopsy turn out to harbor a higher-grade lesion (VIN 3 or invasive carcinoma) in a large proportion of cases [13].

In the same period of time in which the pathophysiologic understanding of, and nomenclature for, vulvar intraepithelial disease has been evolving, a striking increase in the incidence of squamous intraepithelial lesions of the vulva has been observed [14–18]. In addition, there has been a trend towards younger age at presentation [14, 19]. The changing epidemiology has been attributed to a number of factors. Since the majority of lesions are HPV-related, the rise in incidence may simply reflect an increased prevalence of HPV in the same period of time. It is likely that additional factors, such as an increased awareness of vulvar diseases on the part of both patients and physicians and an increased use of biopsy to evaluate lesions of the vulva, have played a role as well. In the case of the differentiated type of VIN (d-VIN), an increased recognition of the previously underdiagnosed lesion has almost certainly contributed to the increase in its diagnosis.

Low-Grade Squamous Intraepithelial Lesions of the Vulva (LSIL)

Clinical Features

Previous classification schemes considered papillomatous-exophytic condyloma acuminata and flat condyloma, commonly known as genital warts, as separate entities from intraepithelial neoplasia, but in the LAST classification both are included in the LSIL category. Condyloma acuminata are considered something of a "special case," as they are such distinctive lesions, and the term has been so long in use, it is unlikely to be easily renamed. The LAST project advises maintaining the term in parenthesis for clarification. Flat lesions with similar cytologic features, while they are occasionally seen on the vulva [20] (Fig. 9.1), are far less common in this site

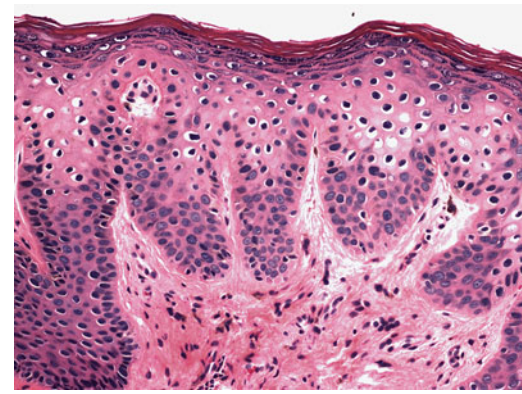


Fig. 9.1 Flat condyloma of vulva. The lesion lacks fibrovascular cores and is not exophytic, but the epithelial changes of mild nuclear crowding at the base, indicative of proliferation, and of koilocytic atypia in the maturing epithelium are identical to those of condyloma acuminatum

than on the cervix and, when present, are usually closely associated with concurrent vulvar HSIL.

The vulva is the most common site for lower genital tract condylomata. The incidence of vulvar condylomata varies according to the population being studied but in Western countries is generally about 1 % of sexually active women, with a peak prevalence occurring between the ages of 16 and 29 years [21, 22]. Although usually asymptomatic, patients may experience itching, pain, or bleeding. Typical exophytic lesions are easily identified grossly, either by the patient herself, who brings it to the attention of her clinician, or by the examining physician. The flat condyloma lesion, including LSIL, is often macular or papular in appearance and may become more apparent on application of a 3 % solution of acetic acid (white vinegar), which will impart a white appearance (acetowhite) to the abnormal epithelium. Lesions are commonly multifocal and may involve the vestibule and perianal areas, as well as the vagina, cervix, urethra, and anal canal. When vulvar lesions are identified, close examination of these adjacent areas is indicated [23]. Lesions may become confluent and involve a significant portion of the vulvar skin or mucosa, particularly during pregnancy [24, 25] (Fig. 9.2).

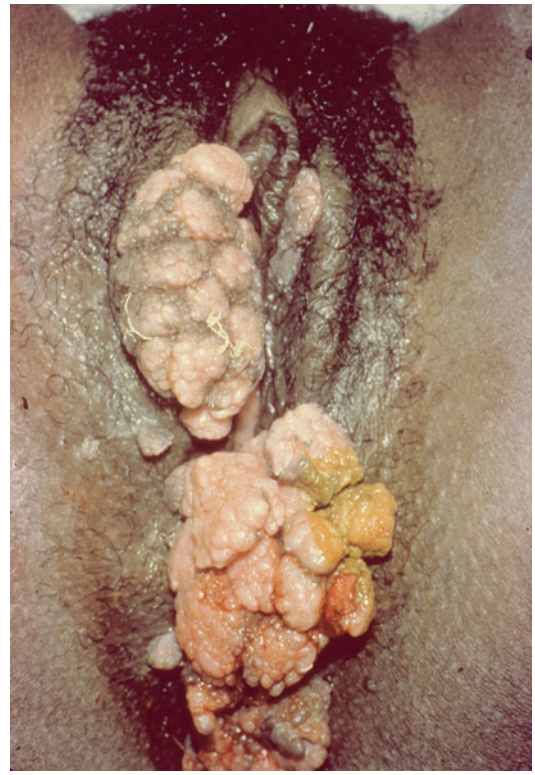


Fig. 9.2 Large condylomas distort the right labia and perineum of this patient. Small "satellite" lesions can also be seen (Photograph courtesy of Dr. Edwin Bowman, Associate Professor of OB-Gyn, retired, Louisiana State University School of Medicine, New Orleans, Louisiana)

Many risk factors have been identified for the development of LSIL of the vulva. The presence of SILs elsewhere in the lower anogenital tract is one; up to 50 % of women with vulvar exophytic condyloma acuminatum also have past, concurrent, or subsequent diagnoses of cervical or vaginal SIL [26, 27]. Other commonly associated conditions are vaginitis, pregnancy, diabetes, oral contraceptive use, and poor hygiene. Immunosuppression is an increasingly common predisposing factor, and women with human immunodeficiency virus (HIV), organ transplants, or autoimmune diseases often struggle with widespread lesions throughout the lower anogenital tract that can be very difficult to eradicate. As a sexually transmitted disease, the risk of SIL or condyloma acuminatum of the vulva is increased with increasing numbers of sexual partners. The presence of vulvar condyloma acuminatum in children should raise the question of sexual activity or abuse, although warts on the genitalia of children may be due to common cutaneous HPV subtypes [28].

All LSILs are HPV related, and the vast majority on the vulva are associated with types 6 and 11, although a significant minority may contain high-risk HPV subtypes [5, 10, 12, 21, 29–31]. Those associated with only low-risk virus are unequivocally benign and have no potential to progress to HSIL or malignancy. There is, however, disagreement as to the biologic behavior of those lesions harboring high-risk viral types [10], although it is clear that if any of these low-grade vulvar lesions do progress to higher-grade lesions, it is a very uncommon occurrence. Due to the as yet unclear evidence as to the biologic behavior of these lesions, as well as the expense of testing so many lesions to identify so few with high-risk virus, HPV typing by in situ hybridization or polymerase chain reaction

(PCR) is not recommended for vulvar LSIL, with the exception of cases occurring in children [20].

Condylomata are highly infectious, and the time between the incident viral infection and the development of a clinically recognizable lesion is estimated at 2.9–5 months [21, 32]. Although spontaneous regression may occur in a minority of lesions [23, 33], most will require treatment. A variety of topical treatments are available, and most patients will require more than one course of treatment to achieve complete eradication of the lesion. The time to clearance varies depending on the treatment used, with a median of 5.9 months [32]. Treatment is often painful and may result in edema, erythema, and ulceration in addition to significant discomfort and associated sexual impairment which may persist following resolution of the lesion [23, 34]. Because treatment is aimed at destruction of the visible lesion rather than the underlying cause, even with no new exposure to HPV, successful treatment cannot preclude a possible recurrence.

Because exophytic condylomata are so distinctive in gross appearance, they are not always biopsied but may be presumptively diagnosed and treated based on appearance. There is lack of consensus among experts as to whether all lesions which appear as typical condylomata ought to be biopsied or not, but it is recommended to biopsy all those occurring in postmenopausal women and those which have failed to regress with topical therapy [35]. The low-grade vulvar lesions encountered in clinical practice of pathology are therefore likely to represent only a minority of cases, those in which the diagnosis was uncertain or which were too large to manage medically.

Histopathology

Typical exophytic condyloma acuminata (Fig. 9.3) are papillary lesions with a central fibrovascular stalk. The epithelial surface is always acanthotic and often hyperkeratotic, parakeratotic, or both. The fibrovascular stalk is lacking in flat lesions. Both papillary and flat lesions exhibit similar epithelial changes, characterized by proliferation of squamous cells with abnormal nuclear features

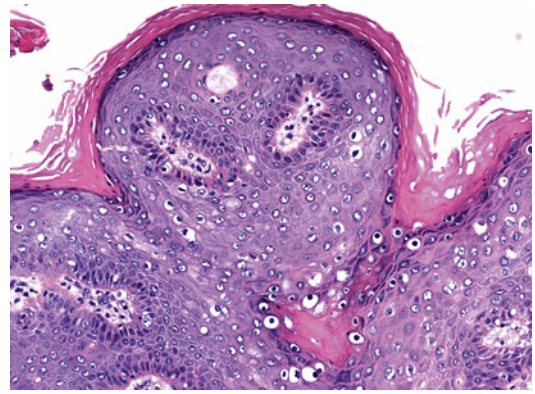


Fig. 9.3 LSIL of the vulva, showing fibrovascular cores of papillary structures in cross section. Surface hyperkeratosis and parakeratosis are also seen

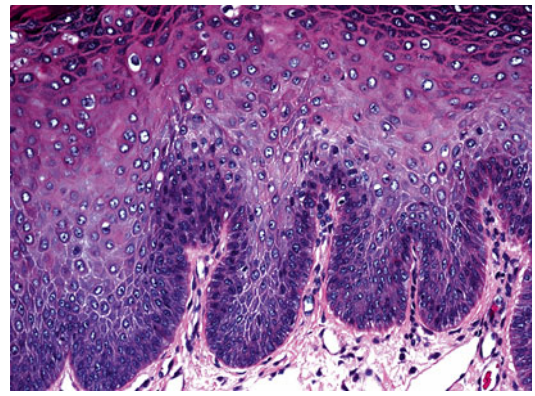


Fig. 9.4 The basal portion of LSIL of the vulva shows expansion of the immature basal cell layer with increased cellularity, crowding, and nuclear overlap, lending a darker appearance overall to the lower third of the epithelium. Cellular maturation appears relatively normal in the upper two-thirds

and little cellular maturation in the lower third of the epithelium (Fig. 9.4). The nuclear abnormalities include increased nuclear size, irregularity of nuclear membranes, multinucleation, nuclear pleomorphism, hyperchromasia, and increased nuclear to cytoplasmic ratios (Fig. 9.5). Koilocytic change is defined by the presence of sharply delineated perinuclear halo encircling the atypical nucleus, the morphologic manifestation of production of viral particles in the nucleus of infected cells, and death of the cell with retraction of the cytoplasm from the nuclear membrane (Fig. 9.5). Koilocytosis is usually present but

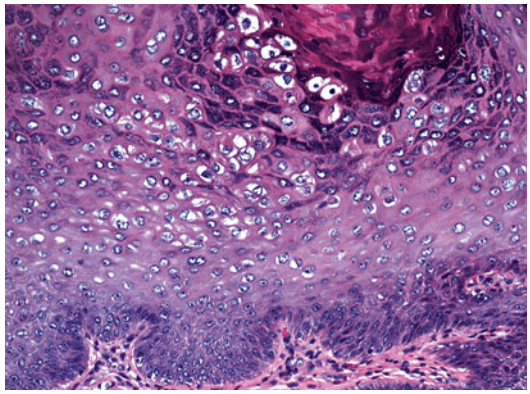


Fig. 9.5 Koilocytic change in LSIL is seen here in the upper layers of the lesion, where numerous cells with irregularly shaped pleomorphic nuclei and sharp perinuclear haloes are evident

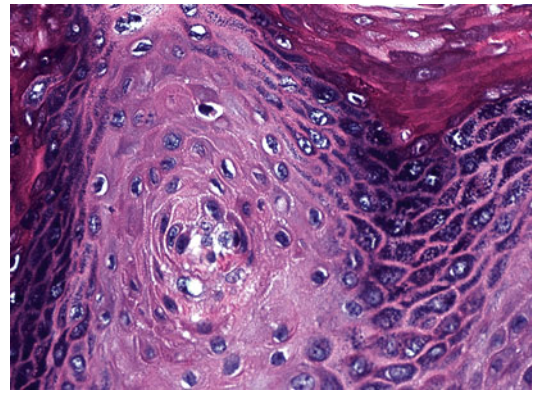


Fig. 9.7 On high power, accentuated intercellular bridges are easily identified in this example of LSIL. Marked surface hypergranulosis and hyperkeratosis are also prominent

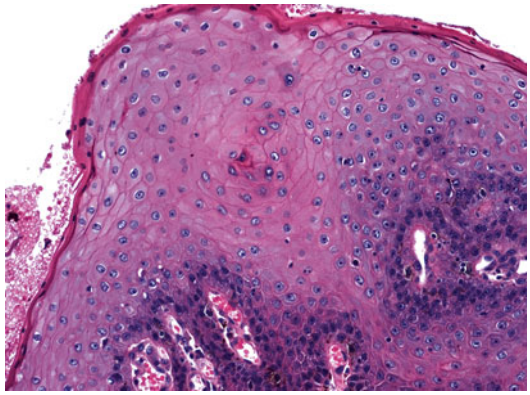


Fig. 9.6 Section shows a condyloma with no koilocytic change. Other areas of this lesion had well-developed koilocytic change, allowing the diagnosis to be made

may be minimal to absent focally (Fig. 9.6) or occasionally throughout entire lesions [20]. Individual cell keratinization, dyskeratosis, and accentuation of intracellular bridges may also be seen (Fig. 9.7). The proliferative nature of the lesion is evidenced by basilar and parabasal stratification, increased mitotic figures confined to the lower third of the epithelium, and cytoplasmic maturation beginning to appear in the middle third and relatively normal maturation of the upper third. Extension of immature cells or mitotic figures into the upper two-thirds of the epithelium or the finding of abnormal mitotic figures in any level precludes the diagnosis of LSIL, and lesions with such features should be interpreted as HSIL. Some nuclear abnormalities are commonly present throughout the full thickness of the epithelium in LSIL, and this finding does not indicate a need to upgrade the diagnosis to HSIL. Severe nuclear atypia, however, is not a typical feature of LSIL, and its presence does warrant upgrading to a diagnosis of HSIL.

Immunohistochemistry for p16INK4a (p16), a cell cycle protein which is upregulated and overexpressed in oncogenic HPV infection, is valuable in the evaluation of HSIL but is of limited use in the evaluation of LSIL of the vulva. While it may be tempting to use p16 as a routine screening tool for dysplasia, it should be noted that this is not an advisable or recommended use for this stain, particularly when the diagnostic impression is of a low-grade lesion. There are, however, certain situations in which staining with p16 may be considered in such lesions, as summarized in Table 9.3. It is recommended [1] for use in cases where there is high clinical suspicion of a high-grade lesion but the initial impression is of a low-grade lesion, in which case it may help to identify occult areas of higher-grade disease [36]. Staining with p16 can also be useful in the triage of the occasional lesions with an intermediate morphology, such as were formerly designated VIN 2, to determine whether the lesion is best interpreted as high or low grade, as positive "en bloc" staining in SIL of uncertain grade correlates with the presence of high-risk HPV types when the mor-

Table 9.3 Recommendations for the use of p16 immunohistochemistry in evaluation of SILs

If the initial impression is:	Use p16 staining:
LSIL	Only if there is high clinical suspicion of an occult HSIL
Intermediate between LSIL and HSIL	To triage the lesion into one or the other (LSIL or HSIL)
HSIL	If needed to differentiate from benign mimic

SIL squamous intraepithelial lesions, *LSIL* low-grade squamous intraepithelial lesions, *HSIL* high-grade squamous intraepithelial lesions

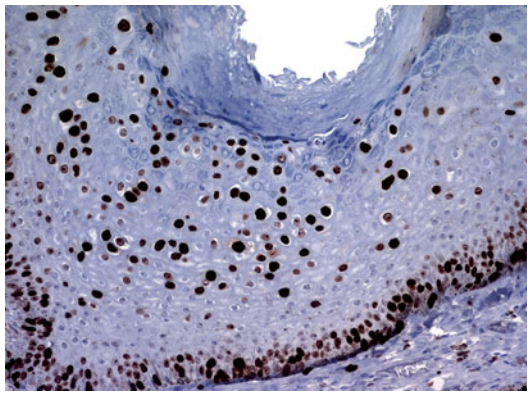


Fig. 9.8 Ki-67 immunohistochemical staining in LSIL demonstrates strong staining in most of the basal and parabasal cells, with numerous cells throughout the upper layers of the epithelium also staining strongly

phology is also supportive [37, 38]. Staining for p16 is of no use in the much more common situation of distinguishing between low-grade lesions and reactive or reparative changes, as LSIL is usually p16 negative [39, 40] and non-HPV-related squamous abnormalities may be p16 positive. Ki-67, a marker of cell cycle activation and cellular proliferation, has a similarly limited role in evaluation of LSIL and is restricted to the more thorough evaluation of proliferation abnormalities in cases in which the cytologic features are equivocal [41]. Because HPV infection in LSIL activates the cell cycle in order to accomplish viral reproduction, and this process is occurring in the maturing squamous cells, Ki-67 will be expressed in some cells in the upper levels of the epithelium in LSIL (Fig. 9.8), while in normally proliferating epithelium, expression of Ki-67 is limited to the basal and parabasal cells.

Differential Diagnosis

The differential diagnoses of vulvar LSIL and condyloma acuminatum include numerous lesions, especially fibroepithelial polyp, squamous papilloma, seborrheic keratosis, warty HSIL, verrucous carcinoma, and warty carcinoma. Polyps, while they do have a fibrovascular core, are typically lacking the acanthosis, hyperkeratosis, and parakeratosis so commonly seen in LSIL of the vulva. Immunohistochemistry for Ki-67 will distinguish LSIL and condyloma acuminatum from fibroepithelial polyps and squamous papilloma in problematic cases, as the staining is limited to the lower third of the epithelium in these lesions but extends into the upper layers in LSIL and condyloma acuminatum [41]. The papillary architecture and hyperkeratosis of seborrheic keratosis can be suggestive of condyloma acuminatum, particularly at low power. Further complicating the differential between these two entities is the fact that seborrheic keratosis, particularly of the vulvar skin, is frequently found to contain HPV DNA [42–44], and some authors have gone so far as to maintain that such lesions on the vulva are, in fact, simply a variant of condyloma [42] although this remains highly controversial. The absence of koilocytic atypia in seborrheic keratosis and of keratin horn cyst in condyloma will usually resolve the diagnosis. Distinction from warty HSIL rests on the identification of increased mitotic activity, abnormal mitotic figures, mitotic figures in the upper two-thirds of the epithelium, and more severe nuclear atypia, in addition to positive staining with p16, all of which are features which point to a high-grade diagnosis. It should be remembered that, although uncommon, lesions with the typical morphologic features of condyloma can contain areas of associated HSIL, in which case it is appropriate to classify it as condyloma acuminatum with HSIL (Fig. 9.9). Although the superficial portion of verrucous and warty types of

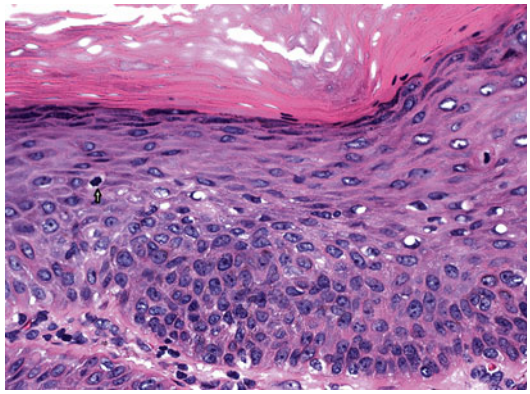


Fig. 9.9 This is a high-power view of a section of a lesion which elsewhere demonstrated typical findings of a condyloma. In this area, immature cells can be seen filling the entire bottom half of the epithelium, and a mitotic figure is evident two-thirds of the way through (arrow). These findings warrant an upgrade to a diagnosis of HSIL

squamous carcinoma may resemble LSIL, identification of invasion at the base of the lesion will indicate the correct diagnosis.

Summary

Clinical Presentation

- Typically exophytic growth
- Asymptomatic
- Range in size from millimeters to many centimeters

Histological Features

- Usually papillary configuration, with central fibrovascular stalks
- Surface with acanthosis
  - +/− Hyperkeratosis
  - +/− Parakeratosis
- Nuclear changes may be seen throughout the epithelium:
  - Enlargement
  - Pleomorphism
  - Hyperchromasia
  - Irregular membranes
- Perinuclear haloes (koilocytic change) common
- Increased mitotic activity confined to lower third of epithelium
- Normal cellular maturation in upper two thirds of epithelium

Differential Diagnosis

- Fibroepithelial polyp
- Squamous papilloma
- Seborrheic keratosis
- Warty HSIL
- Verrucous carcinoma
- Squamous carcinoma, warty type

Takeaway Essentials

Clinical Relevant Pearls

- Most lesions are condylomata
- Flat lesions are rare
- Most cases due to infection with low-risk HPV types 6 and 11

Pathology Interpretation Pearls

- Koilocytic change is characteristic
- Immature cells or mitotic figures must not extend beyond the lower third of the epithelium
- Immunohistochemistry for p16 is of limited use and must not be relied on to differentiate LSIL from benign mimics

High-Grade Squamous Intraepithelial Lesions of the Vulva (VIN 2-3)

Clinical Features

The risk factors for HPV-related HSIL are essentially the same as those for LSIL, and the patient population is similar. Patients with HPV-related HSIL are usually in their 30s or 40s [9, 26, 45], are frequently smokers, and often have a history of or concurrent multifocal vulvar lesions and/or multicentric HPV-related disease or other sexually transmitted diseases [9, 14, 16, 17, 26, 27, 45–49]. Pruritis is the most common symptom, reported in approximately 60 % of patients [14, 23, 41]. Other symptoms may include pain, ulceration, or dysuria, and approximately 20 % of patients have no symptoms at all but may have

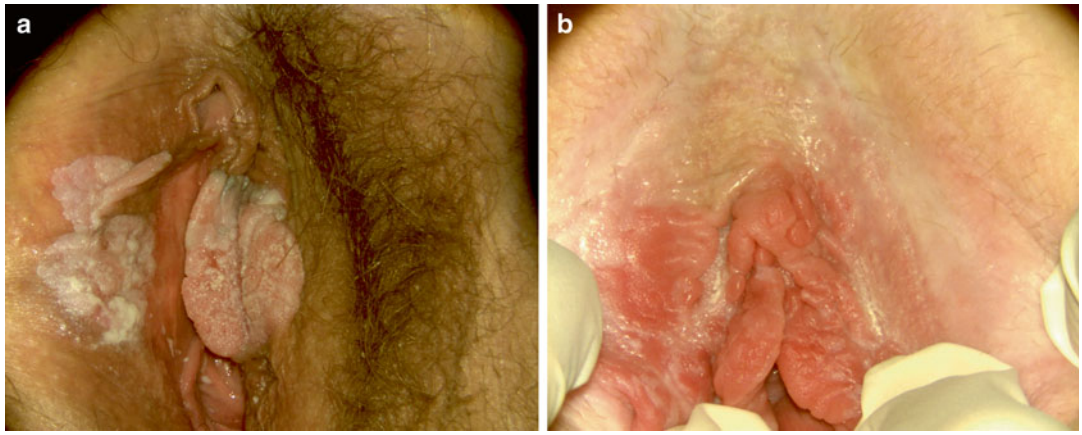


Fig. 9.10 (**a**) Asymmetric white plaques on the bilateral labia minora. Biopsy revealed HSIL (VIN 2–3). (**b**) The HSIL in this patient appeared as irregular red and white patches in the periclitoral region (Photographs courtesy of Dr. Jacqueline Castagno, Assistant Professor of Obstetrics and Gynecology, University of Florida, Gainesville, Florida)

observed an abnormal area on self-examination [12, 14, 45, 49].

The gross appearance of the lesion is variable and does not predict the histological appearance. Lesions may appear red, white, or pigmented and may be raised, flat, or ulcerated (Fig. 9.10a, b). Most lesions are well demarcated and asymmetrical. They are usually multiple but may be unifocal as well, and the incidence of multicentricity decreases with increasing age [49–51]. The most common sites are on the labia majora, labia minora, and fourchette [14], but the lesions may be found anywhere on the vulva and the perineum. Like LSIL, lesions may involve the perianal skin and extend into the anal mucosa.

"Bowenoid papulosis" is a clinical term which has been applied in circumstances in which multifocal, pigmented, or violaceous papular lesions are identified on the vulva and perineum of a young woman, often during pregnancy or postpartum [9, 19]. Histologically, such lesions are indistinguishable from HSIL, but they are thought to have less potential to progress, and indeed many cases have been found to regress spontaneously [19, 49, 52]. Because the diagnosis rests on specific clinical features, bowenoid papulosis is not acceptable as a histopathologic diagnosis, but close communication between the pathologist and clinician in cases which meet the clinical criteria can allow for optimal management decisions.

Most HSILs of the vulva have been found to contain high-risk HPV types [9, 10, 12, 17, 20, 26, 29–31, 53], most commonly HPV type 16. In this HPV-dependent pathway, the estimated time of progression from incident infection to the development of clinical disease has been estimated at 18.5 months [30].

HSIL may recur or progress to invasive carcinoma. The frequency of recurrence on the vulva is higher than for HSIL in other sites in the lower anogenital tract [54], and reported rates vary from 30 % to over 50 % [55–57]. Recurrence is more likely in patients who continue to smoke after initial diagnosis [46, 58], in patients with multifocal disease [45, 59], and in patients with positive margins on the initial resection [35, 58, 59], although the latter association has recently been refuted [14].

Up to 25.7 % of patients with HSIL of the vulva have been found to have prior, concurrent, or subsequent vulvar squamous cell carcinoma [12, 55, 60]. Subsequent carcinoma may occur at the original site of the HSIL or in a new location. Several clinical risk factors for the progression to carcinoma have been identified. In addition to continued smoking, larger size of the initial lesion and basaloid morphology increase the likelihood of progression [26, 58]. Patient age, multicentricity and multifocality of disease, and immunosuppression have all been reported as affecting the

risk of progression, but to date the data remain conflicting on all counts [9, 16, 19]. The reported rates of progression for treated vulvar HSIL have ranged from 2.0 % to 10 % [16, 19, 26, 58, 61], and the median times to a cancer diagnosis have been reported as 2.4–9 years [19, 58]. Untreated lesions have been found to progress to carcinoma with a median of 3.9 years, with all these cases resulting in carcinoma within 8 years [19]. In general, the long-term risk for developing carcinoma in women previously treated for HSIL is 2.5–7 % [62–64]. Early diagnosis and treatment can prevent this occurrence, and data showing that while the diagnosis of HSIL has been increasing in frequency, the diagnosis of invasive vulvar carcinoma has not [15], suggest that this has indeed been the case.

There has been a trend towards more conservative management of HSIL over the last 20 years. While total vulvectomy was once the standard treatment for these patients, most disease is now managed with wide local superficial partial excision or laser ablation [14, 26, 49]. Topical treatments are available and currently under investigation, but to date none have been shown to be as effective as surgical treatment [26]. Close clinical follow-up is very important, as patients with HPV-related HSIL remain at risk for recurrent or new vulvar lesions, as well as lesions elsewhere in the lower anogenital tract for the rest of their lives. The American College of Obstetrics and Gynecology advises follow-up at 6 and 12 months following the initial diagnosis, and if no lesions are discovered in that time, yearly thereafter [35].

Histopathology

HPV-related HSIL is characterized by the presence of nuclear abnormalities similar to those seen in LSIL but with more pronounced proliferation and decreased maturation. Mitotic figures may be found in all layers of the epithelium and abnormal forms may be seen (Figs. 9.11 and 9.12). Little to no maturation is seen in the middle to upper thirds. Marked nuclear pleomorphism and nuclear hyperchromasia are present, especially in cases with warty histological features.

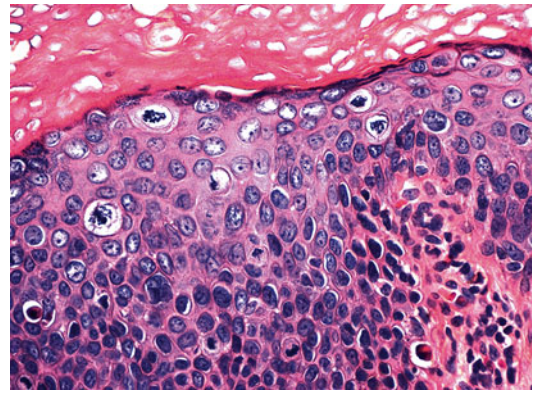


Fig. 9.11 HSIL with several mitotic figures evident in the upper layers of the epithelium

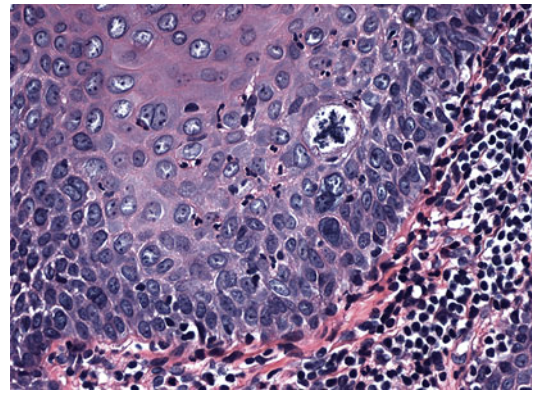


Fig. 9.12 A markedly atypical mitotic figure in HSIL

Two distinct histological patterns may be recognized, alone or mixed. The warty (condylomatous) type of HSIL is characterized by a spiky or undulating surface, giving the lesions a warty or condylomatous gross appearance. The epithelium is markedly thickened, with wide, deep rete pegs separated by thin dermal papillae that often closely approach the surface (Fig. 9.13). The epithelium shows disorganization, but there is evidence of maturation in the upper layers. Hyperkeratosis is prominent, often with accompanying hypergranulosis, parakeratosis, and koilocytic change which may be present towards the surface (Fig. 9.13). Multinucleated cells may be present, as well as dyskeratotic cells (Fig. 9.14), and nuclear atypia may be quite severe (Fig. 9.15). Nuclei are enlarged and nuclear chromatin hyperchromatic, with irregular

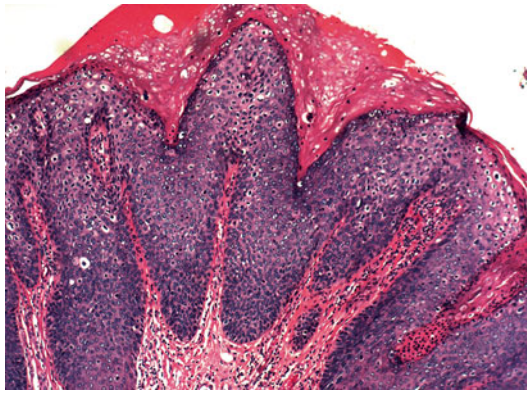


Fig. 9.13 At low magnification, the undulating surface of warty HSIL and deep rete pegs with thin dermal papillae are evident. Hyperkeratosis and parakeratosis are also prominent in this case

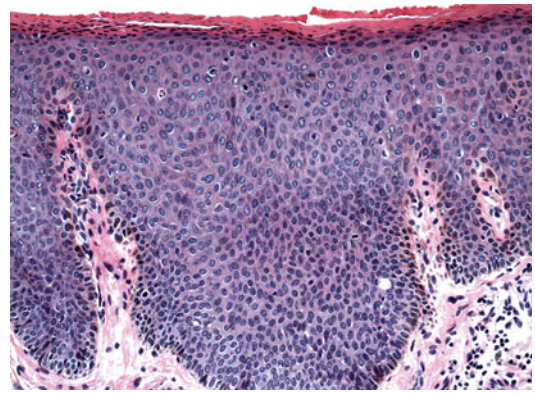


Fig. 9.16 In basaloid HSIL, there is little cellular maturation throughout the entire epithelium. This case shows some surface parakeratosis, but the surface is smooth, without papillary formations

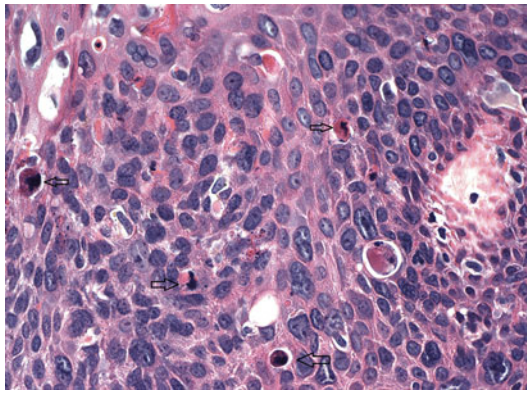


Fig. 9.14 Dyskeratotic cells (*arrows*) are abundant in this case of HSIL

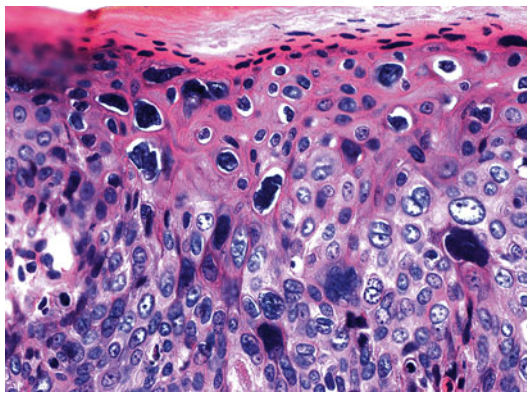


Fig. 9.15 HSIL with striking nuclear atypia and pleomorphism

nuclear membrane contours and prominent pleomorphism. An appreciable amount of eosinophilic cytoplasm is present, and the cell borders are easily delineated.

In the basaloid pattern of HSIL, the surface is relatively flat, and consequently these lesions do not typically appear papillary or exophytic but rather macular to papular. Hyperkeratosis, parakeratosis, and koilocytosis may be present but to a lesser degree than is seen in the warty type. The epithelium is thickened by a relatively uniform population of immature cells with scant cytoplasm, poorly defined cytoplasmic membranes, and enlarged nuclei with chromatin hyperchromasia (Fig. 9.16).

It should be recognized that lesions are not always easily categorized as warty or basaloid. Mixed forms, containing morphologic features of both types, are not uncommon and may be designated as such. Because there is no clinical or outcome differences between warty and basaloid types, it is also acceptable not to specify any subtype, so long as the distinction from differentiated vulvar intraepithelial neoplasia (d-VIN) is made clear.

Immunohistochemical study for Ki-67 in HSIL of the vulva shows widespread reactivity in the basal third and extending upwards into the upper two thirds (Fig. 9.17). Of greater utility in the diagnosis of vulvar HSIL is p16, which has been shown to be a reliable indicator of infection with high-risk HPV in the vulva [47, 53, 65–68].

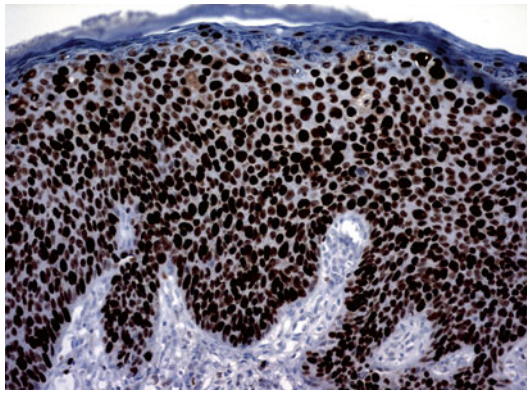


Fig. 9.17 Ki-67 immunostaining in HSIL stains almost all nuclei throughout the entire epithelium

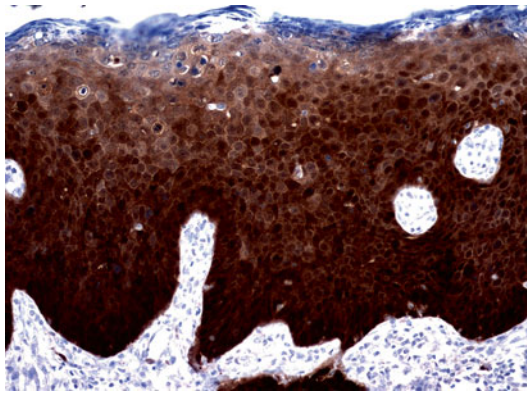


Fig. 9.18 p16 immunostaining in HSIL. Staining is both nuclear and cytoplasmic and strongly positive through the full thickness of the epithelium in a continuous block of cells

As such, it is strongly and diffusely positive throughout the epithelium in the oncogenic HPV-associated warty/basaloid HSILs [40, 53, 68, 69]. The pattern of reactivity may be nuclear or nuclear and cytoplasmic and should be reactive in a continuous "block" pattern throughout at least the lower one third of the epithelium (Fig. 9.18). Focal, patchy staining is nonspecific. Rare cases of HPV-negative basaloid HSIL have been identified in association with HPV-negative cancers and have been found to be HPV negative and p16 negative as well [70]. Unlike in LSIL, p16 immunostaining is useful in, and recommended for, distinguishing between HSIL from potential mimics (Table 9.3) [1] and its use is reported to increase interobserver agreement and accuracy of diagnosis in cases of HSIL [37, 67, 68, 71–73].

Differential Diagnosis

The differential diagnosis of HSIL varies according to the morphology of the lesion. Basaloid lesions are generally easy to recognize as lesions of malignant potential, with their pronounced lack of maturation, but may be mistaken for basal cell carcinoma which shows a similar lack of maturation. The lack of identifiable stromal invasion in HSIL should distinguish it from basal cell carcinoma. It should also be kept in mind that while basaloid HSIL is a relatively common diagnosis on the vulva, basal cell carcinoma in this location is distinctly uncommon. Lesions of warty or mixed morphologies may show overlapping features with numerous other conditions, both benign and malignant. Although both condyloma and warty HSILs may have prominent koilocytic changes, HSIL can be distinguished from condyloma acuminatum by the presence of atypical, pleomorphic cells in the deeper levels of the epithelium and by the presence of increased mitotic activity, including abnormal mitotic figures in the upper layers. Additional features which may be of use in distinguishing low-grade from high-grade lesions are enumerated in Table 9.4. As discussed previously, it should be kept in mind that the two are not mutually exclusive; HSIL may be found within or adjacent to condyloma and in such cases both entities may be diagnosed in the same biopsy. Other lesions that may be confused with warty HSIL include seborrheic keratosis and lichen simplex chronicus, which can show acanthosis and hyperkeratosis, but these lesions lack the nuclear atypia of HSIL. The large atypical cells present in the epithelium in cases of superficial spreading melanoma can sometimes be confused with all types of HSIL. Immunohistochemical studies for

Table 9.4 Additional features distinguishing LSIL from HSIL

	LSIL	HSIL
Abnormal mitoses	No	May be present
Severe nuclear atypia	No	May be present
Papillary architecture	Almost always	Sometimes
HPV types	6 and 11	16, 18, others

LSIL low-grade squamous intraepithelial lesions, *HSIL* high-grade squamous intraepithelial lesions

S-100, HMB-45, and/or Melan-A will resolve this dilemma, as HSIL will be negative for these melanoma markers but will be reactive for cytokeratin, which are negative in melanoma.

Several rare variants of HSIL have been described, usually in association with concurrent invasive carcinoma. Occasional cases have been recognized with a pagetoid morphology, in which clusters and nests of atypical squamous cells are found scattered within an otherwise normal epithelium [74, 75]. Synchronous non-pagetoid SIL was also present in two of three reported cases, providing for easy comparison of the neoplastic pagetoid cells with those in the more typical lesion. When this alone will not resolve the matter, true Paget cells will stain for mucin, but the VIN cells will be negative. More recently, a variant of HSIL has been described containing cells with mucinous cytoplasm admixed throughout the epithelium of an otherwise typical HSIL, which has been termed "VIN with mucinous differentiation" [76]. Both pagetoid VIN and VIN with mucous differentiation are oncogenic HPV related and reported lesions were also positive diffusely reactive for p16INK4a. Distinction from Paget disease may be made on morphologic grounds, but awareness of these Paget-like entities should allow for the proper interpretation.

It is not uncommon to encounter invasion in lesions clinically thought to be intraepithelial; studies have shown invasive squamous carcinoma in 3–31 % of excisions performed for the initial diagnosis of HSIL [5, 13, 59, 61, 64, 77, 78]. In most such cases the tumor is only superficially invasive. When significant invasive disease is encountered, it is usually readily recognized, but early, very superficial invasion is notoriously difficult to identify with certainty. Close attention should be paid to the basal layers of the epithelium and the basement membrane. The border along the basement membrane in VIN should be smooth, while in early invasion irregularly shaped tongues of cells protrude from the basal layer through the basement membrane. Often this is accompanied by apparent maturation of the cells on the invasive front, with enlarged cells with variable degrees of eosinophilic cytoplasm.

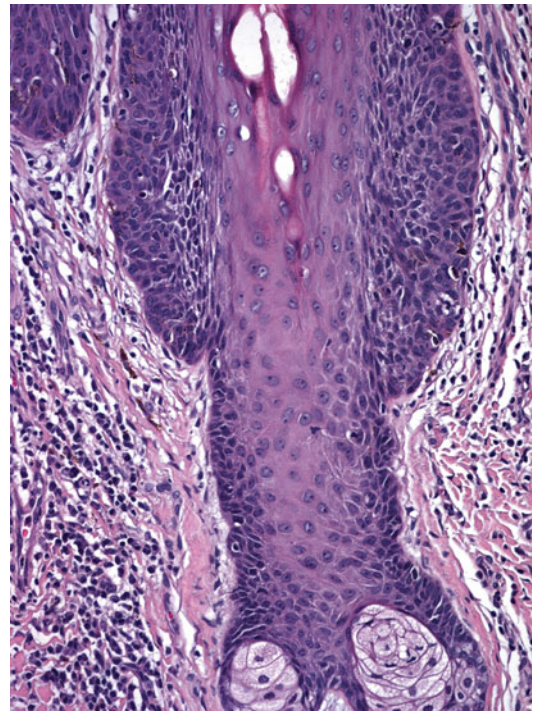


Fig. 9.19 Skin appendage involved by HSIL. The lesion extends downward from the surface along the periphery of this hair shaft. In time, the entire appendage may become lined with HSIL

The early invasive nests of squamous carcinoma are small, irregularly shaped, and usually accompanied by a dermal desmoplastic response. Tangential sections through the basal layers of VIN, on the other hand, will appear as clusters of smaller cells with less cytoplasm and a smooth border. The identification of early invasion can be further complicated by involvement of skin appendages that may extend quite deep into the underlying dermis (Fig. 9.19). The distinction rests on the preservation of a distinct epithelial-dermal junction and the lack of inflammatory response or stromal desmoplasia in appendage involvement. Identification of adjacent uninvolved appendages at the same depth in the dermis may also be helpful. Additional sections of the block with the questionable invasive lesion are often of value and, with skin appendage involvement by HSIL, the uninvolved cutaneous glands or ducts may be evident.

Summary

Clinical Presentation

- Lesions frequently multifocal or multicentric
- Symptoms of pruritis, pain, ulceration
- Variable gross appearance

Histological Features

- Warty type
  - Undulating surface
  - Hyperkeratosis
  - Parakeratosis
  - Nuclear pleomorphism
  - Abundant cytoplasm
- Basaloid type
  - Flat surface
  - Uniform nuclei
  - Scant cytoplasm
- All types
  - Mitotic figures and immature cells present in upper two thirds of the epithelium
  - Abnormal mitotic figures may be present
  - Little to no maturation in the upper layers of the epithelium

Differential Diagnosis

- Warty type
  - Condyloma (LSIL)
  - Seborrheic keratosis
  - Lichen simplex chronicus
- Basaloid type
  - Basal cell carcinoma

Takeaway Essentials

Clinical Relevant Pearls

- Most common type of vulvar intraepithelial lesion
- Associated with high-risk HPV subtypes, most commonly HPV 16
- Prone to recurrence
- Patients at risk for subsequent squamous cell carcinoma

Pathology Interpretation Pearls

- Warty, basaloid, or mixed morphology.
- Immunohistochemical reactivity for p16 in a "block pattern" is useful in the identification of HSIL and in differentiating it from benign mimics.

Vulvar Intraepithelial Neoplasia, Differentiated Type (d-VIN)

Clinical Features

Vulvar intraepithelial neoplasia of the differentiated type (d-VIN) is not classified as an HSIL lesion because it is characteristically not related to oncogenic HPV infection [6], although it is by definition a high-grade lesion in that it has the potential to progress to invasive disease. Differentiated VIN is much less common than HPV-related HSIL of the vulva. Studies have cited differentiated lesions as comprising anywhere from 2 % to 18.3 % of high-grade lesions of the vulva [8, 50, 69, 79, 80]. On average d-VIN can be estimated to comprise approximately 10 % of vulvar noninvasive neoplastic epithelial lesions. There are a number of possible explanations for the relative rarity of this diagnosis. It may be that it is truly a rare occurrence, but it seems most likely that the difficulty in recognizing the lesion, both grossly and microscopically, contributes to underrecognition and underreporting of the condition. It is possible that the relatively short period of time differentiated VIN appears to exist in the in situ phase reflects the apparent faster rate of progression to invasive carcinoma compared to HPV-related HSIL lesions as discussed below. This may also contribute to the less frequent diagnosis of d-VIN in biopsies.

Although the gross appearance of the lesions and the associated symptoms vary little from those of HPV-related HSIL, except in that d-VIN may be deceptively subtle in appearance, patient demographics and associated conditions differ considerably. Patients with d-VIN tend to be older than those with HPV-related lesions, with a

mean age of 67–69.2 years [8, 26]. There is no association with smoking history or other HPV-related lesions of the lower anogenital tract. The d-VIN lesions are less often multifocal [9, 16, 17, 26, 27, 47]. Differentiated VIN is associated with lichen sclerosus and with lichen simplex chronicus. Long-standing lichen sclerosus has been implicated by some authors as a precursor lesion to differentiated VIN and associated carcinoma [9, 17, 26, 47]. It is important not to take the presence of lichen sclerosus as an indication that an associated SIL is of the differentiated type, however, as HPV positive HSIL has also been reported in association with lichen sclerosus [81, 82].

Although the details of the proposed pathway of disease development from lichen sclerosus to differentiated VIN remain unclear, it has been proposed that the chronic tissue damage from inflammation, irritation, and resulting scratching of the lichen sclerosus may be responsible [83]. Molecular evidence that lichen sclerosus and squamous hyperplasia are true precursor lesions of differentiated VIN has been found in the fact that allelic imbalance is detectable in these conditions [84]. Also implicated in the development of d-VIN is p53 mutation, which has been found in both the proposed precursors of squamous hyperplasia and lichen sclerosus as well as in differentiated VIN [85, 86]. Lesions with the differentiated morphology are rarely HPV positive, with reported ranges of 0–12 % [9, 12, 27, 48, 53, 60, 82, 87], and HPV does not appear to have any role in the development of this lesion.

Differentiated VIN is considerably more aggressive than HPV-related HSIL. The evidence strongly suggests that the differentiated type is more likely to progress to invasion than the HPV-related type and appears to do so at a more rapid rate [16, 60]. Differentiated VIN lesions have the highest rate of progression to squamous carcinoma [69] and are rarely diagnosed in the absence of an accompanying carcinoma, suggesting it may progress at a much faster rate as well [16, 47]. The rate of progression to invasive cancer is reported at approximately 33 % for d-VIN [16, 87], and the presence of preceding, concurrent, or subsequent invasive carcinoma in patients with d-VIN has been reported in as high as 85.7 % [8, 12, 60]. The finding of invasive carcinoma in resections performed for an initial diagnosis of d-VIN is also common, reported in 20.5–83.3 % of cases [69, 88].

As the pathogenesis and biologic behavior of HPV-related HSIL and d-VIN lesions appear to be different, treatments also vary according to the histological type. Differentiated VIN lesions are typically managed more aggressively with more extensive excision because of their strong association with squamous cell carcinoma [17, 26].

Histopathology

The histological changes in differentiated VIN can be very subtle, making it notoriously difficult to recognize. This is especially unfortunate because it also has the worst prognosis of all the intraepithelial squamous neoplastic lesions of the vulva, and early detection can make the most difference in the clinical course of these patients. In d-VIN, unlike HPV-related HSIL lesions, the most atypical cells are confined to the basal and parabasal layers of the epithelium, while the superficial layers of the epithelium often appear relatively normal, making it easy to overlook. In contradistinction to HPV-related HSIL, in which little evidence of maturation is seen, d-VIN has a "paradoxical" maturation, and rather than an increase in the proportion of immature, less differentiated cells in the epithelium, as seen in HSIL, there is an increased proportion of mature, more differentiated cells, hence the term "differentiated" used in its nomenclature. The basal cell layer is often expanded by the population of atypical basal and parabasal cells with hyperchromatic, irregular, and variably sized nuclei with coarse nuclear chromatin and prominent macronucleoli, but there is no noticeable proliferation abnormality (Fig. 9.20). These atypical basal and parabasal cells also contain a moderate-to-abundant amount of hypereosinophilic cytoplasm with prominent intracellular bridges (Fig. 9.21), indicating premature keratinization and dysmaturation. These cells may form whorled aggregates with or without keratin pearls in the basal-parabasal portion of the epithelium (Fig. 9.22). The epithelium may be strikingly acanthotic

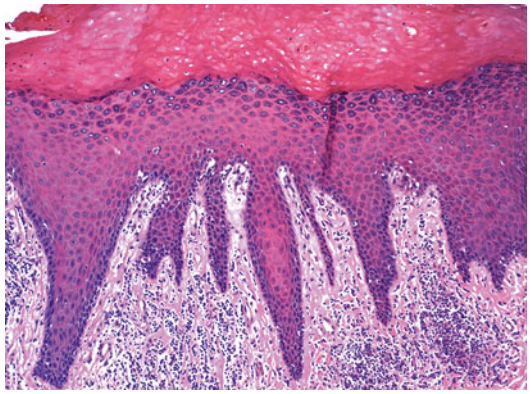


Fig. 9.20 Vulvar intraepithelial neoplasia, differentiated type (d-VIN). Atypical basal cells are hyperchromatic, and prominent nucleoli are seen throughout the lower portions of the epithelium. Even cells located deep within the rete pegs show abundant brightly eosinophilic cytoplasm and clearly demarcated intercellular borders such as is normally seen in cells maturing towards the surface

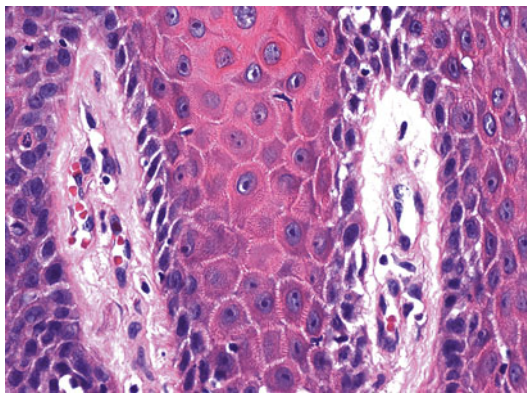


Fig. 9.21 On higher magnification, the cells in d-VIN show clear intracellular bridges, and the abnormal nuclear features with prominent nucleoli are better appreciated

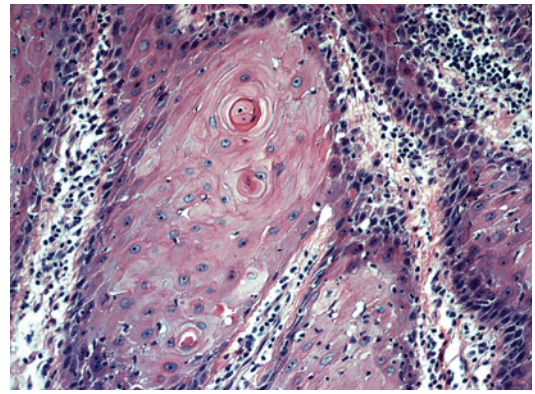


Fig. 9.22 Keratin pearl formation in HSIL, differentiated type (d-VIN)

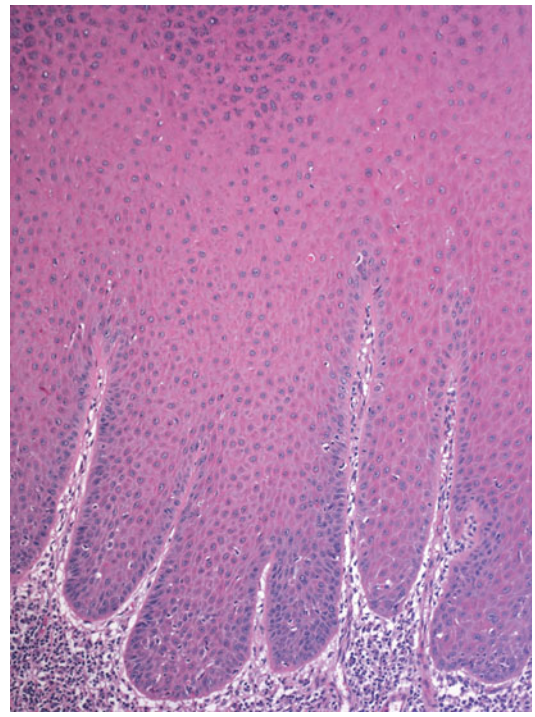


Fig. 9.23 Pronounced acanthosis in a case of d-VIN

(Fig. 9.23) but can also be normal in thickness or even atrophic. The rete pegs are typically elongated and branched and may show an anastomosing pattern (Fig. 9.24a, b).

As discussed above, it is more common for invasive squamous carcinoma to be present in the same or subsequent excisions of lesions of the differentiated type. Identifying early invasion in these cases can be more challenging than in HPV-related HSIL lesions. Close attention to the epithelial-stromal junction is imperative in evaluation of these cases. Early invasion will be evident, as in HPV-related lesions, by disruption of the normally smooth basement membrane border, with tongues and irregularly shaped nests or single cells extruding into the underlying stroma (Fig. 9.25).

Because d-VIN can be so difficult to recognize morphologically, there is tremendous interest in identifying a marker that would help distinguish it. Immunohistochemical study for p53 is reported as immunoreactive in the majority of

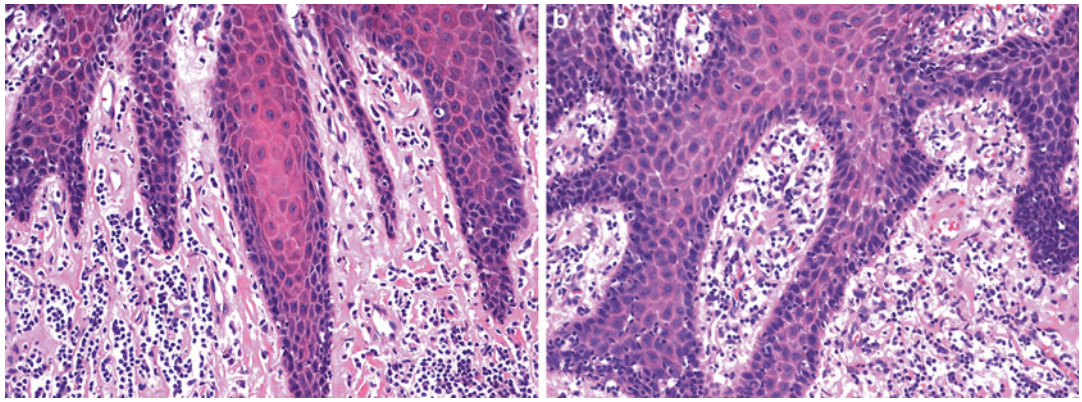


Fig. 9.24 (**a**) Elongation, branching, and narrowing of rete pegs in d-VIN. (**b**) Anastomosing rete pegs in d-VIN

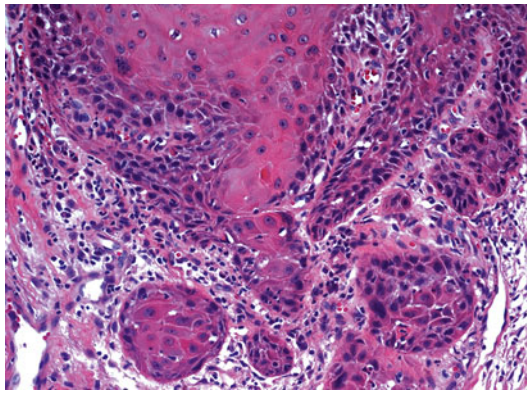


Fig. 9.25 Early invasive squamous carcinoma arising in d-VIN. Nests and tongues of tumor cells disrupt the normally smooth, rounded base of the epithelium

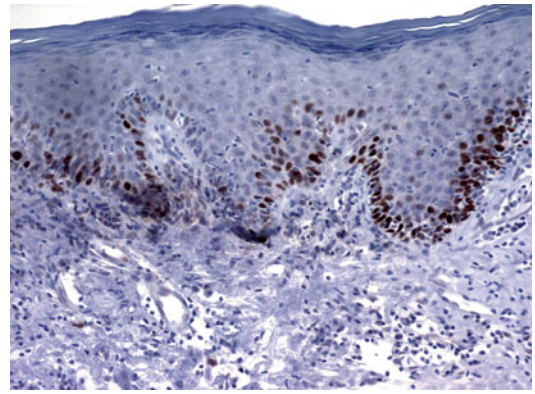


Fig. 9.26 p53 immunostaining in HSIL, differentiated type (d-VIN). There is strong, but discontinuous, nuclear staining in the basal epithelial cells, with scattered reactivity in suprabasilar cells

the basal epithelial cells with superficial extension of this reactivity in 66–84 % of d-VIN (Fig. 9.26) [9, 53, 68, 69, 86, 87]. However, the reactivity for p53 is not always consistent throughout the lesion and a similar reactive pattern can be seen in several other benign and malignant conditions, most significantly in lichen sclerosus, where it is presumed to be due to ischemic stress rather than mutation of the gene [68, 69, 89]. Reactivity for p53 does not necessarily correlate with gene mutation, raising an additional concern as to the significance and utility of this finding [85, 86]. Thus, while the p53 study may be of value in confirming the diagnosis of d-VIN, it appears to be neither sensitive nor specific as a marker, and results must be interpreted with caution. Morphology remains critically important to reaching the proper diagnosis. In d-VIN the abnormal cells are confined to the basal and parabasal areas and Ki-67 reactivity is restricted to these layers as well [41]. Hence, the localization of Ki-67 staining is not informative when the diagnosis is suspected, but it is reported that a higher proportion of cells are reactive in differentiated VIN than in normal epithelium [29, 58]. As d-VIN has no typical association with oncogenic HPV, p16 staining is typically negative. On occasion weak, focal reactivity may be seen [53, 68, 69, 90], however, and this should not be taken as evidence of oncogenic HPV infection or a diagnosis of HPV-related HSIL. A summary of the salient features distinguishing HPV-related HSIL from differentiated lesions (d-VIN) is presented in Table 9.5.

Table 9.5 Comparison of high-grade squamous intraepithelial lesion (HSIL) and d-VIN

HSIL of the vulva		
	VIN2-3	d-VIN
Frequency	82–98 % of vulvar HSIL	2–18 % of vulvar HSIL
Patient age	30s and 40s	60s and 70s
Associated conditions	HPV-related SILs of other anogenital sites	Lichen simplex chronicus Lichen sclerosus
HPV present	Yes	No
Smokers	Yes	No
Maturation disturbance	Decreased to nonexistent cellular maturation	Accelerated maturation in basilar cells
Immunohistochemistry	Diffuse block staining with p16	Basal and suprabasilar staining with p53
Multifocality	Frequent	Infrequent
Reported rates of progression	2–10 %	33 %
Prior, concurrent, or subsequent carcinoma	Up to 25.7 %	Up to 85.7 %

Differential Diagnosis

Vulvar differentiated VIN may be difficult to distinguish from the lichen sclerosus, lichen simplex chronicus, or from other benign reactive changes that may accompany it. These benign conditions, unlike d-VIN, usually display prominent hyperkeratosis and do not show atypia of the basal and parabasal cells, disturbance of cell maturation, or expanded rete ridges, squamous whorls, or keratin pearls. These latter features may be mimicked, to a certain extent, by seborrheic keratosis; however, the lack of significant nuclear atypia should distinguish it from a d-VIN.

Summary

Clinical presentation

- Symptoms of pruritis, pain, ulceration
- Variable gross appearance
- Common association with lichen sclerosus or lichen simplex chronicus

Histological features

- Atypical basal and parabasal cells with enlarged vesiculated nuclei, coarse chromatin, and prominent macronucleoli
- Abundant eosinophilic cytoplasm in the atypical cells, indicating premature maturation of cells
- Expanded, anastomosing rete ridges
- Keratin pearl formation
- Relatively normal upper layers and surface epithelium
- Atypical mitotic figures
- Parakeratosis

Differential diagnosis

- Lichen simplex chronicus
- Lichen sclerosus
- Seborrheic keratosis
- Other reactive inflammatory changes

Takeaway Essentials

Clinical relevant pearls

- Diagnosis is uncommon due to low frequency compounded by difficulty in diagnosis
- No association with HPV
- High frequency of concurrent or subsequent squamous cell carcinoma

Pathology interpretation pearls

- Atypical cells confined to the basal and parabasal layers.
- Often associated with lichen sclerosus or lichen simplex chronicus.
- Suprabasilar staining with p53 may be seen but cannot be relied on for diagnosis as it is neither sensitive nor specific.

Case Vignettes

Vignette 1

Clinical History: A 25-year-old woman with a history of systemic lupus erythematosus (SLE) presents with a large, exophytic mass on the vulva obscuring the labia minora and majora bilaterally. Additional, smaller lesions were observed separate from the mass, extending over the patients inner thighs, upper portions of the vulva, and perianal areas (Fig. 9.27). All visible lesions were excised.

Microscopic Description: At low power sections of the mass as well as of the associated smaller lesions demonstrated a complex papillary architecture, with branching fibrovascular cores lined by acanthotic squamous epithelium (Fig. 9.28). At higher magnification prominent parakeratosis was evident in many areas, and koilocytic atypia was readily identified (Fig. 9.29). There was mild increase in the population of immature cells within the basal few layers of epithelium, but mitotic figures were rare and, when present, were basally located. Cytoplasmic maturation was relatively normal as cells progressed towards the upper layers of epithelium.

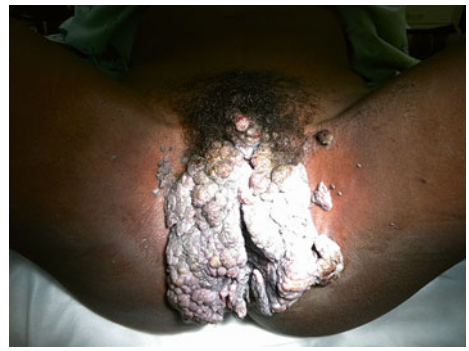


Fig. 9.27 Vignette 1. In this preoperative photograph, extensive exophytic growths are seen almost completely obscuring the normal vulvar anatomy (Photograph courtesy of Dr. Jacqueline Castagno, Assistant Professor of Obstetrics and Gynecology, University of Florida, Gainesville, Florida)

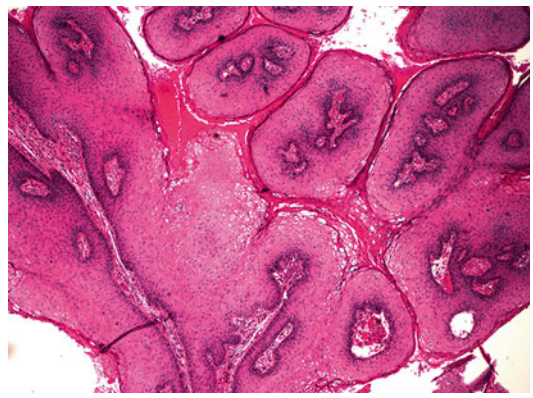


Fig. 9.28 Vignette 1. Low-power microscopy shows the papillary architecture, with fibrovascular cores throughout. Thickening of the epithelium and hyperparakeratosis are also evident

(continued)

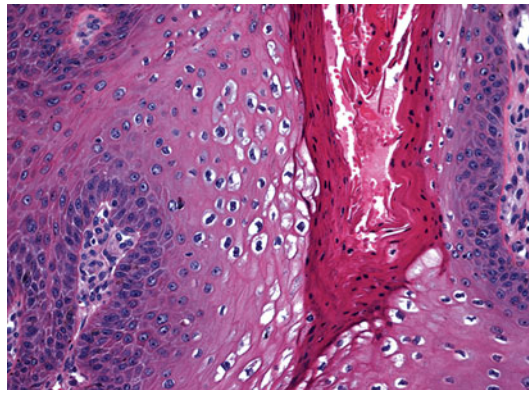


Fig. 9.29 Vignette 1. On higher magnification, koilocytic change in the upper layers of the epithelium was prominent and easily identified throughout the entire lesion

Diagnosis: The above microscopic features are diagnostic of condylomata acuminata.

Discussion: Although HPV-related vulvar disease in immunosuppressed patients has been less well studied than cervical disease, it has been well documented that these patients are at increased risk for HPV-related lesions of the entire lower genital tract. It is obvious why this should be the case. Patients with HIV and transplant recipients are arguably the most famously immunosuppressed patient populations and have received the greatest attention regarding susceptibility to HPV-induced disease. Patients with SLE are a rarer, but particularly interesting, class of immunosuppressed patients, as their immune systems are doubly compromised, first by the effects of the disease itself and second by the immunosuppressive drugs used to treat it.

Patients with SLE are known to have a higher prevalence of HPV infection than the normal population [91, 92], and it is advised that they receive regular gynecologic exams at a greater frequency. Interestingly, a recent study has found a strikingly elevated prevalence of HPV in SLE patients even though the patients had fewer risk factors than the control population [92]. It remains unclear how much of this increased prevalence is due to the disease itself and how much to the immunosuppressive drugs, as few patients with SLE are not taking these drugs, but there is some evidence that at least one of these drugs, azathiaprine, increases the risk to these patients [92, 93]. In the aforementioned study, immunosuppressive drugs were not found to affect prevalence, but they were found to increase the incidence of clinically detectable lesions, which are of more concern than the mere presence of virus [92].

The extensive disease seen in this patient, requiring surgical intervention, is not exclusive to immunosuppressed patients but raises another interesting point. Surgical treatment is not typically necessary for low-grade squamous intraepithelial lesions, including condylomata, and is required only when such extensive disease is present. Most smaller lesions can be treated medically with topical agents, but for immunosuppressed patients, fewer therapies may be effective. Topical imiquimod, for example, which is an immunomodulator, is one of the least unpleasant treatment options, and while there is as yet only limited experience with it in immunocompromised patients, there is reason to be concerned that it may be less effective in patients with defective immune systems [35].

Early data on the effects of HPV vaccination strongly suggests that low-grade vulvar lesions induced by HPV types 6 and 11 are a vanishing breed. In Denmark and Australia, where robust efforts to vaccinate girls have been undertaken with coverage of 70–85 % of the target population, marked reductions in the diagnosis have already been reported [94, 95].

(continued)

There is reason to believe they will be much less common in the future, and with sufficient vaccination rates, they might become quite rare. It remains to be seen, however, whether immunosuppressive diseases or drugs will have any effect on the efficacy of the HPV vaccine, which would be most unfortunate for patients like this one, leaving them as potentially the only remaining victims for this disease.

Vignette 2

Clinical History: A 30-year-old HIV-positive woman with a previous history of multiple high-grade squamous intraepithelial lesions resected from the vulva, vagina, and cervix presented with multiple lesions of the vulva and perianal regions, ranging from papillary in configuration to elevated plaques to flat macules, some of which appeared pigmented (Fig. 9.30). Multiple biopsies were taken, followed by surgical excision.

Microscopic Description: Sections of the resected tissue demonstrated a variety of appearances. In sections of the more exophytic areas, fibrovascular cores were evident, with minimal cellular proliferation, evidence of appropriate cellular maturation throughout the epithelial layers, and prominent koilocytic change (Fig. 9.31). In sections of plaque-like and macular areas, maturation was notably decreased and mitotic activity was marked and evident into the more superficial layers of the epithelium (Fig. 9.32). Some sections showed junctions of the papillary and flatter lesions, with juxtaposition of the two different histological patterns (Fig. 9.33a, b).

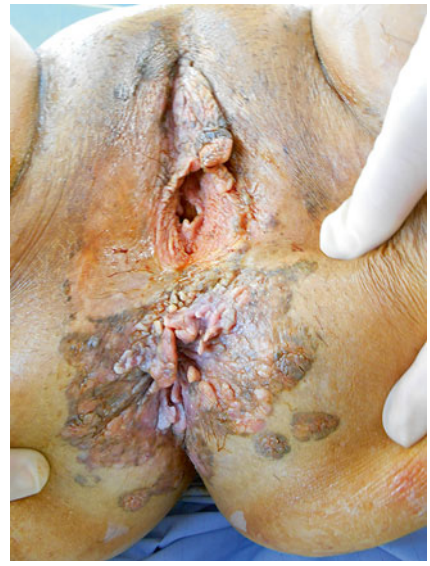


Fig. 9.30 Vignette 2. In this patient, much of the labial tissue is already absent due to prior resections. The lesions in the perineal and perianal regions are most noticeable, due to their warty appearance, but many other areas of raised and otherwise abnormal epithelium, some of them pigmented, can be seen as well (Photo courtesy of Dr. Jacqueline Castagno, Assistant Professor of Obstetrics and Gynecology, University of Florida, Gainesville, Florida)

(continued)

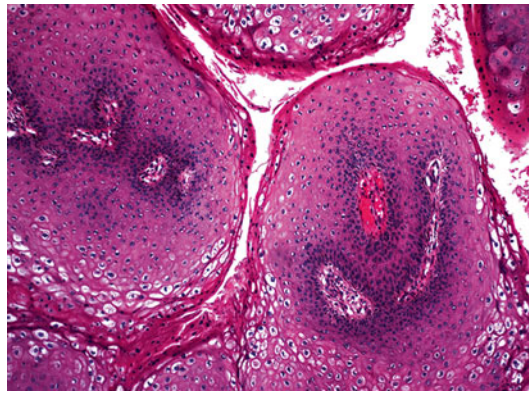


Fig. 9.31 Vignette 2. Some areas of the lesion showed papillary architecture with prominent fibrovascular stalks and koilocytic atypia

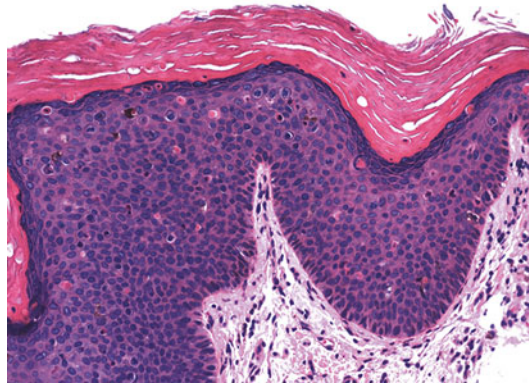


Fig. 9.32 Vignette 2. In other areas, fibrovascular cores and koilocytic change were lacking, cellular maturation was markedly decreased, and numerous mitotic figures and dyskeratotic cells were evident in the mid and upper layers of the epithelium

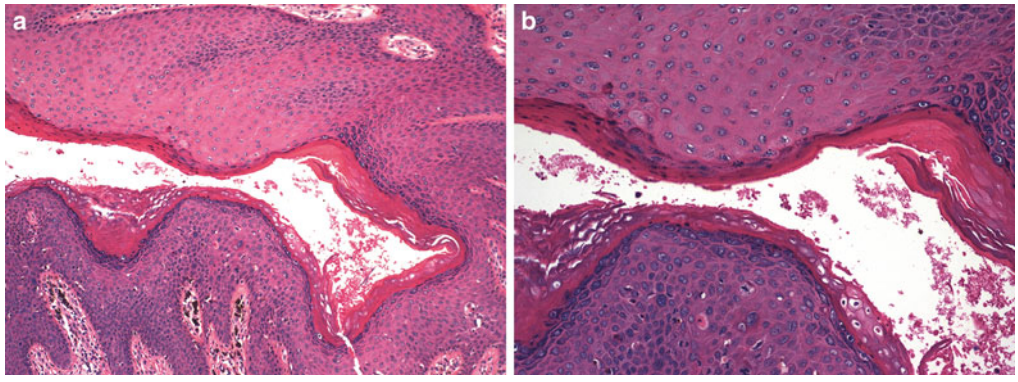


Fig. 9.33 Vignette 2. (**a**) This low-power image shows an area where the two types of lesions were adjacent to each other. The papillary growth (*top*) is folded over a flat lesion (*bottom*). (**b**) On higher power, the difference between the epithelium in the papillary portion (*top*) and flat portion (*bottom*) is evident

(continued)

Diagnosis: Low-grade squamous intraepithelial lesion of vulva (LSIL/Condylomata) with associated high-grade squamous intraepithelial lesion of vulva (VIN 2-3).

Discussion: Cases like this, in which both low-grade condylomatous lesions and high-grade lesions are present at the same time and intimately admixed with one another, are almost exclusively seen in immunosuppressed patients [96], and it is important for both clinicians and pathologists to be aware of such cases for several reasons. For one, the observation of this type of coexistent low- and high-grade disease in the immunosuppressed patients suggests that in these settings even lesions which appear to be benign condylomata ought to be biopsied to ensure the absence of a high-grade component [97]. Another issue to be aware of is that even when these lesions are biopsied, the extensive condylomatous component may be so distant from other abnormal areas that the high-grade component may be missed on sampling. The same may occur on a microscopic level when small foci of adjacent high-grade lesion are missed when the slides are examined due to the relative abundance of condylomatous lesion. In this case, the low- and high-grade lesions were adjacent to each other, but each showed distinct features. In other cases the lesions may be more closely intermingled and cases have been described in which features of both lesions were present, in which lesions with a marked condylomatous architecture had epithelial changes of HSIL [96]. These lesions may be perilous for the clinician and pathologists, as to mistake them for condylomata either clinically or microscopically would result in undertreatment. It is speculated that lesions like these may be responsible for previously reported cases of condyloma containing high-risk HPV.

Immunosuppressed patients are at increased risk for persistent and/or recurrent HSIL, and the progression to carcinoma is more likely to occur in such patients and to do so at an accelerated pace [45, 96], making it especially important to detect high-grade disease in them when it occurs. Cases like this, with mixed high- and low-grade lesions, emphasize the need for very careful examination of the tissue obtained from such patients, even when the architecture is exclusively condylomatous.

Vignette 3

Clinical History: A 78-year-old woman with long-standing lichen sclerosus presents with ill-defined areas of whitened epithelium on the vulva (Fig. 9.34). Multiple biopsies of abnormal-appearing areas were biopsied.

Microscopic Description: Of four biopsies submitted, two showed lichen sclerosus and no other pathologic abnormalities. The other biopsies also had lichen sclerosus but in addition showed further epithelial alterations. These alterations included hyperkeratosis, as well as evidence of increased cytoplasmic maturation, with cells containing abundant eosinophilic cytoplasm found even towards the base of the epithelium (Fig. 9.35). Whorls of keratinized cells were seen deep in the rete ridges (Fig. 9.36). Nuclei showed prominent nucleoli throughout the epithelium, and scattered dyskeratotic cells could be found. The basal epithelium was less eosinophilic but showed more pronounced nuclear atypia, with more nuclear pleomorphism and even more prominent and irregular nucleoli, and anastomosis of rete ridges was present (Fig. 9.37).

(continued)

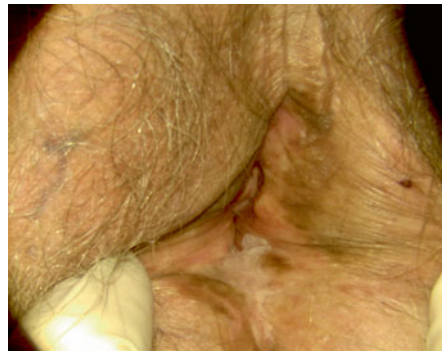


Fig. 9.34 Vignette 3. The vulvar skin is thin and parchment-like, consistent with the history of lichen sclerosus. Areas of pigmentation are also seen, possible secondary to episodes of bleeding from the fragile epithelium. An ill-defined whitened area is present inferiorly (Photo courtesy of Dr. Jacqueline Castagno, Assistant Professor of Obstetrics and Gynecology, University of Florida, Gainesville, Florida)

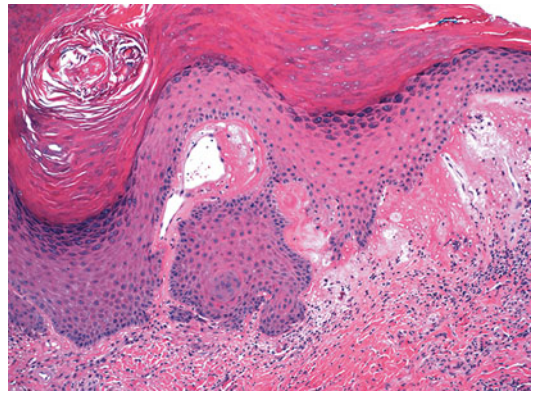


Fig. 9.35 Vignette 3. The lesion showed subepithelial hyalinization, consistent with lichen sclerosus. In addition, the epithelium is thickened and hyperkeratotic, and the pattern of the rete ridges is irregular

(continued)

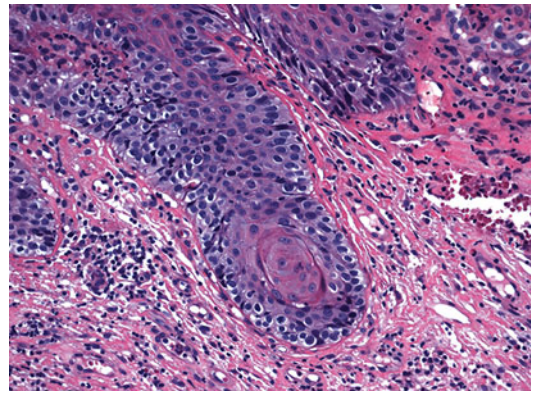


Fig. 9.36 Vignette 3. Deep in this rete ridge is a whorl of mature keratinized cells forming an early "pearl"

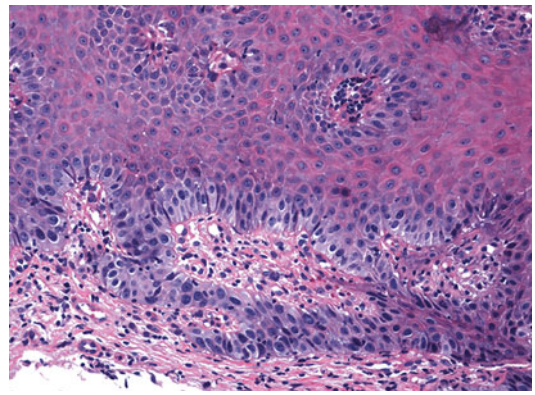


Fig. 9.37 Vignette 3. The base of the lesion shows anastomosis of the thinned and irregular rete ridges, with disorganization of the basilar cells which show nuclear atypia, prominent and irregular nucleoli, and nuclear pleomorphism

Diagnosis: Vulvar intraepithelial neoplasia, differentiated type (d-VIN)

Discussion: This case emphasizes how difficult it can be to distinguish d-VIN from lichen sclerosus and other nonneoplastic conditions on gross examination. The lesion was not at all distinct grossly, and it is conceivable that all four biopsies might have shown only lichen sclerosus and missed the d-VIN entirely. It is fortunate for the patient that she was being followed closely and that the lesions were detected while still confined to the epithelium, since, as discussed in the previous section, d-VIN progresses relatively rapidly to carcinoma and often is not detected until it has done so, at which point more aggressive treatment is required, with the potential for more complications, and the potential for metastatic and even fatal disease exists.

(continued)

The difficulty in making the diagnosis of d-VIN is notorious, and it has always been poorly reproducible. With the shift to a two-tiered classification system and the advent of p16 immunohistochemistry, interobserver variability in interpretation of HPV-related lesions has been steadily decreasing, but with no such advances in the field of d-VIN, interobserver variability remains a significant problem. A recent attempt to address this problem [98] confirmed concordance rates in the diagnosis of d-VIN were dismal and only modestly improved following focused education in the diagnostic criteria. The study was able, however, to identify criteria which were judged most useful in the diagnosis, which included the identification of atypical mitoses in the basal layer, basal cellular atypia, dyskeratosis, prominent nucleoli, and elongation and anastomosis of rete ridges. Of these features, all but the first are seen in this case.

Despite the somewhat disheartening failure of the study to find an effective way to decrease the high rate of interobserver variability in diagnosis of d-VIN, there was at least one positive finding; it did demonstrate that pathologists with subspecialty training in gynecologic pathology were able to achieve a far greater degree of concordance, suggesting that greater experience with the disease does lead to improved diagnostic ability and that patients with a suspected diagnosis of d-VIN might be best served by having their cases reviewed by a specialized gynecologic pathologist.

References

1. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012;16(3):205–42.
2. Crum CP. Vulvar intraepithelial neoplasia: the concept and its application. Hum Pathol. 1982;13(3):187–9.
3. Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD terminology committee. J Reprod Med. 1986;31(10):973–4.
4. Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, Haefner H, Neill S. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISVVD vulvar oncology subcommittee. J Reprod Med. 2005;50(11):807–10.
5. Heller DS. Report of a new ISVVD classification of VIN. J Low Genit Tract Dis. 2007;11(1):46–7.
6. Crum C, Herrington CS, McCluggage WG, Regauer S, Wilkinson EJ. Tumors of the vulva. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH, eds. WHO classification of tumours of female reproductive organs. 4th ed. Lyons. IARC Press; 2014.
7. Preti M, Mezzetti M, Robertson C, Sideri M. Interobserver variation in histopathologic diagnosis and grading of vulvar intraepithelial neoplasia: results of an European collaborative study. BJOG. 2000;107(5):594–9.
8. Scurry J, Campion M, Scury B, Kim SN, Hacker N. Pathologic audit of 164 consecutive cases of vulvar intraepithelial neoplasia. Int J Gynecol Pathol. 2006;25(2):176–81.
9. Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. Int J Gynecol Pathol. 2001;20(1):16–30.
10. Srodon M, Stoler MH, Baber GB, Kurman RJ. The distribution of low and high–risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). Am J Surg Pathol. 2006;30(12):1513–8.
11. McCluggage WG, Walsh MY, Thornton CM, Hamilton PW, Date A, Caughley LM, Bharucha H. Inter- and intra-observer variation in the histopathological reporting of cervical squamous intraepithelial lesions using a modified Bethesda grading system. BJOG. 1998;105(2):206–10.
12. Skapa P, Zamecnik J, Hamsikova E, Salakova M, Smahelova J, Jandova K, Robova H, Rob L, Tachezy R. Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. Am J Surg Pathol. 2007;31(12):1834–43.
13. Polterauer S, Dressler AC, Grimm C, Seebacher V, Tempfer C, Reinthaller A, Hefler L. Accuracy of preoperative vulva biopsy and the outcome of surgery in vulvar intraepithelial neoplasia 2 and 3. Int J Gynecol Pathol. 2009;28(6):559–62.

14. McNally OM, Mulvany NJ, Pagano R, Quinn MA, Rome RM. VIN 3: a clinicopathologic review. Int J Gynecol Cancer. 2002;12(5):490–5.
15. Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. Obstet Gynecol. 2006;107(5): 1018–22.
16. Van de Nieuwenhof HP, Massuger L, van der Avoort I, Bekkers R, Casparie M, Abma W, Van Kempen L, de Hullu JA. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur J Cancer. 2009;45(5):851–6.
17. Terlou A, Blok L, Helmerhorst T, van Buerden M. Premalignant epithelial disorders of the vulva: squamous vulvar intraepithelial neoplasia, vulvar Paget's disease and melanoma in situ. Acta Obstet Gynecol Scand. 2010;89(6):741–8.
18. Saraiya M, Watson M, Wu X, King JB, Chen VW, Smith JS, Giuliano AR. Incidence of in situ and invasive vulvar cancer in the US, 1998–2003. Cancer. 2008;113 Suppl 10:2865–72.
19. Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. Obstet Gynecol. 2005;106(6):1319–26.
20. Medeiros F, Nascimento AF, Crum CP. Early vulvar squamous neoplasia: advances in classification, diagnosis and differential diagnosis. Adv Anat Pathol. 2005;12(1):20–6.
21. Garland SM, Steben M, Sings HL, James M, Lu S, Railkar R, Barr E, Haupt RM, Joura EA. Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (Types 6, 11, 16, and 18) vaccine. J Infect Dis. 2009;199(6):805–14.
22. Hoy T, Singhal PK, Willey VJ, Insinga RP. Assessing incidence and economic burden of genital warts with data from a US commercially insured population. Curr Med Res Opin. 2009;25(10):2343–51.
23. Kennedy CM, Boardman LA. New approaches to external genital warts and vulvar intraepithelial neoplasia. Clin Obstet Gynecol. 2008;51(3):518–26.
24. Mittal P, Prakash V, Gupta R, Dewan R, Singhal S, Suri J. Giant condyloma acuminatum of vulva treated by surgical excision and reconstruction of defect. Arch Gynecol Obstet. 2013;287(5):1047–8.
25. Tan XJ, Wu M, Lang JH. Giant condyloma acuminatum of the vulva. Int J Infect Dis. 2010;14(5):e455–6.
26. Van de Nieuwenhof HP, van der Avoort I, de Hullu JA. Review of squamous premalignant vulvar lesions. Crit Rev Oncol Hematol. 2008;68(2):131–56.
27. De Bie RP, van De Nieuwenhof HP, Bekkers R, Melchers WJG, Siebers AG, Bulten J, Massuger L, deHullu JA. Patients with usual vulvar intraepithelial neoplasia-related vulvar cancer have an increased risk of cervical abnormalities. Br J Cancer. 2009;101(1): 27–31.
28. Aguilera-Barrantes I, Magro C, Nuovo GJ. Verruca vulgaris of the vulva in children and adults: a nonvenereal type of vulvar wart. Am J Surg Pathol. 2007;31(4):529–35.
29. Van der Avoort I, van de Nieuwenhof HP, Otte-Holler I, Nirmala E, Bulten J, Massuger L, van der Laak JA, Slootweg PJ, deHullu JA, van Kempen L. High levels of p53 expression correlate with DNA aneuploidy in (pre)malignancies of the vulva. Hum Pathol. 2010; 41(10):1475–85.
30. Garland SM, Insinga RP, Sings HL, Haupt RM, Joura EA. Human papillomavirus infections and vulvar disease development. Cancer Epidemiol Biomarkers Prev. 2009;18(6):1777–84.
31. Smith JS, Backes DM, Hoots BE, Kurman RJ, Pimenta JM. Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. Obstet Gynecol. 2009;113(4):917–24.
32. Winer RL, Kiviat NB, Hughes JP, Adam DE, Lee S, Kuypers JM, Koutsky LA. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis. 2005;191(5):731–8.
33. Lacey CJ, Lowndes CM, Shah KIV. Burden and management of non-cancerous HPV-related conditions: HPV 6/11 disease. Vaccine. 2006;24 Suppl 3:S3/35–41.
34. Graziottin A, Serafini A. HPV infection in women: psychosexual impact of genital warts and intraepithelial lesions. J Sex Med. 2009;6(3):633–45.
35. ACOG Committee Opinion No. 509. Management of vulvar intraepithelial neoplasia. Obstet Gynecol. 2011;118(5):1192–4.
36. Ordi J, Garcia S, del Pino M, Landolfi S, Alonso I, Quinto L, Torne A. p16INK4a immunostaining identifies occult CIN lesions in HPV-positive women. Int J Gynecol Pathol. 2009;28(1):90–7.
37. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D, Kurman RJ, Schmidt D, Stoler M, von Knebel Doeberitz M. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002;26(11):1389–99.
38. Benevolo M, Mottolese M, Marandino F, Vocatura G, Sindico R, Piperno G, Mariani L, Sperduti I, Canalini P, Donnorso R, Vocatura A. Immunohistochemical expression of p16INK4a is predictive of HR-HPV infection in cervical low grade lesions. Mod Pathol. 2006;19(3):384–91.
39. Rufforny I, Wilkinson EJ, Liu C, Zhu H, Buteral M, Massoll NA. Human papillomavirus infection and p16INK4a protein expression in vulvar intraepithelial neoplasia and invasive squamous carcinoma. J Low Genit Tract Dis. 2005;9(2):108–13.
40. Finegan MM, Han AC, Edelson MI, Rosenblum NG. p16 expression in squamous lesions of the female genital tract. J Mol Histol. 2004;35(2):111–4.
41. Logani S, Lu D, Quint W, Ellenson L, Pirog E. Low-grade vulvar and vaginal intraepithelial neoplasia: correlations of histological features with human papillomavirus DNA detection and MIB-1 immunostaining. Mod Pathol. 2003;16(8):735–41.

42. Li J, Ackerman AB. Seborrheic keratosis that contain human papillomavirus are condyloma acuminata. Am J Dermatopathol. 1994;16(4):398–405.
43. Bai H, Cviko A, Granter S, Yuan L, Betensky R, Crum CP. Immunophenotypic and viral (human papillomavirus) correlates of vulvar seborrheic keratosis. Hum Pathol. 2003;34(6):559–64.
44. Gushi A, Kanekura T, Kanzaki T, Eizuru Y. Detection and sequences of human papillomavirus DNA in nongenital seborrheic keratosis of immunopotent individuals. J Dermatol Sci. 2003;31(2):143–9.
45. van Esch E, Dam M, Osse M, Putter H, Trimbos B, Fleuren G, van der Burg S, van Poelgeest M. Clinical characteristics associated with development of recurrence and progression in usual-type vulvar intraepithelial neoplasia. Int J Gynecol Cancer. 2013;23(8): 1476–83.
46. Khan AM, Freeman-Wang T, Pisal N, Singer A. Smoking and multicentric vulval intraepithelial neoplasia. J Obstet Gynaecol. 2009;29(2):123–5.
47. McCluggage WG. Recent developments in vulvovaginal pathology. Histopathology. 2009;54(2):156–73.
48. Bloss J, Liao S, Wilczynski S, Macri C, Walker J, Peake M, Berman M. Clinical and histological features of vulvar carcinomas analyzed for human papillomavirus status: evidence that squamous cell carcinoma of the vulva has more than one etiology. Hum Pathol. 1991;22(7):711–8.
49. Joura EA. Epidemiology, diagnosis and treatment of vulvar intraepithelial neoplasia. Curr Opin Obstet Gynecol. 2002;14(1):39–43.
50. Preti M, van Seters M, Sideri M, van Buerden M. Squamous vulvar intraepithelial neoplasia. Clin Obstet Gynecol. 2005;48(4):845–61.
51. Wilkinson EJ, Friedrich EG, Fu YS. Multicentric nature of vulvar carcinoma in situ. Obstet Gynecol. 1981;58(1):69–74.
52. Stephenson RD, Denehy TR. Rapid spontaneous regression of acute-onset vulvar intraepithelial neoplasia3 in young women: a case series. J Low Genit Tract Dis. 2012;16(1):56–8.
53. Hoevenaars BM, van der Avoort I, de Wilde P, Massuger L, Melchers W, de Hullu JA, Bulten J. A panel of p16ink4a, MIB1 and p53 proteins can distinguish between the two pathways leading to vulvar squamous cell carcinoma. Int J Cancer. 2008; 123(12):2767–73.
54. Wright VC, Chapman W. Intraepithelial neoplasia of the lower female genital tract: etiology, investigation, and management. Semin Surg Oncol. 1992;8(4): 180–90.
55. Kuppers V, Stiller M, Somville T, Bender HG. Risk factors for recurrent VIN. Role of multifocality and grade of disease. J Reprod Med. 1997;42(3):140–4.
56. Kaufman RH. Intraepithelial neoplasia of the vulva. Gynecol Oncol. 1995;56:8–21.
57. Fehr M, Baumann M, Mueller M, Fink D, Heinzl S, Imesch P, Dedes K. Disease progression and recurrence in women treated for vulvovaginal intraepithelial neoplasia. J Gynecol Oncol. 2013;24(3):236–41.
58. Wallbich JJ, Rhodes HE, Milbourne AM, Munsell MF, Frumovitz M, Brown J, Trimble CL, Schmeler KM. Vulvar intraepithelial neoplasia (VIN 2/3): comparing clinical outcomes and evaluating risk factors for recurrence. Gynecol Oncol. 2012;127(2):312–5.
59. Modessitt SC, Waters AB, Walton L, Fowler WC, Van Le L. Vulvar intraepithelial neoplasia III: occult cancer and the impact of margin status on recurrence. Obstet Gynecol. 1998;92(6):962–6.
60. Eva LJ, Ganesan R, Chan KK, Honest H, Luesly DM. Differentiated-type vulval intraepithelial neoplasia has a high-risk association with vulval squamous cell carcinoma. Int J Gynecol Cancer. 2009;19(4): 741–4.
61. Van Seters M, van Buerden M, de Craen A. Is the assumed natural history of vulvar intraepithelial neoplasia based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol. 2005;97(2):645–51.
62. Iversen T, Tretti S. Intraepithelial and invasive squamous cell neoplasia of the vulva: trends in incidence, recurrence and survival rate in Norway. Obstet Gynecol. 1998;91(6):969–72.
63. Jones RW, Baranyai J, Stables S. Trends in squamous cell carcinoma of the vulva: the influence of vulvar intraepithelial neoplasia. Obstet Gynecol. 1997; 90(3):448–52.
64. Thuis YN, Campion M, Fox H, Hacker NF. Contemporary experience with the management of vulvar intraepithelial neoplasia. Int J Gynecol Cancer. 2000;10(3):223–7.
65. Reithdorf S, Neffen EF, Cvika A, Loning T, Crum CP, Reithdorf L. p16INK4A expression as biomarker for HPV16-related vulvar neoplasias. Hum Pathol. 2004;35(12):1477–83.
66. De Koning M, Quint W, Pirog E. Prevalence of mucosal and cutaneous human papillomaviruses in different histological subtypes of vulvar carcinoma. Mod Pathol. 2008;21(3):334–44.
67. Santos M, Landolfi S, Olivella A, Lloveras B, Klaustermeier J, Suarez H, Alos L, Puig-Tintore L, Campo E, Ordi J. P16 overexpression identified HPV-positive vulvar squamous cell carcinoma. Am J Surg Pathol. 2006;30(11):1347–56.
68. Santos M, Montagut C, Mellado B, Garcia A, Ramon y Cajal S, Cardesa A, Puig-Tintore L, Ordi J. Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. Int J Gynecol Pathol. 2004;23(3):206–14.
69. Mulvany N, Allen D. Differentiated neoplasia of the vulva. Int J Gynecol Pathol. 2008;27(1):125–35.
70. Ordi J, Alejo M, Fuste V, Lloveras B, del Pino M, Alonso I, Torne A. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histological pattern: an unrecognized variant of simplex (differentiated) VIN. Am J Surg Pathol. 2009;33(11):1659–65.

71. Horn LC, Reichert A, Oster A, Arndal SF, Trunk MJ, Ridder R, Rassmussen OF, Bjelkenkrantz K, Christensen P, Eck M, Lorey T, Skovlund VR, Ruediger T, Schneider V, Schmidt D. Immunostaining for p16INK4a used as a conjunctive tool improves interobserver agreement of the histological diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2008;32(4):502–12.
72. Djikstra MG, Heideman DA, deRoy SC, Rozendaal L, Berkhof J, van Krimpen K, van Groningen K, Snijders PJ, Meijer CJ, van Kemenade FJ. P16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. J Clin Pathol. 2010;63(11):972–7.
73. Bergeron C, Ordi J, Schmidt D, Trunk M, Keller T, Ridder R. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. Am J Clin Pathol. 2010; 133(3):395–406.
74. Raju R, Goldblum J, Hart W. Pagetoid squamous cell carcinoma in situ (Pagetoid Bowen's Disease) of the external genitalia. Int J Gynecol Pathol. 2003;22(2): 127–35.
75. Armes JE, Lourie R, Bowlay G, Tabrizi S. Pagetoid squamous cell carcinoma in situ of the vulva: comparison with extramammary Paget disease and nonpagetoid squamous cell neoplasia. Int J Gynecol Pathol. 2008;27(1):118–24.
76. McCluggage WG, Jamison J, Boyde A, Ganesan R. Vulval intraepithelial neoplasia with mucinous differentiation: report of 2 cases of a hitherto undescribed phenomenon. Am J Surg Pathol. 2009;33(6): 945–9.
77. Chafe W, Richards A, Morgan L, Wilkinson E. Unrecognized invasive carcinoma in vulvar intraepithelial neoplasia (VIN). Gynecol Oncol. 1988;31(1):154–65.
78. Lavoue V, Lemarrec A, Bertheuil N, Henno S, Mesbah H, Watier E, Leveque J, Morcel K. Quality of life and female sexual function after skinning vulvectomy with split-thickness skin graft in women with vulvar intraepithelial neoplasia or Paget disease. Eur J Surg Oncol. 2013;39(12):1444–50.
79. Kokka F, Singh N, Faruqi A, Gibbon K, Rosenthal A. Is differentiated vulval intraepithelial neoplasia the precursor lesion of human papillomavirus negative vulval squamous cell carcinoma? Int J Gynecol Cancer. 2011;21(7):1297–305.
80. Van Buerden M, ten Kate FJ, Smits HL, Berkhout RJ, de Craen AJ, van der Vange N, Lammes FB, ter Schegger J. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. Cancer. 1995;17(12): 2879–84.
81. Van Seters M, ten Kate F, Verheijen R, Meijer C, Burger M, Helmerhorst T. In the absence of (early) invasive carcinoma, vulvar intraepithelial neoplasia associated with lichen sclerosus is mainly of undifferentiated type: new insights in histology and aetiology. J Clin Pathol. 2007;60(5):504–9.
82. Haefner H, Tate J, McLachlin C, Crum CP. Vulvar intraepithelial neoplasia: age, morphological phenotype, papillomavirus DNA, and coexisting invasive carcinoma. Hum Pathol. 1995;26(2):147–54.
83. Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. Int J Gynecol Cancer. 1999;9(2):89–97.
84. Pinto A, Lin M, Sheets E, Muto M, Sun D, Crum CP. Allelic imbalance in lichen sclerosus, hyperplasia, and intraepithelial neoplasia of the vulva. Gynecol Oncol. 2000;77(1):171–6.
85. Rolfe KJ, MacLean AB, Crow JC, Benjamin E, Reid WHM, Perrett CW. TP53 mutations in vulval lichen sclerosus adjacent to squamous cell carcinoma of the vulva. Br J Cancer. 2003;89(12):2249–53.
86. Pinto A, Miron A, Yassin Y, Monte N, Woo T, Mehra K, Medeiros F, Crum CP. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. Mod Pathol. 2010;23(3):404–12.
87. Yang B, Hart W. Vulvar intraepithelial neoplasia of the simplex (differentiated) type. A clinicopathologic study including analysis of HPV and p53 expression. Am J Surg Pathol. 2000;24(3):429–41.
88. Husseinzadeh N, Recinto J. Frequency of invasive cancer in surgically excised vulvar lesions with intraepithelial neoplasia (VIN 3). Gynecol Oncol. 1999;73(1):119–20.
89. Liegl B, Regauer S. P53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). Histopathology. 2006;48(3): 268–74.
90. Ruhul Quddus M, Xu C, Steinhoff MM, Zhang C, Lawrence WD, Sung CJ. Simplex (differentiated) type VIN: absence of p16INK4 supports its weak association with HPV and its probable precursor role in non-HPV related vulvar squamous cancers. Histopathology. 2005;46(6):718–20.
91. Santana IU, Gomes A, Lyrio LDC, Grassi MFR, Santiago MB. Systemic lupus erythematosus, human papillomavirus infection, cervical pre-malignant and malignant lesions: a systematic review. Clin Rheumatol. 2011;30(5):665–72.
92. Lyrio LDC, Grassi MFR, Santana IU, Olavarria VG, Gomes A, CostaPinto L, Oliveira RP, Aquino R, Santiago MB. Prevalence of human papillomavirus infection in women with systemic lupus erythematosus. Rheumatol Int. 2013;33(2):335–40.
93. Dicle O, Parmaksizoglu B, Gurkan A, Tuncer M, Demirbas A, Yilmaz E. Choice of immunosuppressants and the risk of warts in renal transplant recipients. Acta Derm Venereol. 2008;88:294–5.

94. Baandrup L, Blomberg M, Dehlendorff C, Sand C, Andersen K, Kjaer S. Significant decrease in the incidence of genital warts in young Danish women after implementation of a national human papillomavirus vaccination program. Sex Transm Dis. 2013; 40(2):130–5.
95. Read TR, Hocking JS, Chen MY. The near disappearance of genital warts in young women 4 years after commencing a national human papillomavirus (HPV) vaccination program. Sex Transm Infect. 2011;87(7): 544–7.
96. Maniar KP, Ronnett BM, Vang R, Yemelyanova A. Coexisting high-grade vulvar intraepithelial neoplasia (VIN) and condyloma acuminatum-independent lesions due to different HPV types occurring in immunocompromised patients. Am J Surg Pathol. 2013;37(1):53–60.
97. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines. MMWR. 2010;59:69–70.
98. van den Einden LCG, deHullu JA, Massuger LFAG, Grefte JMM, Bult P, Wiersma A, Sturm B, Bosch S, Hollema H, Bulten J. Interobserver variability and the effects of education in the histopathological diagnosis of differentiated vulvar intraepithelial neoplasia. Mod Pathol. 2013;26(6):874–80.

Squamous Cell Carcinoma of the Vulva

10

Sarah M. Bean and Rex C. Bentley

Introduction

Squamous cell carcinoma is the most common malignant neoplasm of the vulva but is relatively rare, accounting for 4 % of malignant tumors of the female genital tract. Unlike the cervix where the majority of squamous cell carcinomas are driven by human papillomavirus (HPV), squamous cell carcinomas of the vulva comprise a heterogeneous group (Table 10.1); some of which are associated with HPV infection, while others are associated with an inflammatory vulvar dermatosis, lichen sclerosus (LS). Both warty and basaloid histological types of squamous cell carcinoma have been associated with HPV and are often identified in the context of usual (warty and basaloid)-type vulvar intraepithelial neoplasia (uVIN); these types of squamous cell carcinoma tend to occur in younger women. HPV type 16 is the most common viral type associated with squamous cell carcinoma. Keratinizing squamous cell carcinoma is generally HPV negative and is identified in older, postmenopausal women with a history of LS. Differentiated (simplex) VIN is frequently histologically identified adjacent to keratinizing squamous cell carcinoma in patients with LS. Rare histological variants of squamous cell carcinoma are sometimes identified such as verrucous carcinoma, keratoacanthoma-like squamous cell carcinoma, sarcomatoid carcinoma, and squamous cell carcinoma with tumor giant cells. Regardless of the etiology and histology of the tumor, surgical excision is the mainstay of treatment, which is utilized to determine tumor stage and prognosis. Patients with less than 1-mm depth of tumor invasion have an excellent prognosis and are essentially cured of disease by surgery alone. The presence of lymph node metastasis is the most important prognostic factor.

Clinical Features

Squamous cell carcinoma of the vulva (vSCC) is relatively rare but is the most common malignant tumor of the vulva, comprising 95 % of malignancies in this organ with malignant melanoma being second most common. The incidence is 1–2 per 100,000 women annually [1]. In 2013, the American Cancer Society estimated that there were 4,700 new cases of vulvar cancer and 990 vulvar cancer-related deaths in the United States [2]. Some have shown that the incidence of VIN has increased over time while the incidence of vSCC has remained stable [3, 4]. Others report that incidence of vSCC has increased over time [5, 6]. White and non-Hispanic women are more frequently affected,

S.M. Bean, M.D. (✉)
Department of Pathology, Duke University Health System, Durham, NC, USA
e-mail: Sarah.bean@duke.edu

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_10,

Table 10.1 Vulvar squamous cell carcinoma is a heterogeneous disease

Type of vulvar SCC	HPV-positive SCC	HPV-negative SCC
Age	35–65 years	55–85 years
Risk factors	Anogenital warts Smoking Alcohol use	Lichen sclerosus
Focality	Multifocal	Single focus
Associated VIN	Usual (warty/basaloid) types	Differentiated
Histologic type	Warty/basaloid SCC	Keratinizing SCC
p16	+	–
p53	–	+

with the incidence among African-American and Hispanic women being one third lower [7]. African-American women present at a significantly younger age than white women [8]. Incidence increases with age at diagnosis such that women 70 and older are most at risk [9]. However, there is a trend toward decreased age at diagnosis of VIN and vSCC [10, 11].

The development of vSCC can frequently be attributed to one of two distinct etiologic pathways: HPV related or non-HPV related. Women who develop HPV-related vSCC are typically younger (35–65 years) than those women who develop non-HPV-related vSCC (55–85 years) [12]. Risk factors for developing HPV-related squamous cell carcinoma include known indicators of high-risk HPV exposure: infection with HPV type 16 [13], history of anogenital warts [9], smoking [9, 13, 14], and alcohol consumption [9]. Infection with herpes simplex virus (HSV) type 2 is a risk factor [13]. Number of years in school is inversely associated with risk [9]. Non-HPV-related vulvar squamous cell carcinoma is associated with vulvar dermatoses, especially lichen sclerosus (LS). LS is a chronic dermatologic condition commonly affecting perimenopausal women that causes vulvar pruritus and is associated with epithelial thinning and distinctive dermal changes (Fig. 10.1). Chronic LS often alters vulvar anatomy with loss of distinction between the labia majora and minora and loss of the clitoral hood. Women with a long-standing history of LS are at risk for developing

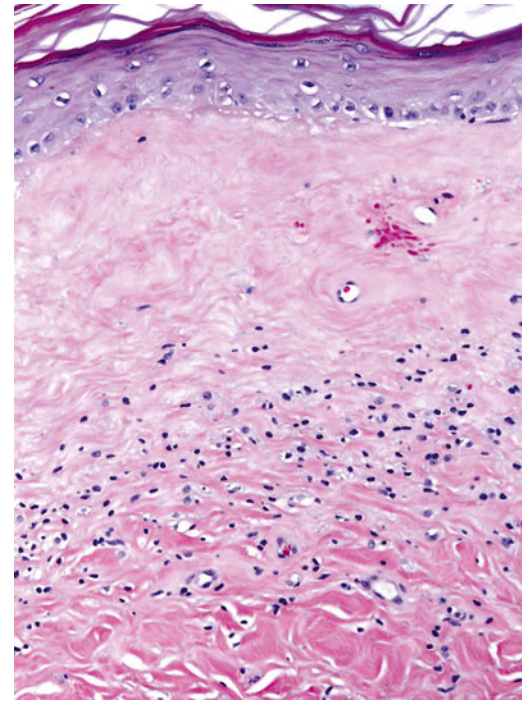


Fig. 10.1 Lichen sclerosus is a chronic dermatologic disease characterized microscopically by epithelial thinning with loss of the rete ridges, homogenization of the superficial dermis, and underlying band of chronic inflammatory infiltrate

vSCC. Approximately 3–15 % of women with LS will eventually develop invasive squamous cell carcinoma [15–19]. In women with LS, the risk of invasive squamous cell carcinoma increases with age and clinical evidence of localized hyperkeratosis [20, 21].

Patients with vSCC most commonly present with one or more of the following vulvar symptoms and signs: pruritus, pain, discharge, bleeding, dysuria, foul odor, and mass. The presenting signs and symptoms are nonspecific, overlapping with numerous benign vulvar diseases including infectious diseases and vulvar dermatoses. Vulvar squamous cell carcinoma can appear as exophytic or papillomatous masses, occasionally mimicking condyloma acuminatum. A small percentage of women (13.8 %) presents with persistent vulvar ulcers that ultimately prove to be squamous cell carcinoma (Fig. 10.2) [22]. As a result, clinicians should have a low threshold for biopsy of patients (especially postmenopausal women) with persistent ulcers and other atypical

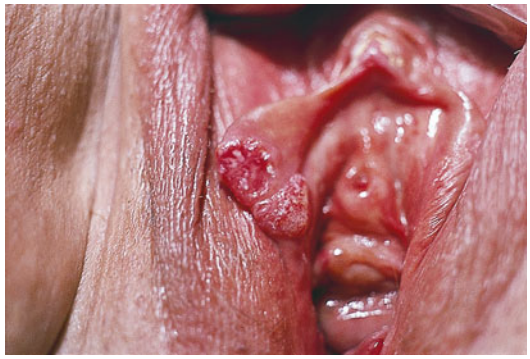


Fig. 10.2 This patient presented with an ulcerated squamous cell carcinoma and no prior history of predisposing disease (Reprinted with permission of Stanley J. Robboy and Robboy Associates LLC)

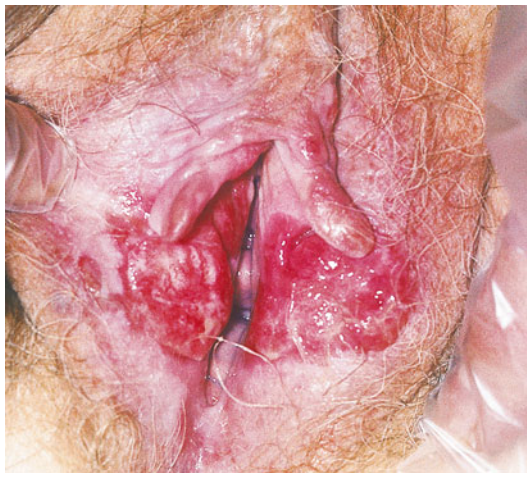


Fig. 10.3 Bilateral vulvar squamous cell carcinoma was identified in the bilateral labia majora of a woman with a history of lichen sclerosus (Reprinted with permission of Stanley J. Robboy and Robboy Associates LLC)

vulvar lesions. Definitive diagnosis of vSCC is frequently delayed either because patients do not seek medical attention when signs and symptoms first appear or their lesions are not biopsied at the time of initial clinical presentation.

The labia majora, labia minora, and clitoris are the most commonly affected sites. The majority of patients present with a solitary localized mass; however, patients occasionally present with more than one focus of invasive squamous cell carcinoma (Fig. 10.3). Multifocal vSCC is more frequently seen in patients with HPV-related tumors. Many tumors are larger than 2 cm at presentation. Tumors are locally destructive and can invade adjacent perineal structures such as the urethra, vagina, bladder, and rectum. The primary lymph node group that drains the vulva is the superficial inguinal lymph nodes. The deep femoral lymph nodes are the secondary lymph nodes, while the deep pelvic lymph nodes are the last group. The incidence of positive inguinal or pelvic lymph nodes ranges from 21 % to 50 %, and the incidence of positive pelvic lymph nodes decreases to 4.6–16.1 % [23]. Surgery (wide local excision/partial vulvectomy) is the mainstay of treatment for patients with resectable disease, corresponding to patients with International Federation of Gynecology and Obstetrics (FIGO) Stage I-II disease. Sentinel lymph node mapping can be used in the setting of early stage disease to determine which patients require locoregional lymphadenectomy [24]. Intraoperative pathologic assessment of sentinel lymph nodes is optional but allows the surgeon to perform completion lymphadenectomy in one procedure when the sentinel lymph node is positive at frozen section. In one study, up to one third of women with T1-T2 vSCC had a positive sentinel lymph node [25]. The risk of non-sentinel lymph node metastasis increases with the size of sentinel lymph node metastasis [25]. There are no standard protocols for pathologic assessment of sentinel lymph nodes. One group bivalved then quadrisected each sentinel lymph node half for routine histologic examination; this was followed by ultrastaging for all negative sentinel lymph nodes [25, 26]. For ultrastaging, three slide pairs were taken per millimeter. A hematoxylin and eosin (H&E) slide and a cytokeratin slide were evaluated for each pair. There is currently no evidence to suggest that ultrastaging of sentinel lymph nodes or special handling of sentinel lymph nodes is necessary. Years of experience with sentinel lymph node biopsy in women with breast cancer have shown that while ultrastaging does indeed reveal small metastatic tumor deposits that may have otherwise been missed by usual pathologic examination, the tumor deposits identified as a result of ultrastaging do not affect prognosis or change clinical management. Women with vSCC in the midline have an increased risk of bilateral lymph node metastasis,

representing a large proportion of false-negative sentinel lymph nodes. Patients with metastasis to two or more lymph nodes are treated with adjuvant radiation or chemoradiation, while patients with locally advanced/metastatic disease may be treated with surgical resection (including rarely pelvic exenteration) if resectable in addition to chemoradiation therapy [27].

Etiology and/or Pathogenesis

Two distinct pathways for development of vulvar squamous cell carcinoma have been delineated: HPV related and non-HPV related. Regardless of the pathway, squamous cell carcinoma is frequently identified adjacent to its precursor lesion, VIN. The progression of VIN to squamous cell carcinoma is not well understood. One systematic review of 3,322 patients reported a 6.5 % progression rate of VIN III/high-grade squamous intraepithelial lesion (HSIL) to squamous cell carcinoma [28].

HPV-related vSCCs are most commonly histologically non-keratinizing squamous cell carcinomas, warty or basaloid type [29], frequently identified adjacent to usual (warty and basaloid)-type VIN. The proportion of HPV-related squamous cell carcinomas ranges from 19 % to 79 % [9, 29–40]. The largest study to date reported 25 % of the more than 1,700 cases of vSCC were HPV related [29]. The variable distribution of HPV-related vSCCs can be attributed to geographic variances of viral incidence and technical differences of molecular diagnostic testing methods. HPV type 16 is the most common HPV type detected in vSCC [30–32, 34–38, 40]. HPV types 18, 31, 33, 45, 52, 53, and 62 have also been detected in these tumors [29, 39, 41].

Non-HPV-related vSCCs are most commonly histologically keratinizing squamous cell carcinomas and can be identified in association with differentiated (simplex)-type VIN and/or LS. The specific mechanism of carcinogenesis in non-HPV-related squamous cell carcinoma is not completely understood, but *TP*53, a tumor suppressor gene on chromosome 17, is thought to play a role. Lichen sclerosus shows p53 overexpression as demonstrated by immunohistochemistry, which has been attributed by one group as an ischemic stress response [42]. The association between LS and squamous cell carcinoma has largely been established based on the frequent observation of LS adjacent to squamous cell carcinoma [19, 37, 43, 44]. Women with LS develop vulvar cancer at a rate more than 300-fold higher than women without LS of similar age [45]. Differentiated (simplex)-type VIN is the precursor of non-HPV-related squamous cell carcinoma [46]. Because differentiated (simplex)-type VIN is infrequently diagnosed, the data on progression to invasive squamous cell carcinoma is limited. However, the few available reports suggest a higher rate of progression to invasive carcinoma and a shorter time interval between diagnosis of differentiated (simplex)-type VIN and detection of invasion than uVIN. Compared to usual VIN, women with differentiated VIN are reported to have a 5.6-fold higher rate of developing squamous cell carcinoma [21]. The majority of differentiated (simplex)-type VIN is HPV negative [46] and expresses increased p53 nuclear expression by immunohistochemistry in the basal and suprabasal epithelial layers [46, 47]. Vulvar squamous cell carcinoma also contains *TP*53 mutations and expresses p53 antigen by immunohistochemistry [47, 48]. Allelic losses at the *TP*53 locus are frequently identified in HPV-negative squamous cell carcinomas with loss of heterozygosity being more common in HPV-negative carcinomas [49]. Further, common *TP*53 mutations identified both in differentiated (simplex)-type VIN and in squamous cell carcinoma have been demonstrated [48]. Alteration of the *TP*53 gene is therefore thought to play a role in development of differentiated (simplex)-type VIN and subsequent keratinizing squamous cell carcinomas. This interpretation is not universally accepted, and it has alternately been suggested that p53 overexpression is reactive rather than causal [50].

Additional possible precursor markers of malignant transformation of LS to vSCC have been suggested [50]. Ki-67, a marker of cellular proliferation, has increased expression in LS and vSCC but is also increased in HPV-related uVIN and vSCC [50]. γ-H2AX expression, a molecule involved in DNA repair, is significantly increased in LS, HSIL, differentiated (simplex)-type VIN,

and vSCC compared with normal vulva, suggesting that γ-H2AX may represent an early event in carcinogenesis [51]. Other markers such as MCM3, an essential protein for DNA function and replication; Cyclin D1, a cell cycle regulation protein; and angiogenesis may be useful markers of malignant transformation, but additional studies are warranted [50].

Prognosis

Prognosis in vSCC is primarily determined by tumor stage, based upon surgical and pathological findings. Tumor size, depth of invasion, tumor extension to adjacent structures, presence of lymph node metastasis, and/or presence of distant metastasis are all assessed to assign a pathologic tumor stage. Inguinal lymph node status and tumor size are primary independent prognostic factors [52] with the most important prognostic factor being presence of lymph node metastasis [52–54]. Staging information is commonly reported using the tumor-node-metastasis (TNM) system outlined in the American Joint Commission on Cancer (AJCC) staging manual, seventh edition (Table 10.2) [55]. Alternatively, the FIGO staging system can be used (Table 10.2). Survival data is presented in Table 10.3. Patients with disease localized to the vulva (FIGO stage I or II) have a 5-year survival of 86 %; involvement of the regional lymph nodes (FIGO stage III) lowers the 5-year survival to 54 %. Patients with FIGO stage IV disease have a dismal prognosis, with a 5-year survival of only 15 %. Only 5 % of patients are FIGO stage IV at presentation, however [56].

Patients with tumor depth of invasion less than 1 mm have essentially no risk for lymph

Table 10.2 Staging of vulvar malignancy

TNM	FIGO	Description
Primary tumor (T)		
TX	–	Primary tumor cannot be assessed
T0	–	No evidence of primary tumor
Tis	–	Carcinoma in situ
T1a	IA	Lesions 2 cm or smaller confined to the vulva perineum and with stromal invasion 1.0 mm or less
T1b	IB	Lesions larger than 2 cm or any size tumor with greater than 1 mm stromal invasion
T2	II	Any size tumor with extension to adjacent perineal structures (lower/distal 1/3 urethra, lower/distal 1/3 vagina, anal involvement)
T3	IVA	Any size tumor with extension to any of the following: upper/proximal 2/3 urethra, upper/proximal 2/3 vagina, bladder mucosa, rectal mucosa, or fixed to pelvic bone
Regional lymph nodes (N)		
NX	–	Regional lymph nodes cannot be assessed
N0	–	No regional lymph node metastasis
N1	–	One or two regional lymph nodes with the following features:
N1a	IIIA	One lymph node metastasis 5 mm or smaller
N1b	IIIA	One lymph node metastasis 5 mm or larger
N2	–	Regional lymph node metastasis with the following features:
N2a	IIIB	Three or more lymph node metastases each smaller than 5 mm
N2b	IIIB	Two or more lymph node metastases 5 mm or larger
N2c	IIIC	Lymph node metastasis with extracapsular spread
N3	IVA	Fixed or ulcerated regional lymph node metastasis
Distant metastasis (M)		
M0	–	No distant metastasis
M1	IVB	Distant metastasis (including pelvic lymph node metastasis)

Used with permission from Edge et al. [55]
TNM tumor-node-metastasis, *FIGO* International Federation of Gynecology and Obstetrics

Table 10.3 Vulvar cancer survival rates

Stage	Relative 5-year survival rate (%)	Relative 10-year survival rate (%)
I	93	87
II	79	69
III	53	46
IV	29	16

Source: National Cancer Institute [56]

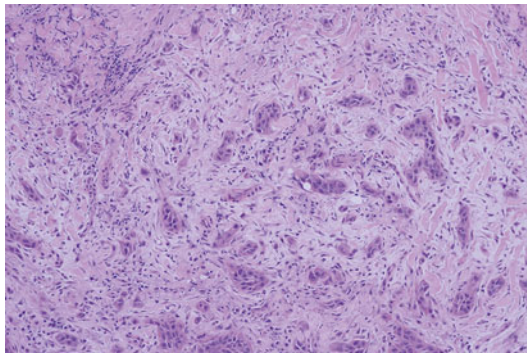


Fig. 10.4 Malignant cells invade the stroma in small nests and individually in a spray pattern. Note the marked desmoplastic stromal response and chronic inflammatory infiltrate

node metastasis and are almost always cured by local excision [57]. Patients with a tumor depth of invasion greater than 1 mm have an associated increased risk of lymphatic/vascular invasion, lymph node metastasis, and recurrence leading to worse survival rates [57]. Histologic patterns of invasion are also prognostic factors. Tumors with a spray or finger-like invasion pattern portend poorer survival compared to pushing invasion (Fig. 10.4) [58]. Risk of lymph node metastasis increases with age, tumor size, depth of invasion, and tumor grade [54]. Extracapsular extension of lymph node metastases and size of metastatic vSCC are predictors of poor survival [59–61]. Women with two or more positive lymph nodes also have poor survival [59]. Bilateral lymph node involvement in vSCC is not more important than number of lymph nodes with metastases [62].

Local recurrences are common in vSCC. Regular follow-up for early detection is important. Approximately 23–33 % develop local recurrences [63–65], and older age (age > 74 years) is an independent risk factor for recurrence [65]. Recurrences detected at regularly scheduled posttreatment follow-up appointments (every 3 months for 2 years, biannually until the 5th year, and then annually thereafter) were significantly smaller than recurrences detected at interval appointments [63]. Patients who develop one recurrence are at a significantly increased risk for development of a second recurrence (72 %) and should be closely monitored for subsequent recurrences [65].

Table 10.4 Recommended elements of synoptic reporting of vulvar squamous cell carcinoma

Specimen type and size
Procedure
Tumor site, size, thickness, and focality
Histologic type and grade
Depth of invasion
Tumor borders
Margin status
Lymphatic/vascular invasion
Lymph node status

The College of American Pathologists (CAP) has developed recommendations to unify the information included in vSCC pathology reports; these are listed in Table 10.4 [66]. Both depth of invasion and tumor thickness are among the recommended criteria. It is important that these measurements be performed in a standardized fashion. The World Health Organization (WHO) defines depth of invasion as the distance (in millimeters) from the epithelial-stromal junction of the nearest adjacent superficial dermal papilla to the deepest point of invasion [66, 67] and tumor thickness as the distance (in millimeters) from the surface of the tumor to the deepest point of invasion [66, 67]. Tumor thickness can be considerably larger than depth of invasion in a given tumor, particularly if it is exophytic, but depth of invasion is the measurement used for staging. In practice, determining the depth of invasion can be challenging. Figure 10.5 demonstrates both tumor thickness (left) and depth of invasion (right). One could argue that the measurement for depth of invasion could logically be taken in several different ways, highlighting the difficulty of determining accurate depth of invasion

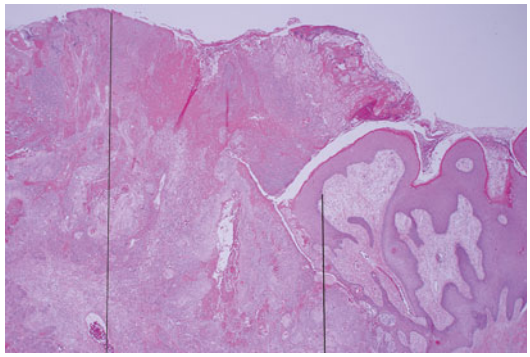


Fig. 10.5 Tumor thickness is measured from the tumor surface to the deepest point of invasion (*left line*). Depth of invasion is measured from the epithelial-stromal junction of the nearest adjacent superficial dermal papilla to the deepest point of invasion (*right line*); this measurement is difficult and could arguably be made in several different places in this particular tumor

in some cases. Even tumors with very early invasion can arguably be greater than 1 mm deep if the depth of invasion definition is strictly followed. Close communication with surgeons and oncologists is necessary in these situations to prevent overly aggressive treatment of very small tumors.

Histologic Features and Differential Diagnosis

Keratinizing Squamous Cell Carcinoma

Keratinizing squamous cell carcinoma is the most common histologic subtype of vSCC. As noted above, keratinizing squamous cell carcinoma is frequently identified in older women with LS and differentiated (simplex)-type VIN.

Invasive squamous cell carcinoma is defined by stromal invasion of malignant-appearing squamous epithelium (Fig. 10.6). In keratinizing squamous cell carcinoma, the malignant cells frequently have eosinophilic cytoplasm, enlarged nuclei with prominent nucleoli, increased nucleus to cytoplasm ratio (N/C), and increased mitotic activity. Well-differentiated squamous cell carcinoma commonly displays paradoxical maturation characterized by abundant eosinophilic

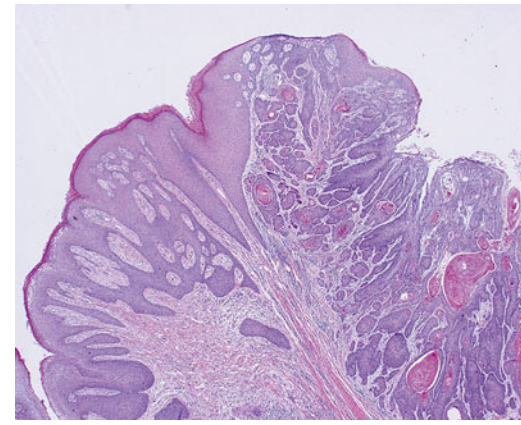


Fig. 10.6 Keratinizing squamous cell carcinoma demonstrates keratin production. Abundant keratin pearls are surrounded by nests of malignant squamous epithelium. At the leading edge of invasion, small nests of invasive tumor are identified in a background of desmoplastic stromal response

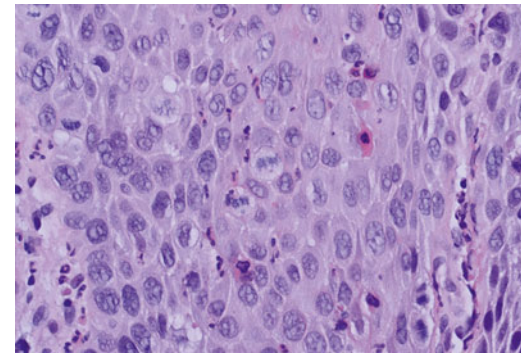


Fig. 10.7 Individual dyskeratotic cells may be identified in keratinizing and non-keratinizing squamous cell carcinomas. Dyskeratotic cells are small cells with brightly eosinophilic and dense cytoplasm and a hyperchromatic, pyknotic nucleus

cytoplasm within the tumor cells; increased mitotic activity may only be identified at the leading edge of invasion. The histologic hallmark of keratinizing squamous cell carcinoma is demonstration of keratin production. Keratin production can be in the form dyskeratotic cells (Fig. 10.7), keratin pearls (Fig. 10.8), or parakeratotic cells. Keratinizing squamous cell carcinomas are defined by the presence of keratin production in the absence of features of warty carcinoma such as fibrovascular papillary architecture and koilocyte-like malignant cells.

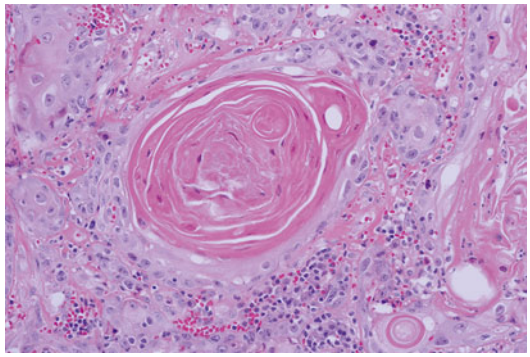


Fig. 10.8 Keratin pearl formation is the hallmark of keratin production, identified in keratinizing squamous cell carcinomas but is absent in non-keratinizing squamous cell carcinomas. Keratin pearls are characterized by whorls of brightly eosinophilic keratin material which may or may not contain parakeratotic nuclei. The nests of keratin are surrounded by malignant-appearing squamous epithelium

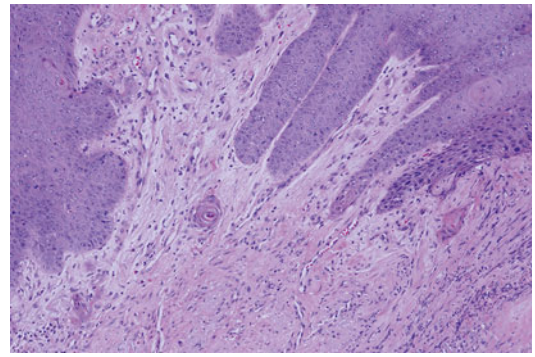


Fig. 10.9 One microscopic nest of atypical cells with a central keratin pearl is seen separate from the overlying atypical squamous epithelium containing cytological atypia and mitotic activity. A subtle possible stromal desmoplastic response and a mild chronic inflammatory infiltrate are seen in the intervening tissue between the overlying epithelium and the abnormal squamous nest suspicious for invasion

Table 10.5 Differential diagnosis of squamous cell carcinoma (SCC)

Keratinizing SCC	Basaloid SCC	Warty SCC
VIN	Basal cell carcinoma	Verrucous carcinoma
Malignant melanoma	Small cell carcinoma	Keratinizing SCC
Metastatic SCC	Merkel cell carcinoma	Condyloma acuminatum
Keratoacanthoma-like SCC	–	–
PEH	–	–
Warty dyskeratoma	–	–

PEH pseudoepitheliomatous hyperplasia

Table 10.6 Histologic features of invasive squamous cell carcinoma

Paradoxical squamous maturation
Dyskeratosis and keratin pearls
Irregularly shaped squamous nests within the dermis
Desmoplastic stromal reaction

Keratinizing squamous cell carcinoma should be differentiated from several benign and malignant entities (Table 10.5).

Patients with VIN may or may not be symptomatic. Those with symptoms present with pruritus and possibly dyspareunia. Lesions are clinically identified using visual inspection with the aid of colposcopy. Biopsies are taken to establish a diagnosis and to rule out invasive squamous cell carcinoma. Treatment depends on extent of disease and is either ablative or surgical.

The histological distinction between VIN and invasive squamous cell carcinoma can be challenging. When frankly invasive squamous cell carcinoma is identified, the diagnosis is straightforward. In cases with patchy VIN and epithelial acanthosis or cases with atypical epithelial nests suspicious for invasion (Fig. 10.9), distinguishing between VIN and well-differentiated or superficially invasive squamous cell carcinoma can be extremely difficult. In fact, interobserver agreement between 11 gynecologic pathologists for diagnosis of the presence of early invasion was only fair (kappa = 0.24) [68], reflecting the diagnostic dilemma commonly encountered.

Several histologic findings can aid in establishing evidence of invasion (Table 10.6, Fig. 10.10). Identification of epithelium with eosinophilic cytoplasm and atypical nuclei characterized by prominent nucleoli, so-called paradoxical maturation, is identified within well-differentiated squamous cell carcinoma. Furthermore, the presence of dyskeratosis and keratin pearls should increase suspicion for invasive squamous cell carcinoma. Evaluation of

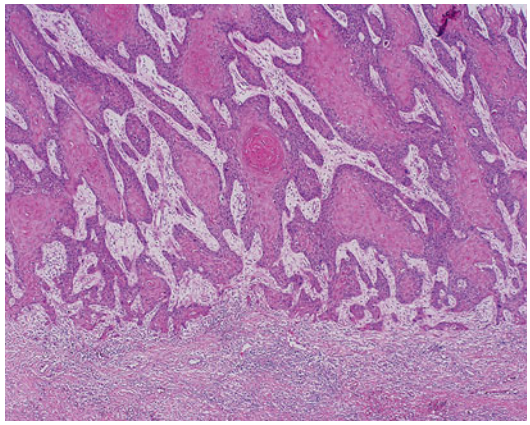


Fig. 10.10 This invasive squamous cell carcinoma displays all of the morphologic features of invasion. The tumor is composed of irregularly shaped anastomosing nests within the dermis that are surrounded by a pale stroma, indicative of stromal desmoplastic response. A single keratin pearl is observed. Within the invasive nests, the more centrally located malignant cells have minimal cytologic atypia and abundant brightly eosinophilic cytoplasm, so-called paradoxical maturation

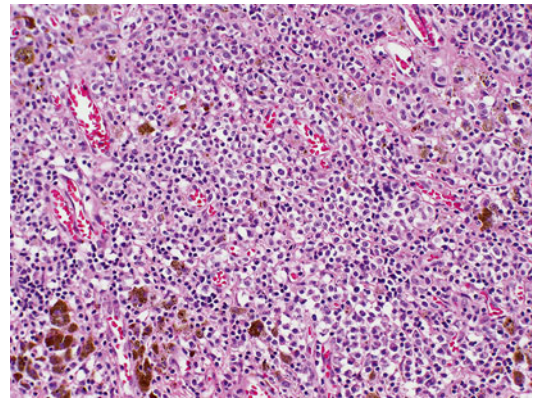


Fig. 10.11 Malignant melanoma is infamously known as the great mimicker and can have varied histologic appearances. This example is characterized by sheets of epithelioid cells with clear cytoplasm and high nuclear/cytoplasmic ratio. There is no evidence of maturation. Pigment-laden macrophages are identified in the background, providing a useful clue

squamous nests within the dermis is also helpful. Small irregularly shaped nests with disordered orientation within the dermis point toward an invasive squamous cell carcinoma. The presence of single atypical malignant cells can occasionally be identified (Fig. 10.4). Finally, the presence of a desmoplastic stromal response, a loose and gray-blue stroma often with an associated inflammatory reaction, can also be useful. In difficult cases, these morphologic features may not be readily identified. Evaluation of additional histologic step sections can be beneficial to distinguish between VIN and invasive squamous cell carcinoma. Occasionally, rare cases may require a descriptive diagnosis stating that the possibility of a superficially invasive squamous cell carcinoma cannot be excluded (Fig. 10.9). In such cases, a description of the microscopic findings including maximal depth of possible invasive focus should be clearly stated within the diagnostic report. See also Vignette 3 at the end of this chapter.

Malignant melanoma comprises approximately 10 % of malignant vulvar tumors and is the second most common malignant tumor of the vulva [69]. Postmenopausal Caucasian women are most frequently affected and present with an ulcer, nodule, or abnormal pigmented lesion. Melanomas have varied histological appearances. In this case, a sheet of tumor cells invades the underlying dermis (Fig. 10.11). The tumor cells are epithelioid with clear cytoplasm. There is no evidence of maturation. Pigment-laden macrophages are observed in the background. Malignant melanoma can, however, closely mimic squamous cell carcinoma, especially in amelanotic melanoma [70] or in melanomas with an intraepithelial component mimicking VIN. Careful scrutiny may reveal focal evidence of squamous differentiation, even in poorly differentiated squamous cell carcinomas, to exclude the possibility of melanoma. Occasionally a panel of immunoperoxidase stains will be necessary to exclude malignant melanoma. Squamous cell carcinomas are reactive with epithelial antibodies, such as high molecular weight cytokeratins (CK903 and CK5/6) and p63, and are nonreactive with S-100, HMB-45, and Mart-1. Occasionally melanoma reacts with epithelial antibodies; however, its characteristic reactivity with S-100, HMB-45, and melan-A will confirm the melanocytic origin of the tumor.

Distinguishing metastatic squamous cell carcinoma from primary vulvar squamous cell carcinoma can be difficult. Currently available immunoperoxidase stains cannot be used to

localize squamous cell carcinomas to a primary site of origin. Therefore, a careful review of clinical history is critical and may provide useful clues for the site of origin. In addition to clinical history, identification of nearby VIN supports a primary carcinoma, whereas absence of VIN and the presence of an invasive carcinoma in the deep soft tissues without connection to the surface epidermis suggest a metastasis (with the caveat that squamous cell carcinoma arising in Bartholin's glands can mimic this appearance).

Keratoacanthoma-like squamous cell carcinoma (KA) is a rapidly growing crater-like lesion that only rarely occurs on the vulva. Microscopically, this tumor is composed of a well-circumscribed squamous proliferation with a characteristic keratin-filled center (Figs. 10.15 and 10.16). The proliferating squamous cells have bland nuclei and have characteristic eosinophilic cytoplasm with a pushing border. Differentiating a KA from an invasive squamous cell carcinoma can be difficult, especially in the absence of pertinent clinical history, but the absence of VIN in the adjacent epithelium [71, 72] and absence of HPV DNA [71] favors a diagnosis of KA.

Keratinizing squamous cell carcinoma should also be distinguished from its benign mimics including pseudoepitheliomatous hyperplasia (PEH) and warty dyskeratoma. PEH is a benign, reactive proliferation of the epithelium seen in a variety of clinical conditions, including vulvar *Enterobius vermicularis* (pinworm) infection [73] and lichen sclerosus [74], and can easily mimic vSCC [75]. PEH is also identified in the setting of hypertrophic herpes infections, a rare manifestation of herpes simplex virus (HSV) that clinically mimics vSCC in immunocompromised patients [76–79]. Irregular nests of squamous epithelium extend into the dermis. Cytologic atypia is usually minimal. Rare mitotic figures can be identified. Warty dyskeratoma is a benign keratotic nodule usually confined to the head and neck but has been rarely described on the vulva [80]. It is characterized by suprabasal epidermal acantholysis with a keratotic plug overlying an epidermal invagination with a dermal villous-like architectural pattern.

Non-keratinizing Squamous Cell Carcinoma

Non-keratinizing squamous cell carcinoma (Fig. 10.12) is a squamous cell carcinoma also identified in postmenopausal women with a long-standing history of LS. It can also, however, be identified in the setting of HPV infection. It is defined by an absence of keratin production. Individual dyskeratotic cells may be seen. Keratin pearls are, however, by definition not identified within non-keratinizing squamous cell carcinoma.

Rarely, spindle cell morphology resembling a sarcomatoid carcinoma can be identified, and the tumor can be either monophasic or biphasic. Sarcomatoid carcinomas are exquisitely rare in the female genital tract and are usually clinically aggressive tumors [81]. Very few cases have been described in the vulva [81–85], the majority of which show biphasic tumor morphology composed of areas of conventional squamous cell carcinoma as well as sarcomatoid carcinoma; rare tumors show heterologous differentiation including osteosarcoma and chondrosarcoma [82, 86].

Non-keratinizing vSCC should be distinguished from epithelioid sarcoma, an uncommon malignant soft tissue tumor that occurs in the distal extremities of young adults. Proximal/axial

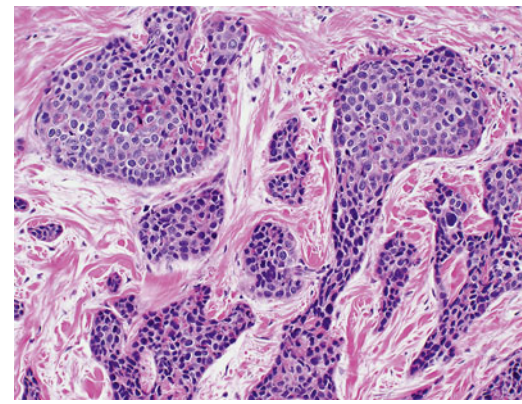


Fig. 10.12 Non-keratinizing squamous cell carcinoma does not produce keratin pearls although individual dyskeratotic cells may be identified within the tumor. This tumor is composed of invasive nests of malignant squamous cells nuclear enlargement, hyperchromasia, increased nuclear/cytoplasmic ratio, and stromal desmoplastic response. An absence of keratin production is noted

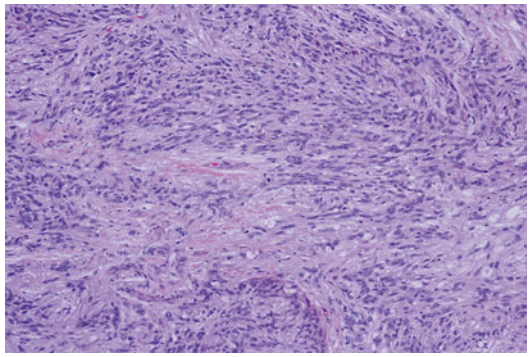


Fig. 10.13 Epithelioid sarcoma is a rare sarcoma that may have areas resembling a squamous cell carcinoma, especially sarcomatoid carcinomas

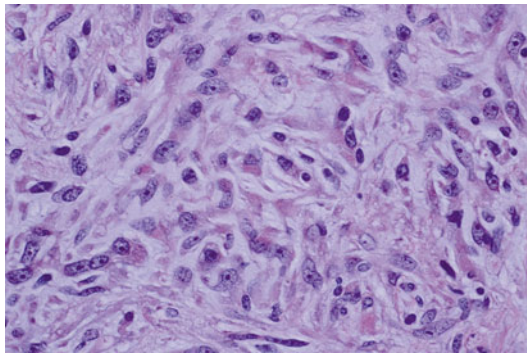


Fig. 10.14 Epithelioid sarcoma, unlike squamous cell carcinoma, contains malignant cells with eosinophilic cytoplasmic inclusions, resembling rhabdomyoblasts

variants portend a worse prognosis and most commonly occur in the deep soft tissues or superficial inguinal soft tissues and rarely in the vulva [87]. Microscopically, proximal epithelioid sarcomas are composed of acidophilic epithelioid tumor cells that may mimic invasive squamous cell carcinoma (Fig. 10.13) and contain cells with intracytoplasmic hyaline inclusions, resembling rhabdoid cells (Fig. 10.14). Large areas of necrosis are commonly identified. Both squamous cell carcinoma and epithelioid sarcoma express epithelial markers (keratin, EMA). Epithelioid sarcoma can be differentiated from squamous cell carcinoma based on its positive reaction with vimentin and variable expression of CD34, desmin, and smooth muscle actin (SMA).

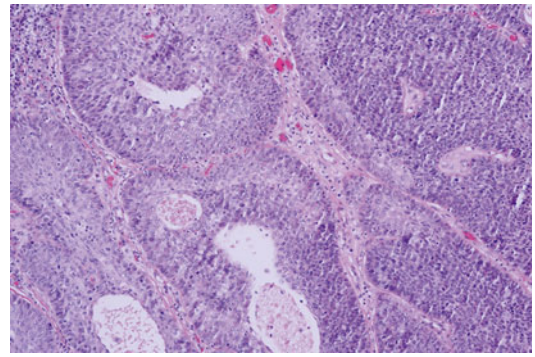


Fig. 10.15 Basaloid squamous cell carcinoma is HPV related and is composed of small basaloid-type squamous cells, which are cells that resemble the basal/parabasal cells of normal squamous epithelium. The cells are small and have a high nuclear/cytoplasmic ratio. Evidence of keratin production is usually not identified. Koilocyte-like cells are generally absent

Basaloid Squamous Cell Carcinoma

Basaloid squamous cell carcinoma is a non-keratinizing squamous cell carcinoma seen in association with HPV [88]. The tumor is characterized by irregular nests of immature tumor cells resembling the basal-type cells identified within normal squamous epithelium (Fig. 10.15). The tumor cells are small with minimal cytoplasm and have a high N/C ratio. The chromatin is coarse but evenly distributed within the nucleus. These tumors typically fail to show evidence of squamous maturation. Rarely, squamous maturation and even keratinization can be identified within the center of tumor nests. Koilocytes are usually not identified.

Basaloid squamous cell carcinoma must be distinguished from basal cell carcinoma, metastatic small cell carcinoma, and Merkel cell carcinoma.

Basal cell carcinoma (BCC) is a common skin cancer identified on sun-exposed areas but is rarely seen on the vulva, comprising approximately 5 % of vulvar malignancies (Fig. 10.16) [89]. The tumors have invasive nests of basaloid-type cells with characteristic peripheral palisading. Stromal desmoplasia and retraction artifact are also typically identified. Compared with basaloid squamous cell carcinoma, BCC is more

circumscribed and is usually not associated with VIN. BCC is immunoreactive with BerEP4 (Table 10.7). Basaloid squamous cell carcinoma can also be BerEP4 reactive [90]. One recent study found, however, that basaloid vSCC is BerEP4 negative [91]. p16 is frequently reactive in both BCC and basaloid squamous cell carcinoma [91, 92]. BCC has patchy p16 expression with fewer than 30 % of tumor cells showing reactivity, which is in sharp contrast with the strong, diffuse p16 reaction observed in HPV-related basaloid vSCC [91]. BerEP4 and p16 may not always be helpful in differentiating BCC and basaloid vSCC. In difficult cases, HPV in situ hybridization may be helpful, as BCC is HPV negative while basaloid vSCC is HPV positive [91]. BCC is an indolent tumor that is locally invasive and is treated with complete surgical excision. See also Vignette 2 at the end of this chapter.

Primary vulvar neuroendocrine tumors rarely occur. A few cases of small cell carcinoma have been reported, including small cell undifferentiated carcinoma of the Bartholin's gland in a 30-year-old woman [93]. Merkel cell carcinoma commonly affects sun-exposed skin, especially on the face, upper limb and shoulder, and lower limb and hip, and only rarely is identified on the vulva [94, 95]. Metastatic small cell carcinoma can also be identified. All of these malignancies are clinically aggressive with a poor prognosis and share common morphologic features characterized by overtly malignant small round blue cells with a high N/C ratio, nuclear molding, crush artifact, and stippled chromatin (Fig. 10.17). Evidence of neuroendocrine differentiation can be demonstrated in each of these tumors using CD56, neuron-specific enolase (NSE), synaptophysin, and chromogranin (Table 10.7). Metastatic (pulmonary) small cell carcinoma will additionally react with TTF-1 and CK7, supporting its pulmonary origin. Merkel cell carcinoma characteristically reacts with CK20 in a dot-like perinuclear pattern.

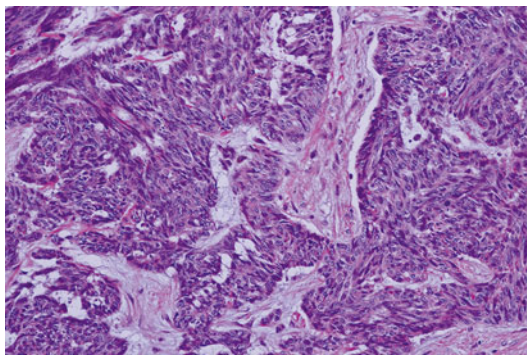


Fig. 10.16 Basal cell carcinoma is characterized by invasive nests of basaloid cells that have peripheral nuclear palisading. Retraction artifact surrounding the invasive tumor nests is frequently identified. The stroma has a gray-blue characteristic

Warty Squamous Cell Carcinoma

Warty squamous cell carcinoma is a non-keratinizing squamous cell carcinoma seen in association with HPV [88]. The tumor is characterized by a papillary, undulating architecture (Fig. 10.18). Fibrovascular cores covered by malignant squamous epithelium are characteristics (Fig. 10.19). The surface may show hyperkeratosis. Morphological evidence of HPV infection, such as koilocytes or koilocyte-like cells, can be identified (Fig. 10.20). The diagnosis of invasion is based on the presence of abnormal, irregularly shaped nests of epithelial cells within the stroma, some of which may contain keratin pearls. Cytological atypia ranges from mild to marked.

Table 10.7 Immunoperoxidase stains can differentiate basaloid squamous cell carcinoma from its mimics

Tumor	CK7	CK20	p16	CD56	TTF-1	BerEP4
Basaloid SCC	–	–	+	–	–	+/–
Basal cell carcinoma	+/–	–	+	–	–	+
Small cell carcinoma	+/–	–	+	+	+	–
Merkel cell carcinoma	–	+	–	+	–	–

+/– positive or negative, – negative, + positive

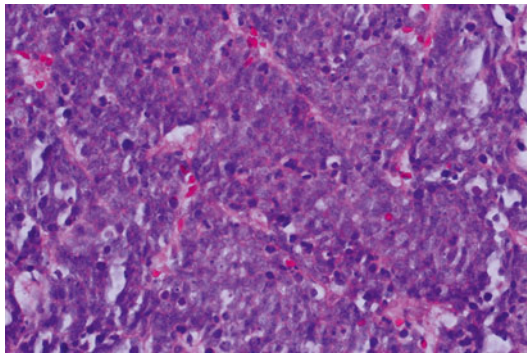


Fig. 10.17 Vulvar neuroendocrine tumors are aggressive and are composed of malignant cells with a high nuclear/cytoplasmic ratio, stippled chromatin, nuclear molding, crush artifact, apoptotic debris, and frequent mitotic figures

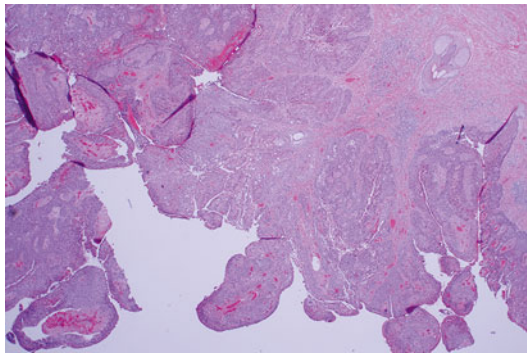


Fig. 10.18 Warty squamous cell carcinoma is HPV related and has an undulating verrucous surface with papillary architecture

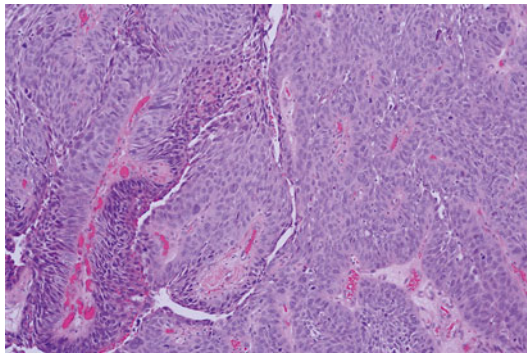


Fig. 10.19 Fibrovascular cores are lined by malignant squamous epithelium in warty squamous cell carcinoma

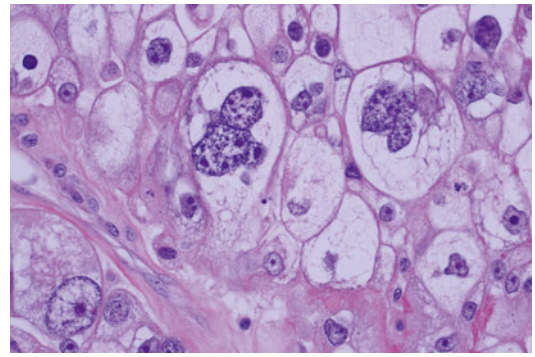


Fig. 10.20 Koilocyte-like malignant cells are present in warty squamous cell carcinomas, which are HPV-related squamous cell carcinomas of the vulva

Distinguishing warty squamous cell carcinoma from its malignant morphological mimics, keratinizing squamous cell carcinoma and verrucous carcinoma, can be quite difficult. Warty squamous cell carcinoma is associated with HPV, specifically HPV type 16, and occurs in younger women, generally less than 60 years old [88]. In contrast, keratinizing squamous cell carcinoma is associated with LS and occurs in older women, generally over 65 years old [88]. Warty squamous cell carcinoma closely resembles keratinizing squamous cell carcinoma but differs based on the abundant presence of koilocytic atypia as well as adjacent warty- or basaloid-type VIN [88].

Verrucous carcinoma is a well-differentiated squamous carcinoma that has an undulating appearance similar to that of warty carcinoma. Fibrovascular cores are identified within warty carcinoma, whereas they are absent in verrucous carcinoma. Koilocytes are also absent in verrucous carcinoma. Differentiation from well-differentiated squamous cell carcinoma can be made based on the presence of small nests of cytologically atypical squamous cells with readily identified mitotic figures at the leading infiltrative edge. Warty squamous cell carcinoma has similar invasive tumor nests within the dermis but is additionally characterized by the presence of papillae and koilocyte-like tumor cells, whereas these features are distinctly absent in verrucous carcinoma. Condyloma acuminatum should also be considered and is distinguished from warty vSCC and verrucous carcinoma based on the presence of typical features of condyloma acuminatum in the absence of dermal invasion. Koilocytes are readily identified in condyloma

acuminatum but are absent in verrucous carcinoma. Condyloma acuminatum is an exophytic mass with true fibrovascular cores. In contrast, fibrovascular cores are absent in verrucous carcinoma, and the leading invasive edge of the tumor is composed of broad bulbous nests.

Verrucous Carcinoma

Verrucous carcinoma of the vulva is an extremely differentiated squamous cell carcinoma characterized by specific morphologic features that may be difficult to distinguish from its morphological mimics, condyloma acuminatum and warty squamous cell carcinoma. By definition, all of these lesions have a verrucous clinical and microscopic appearance, producing warty growths on the vulva. To avoid diagnostic and prognostic confusion, the term giant condyloma of Buschke-Lowenstein should not be used as a synonym for verrucous carcinoma. The term condyloma should be reserved for noninvasive HPV-related warty lesions.

Verrucous carcinoma occurs in women from ages 50 to 83 and is generally not an HPV-associated lesion [96] although low-risk HPV (typically HPV type 6) has been implicated in rare cases [97, 98]. It has been suggested that vulvar acanthosis with altered differentiation (VAAD) is the precursor lesion, characterized by marked acanthosis with variable verruciform architecture, loss of the granular cell layer with superficial epithelial pallor, and multilayered parakeratosis [99]. Microscopically, verrucous carcinoma is characterized by a thick undulating extremely well-differentiated malignant squamous epithelium with bulbous and pushing squamous borders that meet an often inflamed dermis (Fig. 10.21). The thickened neoplastic squamous epithelium often displays parakeratosis and/or hyperkeratosis. The infiltrating margin of verrucous carcinoma is composed of large bulbous nests of cytologically bland squamous cells that display the greatest nuclear atypia at the advancing edge (Fig. 10.22). Cytological findings are often subtle, but verrucous carcinoma can be identified by the presence of keratinocytes with prominent nucleoli, coarse chromatin, and eosinophilic cytoplasm. Koilocytes are typically not observed. Mitotic figures are rarely identified. Small biopsies of verrucous carcinomas are frequently

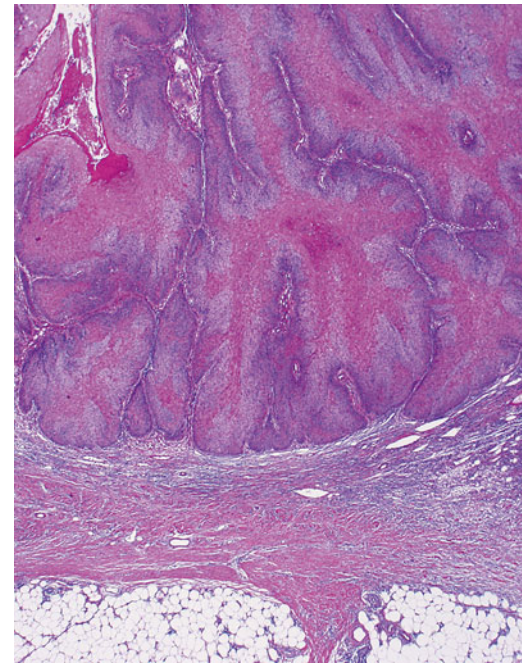


Fig. 10.21 Verrucous carcinoma has undulating broad nests of highly differentiated malignant squamous epithelium. The cells frequently have brightly eosinophilic cytoplasm

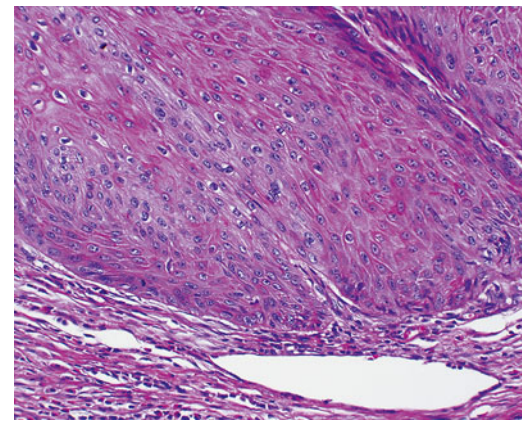


Fig. 10.22 The leading edge of invasion in verrucous carcinoma has broad-based tumor nests with pushing borders. Cytologic atypia is most pronounced at the leading edge of the tumor. The underlying stroma frequently contains an inflammatory infiltrate

impossible to recognize as malignant; it is important to include the base of the lesion in the biopsy.

Verrucous carcinoma should be distinguished from warty vSCC and condyloma acuminatum (see section "Warty Squamous Cell Carcinoma"). Verruciform xanthoma, a rare benign tumor usually seen in the oral cavity, should also be a diagnostic consideration as rare vulvar cases have been reported [100–105]. Verruciform xanthomas appear as yellow-orange verrucous plaques clinically and have parakeratosis, acanthosis with elongate rete ridges, cytologic atypia, and xanthomatous cell aggregates in the papillary dermis.

Verrucous carcinoma is locally invasive and can recur if not completely excised. This tumor rarely metastasizes. Thus, wide local excision is the most common therapy. Prognosis is excellent if the primary tumor is completely resected [106].

See also Vignette 1 at the end of this chapter.

Squamous Cell Carcinoma, Keratoacanthoma Type

Squamous cell carcinoma, keratoacanthoma (SCC-KA) type, is a rapidly growing often umbilicated lesion of the epidermis that invades the dermis (Fig. 10.23). SCC-KA most commonly occurs on sun-exposed skin surfaces and only rarely on the vulva. Microscopically, SCC-KA is a well-circumscribed squamous proliferation with a characteristic central keratin-filled crater (Fig. 10.24). The proliferating squamous cells have bland nuclei and have characteristic eosinophilic cytoplasm with a pushing border. Lymphatic/vascular invasion and/or perineural invasion [107] may be seen [108]. Complete excision of SCC-KA is the treatment of choice and is usually curative, although rare reports of metastasis from this tumor have been reported [109].

Squamous Cell Carcinoma with Tumor Giant Cells

Squamous cell carcinoma with tumor giant cells is a distinct variant of squamous cell carcinoma that is also only rarely identified. It is characterized by the presence of multinucleated tumor giant cells and nuclear pleomorphism and should be distinguished from melanoma [110]. These tumors have a poor prognosis.

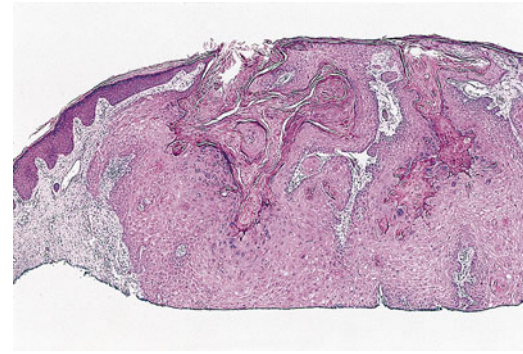


Fig. 10.23 Keratoacanthoma is a rapidly growing umbilicated squamous proliferation that invades the dermis (Reprinted with permission of Stanley J. Robboy and Robboy Associates LLC)

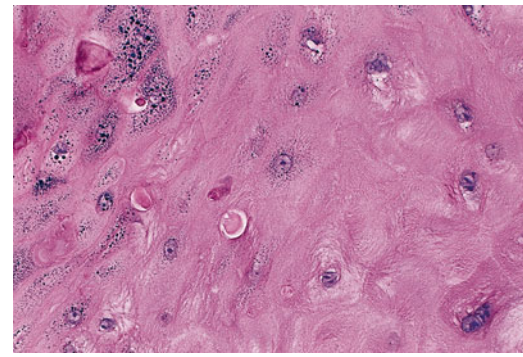


Fig. 10.24 A keratoacanthoma has a central crater that is filled with keratinous debris and bland squamous cells (Reprinted with permission of Stanley J. Robboy and Robboy Associates LLC)

Histologic Grade

The College of American Pathologists (CAP) recommends that histologic grade should be determined. According to the AJCC, a two-, three-, or four-grade system can be used; however, if the grading system is not specified within a report, it is understood that a four-grade system was used (Table 10.8) [55]. The Gynecologic

Table 10.8 Histologic grading of squamous cell carcinoma

Grade	AJCC	GOG
X	Cannot be assessed	–
1	Well differentiated	No undifferentiated cells
2	Moderately differentiated	Less than 50 % undifferentiated cells
3	Poorly differentiated	50 % or more undifferentiated cells
4	Undifferentiated	–

AJCC American Joint Commission on Cancer, *GOG* Gynecology Oncology Group

Oncology Group (GOG) suggests that the grade of squamous cell carcinomas be determined based upon the percentage of undifferentiated cells that comprise the tumor such that a grade 1 tumor has no undifferentiated cells, a grade 2 has 1–49 % undifferentiated cells, and a grade 3 tumor has 50 % or more undifferentiated cells (Table 10.8) [111]. Low-grade tumors do not recur or have metastases [111]. Lymph node metastases are observed with increasing frequency as histologic grade increases [112].

Immunophenotype

Morphological evaluation alone will suffice to make a confident pathologic diagnosis of most vSCCs. Occasionally, the use of immunoperoxidase stains may be helpful, especially in the cases of poorly differentiated malignancies or malignancies with basaloid morphology. Like squamous cell carcinomas from other body sites, vSCCs are reactive with the usual epithelial markers including epithelial membrane antigen (EMA, cytoplasmic reaction) and pancytokeratin (cytoplasmic reaction) as well as CK5/6 (cytoplasm reaction) and p63 (nuclear reaction). Many vSCCs can be subdivided based on the two distinct etiologic pathways as either HPV related or non-HPV related using p16 and p53. In daily practice, p16 and p53 are, however, not routinely used to classify vulvar squamous cell carcinoma.

p16 is a well-established surrogate biomarker for HPV. Integration of HPV DNA into host DNA has been demonstrated both in the cervix [113] as well as in the vulva [114] as initial steps in squamous HPV-related carcinogenesis. In the cervix, high-risk HPV viral gene products E6 and E7 interact with p53 and retinoblastoma (Rb) proteins, respectively, disrupting the normal cell cycle leading to absence of cell cycle arrest and proliferation of the tumor cells. As a result of p53 degradation and Rb inactivation, p16INK4A and p14ARF are overexpressed [115, 116] and can therefore be used as surrogate biomarkers for high-risk HPV viral types (such as the commonly encountered HPV types 16 and 18). A positive p16 stain is defined as strong and diffuse block nuclear or nuclear and cytoplasmic staining in the basal layer extending upward involving at least one third of the epithelial thickness. In VIN, this manifests as a diffuse, band-like staining pattern in the dysplastic epithelium, whereas in a squamous cell carcinoma, the pattern may be focal but highlights the abnormal architectural patterns of the invasive tumor. p16 is widely utilized for routine diagnosis of anogenital tissue samples to aid in the diagnosis of HPV-related lesions and is useful for detecting and distinguishing high-grade squamous intraepithelial lesion (HSIL) from its histological mimics [117].

A majority of HPV related vulvar squamous cell carcinomas are reactive with p16 [34, 37, 118], while non-HPV-related tumors are generally nonreactive with p16 [35, 119, 120]. Normal vulvar squamous epithelium, squamous cell hyperplasia, lichen sclerosus, and simplex (differentiated)-type VIN are p16 negative [92].

*TP*53 is a tumor suppressor gene located on chromosome 17 at 17p13. Expression of p53 as detected by immunohistochemistry is thought to be indicative of a stabilizing mutation [121]. *TP*53 gene mutations with loss of heterozygosity at 17p13.1 are more commonly seen in HPV-negative vulvar carcinomas than in HPV-related vulvar carcinomas [49]. *TP*53 missense mutations are p53 positive by immunohistochemistry, while *TP*53 nonsense and deletion mutations may be p53 negative [48]. This is in contrast to

degradation of p53 in the HPV-related pathway (and not mutation), which is p53 negative.

In the setting of HPV-negative vulvar carcinoma, p53 expression is frequently identified but is variably reactive with p53 (55–64.5 %) [120, 122, 123]. Squamous cell carcinoma arising in the setting of LS more frequently expresses p53 than tumors without LS [124]. Rare non-HPV-related vulvar squamous cell carcinomas are positive for p16 [35]. Conversely, rare HPV-related tumors are positive for p53 [120].

Genetics and Molecular Findings

To date, the molecular mechanism of carcinogenesis in the vulva is not well understood. Numerous comparative genomic hybridization (CGH) studies have reported chromosomal abnormalities including: losses in 2p [125], 4p13-pter [125, 126], 3p [125–128], 5q, 6q, 8p [128], 11p [125], 11q, 13q, and 22q [128] and gains at 1q [125], 3q [125, 126, 128, 129], 8p, 8q [125, 129], 11q [128], 14, 17, and 20q [126]. Gains in 3q [129, 130] and 12q have been more commonly identified in HPV-positive tumors, while gains in 8q [129, 130] have been more commonly identified in HPV-negative tumors [129]. Using karyotyping, DNA ploidy analysis, array CGH, and expression arrays one gene, *FHIT* (3p14) was identified that possibly plays a role in carcinogenesis [131]. Evidence of microsatellite instability (MSI) does not appear to play a significant role [132]. Additional work in this area is necessary to elucidate the molecular pathways of vulvar carcinogenesis.

Vulvar squamous cell carcinoma is a rare tumor of the female genital tract but is the most common malignant tumor of the vulva. Unlike the cervix where essentially all squamous cell carcinomas are associated with HPV, vulvar squamous cell carcinoma is a heterogeneous disease, composed of HPV-positive tumors and HPV-negative tumors. Patients with HPV-associated disease tend to be younger and frequently have a history of and/or concurrent usual-type VIN (warty or basaloid types), whereas women with HPV-negative disease are older often with a long-standing history of LS. Simplex (differentiated)-type VIN is frequently observed adjacent to squamous cell carcinoma in women with LS. Warty and basaloid histologic types of squamous cell carcinoma are generally associated with HPV-positive tumors, while keratinizing squamous cell carcinoma is identified in HPV-negative squamous cell carcinoma associated with LS. Immunoperoxidase stains for p16 and p53 can be used to subtype squamous cell carcinomas. HPV-associated tumors are frequently p16 positive and p53 negative. The converse is frequently true of HPV-negative vulvar squamous cell carcinoma. Additional histologic subtypes of squamous cell carcinoma are infrequently identified including: verrucous carcinoma, keratoacanthoma-like squamous cell carcinoma, and sarcomatoid carcinoma. Regardless of the histologic subtype of squamous cell carcinoma, patients are staged and treated the same. Surgery is the first line of therapy and is used to determine stage. Patients with small tumors (<2 cm) and with depth of invasion less than 1 mm tend to have an excellent prognosis. Patients with lymph node metastasis have a worse prognosis.

Summary

Clinical Presentation

- vSCC is a heterogeneous tumor.
- vSCC affects premenopausal and postmenopausal patients.
- Patients may present with vulvar pruritus, pain, discharge, bleeding, dysuria, foul odor, persistent ulcer, or clinically apparent mass.
- Signs and symptoms are nonspecific, often resulting in a delay of diagnosis.

Histologic Features

- Keratinizing squamous cell carcinoma is the most common histologic type and is defined by the presence of keratin (keratin pearls, dyskeratotic cells, and parakeratosis) within the tumor in the

absence of warty/basaloid features and is usually HPV negative.
- Warty/basaloid squamous cell carcinomas are also frequently identified and are associated with HPV.
- Other variants of vSCC include: nonkeratinizing squamous cell carcinoma; verrucous carcinoma; squamous cell carcinoma, keratoacanthoma type, and squamous cell carcinoma with tumor giant cells.

Differential Diagnosis
- Keratinizing squamous cell carcinoma should be distinguished from: VIN, malignant melanoma, metastatic squamous cell carcinoma, keratoacanthoma-like squamous cell carcinoma, pseudoepitheliomatous hyperplasia, and warty dyskeratoma.
- Basaloid squamous cell carcinoma differential diagnosis includes: basal cell carcinoma, small cell carcinoma, and Merkel cell carcinoma.
- Warty squamous cell carcinoma differential diagnosis includes: verrucous carcinoma, keratinizing squamous cell carcinoma, and condyloma acuminatum.

Takeaway Essentials

Clinically Relevant Pearls
- HPV-positive squamous cell carcinoma is generally identified in younger women (35–65 years old) often in association with warty/basaloid (usual)-type VIN.
- HPV-negative squamous cell carcinoma is generally identified in older women (55–85 years old) often in association with LS and differentiated (simplex)-type VIN.
- Signs and symptoms are nonspecific. As a result, patients with atypical vulvar lesions including persistent ulcers should have biopsies for definitive pathological diagnosis.
- Care must be taken to distinguish vSCC from its benign mimics like pseudoepitheliomatous hyperplasia, verruciform xanthoma, and infection (such as herpes simplex virus), among others.
- Lymph node status is the most predictive prognostic factor.
- Recurrences can occur 5 years or more after initial treatment.

Pathology Interpretation Pearls
- The most helpful clues to identify early invasion include: paradoxical squamous maturation, dyskeratosis, keratin pearls, irregular epithelial nests in the dermis, and desmoplastic stromal response.
- Depth of invasion is an important prognostic factor and is measured from the epithelial-stromal junction of the nearest adjacent superficial dermal papilla to the deepest point of stromal invasion.
- Recommended information to be included in the pathology report to guide treatment includes:
  - Specimen type and size
  - Procedure
  - Tumor site, size, thickness, and focality
  - Histologic type and grade
  - Depth of invasion
  - Tumor borders
  - Margin status
  - Lymphatic/vascular invasion
  - Lymph node status.

Immunohistochemical/Molecular Findings
- HPV-positive tumors are usually p16 positive and p53 negative.
- HPV-negative tumors are commonly p16 negative and p53 positive.

Case Vignettes

Vignette 1

Clinical History: A 47-year-old woman with a long-standing history of vulvar and vaginal condylomata presented with a large 7 cm firm white-tan, sessile warty mass on the right labia majora.

Microscopic Description: A biopsy was performed. Microscopically, the tumor has an undulating, papillomatous surface with areas of marked hyperkeratosis (Fig. 10.25). Fibrovascular cores are not identified. Koilocyte-like tumor cells are also not identified in this mass. The tumor is composed of broad, bulbous nests of squamous epithelium with prominent acanthosis (Fig. 10.26). The tumor nests have a pushing border rather than overtly invasive border with the underlying dermis, which contains an inflammatory infiltrate. The tumor cells contain brightly eosinophilic cytoplasm and have minimal cytologic atypia with some of the nuclei containing conspicuous nucleoli (Fig. 10.27). Mitotic figures are not identified. The differential diagnosis includes: keratinizing squamous cell carcinoma, warty squamous cell carcinoma, condyloma acuminatum, and verrucous carcinoma.

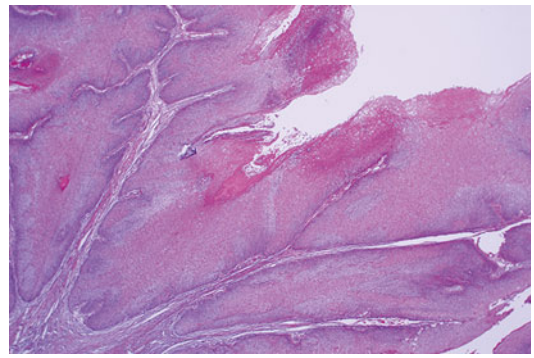


Fig. 10.25 Vignette 1. This 47-year-old woman presented with a warty vulvar mass that has an undulating papillomatous surface with areas of hyperkeratosis

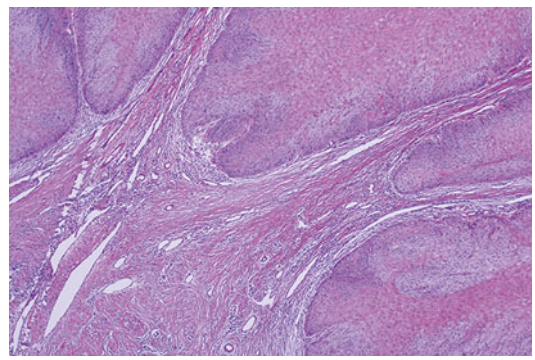


Fig. 10.26 Vignette 1. The tumor is composed of broad, bulbous nests of well-differentiated squamous epithelium

(continued)

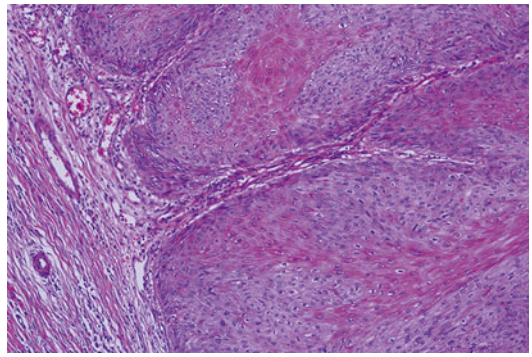


Fig. 10.27 Vignette 1. The tumor-dermal interface has a pushing border, and there is a mild dermal inflammatory infiltrate. The cells have minimal cytologic atypia and brightly eosinophilic cytoplasm

Diagnosis: The clinical and microscopic features in this case are diagnostic of verrucous carcinoma.

Discussion: Keratinizing squamous cell carcinoma is commonly identified in older women with a long-standing history of LS; these tumors can present as papillomatous or exophytic lesions. Unlike verrucous carcinomas, keratinizing squamous cell carcinomas have overtly invasive nests of atypical squamous epithelium, characterized by irregular nests, single cell patterns, and stromal desmoplasia, whereas verrucous carcinoma invades the underlying dermis in broad, bulbous squamous nests with minimal cytologic atypia both throughout the tumor and at the leading invasive edge. Cytologic atypia is readily identified as are mitotic figures in keratinizing squamous cell carcinomas. The presence of keratin pearls and dyskeratotic cells is also helpful in distinguishing the two tumors. While verrucous carcinomas may have marked hyperkeratosis and parakeratosis, keratin pearls are generally not seen in verrucous carcinoma.

Warty squamous cell carcinoma and condyloma acuminatum are both HPV-related lesions with papillary gross and microscopic architecture. Both display fibrovascular cores lined by abnormal squamous epithelium. Verrucous carcinoma does not have true microscopic papillary architecture. Warty squamous cell carcinoma is composed of malignant squamous cells including koilocyte-like malignant cells, and at the tumor-dermal interface, irregular nests of squamous epithelium are seen. Koilocyte-like cells are not identified in verrucous carcinoma, and the tumor cells are extremely well differentiated with very minimal cytologic atypia. Condyloma acuminatum is an exophytic benign neoplasm with complex papillary architecture lined by a dysplastic squamous epithelium containing koilocytes. No stromal invasion is identified.

Complete excision of the mass was subsequently performed. The patient has done well and is without recurrent disease.

Vignette 2

Clinical History: A 72-year-old woman presented with a persistent pruritus on the right labia majora. Physical examination showed a pale, slightly raised plaque on the labia.

Microscopic Description: A punch biopsy was performed (Fig. 10.28). Sections show an abnormal basaloid proliferation extending from the squamous epithelium into the underlying dermis. The tumor nests have peripheral palisading of the nuclei and are composed of small cells with a high N/C ratio (Fig. 10.29). A cleft-like space intervenes focally between the tumor nest and the surrounding dermis, so-called retraction artifact. The differential diagnosis includes: basal cell carcinoma, basaloid squamous cell carcinoma, small cell carcinoma, and Merkel cell carcinoma.

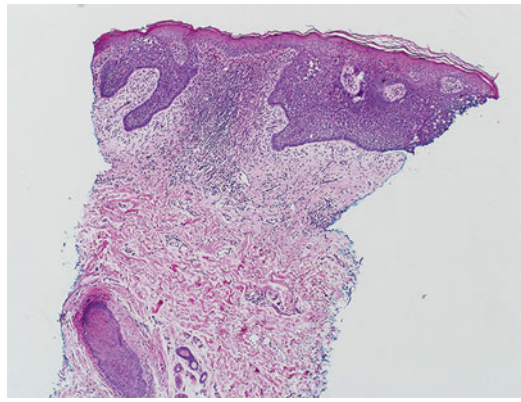


Fig. 10.28 Vignette 2. A punch biopsy was performed in this 72-year-old woman who presented with vulvar pruritis

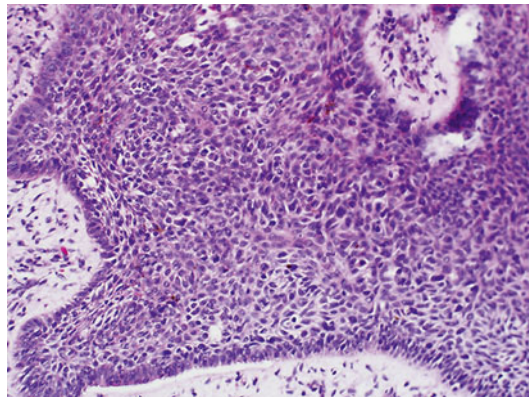


Fig. 10.29 Vignette 2. The tumor is composed of nests of basaloid tumor cells with peripheral palisading and focal retraction artifact in the adjacent stroma

Diagnosis: The clinical and microscopic features in this case are diagnostic of basal cell carcinoma.

Discussion: Basal cell carcinoma is rarely identified on the vulva and is not associated with VIN-like basaloid squamous cell carcinoma. It is frequently seen in older women. Basaloid squamous cell carcinoma is a squamous cell carcinoma composed of basaloid-type malignant cells eliciting

(continued)

the usual stromal desmoplastic response. The tumor nests of basaloid squamous cell carcinoma lack the characteristic peripheral nuclear palisading seen in BCC. In addition, retraction artifact and gray-blue stroma are not generally identified in basaloid squamous cell carcinoma. Immunoperoxidase stains for BerEP4 and p16 may be helpful in differentiating a BCC from a basaloid squamous cell carcinoma. BerEP4 can be reactive in both BCC and basaloid squamous cell carcinoma but is generally positive in BCC. Basaloid squamous cell carcinomas are diffusely and strongly reactive with p16, whereas BCC shows patchy p16 reactivity. For difficult cases, HPV in situ hybridization for high-risk HPV viral types can be helpful as basaloid squamous cell carcinoma is HPV positive. Both small cell carcinoma and Merkel cell carcinoma are high grade, aggressive neuroendocrine carcinomas, and morphologically similar and distinct from that of BCC. They are characterized by malignant cells with a high N/C ratio, stippled chromatin, nuclear molding, and crush artifact. Mitotic figures and apoptotic debris are readily identified. Immunoperoxidase stains can also be used to distinguish these entities from BCC (Table 10.7).

Complete excision of the lesion was subsequently performed. The patient has done well and is without recurrences.

Vignette 3

Clinical History: A 44-year-old asymptomatic woman with a history of VIN presented for routine follow-up. Colposcopic examination of the vulva revealed focal abnormal acetowhite epithelium suspicious clinically for a high-grade squamous intraepithelial lesion (HSIL).

Microscopic Description: A biopsy was taken (Fig. 10.30). Sections show several irregularly shaped nests of squamous epithelium in a desmoplastic stroma with associated inflammatory infiltrate underlying VIN3/HSIL. The squamous epithelium within the nests has eosinophilic cytoplasm and minimal cytologic atypia. The differential diagnosis includes VIN 3/HSIL versus a superficially invasive squamous cell carcinoma.

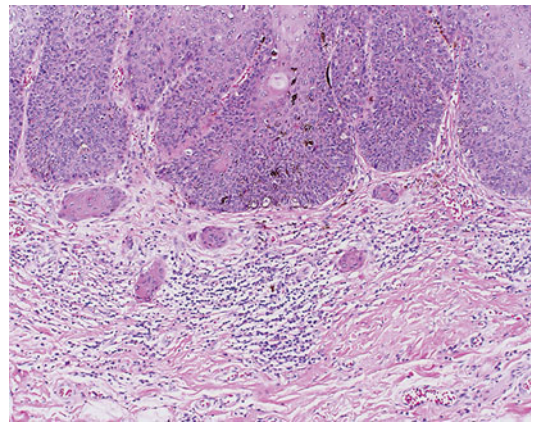


Fig. 10.30 Vignette 3. A 44-year-old woman with a history of VIN was found to have a new acetowhite area. Abnormal nests of squamous epithelium with minimal cytologic atypia and eosinophilic cytoplasm are identified in a desmoplastic stroma with associated inflammatory infiltrate and underlie an abnormal epithelium with VIN3/HSIL

(continued)

Diagnosis: The microscopic features are diagnostic of a superficially invasive squamous cell carcinoma.

Discussion: Differentiating between VIN and a superficially invasive squamous cell carcinoma can be challenging. The dermal-epidermal junction of normal and abnormal vulva can have an undulating appearance complicating the distinction between an elongated rete ridge versus a microscopic focus of invasive squamous cell carcinoma. Table 10.6 outlines several histologic findings that favor a diagnosis of invasion. In this case, the invasive tumor nests have paradoxical maturation and appear in a desmoplastic stroma with associated inflammatory infiltrate, supporting the diagnosis.

This patient had complete excision of the lesion with sentinel lymph node biopsy, which was negative. She has since had recurrent VIN but no other foci of invasive squamous cell carcinoma to date.

Abbreviations

AJCC	American Joint Commission on Cancer
BCC	Basal cell carcinoma
CAP	College of American Pathologists
CGH	Comparative genomic hybridization
EMA	Epithelial membrane antigen
FIGO	International Federation of Gynecology and Obstetrics
GOG	Gynecology Oncology Group
H&E	Hematoxylin and eosin
HMB-45	Human melanoma black-45
HPV	Human papilloma virus
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes simplex virus
KA	Keratoacanthoma
MSI	Microsatellite instability
LS	Lichen sclerosus
N/C	Nucleus to cytoplasm ratio
PEH	Pseudoepitheliomatous hyperplasia
Rb	Retinoblastoma
SCC	Squamous cell carcinoma
SMA	Smooth muscle actin
TNM	Tumor-Node-Metastasis
TTF-1	Thyroid transcription factor-1
uVIN	Usual vulvar intraepithelial neoplasia
VAAD	Vulvar acanthosis with altered differentiation
VIN	Vulvar intraepithelial neoplasia
vSCC	Vulvar squamous cell carcinoma
WHO	World Health Organization

References

1. Ansink AC, Heintz AP. Epidemiology and etiology of squamous cell carcinoma of the vulva. Eur J Obstet Gynecol Reprod Biol. 1993;48(2):111–5.
2. American Cancer Society. Cancer facts and figures 2013. American Cancer Society: Atlanta; 2013.
3. Lanneau GS, Argenta PA, Lanneau MS, Riffenburgh RH, Gold MA, McMeekin DS, et al. Vulvar cancer in young women: demographic features and outcome evaluation. Am J Obstet Gynecol. 2009;200(6):645. e1-5.
4. van de Nieuwenhof HP, van der Avoort IA, de Hullu JA. Review of squamous premalignant vulvar lesions. Crit Rev Oncol Hematol. 2008;68(2):131–56.
5. Hampl M, Deckers-Figiel S, Hampl JA, Rein D, Bender HG. New aspects of vulvar cancer: changes in localization and age of onset. Gynecol Oncol. 2008;109(3):340–5.
6. Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. Obstet Gynecol. 2006;107(5):1018–22.
7. Saraiya M, Watson M, Wu X, King JB, Chen VW, Smith JS, et al. Incidence of in situ and invasive vulvar cancer in the US, 1998–2003. Cancer. 2008;113 Suppl 10:2865–72.

8. Rauh-Hain JA, Clemmer J, Clark RM, Bradford LS, Growdon WB, Goodman A, et al. Racial disparities and changes in clinical characteristics and survival for vulvar cancer over time. Am J Obstet Gynecol. 2013;209(5):468. e1–e10.
9. Madsen BS, Jensen HL, van den Brule AJ, Wohlfahrt J, Frisch M. Risk factors for invasive squamous cell carcinoma of the vulva and vagina–population-based case-control study in Denmark. Int J Cancer. 2008;122(12):2827–34.
10. Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. Obstet Gynecol. 2005; 106(6):1319–26.
11. McNally OM, Mulvany NJ, Pagano R, Quinn MA, Rome RM. VIN 3: a clinicopathologic review. Int J Gynecol Cancer. 2002;12(5):490–5.
12. Ueda Y, Enomoto T, Kimura T, Yoshino K, Fujita M, Kimura T. Two distinct pathways to development of squamous cell carcinoma of the vulva. J Skin Cancer. 2011;2011:951250.
13. Madeleine MM, Daling JR, Carter JJ, Wipf GC, Schwartz SM, McKnight B, et al. Cofactors with human papillomavirus in a population-based study of vulvar cancer. J Natl Cancer Inst. 1997;89(20): 1516–23.
14. Daling JR, Sherman KJ, Hislop TG, Maden C, Mandelson MT, Beckmann AM, et al. Cigarette smoking and the risk of anogenital cancer. Am J Epidemiol. 1992;135(2):180–9.
15. Friedrich Jr EG. Vulvar dystrophy. Clin Obstet Gynecol. 1985;28(1):178–87.
16. Hart WR, Norris HJ, Helwig EB. Relation of lichen sclerosus et atrophicus of the vulva to development of carcinoma. Obstet Gynecol. 1975;45(4):369–77.
17. Maclean AB. Vulval cancer: prevention and screening. Best Pract Res Clin Obstet Gynaecol. 2006; 20(2):379–95.
18. Meffert JJ, Davis BM, Grimwood RE. Lichen sclerosus. J Am Acad Dermatol. 1995;32(3):393–416.
19. Carli P, Cattaneo A, De Magnis A, Biggeri A, Taddei G, Giannotti B. Squamous cell carcinoma arising in vulval lichen sclerosus: a longitudinal cohort study. Eur J Cancer Prev. 1995;4(6):491–5.
20. Jones RW, Sadler L, Grant S, Whineray J, Exeter M, Rowan D. Clinically identifying women with vulvar lichen sclerosus at increased risk of squamous cell carcinoma: a case-control study. J Reprod Med. 2004;49(10):808–11.
21. van de Nieuwenhof HP, Massuger LF, van der Avoort IA, Bekkers RL, Casparie M, Abma W, et al. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur J Cancer. 2009;45(5):851–6.
22. Spencer RJ, Young RH, Goodman A. The risk of squamous cell carcinoma in persistent vulvar ulcers. Menopause. 2011;18(10):1067–71.
23. DiSaia PJ, Creasman WT, editors. Clinical gynecologic oncology. 7th ed. Philadelphia: Mosby/ Elsevier; 2007.
24. De Cicco C, Sideri M, Bartolomei M, Grana C, Cremonesi M, Fiorenza M, et al. Sentinel node biopsy in early vulvar cancer. Br J Cancer. 2000; 82(2):295–9.
25. Oonk MH, van Hemel BM, Hollema H, de Hullu JA, Ansink AC, Vergote I, et al. Size of sentinel-node metastasis and chances of non-sentinel-node involvement and survival in early stage vulvar cancer: results from GROINSS-V, a multicentre observational study. Lancet Oncol. 2010;11(7):646–52.
26. Van der Zee AG, Oonk MH, De Hullu JA, Ansink AC, Vergote I, Verheijen RH, et al. Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. J Clin Oncol. 2008;26(6):884–9.
27. Woelber L, Trillsch F, Kock L, Grimm D, Petersen C, Choschzick M, et al. Management of patients with vulvar cancer: a perspective review according to tumour stage. Ther Adv Med Oncol. 2013; 5(3):183–92.
28. van Seters M, van Beurden M, de Craen AJ. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol. 2005;97(2):645–51.
29. de Sanjose S, Alemany L, Ordi J, Tous S, Alejo M, Bigby SM, et al. Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. Eur J Cancer. 2013;49(16):3450–61.
30. Kim YT, Thomas NF, Kessis TD, Wilkinson EJ, Hedrick L, Cho KR. p53 mutations and clonality in vulvar carcinomas and squamous hyperplasias: evidence suggesting that squamous hyperplasias do not serve as direct precursors of human papillomavirus-negative vulvar carcinomas. Hum Pathol. 1996;27(4):389–95.
31. Monk BJ, Burger RA, Lin F, Parham G, Vasilev SA, Wilczynski SP. Prognostic significance of human papillomavirus DNA in vulvar carcinoma. Obstet Gynecol. 1995;85(5 Pt 1):709–15.
32. Carter JJ, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC, et al. Human papillomavirus 16 and 18L1 serology compared across anogenital cancer sites. Cancer Res. 2001;61(5): 1934–40.
33. Pinto AP, Lin MC, Sheets EE, Muto MG, Sun D, Crum CP. Allelic imbalance in lichen sclerosus, hyperplasia, and intraepithelial neoplasia of the vulva. Gynecol Oncol. 2000;77(1):171–6.
34. Riethdorf S, Neffen EF, Cviko A, Loning T, Crum CP, Riethdorf L. p16INK4A expression as biomarker for HPV 16-related vulvar neoplasias. Hum Pathol. 2004;35(12):1477–83.
35. Alonso I, Fuste V, del Pino M, Castillo P, Torne A, Fuste P, et al. Does human papillomavirus infection imply a different prognosis in vulvar squamous cell carcinoma? Gynecol Oncol. 2011;122(3):509–14.
36. Kowalewska M, Szkoda MT, Radziszewski J, Ptaszynski K, Bidzinski M, Siedlecki JA. The frequency of human papillomavirus infection in polish

patients with vulvar squamous cell carcinoma. Int J Gynecol Cancer. 2010;20(3):434–7.
37. van de Nieuwenhof HP, van Kempen LC, de Hullu JA, Bekkers RL, Bulten J, Melchers WJ, et al. The etiologic role of HPV in vulvar squamous cell carcinoma fine tuned. Cancer Epidemiol Biomarkers Prev. 2009;18(7):2061–7.
38. Gargano JW, Wilkinson EJ, Unger ER, Steinau M, Watson M, Huang Y, et al. Prevalence of human papillomavirus types in invasive vulvar cancers and vulvar intraepithelial neoplasia 3 in the United States before vaccine introduction. J Low Genit Tract Dis. 2012;16(4):471–9.
39. Sutton BC, Allen RA, Moore WE, Dunn ST. Distribution of human papillomavirus genotypes in invasive squamous carcinoma of the vulva. Mod Pathol. 2008;21(3):345–54.
40. Tsimplaki E, Argyri E, Michala L, Kouvousi M, Apostolaki A, Magiakos G, et al. Human papillomavirus genotyping and e6/e7 mRNA expression in Greek women with intraepithelial neoplasia and squamous cell carcinoma of the vagina and vulva. J Oncol. 2012;2012:893275.
41. Bonvicini F, Venturoli S, Ambretti S, Paterini P, Santini D, Ceccarelli C, et al. Presence and type of oncogenic human papillomavirus in classic and in differentiated vulvar intraepithelial neoplasia and keratinizing vulvar squamous cell carcinoma. J Med Virol. 2005;77(1):102–6.
42. Liegl B, Regauer S. p53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). Histopathology. 2006;48(3):268–74.
43. van de Nieuwenhof HP, Bulten J, Hollema H, Dommerholt RG, Massuger LF, van der Zee AG, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. Mod Pathol. 2011;24(2):297–305.
44. Carlson JA, Ambros R, Malfetano J, Ross J, Grabowski R, Lamb P, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. Hum Pathol. 1998;29(9):932–48.
45. Jones RW, Scurry J, Neill S, MacLean AB. Guidelines for the follow-up of women with vulvar lichen sclerosus in specialist clinics. Am J Obstet Gynecol. 2008;198(5):496. e1–3.
46. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. Am J Surg Pathol. 2000;24(3):429–41.
47. Hantschmann P, Sterzer S, Jeschke U, Friese K. p53 expression in vulvar carcinoma, vulvar intraepithelial neoplasia, squamous cell hyperplasia and lichen sclerosus. Anticancer Res. 2005;25(3A):1739–45.
48. Pinto AP, Miron A, Yassin Y, Monte N, Woo TY, Mehra KK, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. Mod Pathol. 2010;23(3):404–12.
49. Flowers LC, Wistuba II, Scurry J, Muller CY, Ashfaq R, Miller DS, et al. Genetic changes during the multistage pathogenesis of human papillomavirus positive and negative vulvar carcinomas. J Soc Gynecol Investig. 1999;6(4):213–21.
50. Carlson BC, Hofer MD, Ballek N, Yang XJ, Meeks JJ, Gonzalez CM. Protein markers of malignant potential in penile and vulvar lichen sclerosus. J Urol. 2013;190(2):399–406.
51. Brustmann H, Hinterholzer S, Brunner A. Immunohistochemical expression of survivin and gamma-H2AX in vulvar intraepithelial neoplasia and low-stage squamous cell carcinoma. Int J Gynecol Pathol. 2011;30(6):583–90.
52. Homesley HD, Bundy BN, Sedlis A, Yordan E, Berek JS, Jahshan A, et al. Assessment of current International Federation of Gynecology and Obstetrics staging of vulvar carcinoma relative to prognostic factors for survival (a Gynecologic Oncology Group study). Am J Obstet Gynecol. 1991;164(4):997–1003.
53. Hacker NF. Surgery for gynaecological cancer: results since the introduction of radical operations. Aust N Z J Obstet Gynaecol. 1990;30(1):24–8.
54. Woelber L, Eulenburg C, Choschzick M, Kruell A, Petersen C, Gieseking F, et al. Prognostic role of lymph node metastases in vulvar cancer and implications for adjuvant treatment. Int J Gynecol Cancer. 2012;22(3):503–8.
55. Edge S, Fritz A, Byrd D, Greene F, Compton C, Trotti A, editors. AAJCC cancer staging handbook. 7th ed. New York: Springer; 2010.
56. National Cancer Institute. [cited 2014 April 12, 2014]. Available from: http://seer.cancer.gov/statfacts/html/vulva.html
57. Kurman RJ, Ronnett BM, editors. Tumors of the cervix, vagina, and vulva. 1st ed. Silver Spring: ARP Press; 2011.
58. Drew PA, al-Abbadi MA, Orlando CA, Hendricks JB, Kubilis PS, Wilkinson EJ. Prognostic factors in carcinoma of the vulva: a clinicopathologic and DNA flow cytometric study. Int J Gynecol Pathol. 1996;15(3):235–41.
59. van der Velden J, van Lindert AC, Lammes FB, ten Kate FJ, Sie-Go DM, Oosting H, et al. Extracapsular growth of lymph node metastases in squamous cell carcinoma of the vulva. The impact on recurrence and survival. Cancer. 1995;75(12):2885–90.
60. Origoni M, Sideri M, Garsia S, Carinelli SG, Ferrari AG. Prognostic value of pathological patterns of lymph node positivity in squamous cell carcinoma of the vulva stage III and IVA FIGO. Gynecol Oncol. 1992;45(3):313–6.
61. Raspagliesi F, Hanozet F, Ditto A, Solima E, Zanaboni F, Vecchione F, et al. Clinical and pathological prognostic factors in squamous cell carcinoma of the vulva. Gynecol Oncol. 2006;102(2):333–7.
62. Fons G, Hyde SE, Buist MR, Schilthuis MS, Grant P, Burger MP, et al. Prognostic value of bilateral positive

nodes in squamous cell cancer of the vulva. Int J Gynecol Cancer. 2009;19(7):1276–80.
63. Oonk MH, de Hullu JA, Hollema H, Mourits MJ, Pras E, Wymenga AN, et al. The value of routine follow-up in patients treated for carcinoma of the vulva. Cancer. 2003;98(12):2624–9.
64. Preti M, Ronco G, Ghiringhello B, Micheletti L. Recurrent squamous cell carcinoma of the vulva: clinicopathologic determinants identifying low risk patients. Cancer. 2000;88(8):1869–76.
65. Woolderink JM, de Bock GH, de Hullu JA, Davy MJ, van der Zee AG, Mourits MJ. Patterns and frequency of recurrences of squamous cell carcinoma of the vulva. Gynecol Oncol. 2006;103(1):293–9.
66. Wilkinson EJ. Protocol for the examination of specimens from patients with carcinomas and malignant melanomas of the vulva: a basis for checklists Cancer Committee of the American College of Pathologists. Arch Pathol Lab Med. 2000; 124(1):51–6.
67. Tavassoli FA, Devilee P, editors. Pathology and genetics of tumours of the breast and female genital organs. 1st ed. Lyon: IARC; 2003.
68. Abdel-Mesih A, Daya D, Onuma K, Sur M, Tang S, Akhtar-Danesh N, et al. Interobserver agreement for assessing invasion in stage 1A vulvar squamous cell carcinoma. Am J Surg Pathol. 2013;37(9):1336–41.
69. Irvin Jr WP, Legallo RL, Stoler MH, Rice LW, Taylor Jr PT, Andersen WA. Vulvar melanoma: a retrospective analysis and literature review. Gynecol Oncol. 2001;83(3):457–65.
70. Baderca F, Cojocaru S, Lazar E, Lazureanu C, Lighezan R, Alexa A, et al. Amelanotic vulvar melanoma: case report and review of the literature. Rom J Morphol Embryol. 2008;49(2):219–28.
71. Chen W, Koenig C. Vulvar keratoacanthoma: a report of two cases. Int J Gynecol Pathol. 2004; 23(3):284–6.
72. Ozkan F, Bilgic R, Cesur S. Vulvar keratoacanthoma. APMIS. 2006;114(7–8):562–5.
73. Konanahalli P, Menon P, Walsh MY, McCluggage WG. *Enterobious vermicularis* (pinworm) infestation of the vulva: report of 2 cases of a pseudoneoplastic lesion mimicking squamous carcinoma. Int J Gynecol Pathol. 2010;29(5):490–3.
74. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. Int J Gynecol Pathol. 2003;22(1):57–62.
75. Frimer M, Chudnoff S, Hebert T, Shahabi S. Pseudoepitheliomatous hyperplasia mimicking vulvar cancer in a patient with AIDS. J Low Genit Tract Dis. 2011;15(1):66–8.
76. do Amaral RL, Giraldo PC, Cursino K, Goncalves AK, Eleuterio Jr J, Giraldo H. Nodular vulvar herpes in an HIV-positive woman. Int J Gynecol Obstet. 2009;107(3):255.
77. Domfeh AB, Silasi DA, Lindo F, Parkash V. Chronic hypertrophic vulvar herpes simulating neoplasia. Int J Gynecol Pathol. 2012;31(1):33–7.
78. Mosunjac M, Park J, Wang W, Tadros T, Siddiqui M, Bagirov M, et al. Genital and perianal herpes simplex simulating neoplasia in patients with AIDS. AIDS Patient Care STDS. 2009;23(3):153–8. doi:10.1089/apc.2008.0143.
79. Ranu H, Lee J, Chio M, Sen P. Tumour-like presentations of anogenital herpes simplex in HIV-positive patients. Int J STD AIDS. 2011;22(4):181–6.
80. Duray PH, Merino MJ, Axiotis C. Warty dyskeratoma of the vulva. Int J Gynecol Pathol. 1983;2(3):286–93.
81. Steeper TA, Piscioli F, Rosai J. Squamous cell carcinoma with sarcoma-like stroma of the female genital tract. Clinicopathologic study of four cases. Cancer. 1983;52(5):890–8.
82. Bigby SM, Eva LJ, Jones RW. Spindle cell carcinoma of the vulva: a series of 4 cases and review of the literature. Int J Gynecol Pathol. 2014;33(2): 203–12.
83. Choi DS, Lee JW, Lee SJ, Choi CH, Kim TJ, Lee JH, et al. Squamous cell carcinoma with sarcomatoid features of the vulva: a case report and review of literature. Gynecol Oncol. 2006;103(1):363–7.
84. Cooper WA, Valmadre S, Russell P. Sarcomatoid squamous cell carcinoma of the vulva. Pathology. 2002;34(2):197–9.
85. Santeusanio G, Schiaroli S, Anemona L, Sesti F, Valli E, Piccione E, et al. Carcinoma of the vulva with sarcomatoid features: a case report with immunohistochemical study. Gynecol Oncol. 1991;40(2): 160–3.
86. Parham DM, Morton K, Robertson AJ, Philip WD. The changing phenotypic appearance of a malignant vulval neoplasm containing both carcinomatous and sarcomatous elements. Histopathology. 1991;19(3):263–8.
87. Hasegawa T, Matsuno Y, Shimoda T, Umeda T, Yokoyama R, Hirohashi S. Proximal-type epithelioid sarcoma: a clinicopathologic study of 20 cases. Mod Pathol. 2001;14(7):655–63.
88. Kurman RJ, Toki T, Schiffman MH. Basaloid and warty carcinomas of the vulva. Distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses. Am J Surg Pathol. 1993;17(2):133–45.
89. Feakins RM, Lowe DG. Basal cell carcinoma of the vulva: a clinicopathologic study of 45 cases. Int J Gynecol Pathol. 1997;16(4):319–24.
90. Linskey KR, Gimbel DC, Zukerberg LR, Duncan LM, Sadow PM, Nazarian RM. BerEp4, cytokeratin 14, and cytokeratin 17 immunohistochemical staining aid in differentiation of basaloid squamous cell carcinoma from basal cell carcinoma with squamous metaplasia. Arch Pathol Lab Med. 2013;137(11):1591–8.
91. Elwood H, Kim J, Yemelyanova A, Ronnett BM, Taube JM. Basal cell carcinomas of the vulva: high-risk human papillomavirus DNA detection, p16 and BerEP4 expression. Am J Surg Pathol. 2014;38(4): 542–7.

92. Santos M, Montagut C, Mellado B, Garcia A, Ramon y Cajal S, Cardesa A, et al. Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. Int J Gynecol Pathol. 2004;23(3):206–14.
93. Jones MA, Mann EW, Caldwell CL, Tarraza HM, Dickersin GR, Young RH. Small cell neuroendocrine carcinoma of Bartholin's gland. Am J Clin Pathol. 1990;94(4):439–42.
94. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol. 2010;37(1):20–7.
95. Eichhorn JH, Young RH. Neuroendocrine tumors of the genital tract. Am J Clin Pathol. 2001; 115(Suppl):S94–112.
96. Gualco M, Bonin S, Foglia G, Fulcheri E, Odicino F, Prefumo F, et al. Morphologic and biologic studies on ten cases of verrucous carcinoma of the vulva supporting the theory of a discrete clinico-pathologic entity. Int J Gynecol Cancer. 2003;13(3):317–24.
97. Crowther ME, Lowe DG, Shepherd JH. Verrucous carcinoma of the female genital tract: a review. Obstet Gynecol Surv. 1988;43(5):263–80.
98. Dvoretsky PM, Bonfiglio TA. The pathology of vulvar squamous cell carcinoma and verrucous carcinoma. Pathol Annu. 1986;21(Pt 2):23–45.
99. Nascimento AF, Granter SR, Cviko A, Yuan L, Hecht JL, Crum CP. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinoma? Am J Surg Pathol. 2004;28(5):638–43.
100. de Rosa G, Barra E, Gentile R, Boscaino A, Di Prisco B, Ayala F. Verruciform xanthoma of the vulva: case report. Genitourin Med. 1989;65(4):252–4.
101. Fite C, Plantier F, Dupin N, Avril MF, Moyal-Barracco M. Vulvar verruciform xanthoma: ten cases associated with lichen sclerosus, lichen planus, or other conditions. Arch Dermatol. 2011;147(9):1087–92.
102. Frankel MA, Rhodes HE, Euscher ED. Verruciform xanthoma in an adolescent: a case report. J Low Genit Tract Dis. 2012;16(1):70–4.
103. Leong FJ, Meredith DJ. Verruciform xanthoma of the vulva. A case report. Pathol Res Pract. 1998;194(9):661–5.
104. Reich O, Regauer S. Recurrent verruciform xanthoma of the vulva. Int J Gynecol Pathol. 2004;23(1):75–7.
105. Santa Cruz DJ, Martin SA. Verruciform xanthoma of the vulva. Report of two cases. Am J Clin Pathol. 1979;71(2):224–8.
106. Andreasson B, Bock JE, Strom KV, Visfeldt J. Verrucous carcinoma of the vulval region. Acta Obstet Gynecol Scand. 1983;62(2):183–6.
107. Godbolt AM, Sullivan JJ, Weedon D. Keratoacanthoma with perineural invasion: a report of 40 cases. Australas J Dermatol. 2001;42(3):168–71.
108. Beham A, Regauer S, Soyer HP, Beham-Schmid C. Keratoacanthoma: a clinically distinct variant of well differentiated squamous cell carcinoma. Adv Anat Pathol. 1998;5(5):269–80.
109. Hodak E, Jones RE, Ackerman AB. Solitary keratoacanthoma is a squamous-cell carcinoma: three examples with metastases. Am J Dermatopathol. 1993;15(4):332–42.
110. Wilkinson EJ, Croker BP, Friedrich Jr EG, Franzini DA. Two distinct pathologic types of giant cell tumor of the vulva. A report of two cases. J Reprod Med. 1988;33(6):519–22.
111. Kabulski Z, Frankman O. Histologic malignancy grading in invasive squamous cell carcinoma of the vulva. Int J Gynecol Obstet. 1978;16(3):233–7.
112. Homesley HD, Bundy BN, Sedlis A, Yordan E, Berek JS, Jahshan A, et al. Prognostic factors for groin node metastasis in squamous cell carcinoma of the vulva (a Gynecologic Oncology Group study). Gynecol Oncol. 1993;49(3):279–83.
113. Ueda Y, Enomoto T, Miyatake T, Ozaki K, Yoshizaki T, Kanao H, et al. Monoclonal expansion with integration of high-risk type human papillomaviruses is an initial step for cervical carcinogenesis: association of clonal status and human papillomavirus infection with clinical outcome in cervical intraepithelial neoplasia. Lab Invest. 2003;83(10):1517–27.
114. Ueda Y, Enomoto T, Miyatake T, Shroyer KR, Yoshizaki T, Kanao H, et al. Analysis of clonality and HPV infection in benign, hyperplastic, premalignant, and malignant lesions of the vulvar mucosa. Am J Clin Pathol. 2004;122(2):266–74.
115. Kanao H, Enomoto T, Ueda Y, Fujita M, Nakashima R, Ueno Y, et al. Correlation between p14(ARF)/p16(INK4A) expression and HPV infection in uterine cervical cancer. Cancer Lett. 2004;213(1):31–7.
116. Sano T, Masuda N, Oyama T, Nakajima T. Overexpression of p16 and p14ARF is associated with human papillomavirus infection in cervical squamous cell carcinoma and dysplasia. Pathol Int. 2002;52(5–6):375–83.
117. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol. 2013;32(1):76–115.
118. Rufforny I, Wilkinson EJ, Liu C, Zhu H, Buteral M, Massoll NA. Human papillomavirus infection and p16(INK4a) protein expression in vulvar intraepithelial neoplasia and invasive squamous cell carcinoma. J Low Genit Tract Dis. 2005;9(2):108–13.
119. Hoevenaars BM, van der Avoort IA, de Wilde PC, Massuger LF, Melchers WJ, de Hullu JA, et al. A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell carcinoma. Int J Cancer. 2008;123(12):2767–73.
120. Santos M, Landolfi S, Olivella A, Lloveras B, Klaustermeier J, Suarez H, et al. p16 overexpression identifies HPV-positive vulvar squamous cell carcinomas. Am J Surg Pathol. 2006;30(11):1347–56.

121. May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene. 1999;18(53):7621–36.
122. Kagie MJ, Kenter GG, Tollenaar RA, Hermans J, Trimbos JB, Fleuren GJ. p53 protein overexpression is common and independent of human papillomavirus infection in squamous cell carcinoma of the vulva. Cancer. 1997;80(7):1228–33.
123. Scheistroen M, Trope C, Pettersen EO, Nesland JM. p53 protein expression in squamous cell carcinoma of the vulva. Cancer. 1999;85(5):1133–8.
124. Carlson JA, Lamb P, Malfetano J, Ambros RA, Mihm Jr MC. Clinicopathologic comparison of vulvar and extragenital lichen sclerosus: histologic variants, evolving lesions, and etiology of 141 cases. Mod Pathol. 1998;11(9):844–54.
125. Huang FY, Kwok YK, Lau ET, Tang MH, Ng TY, Ngan HY. Genetic abnormalities and HPV status in cervical and vulvar squamous cell carcinomas. Cancer Genet Cytogenet. 2005;157(1):42–8.
126. Jee KJ, Kim YT, Kim KR, Kim HS, Yan A, Knuutila S. Loss in 3p and 4p and gain of 3q are concomitant aberrations in squamous cell carcinoma of the vulva. Mod Pathol. 2001;14(5):377–81.
127. Raitanen M, Worsham MJ, Lakkala T, Carey TE, Van Dyke DL, Grenman R, et al. Characterization of 10 vulvar carcinoma cell lines by karyotyping, comparative genomic hybridization and flow cytometry. Gynecol Oncol. 2004;93(1):155–63.
128. Worsham MJ, Van Dyke DL, Grenman SE, Grenman R, Hopkins MP, Roberts JA, et al. Consistent chromosome abnormalities in squamous cell carcinoma of the vulva. Genes Chromosomes Cancer. 1991;3(6):420–32.
129. Allen DG, Hutchins AM, Hammet F, White DJ, Scurry JP, Tabrizi SN, et al. Genetic aberrations detected by comparative genomic hybridisation in vulvar cancers. Br J Cancer. 2002;86(6):924–8.
130. Yangling O, Shulang Z, Rongli C, Bo L, Lili C, Xin W. Genetic imbalance and human papillomavirus states in vulvar squamous cell carcinomas. Eur J Gynaecol Oncol. 2007;28(6):442–6.
131. Micci F, Panagopoulos I, Haugom L, Dahlback HS, Pretorius ME, Davidson B, et al. Genomic aberration patterns and expression profiles of squamous cell carcinomas of the vulva. Genes Chromosomes Cancer. 2013;52(6):551–63.
132. Bujko M, Kowalewska M, Zub R, Radziszewski J, Bidzinski M, Siedlecki JA. Lack of microsatellite instability in squamous cell vulvar carcinoma. Acta Obstet Gynecol Scand. 2012;91(3):391–4.

Part V

Cysts, Glandular Lesions, and Anogenital Mammary-Like Lesions of the Vulva

11 Lesions of Anogenital Mammary-Like Glands, Adnexal Neoplasms, and Metastases

Mai P. Hoang and Dmitry V. Kazakov

Introduction

The vulva contains a wide range of constituents including adnexal structures as seen in nongenital skin (e.g., apocrine and eccrine glands and folliculosebaceous units) and glands only seen in the anogenital region (e.g., anogenital mammary-like glands, major and minor vestibular glands). The modified mucous membrane of the labia minora contains apocrine glands and ectopic sebaceous glands. Other glandular elements that are present in the vulva include the Skene glands (paraurethral glands), the major vestibular glands or Bartholin glands (mucus-producing vestibular glands), and minor vestibular glands [1]. Mammary-type tissue in the vulva was first described by Hartung in 1892 [2]. Previously thought to represent ectopic breast tissue, anogenital mammary-like glands are now regarded by many as normal structures of the anogenital region. They are typically found in the sulcus between the labia minora and majora and extend through the perineum to the anal region [2, 3]. The superficial portion of the gland's excretory duct possesses an outer myoepithelial layer and transitional cell epithelium [4]. As the duct enters the epidermis, the myoepithelial layer is lost, and it is lined by squamous epithelium [4]. Toker cells (cytokeratin 7-positive clear cells which may occur singly and in small clusters in the lower epidermis), similar to those described by Toker in the normal nipples, may be apparent within the ductal epithelium of the anogenital mammary-like glands at the site of insertion [5]. These Toker cells were first documented in the vulva by Willman et al. [6].

M.P. Hoang, M.D. (✉)
Department of Pathology, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA
e-mail: mhoang@mgh.harvard.edu

Lesions of Anogenital Mammary-Like Glands

Fibroadenoma and Phyllodes Tumor

Vulvar fibroadenoma was first reported in 1932 by Friedel [7]. These rare fibroepithelial lesions and phyllodes tumors arise from the anogenital mammary-like glands which microscopically appears identical to their more common mammary counterparts [8–13] (see Chap. 1, Fig. 1.8). They present as asymptomatic nodules with sizes ranging from 0.8 to 6 cm. The ages of the patients ranged from 20 to 69 years [8–13]. Local recurrence has been reported in phyllodes tumors [11]. Exceptionally, simultaneous appearance of vulvar and mammary fibroadenoma as well as bilateral presentation has been reported [14, 15]. Local excision appears to be the treatment of choice.

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_11,

Histologic Features

Fibroadenoma is a circumscribed tumor composed of branching and anastomosing glandular structures surrounded by a fibrous paucicellular stroma (Fig. 11.1). Cystic dilatation and intraluminal papillary projections can be seen. Although with a similarly biphasic pattern, phyllodes tumor exhibits leaflike projections and a more cellular stromal component, often with periductal stromal condensation (Fig. 11.2a, b). Similar to the mammary counterpart, depending on the degree of pleomorphism of the stroma, phyllodes tumor in the anogenital areas can be classified into benign, low-grade, and high-grade variants. It appears that most reported cases belonged to either benign or low-grade variant, with a single case of high-grade neoplasm showing a rhabdomyosarcomatous stroma being documented [16]. In both fibroadenoma and phyllodes tumor, unusual features can be seen in the form of pseudoangiomatous stromal hyperplasia (PASH), hyperplastic and metaplastic epithelial and stromal changes, and lactation-like changes [11]. PASH characterized by open, slit-like, often anastomosing channels devoid of erythrocytes and lined by discontinuous, often attenuated, inconspicuous cells without atypia or mitotic activity may simulate low-grade angiosarcoma [17].

Similar to the mammary counterpart, the epithelial component expresses epithelial markers (AE1/AE3, CK7), estrogen receptor (ER) and progesterone receptor (PR); and smooth muscle markers highlight the myoepithelial layer [8, 13]. Human papillomavirus (HPV) DNA has been shown to be negative in three vulvar fibroadenomas [18].

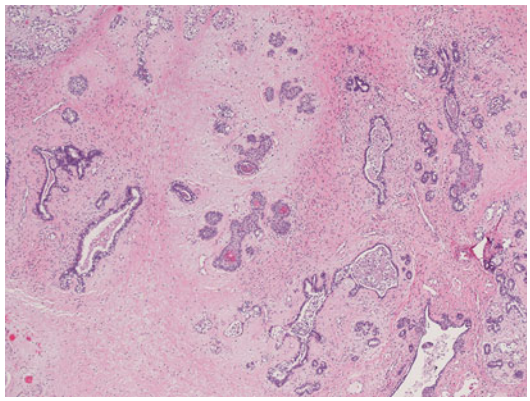


Fig. 11.1 *Fibroadenoma*. A biphasic tumor comprised of branching glandular structures within a fibrous stroma (Courtesy of Dr. Payal Kapur, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas)

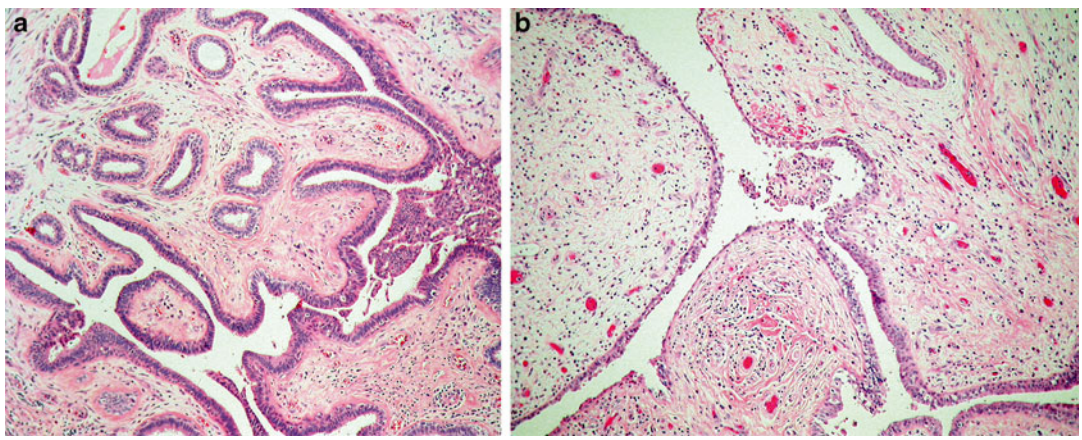


Fig. 11.2 (**a**, **b**) *Phyllodes tumor*. (**a**). Leaflike projections are seen at low magnification. (**b**). A cellular stroma is seen at higher magnification (Courtesy of Dr. Venetia Sarode, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas)

Differential Diagnosis

The architecture and stroma would be the main differentiating features between fibroadenoma and benign phyllodes tumor. Fibroadenoma would exhibit a fibrous paucicellular stroma, whereas benign phyllodes tumor has a more cellular stroma. PASH should be differentiated from its mimicker, the low-grade angiosarcoma, by the lack of cytologic atypia, mitotic activity, and erythrocytes [17].

Summary

Clinical Presentation

- Present as asymptomatic nodules

Histologic Features

- Fibroadenoma: a circumscribed tumor composed of branching glandular structures surrounded by a fibrous paucicellular stroma
- Phyllodes tumor: leaflike projections and cellular stromal component with variable number of pleomorphic cells and mitosis

Differential Diagnosis

- Vascular neoplasm for lesion with pseudoangiomatous stromal hyperplasia

Takeaway Essentials

Clinical Relevant Pearls

- Vulvar fibroadenoma and phyllodes tumor are rare lesions arising from the anogenital mammary-like glands.
- Enlargement during pregnancy may be seen due to lactating-like changes.

Pathology Interpretation Pearls

- The number of stromal mitosis is the determining criterion in distinguishing benign from low-grade phyllodes tumor.

Immunohistochemical/Molecular Findings

- The epithelial component expresses estrogen receptor.

Lactating Adenoma

Associated with pregnancy, lactating adenoma arises from the anogenital mammary-like glands in rare occasions [19]. They can be solitary or multiple and present as masses [20, 21].

Histologic Features

The lesion is circumscribed and comprised of densely packed round tubules lined by cells with cytoplasmic vacuoles and intraluminal eosinophilic secretion (Fig. 11.3a, b). The stromal component does not compress the ducts. Mitotic figures may be identified.

Differential Diagnosis

Other lesions of anogenital mammary-like glands can exhibit lactational changes such as a fibroadenoma [11, 22].

Summary

Clinical Presentation

- Rare lesion that presents as a mass during pregnancy

Histologic Features

- Well-circumscribed lesion
- Epithelial cells with cytoplasmic vacuoles

Differential Diagnosis

- Other lesions of anogenital mammary-like glands with lactational changes

Takeaway Essentials

Clinical Relevant Pearls

- Most of these lesions are alarming because of the detection of significant clinical changes in a short period of time.

Pathology Interpretation Pearls

- Mitotic figures are not associated with aggressive behavior.

Immunohistochemical/Molecular Findings

- In practice rarely are immunohistochemical stains used to diagnose these lesions.

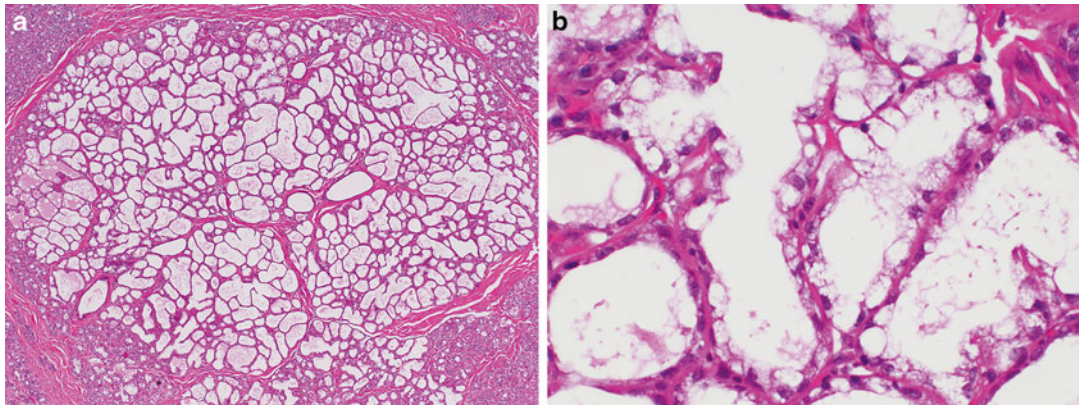


Fig. 11.3 (**a**, **b**) *Lactating adenoma*. A circumscribed proliferation of round tubules which are lined by cells with cytoplasmic vacuoles (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

Hidradenoma Papilliferum and Hidradenocarcinoma Papilliferum

Hidradenoma papilliferum was first reported by Worth in 1878 and characteristically occurs in the vulvar and perianal regions [23]. Initially thought to be derived from the apocrine glands [24], subsequent reports support that hidradenoma papilliferum (as well as tubular adenoma) are derived from anogenital mammary-like glands [25, 26]. Therefore, the term "mammary-like gland adenoma of the vulva" has been proposed; however, hidradenoma papilliferum is best conceptually compared to intraductal papilloma of the breast [4, 27]. In a recent review [28], hidradenoma papilliferum was the most common benign vulvar glandular neoplasm accounting for 60 % of the benign lesions.

It often presents as a solitary and asymptomatic nodule. The ages of the patients in large series range from 29 to 90 years with the mean of 50 years [27]. The sites of involvement include labia minora (50 %), labia majora (40 %), fourchette (7 %), and clitoris (3 %) [27]. This distribution mirrors that of anogenital mammary-like glands. Although HPV types 16, 31, 33, 53, and 56 have been identified within lesional tissue, the virus does not appear to play a causative role [17].

Histologic Features

Histopathologically, the tumor exhibits both a papillary and glandular architecture with interconnected anastomosing tubules. The inner epithelial cells are typically columnar with pale eosinophilic cytoplasm and are surrounded by a myoepithelial layer (Fig. 11.4a, b). Oncocytic (oxyphilic) metaplasia, clear cell change, predominantly solid growth, cystic change, sclerosing adenosis-like changes, and others can be seen [4, 17, 29]. Mitoses can be identified in both the epithelial as well as the myoepithelial components and are not indicative of aggressive behavior [30].

The epithelial component is highlighted by a variety of keratins (AE1/AE3, CK5/6, CK15) [31, 32], ER [33], and GCDFP-15 [34]. The myoepithelial component would be stained with a number of myoepithelial markers (S100, smooth muscle actin, calponin) and interestingly nestin [32].

The literature cites five cases of ductal carcinoma in situ arising in association with hidradenoma papilliferum [35–38]. Histopathologically, these tumors are described to possess foci of crowded pleomorphic epithelial cells with hyperchromatic nuclei, prominent nucleoli, and abnormal mitotic figures. However, the retention of a myoepithelial layer is indicative of its in situ nature [36, 38].

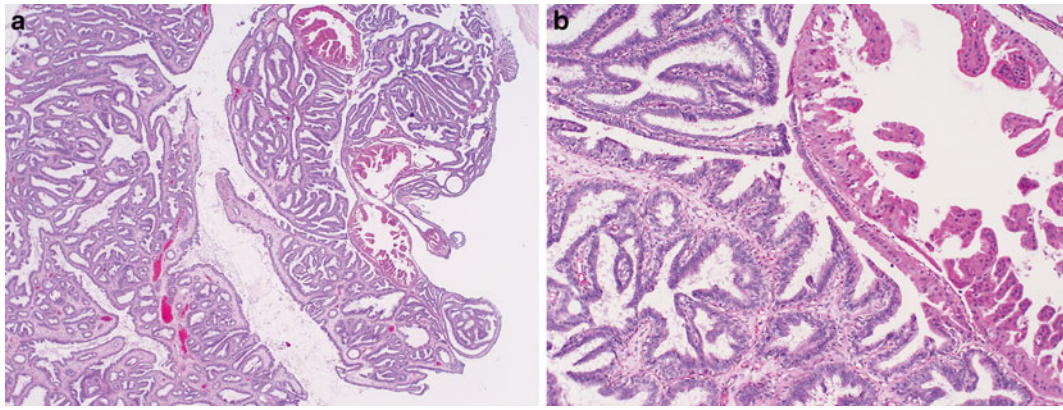


Fig. 11.4 (**a**, **b**) *Hidradenoma papilliferum*. (**a**). An intradermal tumor comprised of branching and anastomosing tubules which are lined by inner epithelial columnar cells and an outer myoepithelial cells. (**b**). Prominent apocrine metaplasia can be seen (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

Differential Diagnosis

When there is connection to the overlying epidermis, epidermal hyperplasia and prominent plasma cells infiltrate can mimic syringocystadenoma papilliferum.

Summary

Clinical Presentation

- A solitary and asymptomatic nodule

Histologic Features

- A papillary and glandular architecture with interconnected anastomosing tubules.
- An inner epithelial and an outer myoepithelial layer.
- Oncocytic metaplasia, clear cell change, predominantly solid growth, and cystic change can be seen.

Differential Diagnosis

- Syringocystadenoma papilliferum

Takeaway Essentials

Clinical Relevant Pearls

- Derived from anogenital mammary-like glands

Pathology Interpretation Pearls

- Mitoses can be seen in both the epithelial as well as the myoepithelial components and are not indicative of aggressive behavior.
- When cellular pleomorphism and severe cytologic atypia are seen, the diagnosis of hidradenocarcinoma papilliferum should be considered.

Immunohistochemical/Molecular Findings

- The retention of a myoepithelial layer highlighted by smooth muscle actin or calponin is indicative of the in situ nature of the lesion.

Extramammary Paget Disease

First reported by Crocker [39] to affect the scrotum and penis, extramammary Paget Disease (EMPD) has since been documented in the vulva and perianal region [40]. EMPD can be either primary or secondary. Primary EMPD can be either an intraepithelial neoplasm with or without invasion, or as a manifestation of an underlying primary adenocarcinoma. The implicated cells of origin for primary EMPD include skin adnexa (eccrine or apocrine glands) and anogenital

mammary-like glands [6], or possibly pluripotential stem cells [4, 41–43]. Chanda et al. [44] identified that 29 % of 197 EMPD cases were associated with an underlying malignancy arising in organs in proximity to the vulva. Through a mechanism reminiscent of mammary Paget disease, secondary EMPD is thought to originate from epidermotropic spread of malignant cells or within contiguous epithelium [45]. Many cases of vulvar secondary EMPD have been described in association with other gynecologic and genitourinary (bladder) neoplasms [40]. Furthermore, perianal Paget disease, which accounts for 20 % of EMPD cases [46], is strongly associated with adenocarcinoma of the anus and colorectum [47].

EMPD of the vulva predominantly affects women in the seventh decade [48–50]. The primary symptoms are pruritus and burning [48]. The labia majora is the most frequently involved site followed by labia minora and clitoris [50]. It presents as a relatively well-demarcated flat or slightly elevated erythematous or gray-white lesion, 1–20 cm in size [50, 51] (Fig. 11.5). "Triple" EMPD is a rare presentation in which the genital lesions are associated with synchronous or metachronous, uni- or bilateral axillary involvement [52]. The primary EMPD is with a high recurrence rate and rarely metastasizes, contrary to the secondary form [48]. Noninvasive EMPD has an excellent prognosis [53]. Studies have shown conflicting data regarding the presence of invasion and prognosis [54, 55]. A recent study using the SEER (Surveillance, Epidemiology, and End Results) Program identified a long-term increased risk of developing secondary malignancies in patients with invasive EMPD [56]. Surgical excision is currently the standard treatment [49].

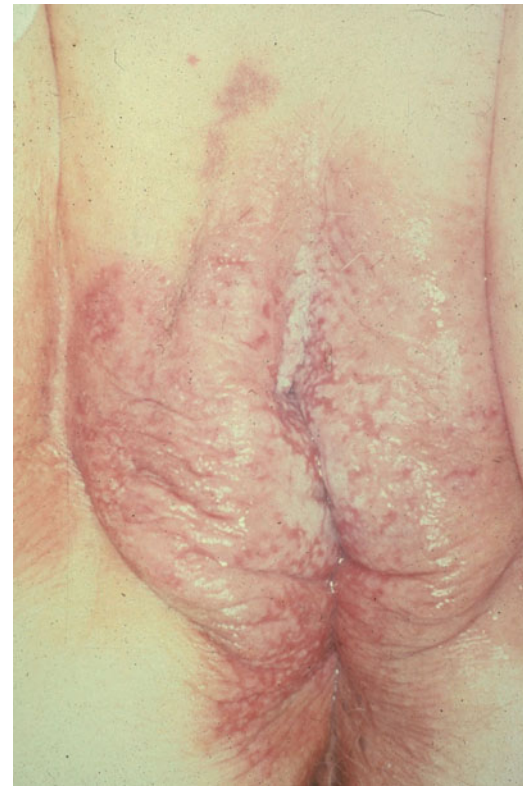


Fig. 11.5 *Extramammary Paget disease*. An erythematous plaque is seen bilaterally on the vulva and extends to the perianal region

Histologic Features

The histologic features are similar in both primary and secondary forms, characterized by large pale cells arranged as single units and clusters or glands formed within the epidermis (Fig. 11.6a–c). The involvement of adnexa, most commonly hair follicles and ducts, is frequently seen in EMPD. A signet-ring appearance can be seen due to marked intracytoplasmic mucin accumulation. In EMPD in situ, the neoplastic cells are localized within the epidermis. Invasive EMPD is classified as microinvasive or invasive. In microinvasive cases, the tumor cells are seen in the stroma no deeper than 1 mm below the basement membrane [57].

Rarely, mammary-type ductal carcinoma, invasive or in situ, can be seen in EMPD. In the latter case, ductal carcinoma in situ (DCIS) appears to involve anogenital mammary-like glands, thus complicating the accepted terminology EMPD in situ used for designation of carcinoma cells confined to the epidermis.

The tumor cells in EMPD are usually positive for mucicarmine, Alcian blue, and periodic acid-Schiff with diastase. Primary EMPD lesions would typically exhibit a sweat gland phenotype (cytokeratin (CK) 7+/CK20-/gross cystic disease fluid protein (GCDFP)-15+), whereas secondary EMPD lesions would exhibit an endodermal phenotype (CK7+/CK20+/

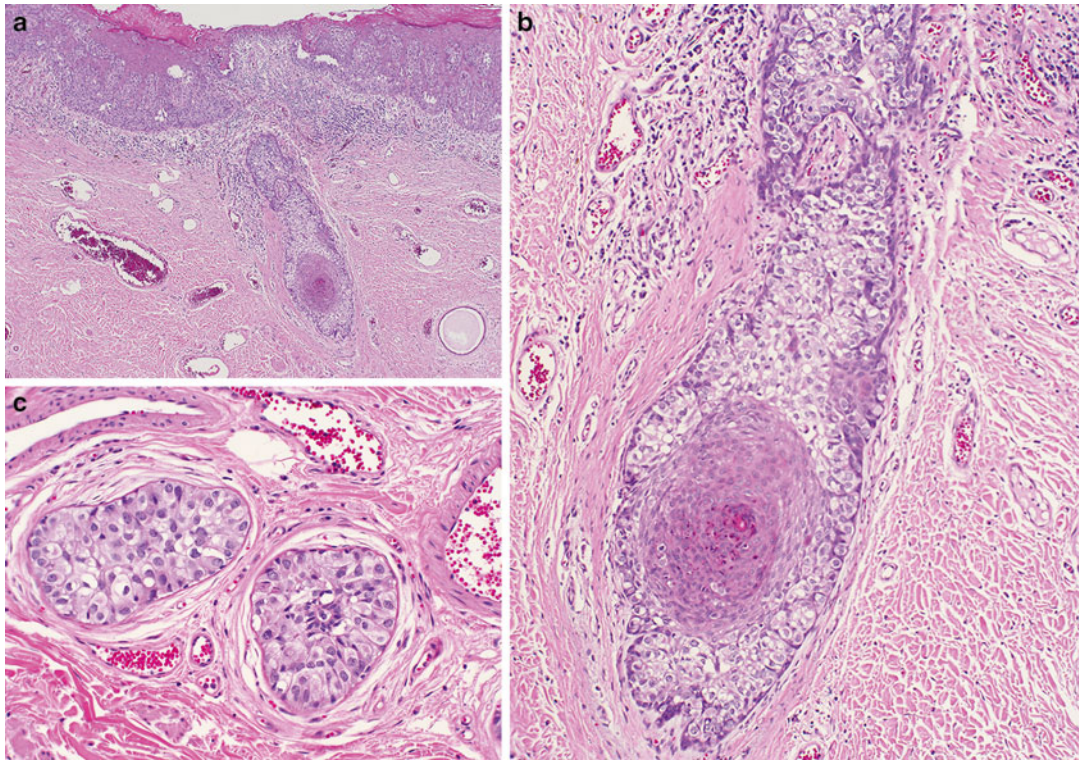


Fig. 11.6 (**a**–**c**) *Extramammary Paget disease*. (**a**, **b**). A proliferation of polygonal neoplastic cells is seen within the epidermis and extending into hair follicular epithelium. (**c**). Tumor cells with abundant pale cytoplasm and vesicular nuclei are seen replacing the eccrine glands. Residual lumen is still visible in one of the glands – a clue for not misdiagnosis adnexal extension as invasion (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

GCDFP-15-) [58] (Fig. 11.7). Co-expression of CK20 and CDX2 is indicative of secondary EMPD of colorectal origin [59, 60]. HER-2/neu and CDX2 have been found to be useful in distinguishing primary EMPD from secondary EMPD of anorectal adenocarcinoma [61, 62]. Although the expression can be variable, mucin core proteins such as MUC2 and MUC5AC might be helpful in distinguishing primary from secondary EMPD (see *Vignette 3* at the end of this chapter) (Table 11.1). Uroplakin III has been demonstrated to be of help in identifying metastatic urothelial carcinomas [63]. It should be emphasized however that the burden of

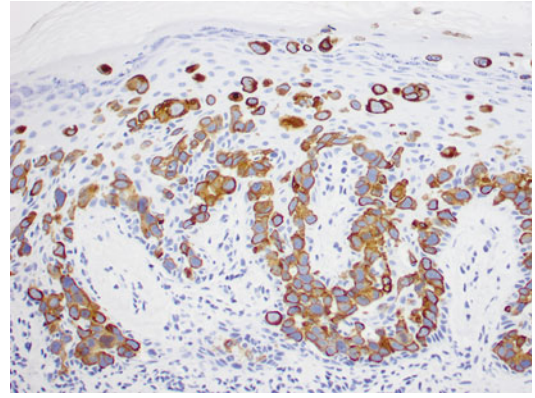


Fig. 11.7 *Extramammary Paget disease*. The intraepidermal tumor cells strongly express cytokeratin 7

Table 11.1 Immunoprofile of primary and secondary extramammary Paget disease

Immunostain	Primary	Secondary colorectal	Secondary urothelial
Cytokeratin 7	+	−/+	−/+
Cytokeratin 20	−	+	+
GCDFP-15	+	−	−
HER-2/neu	+	−	−
CDX2	−	+	−
Uroplakin III	−	−	+
p63	−	−	+
MUC5AC	+	−	−
MUC-2	−	+	−

GCDFP-15 gross cystic disease fluid protein-15

delineation between primary and secondary EMPD lies on the clinician.

Our understanding of the genetic basis for EMPD is currently limited. The expression of HER-2/neu and androgen receptor indicates potential benefit from targeted therapy [62, 64]. However, fluorescence in situ hybridization demonstrates no evidence of HER-2 gene amplification [65]. Recently, the deleted in liver cancer-1 gene (*DLC1*) hypermethylation and mutations in oncogene *PIK3CA* with overexpression of PI3K protein have been shown in EMPD cases [66–68].

Differential Diagnosis

The differential diagnosis of EMPD includes both benign conditions such as vulvar Toker cells, mucinous metaplasia, vulva intraepithelial neoplasia, and malignant melanoma [69]. In a recent study of 56 patients, Shaco-Levy et al. [50] reported that a panel of pan-cytokeratin, CK7, CEA, and EMA labels 100 % of primary EMPD cases, while no cases stained with S100, HMB-45, and MART-1 distinguishing EMPD from malignant melanoma. The mucinous cells in mucinous metaplasia would lack cytologic atypia and replace rather than infiltrate the squamous epithelium as in EMPD (see Chap. 12). Careful examination would show that the neoplastic cells in EMPD are round and often with cytoplasmic mucin in contrast to the dysplastic keratinocytes seen in VIN.

Summary

Clinical Presentation

- Affects mainly women in the seventh decade.
- The labia majora is the most frequently involved site followed by labia minora and clitoris.
- It presents as a relatively well-demarcated flat or slightly elevated erythematous or gray-white lesion.

Histologic Features

- Large pale cells arranged in singly or in clusters within the epidermis.
- Extension along the adnexal structures can be seen.
- Microinvasion is defined when the tumor cells are seen less than one millimeter below the basement membrane.

Differential Diagnosis

- Vulvar Toker cells
- Mucinous metaplasia
- Vulvar intraepithelial neoplasia with mucinous differentiation
- Secondary EMPD
- Malignant melanoma

Takeaway Essentials

Clinical Relevant Pearls

- Primary EMPD is slowly progressive and rarely metastasizes, in contrast to the secondary form.
- EMPD is frequently confused with eczematous process delaying the diagnosis.

Pathology Interpretation Pearls

- Minimal inflammation is seen in association with EMPD.
- Cytoplasmic mucin is seen only in EMPD and not in squamous cell carcinoma or malignant melanoma.

Immunohistochemical/Molecular Findings

- Panel for primary EMPD: CK7, CK903, and S100
- Panel for secondary EMPD: CK7, CK20, CDX2, and p63

Other Lesions of Anogenital Mammary-Like Glands

Uncommon benign lesions of the anogenital mammary-like glands include fibrocystic-like changes in the form of sclerosing adenosis, columnar cell lesions, ductal lesions, and various metaplastic changes affecting the epithelium and myoepithelium.

Other Carcinomas of Anogenital Mammary-Like Glands

Adenocarcinoma of Anogenital Mammary-Like Glands

First reported by Greene in 1936, there have been approximately 30–40 cases of adenocarcinoma of anogenital mammary-like glands reported in the literature to date [4, 70–76]. Anogenital mammary-like glands have been suggested in those cases as a likely origin due to their marked similarity to homologous mammary carcinomas. The labia majora is the most commonly involved site, and the mean tumor size is 2.5 cm [71]. Because of the rarity, varying treatment modalities, and relatively short follow-up periods in the original publications, it is difficult to draw firm conclusions regarding the natural history and prognosis of these neoplasms. As a group, primary mammary-type adenocarcinomas appear to be locally aggressive tumors. In one review, 60 % (12/20) of patients were found to have regional lymph node metastasis at the time of presentation [71]. Radical or hemivulvectomy has been the primary treatment in most cases [71].

Histologic Features

The presence of an in situ component, remarkable similarity to a mammary counterpart, or a transition zone between normal mammary-like glands and the carcinoma component is necessary to establish a diagnosis. The histopathology of these tumors varies from predominantly ductal to lobular, mixed ductal and lobular, tubulolobular, mucinous, and adenoid cystic-like [71, 73, 74, 77, 78]. The morphology of most cases is that of an invasive ductal carcinoma [71] (Fig. 11.8a, b).

Differential Diagnosis

The differential diagnosis includes extramammary Paget disease, sweat gland carcinoma, and metastatic adenocarcinoma to the vulva. Without clinical history, it would be difficult to distinguish adenocarcinoma of anogenital mammary-like glands from metastatic carcinoma from the breast since both have similar histology. Expression of ER and PR has been variable in adenocarcinoma of mammary-like glands [71, 76].

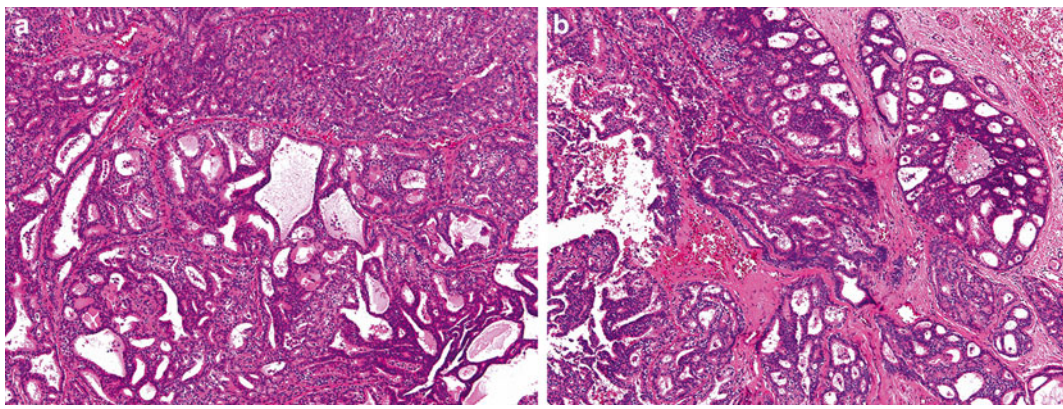


Fig. 11.8 (**a**, **b**) *Adenocarcinoma of mammary-like glands*. An infiltrative carcinoma resembling mammary ductal carcinoma is seen

Summary

Clinical Presentation

- The labia majora is the most commonly involved site.

Histologic Features

- Varied from predominantly ductal to lobular, mixed ductal and lobular, tubulolobular, mucinous, and adenoid cystic-like

Differential Diagnosis

- Extramammary Paget disease, sweat gland carcinoma, and metastatic adenocarcinoma to the vulva

Takeaway Essentials

Clinical Relevant Pearls

- An aggressive tumor with 60 % of patients presented with nodal metastasis

Pathology Interpretation Pearls

- Transition zone between normal mammary-like glands and the carcinoma component confirms the diagnosis.
- The histology of most cases is that of an invasive ductal carcinoma.

Immunohistochemical/Molecular Findings

- Can express estrogen and progesterone receptors

Other Adnexal Neoplasms

Syringoma

Syringoma was first described in 1874 by Kaposi and Biesiadeki as "lymphangioma tuberosum multiplex"; however vulvar involvement was not reported until 1972 [79]. Since then isolated cases and small case series of syringomas on the genital region have been reported [80–88].

Vulvar syringomas mostly occurred in young women in the third decade [81]. In a series of 18 vulvar syringomas from Taiwan, 11 lesions presented as multiple skin-colored, smooth-surfaced or brownish papules on the labia majora [81]. Discrete whitish cystic papules were seen in three patients [81]. Pruritus was the most commonly presenting symptom [81]. The size has been reported to increase during summer month or menstruation. Rare report of familial history has been documented [80].

Some have hypothesized that the growth of syringoma is under hormonal influence [82, 83]; however, staining for estrogen receptor (ER) and progesterone receptor (PR) was negative for all 15 studied cases by Huang et al. [81] and the case reported by Trager et al. [84]. Reported treatment for vulvar syringomas includes carbon dioxide laser treatment [81, 86], cryotherapy [87], electrosurgery [88], and excision [83].

Histologic Features

The lesion is typically a well-circumscribed proliferation of small ductal structures that are evenly distributed in a collagenous stroma (Fig. 11.9). The geometric ductal structures such as "comma-like" or "tadpole-like" are characteristic features. Clear cell change can be seen in tumors associated with diabetes mellitus and Down syndrome [89]. Extension into the deep dermis and subcutaneous tissue is typically seen in the plaque-type syringoma. Vulvar syringoma can be encountered as an incidental finding in association with melanocytic nevi, lichen sclerosus, and lichen simplex chronicus.

Syringoma are reported to express cytokeratin (CK) 6 and CK10 [90] and variable expression

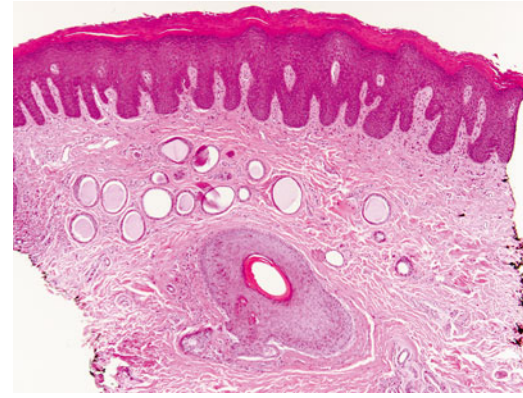


Fig. 11.9 *Syringoma*. A proliferation of small ductal structures is seen evenly distributed in a collagenous stroma. The overlying epidermis exhibits lichen simplex chronicus changes – hyperkeratosis, epidermal hyperplasia, and hypergranulosis

for epithelial membrane antigen, CK1, CK5, CK11, CK14, and CK19 [91–94].

Differential Diagnosis

The differential diagnosis of deep or plaque-type vulvar syringoma would include microcystic adnexal carcinoma [95, 96]. (See *Vignette 1* at the end of this chapter.) A sharp demarcation between the deep syringoma and the adjacent dermis or subcutaneous tissue would not be seen in microcystic adnexal carcinoma.

Summary

Clinical Presentation

- Multiple skin-colored or brownish papules on the labia majora

Histologic Features

- A well-circumscribed superficial proliferation of small ductal structures that are evenly distributed in a collagenous stroma

Differential Diagnosis

- Microcystic adnexal carcinoma

Takeaway Essentials

Clinical Relevant Pearls

- Pruritus is the most common presenting symptom worsening in summertime and menstruation.
- It can be confused with HPV lesions.

Pathology Interpretation Pearls

- Often an incidental finding in association with melanocytic nevi, lichen sclerosus, and lichen simplex chronicus

Hidradenoma

Clear cell hidradenoma usually presents as a solid and cystic nodule with no site predilection, though is only rarely reported to occur on the vulva [28, 97–99]. There have been rare case reports of vulvar hidradenocarcinoma [100–102]. Approximately 50 % of hidradenoma possesses the t(11;19) translocation [102]. HER-2/neu amplification has been reported in a case of metastasizing hidradenocarcinoma [103]; however, this finding has not been confirmed by subsequent studies [102, 104, 105]. Mutations of *TP53* [102, 104, 105], *PIK3CA* [105], and *AKT-1* [105] have been documented in rare cases of hidradenocarcinomas.

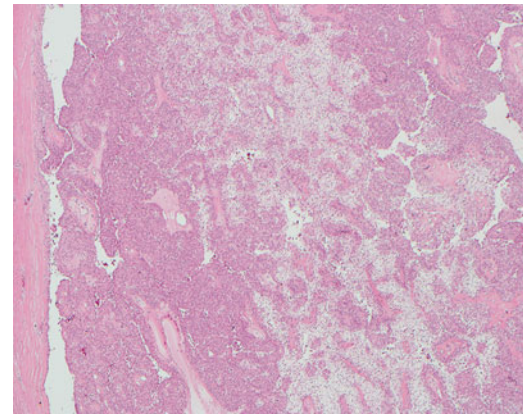


Fig. 11.10 *Hidradenoma*. A cystic and solid neoplasm comprised of clear as well as eosinophilic neoplastic cells is seen

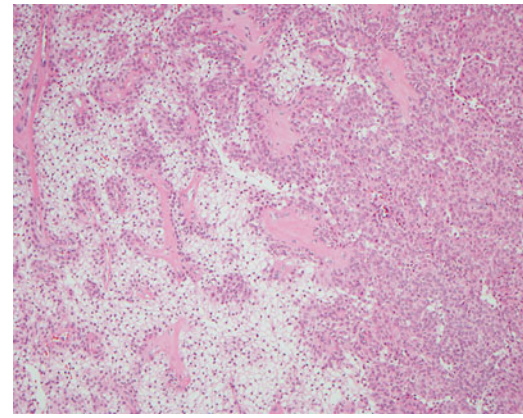


Fig. 11.11 *Hidradenoma*. A cystic and solid neoplasm comprised of clear as well as eosinophilic neoplastic cells is seen

Histologic Features

Hidradenoma is characterized by multilobulated, circumscribed yet unencapsulated tumor (Figs. 11.10 and 11.11). Glandular structures can be seen, and in some cases these are striking. The tumor cells are diverse and can be clear, eosino-

philic, squamoid/epidermoid, mucinous, oxyphilic/oncocytic, and transitional/intermediate [24]. Stroma is often hyalinized and sclerotic.

Differential Diagnosis

When focal infiltrative architecture and increased mitotic figures are seen, it is classified as atypical hidradenoma [106], whereas hidradenocarcinomas are characterized by infiltrative growth pattern, deep extension, necrosis, nuclear pleomorphism, and greater than 4 mitoses per 10 high-power fields [106]. Although in some rare instances, metastasizing hidradenocarcinomas do not show these histopathologic features [102]. Apparently, both high-grade and low-grade variants of hidradenocarcinoma exist [107].

Summary

Clinical Presentation

- A solid and cystic nodule

Histologic Features

- Glandular structures and tumor cells with eosinophilic, clear, or mucinous cytoplasm.
- Stroma is frequently sclerotic and hyalinized.

Differential Diagnosis

- Atypical hidradenoma
- Hidradenocarcinoma

Takeaway Essentials

Pathology Interpretation Pearls

- Hidradenocarcinomas are characterized by infiltrative growth pattern, deep extension, necrosis, nuclear pleomorphism, and greater than 4 mitoses per 10 high-power fields.

Immunohistochemical/Molecular Findings

- Ki-67 index can be helpful in distinguishing atypical and malignant from benign hidradenoma.

Sebaceous Carcinoma

Although sebaceous glands can be prominent on the vulva, there have been only few cases of sebaceous carcinoma reported at this site [108–114]. The case reported by Jacobs et al. [110] was associated with colonic adenocarcinoma, likely in the setting of Muir-Torre syndrome. Two cases were associated with overlying Bowen's disease [110, 111].

Histologic Features

Histologically, sebaceous carcinoma is characterized by an infiltrative proliferation comprised of pleomorphic basaloid cells with focal sebaceous differentiation. Ductal structures with eosinophilic cuticle, characteristic of sebaceous ducts, can be seen in well-differentiated sebaceous tumors (Fig. 11.12a, b). Vascular and perineural

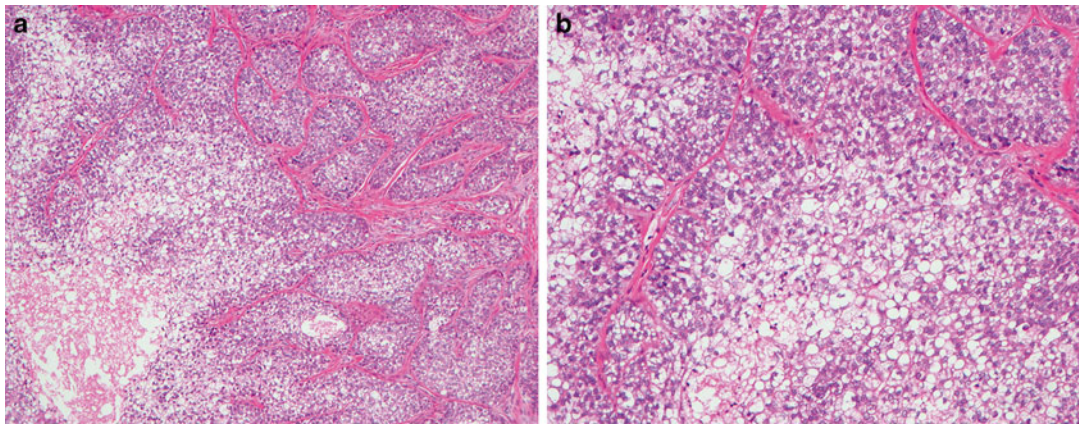


Fig. 11.12 (**a**, **b**) *Sebaceous carcinoma*. (**a**). An infiltrative carcinoma with sebaceous differentiation is seen. (**b**). Cytoplasmic lipid vacuoles are noted at higher magnification

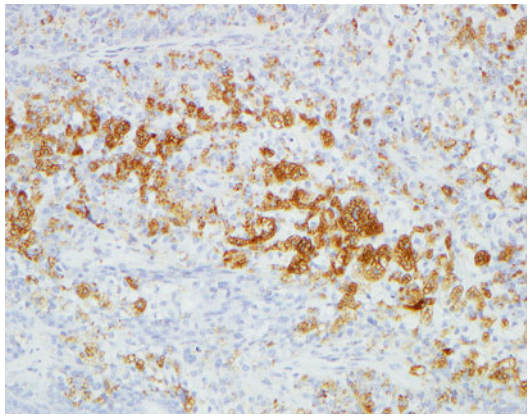


Fig. 11.13 *Sebaceous carcinoma*. The abundant cytoplasmic lipid vacuoles are highlighted by adipophilin immunostain

invasion can be seen. A panel of 2 mismatch repair proteins (PMS2 and MSH6) has been shown by one group to be as effective as a four-antibody panel (PMS2, MSH6, MSH2, and MLH1) as screening panel in detecting Lynch or Muir-Torre syndromes [115]. Vulvar sebaceous lesions however are rarely, if ever, associated with the syndrome. Recently, adipophilin has been shown to be a helpful marker in distinguishing sebaceous carcinomas from mimics [116, 117] (Fig. 11.13).

Differential Diagnosis

Sebaceoma has rarely been reported in the vulva [28]. When a tumor exhibits multiple nests of basaloid cells with scattered mitoses but lacks the atypia of sebaceous carcinoma, it is classified as a sebaceoma.

Summary

Clinical Presentation

- Rarely present on the vulva

Histologic Features

- An infiltrative tumor comprised predominantly of basaloid cells and focally sebaceous differentiation

Differential Diagnosis

- Sebaceoma

Takeaway Essentials

Clinical Relevant Pearls

- Vulvar sebaceous lesions however are rarely, if ever, associated with Muir-Torre syndrome.

Pathology Interpretation Pearls

- The sebaceous changes can be focal; therefore, examination of the entire lesion increases the chance of finding the diagnostic areas.

Immunohistochemical/Molecular Findings

- PMS2 and MSH6 have been shown to be as effective as a four-antibody panel (PMS2, MSH6, MSH2, and MLH1) as screening panel in detecting Lynch or Muir-Torre syndrome.
- Adipophilin can be helpful in highlighting the sebaceous differentiation.

Poroma

Poroma is often present on plantar or palmar skin; however, it may be found on any area containing sweat glands [118]. Rare cases of vulvar poroma have been reported with the frequency of 2.2 % of benign vulvar adnexal neoplasms [28].

Histologic Features

The tumor exhibits a proliferation of uniformly round (poroid) cells and eosinophilic squamoid (cuticular) cells (Fig. 11.14). Small ductal structures lined by an eosinophilic cuticle (highlighted by periodic acid-Schiff (PAS), epithelial membrane antigen (EMA), and carcinoembryonic antigen (CEA)) are often seen. It is generally accepted that poromas encompass the following tumors: classic poroma, hidroacanthoma simplex, dermal duct tumor, and poroid hidradenoma depending on the architecture of the neoplasm.

Studies have suggested that poroma derives from the basal keratinocytes of the sweat duct ridge and of the lower acrosyringium [119]. A panel of CK7 and CK19 has been shown to be helpful in differentiating porocarcinoma from squamous cell carcinoma [120].

Loss of heterozygosity in the *APC* gene has been shown in 3/7 poromas; however, this finding is of uncertain significance [121].

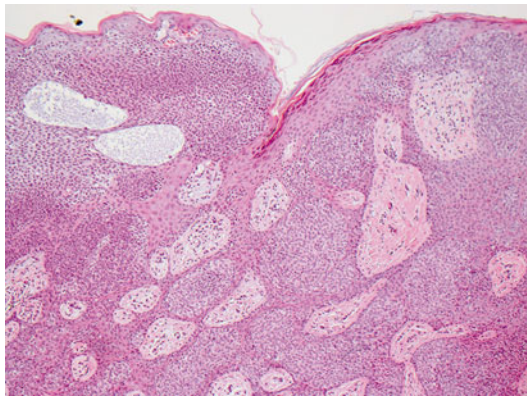


Fig. 11.14 *Poroma*. An intraepidermal proliferation of uniformly round cells

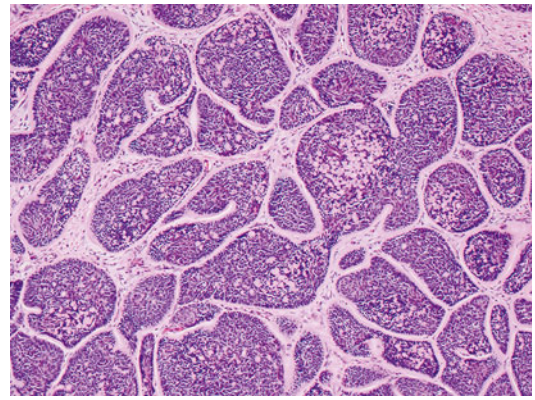


Fig. 11.15 *Cylindroma*. Nodules of basaloid tumor cells are surrounded by eosinophilic basement membrane material

Differential Diagnosis

Porocarcinoma of the vulva is very rare with few cases reported in the literature [122–125]. They often affect women in the sixth decade. Similar to porocarcinomas at nongenital sites, they are associated with frequent nodal metastases [122–125]. One should keep in mind that porocarcinoma is likely to be overdiagnosed or even misdiagnosed, as the analysis of the histological images and descriptions of published "porocarcinomas" strongly suggests.

Summary

Clinical Presentation

- Poroma may be found on any skin area with sweat glands.

Histologic Features

- Comprised of uniform poroid cells and squamoid cuticular cells

Differential Diagnosis

- Porocarcinoma

Takeaway Essentials

Clinical Relevant Pearls

- This tumor needs to be in the differential diagnosis of a mass in postmenopausal women.

Pathology Interpretation Pearls

- Small cytoplasmic vacuoles within the cuticular cells are evidence of primitive ductal differentiation.

Immunohistochemical/Molecular Findings

- EMA and CEA are useful diagnostic tools in highlighting the ductal differentiation.

Cylindroma

Cylindromas are typically found on the head and neck region [126]. Multiple lesions together with spiradenoma and/or trichoepitheliomas are found in the inherited Brooke-Spiegler syndrome [127, 128]. In the vulva, rare cases of cylindroma including those occurring in the setting of Brooke-Spiegler syndrome have been reported [28, 126].

Histologic Features

Nodules of basaloid cells are surrounded by eosinophilic basement membrane material arranged in a jigsaw pattern (Fig. 11.15).

Differential Diagnosis

The malignant counterpart, commonly termed cylindrocarcinoma, has been documented in one rare case at this site [129]. Histologically, several malignant patterns have been documented in cases of malignant transformation of a preexisting benign cylindroma (please refer to differential

diagnosis section of spiradenoma). Cylindroma-like or spiradenocylindroma-like basaloid (cloacogenic) carcinoma can also be a differential diagnostic consideration from a histological point of view, but this lesion usually occurs on the perianal and anal area [130].

Summary

Clinical Presentation

- Solitary or multiple skin-colored nodules

Histologic Features

- Nodules of basaloid cells are surrounded by eosinophilic basement membrane material arranged in a jigsaw pattern.

Differential Diagnosis

- Spiradenoma

Takeaway Essentials

Clinical Relevant Pearls

- Multiple lesions together with spiradenoma and/or trichoepitheliomas are indicative of Brooke-Spiegler syndrome.

Pathology Interpretation Pearls

- Transition between the preexisting benign tumor and the malignant neoplasm is typically gradual.

Spiradenoma

Spiradenoma often presents as a nodule on the head and neck, trunk, or extremities [131]. Rare cases of spiradenoma have been reported in the vulva [28].

Histologic Features

Histologically, spiradenoma presents as a well-circumscribed tumor is composed of two cell populations – central cluster of page large cells surrounded by small dark basaloid cells with hyperchromatic nuclei (Fig. 11.16a, b). Ductal differentiation can often be seen, and in rare cases, authentic adenomatous or adenomyoepitheliomatous structures with glands possessing a peripheral myoepithelial cell layer can be recognized [132, 133]. Intratumoral lymphocytes are an essential component of the neoplasm.

Differential Diagnosis

The main differential diagnosis would be a related tumor, the cylindroma. In fact, cylindroma and spiradenoma are thought to comprise a morphological spectrum, with a hybrid or intermediate lesion, the spiradenocylindroma in between, making the strict separation of cylindroma and spiradenoma at some point artificial. On the other hand, considering the microscopic heterogeneity of the malignant counterpart, the terms spiradenocarcinoma and malignant spiradenoma, although often used, are too generic and fail to reflect the range of microscopic appearances that may be indicative

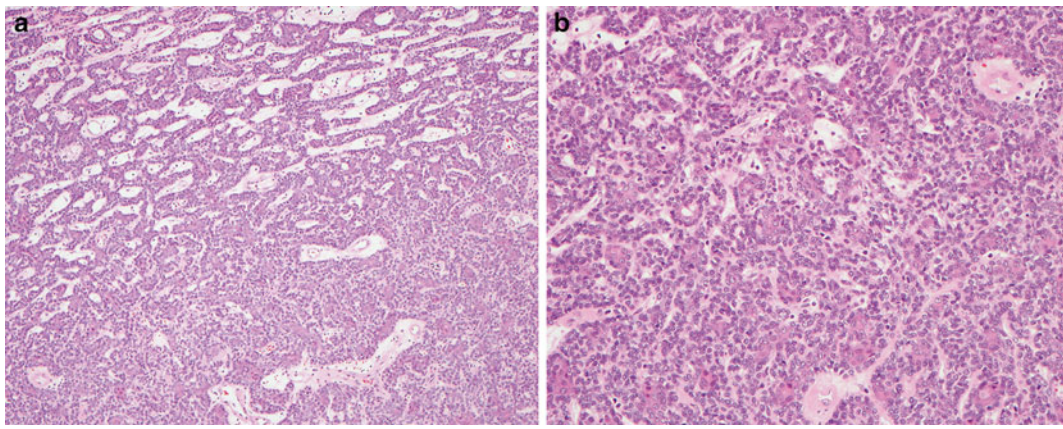


Fig. 11.16 (**a**, **b**) *Spiradenoma*. A tumor comprised of central pale large cells and outer small dark basaloid cells with hyperchromatic nuclei

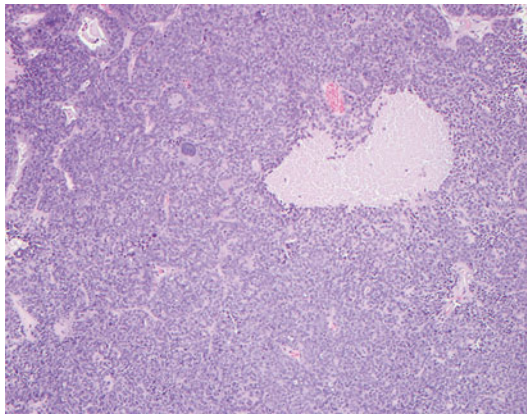


Fig. 11.17 *Spiradenocarcinoma*. Necrosis is noted in this cellular tumor

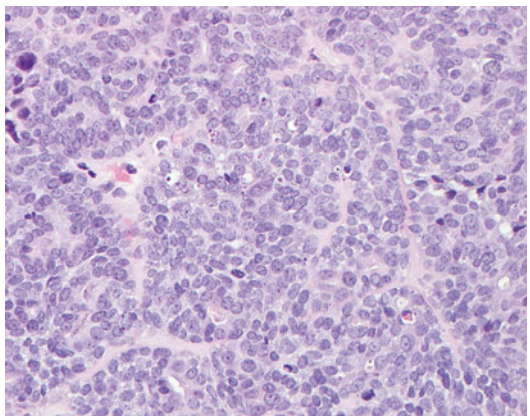


Fig. 11.18 *Spiradenocarcinoma*. Prominent mitotic figures are noted

of behavior and prognosis. Malignant lesions evolving from spiradenoma are classified based on at least four histopathological patterns: salivary gland-type basal cell adenocarcinoma, low grade; salivary gland-type basal cell adenocarcinoma, high grade; apocrine adenocarcinoma, not otherwise specified, in situ or infiltrative; and sarcomatoid (metaplastic) carcinoma [134] (Figs. 11.17 and 11.18). The lesions mostly occur as sporadic solitary neoplasms, or as a component of Brooke-Spiegler syndrome. Only rare cases have been documented arising in the vulva [135–137]. The clinical course of malignant tumors correlates to some extent to the histological pattern and the clinical phenotype. Low-grade neoplasms resembling basal cell adenocarcinoma of salivary glands have been shown to have a less aggressive course, with local recurrences but no distant metastases, whereas analogous high-grade lesions followed a highly aggressive course. Patients with sarcomatoid (metaplastic) carcinoma had a relatively good survival, an unexpected feature noted by various authors. It appears that the tumor occurring in patients with Brooke-Spiegler syndrome demonstrates a more aggressive behavior compared to their sporadic counterparts, but this should be validated in large series.

Summary

Clinical Presentation

- A nodule on the head and neck, trunk, or extremities

Histologic Features

- A well-circumscribed tumor composed of two cell populations – central cluster of pale large cells surrounded by small dark basaloid cells with hyperchromatic nuclei
- Intratumoral presence of lymphocytes

Differential Diagnosis

- Spiradenocylindroma

Takeaway Essentials

Clinical Relevant Pearls

- The lesions mostly occur as sporadic solitary neoplasms, or as a component of Brooke-Spiegler syndrome

Pathology Interpretation Pearls

- Cylindroma and spiradenoma are thought to comprise a morphological spectrum, with a hybrid or intermediate lesion, the spiradenocylindroma in between.
- The malignant component of spiradenocarcinoma can be:
  - Salivary gland-type basal cell adenocarcinoma, low grade
  - Salivary gland-type basal cell adenocarcinoma, high grade
  - Apocrine adenocarcinoma, not otherwise specified, in situ or infiltrative
  - Sarcomatoid (metaplastic) carcinoma

Mixed Tumors (Chondroid Syringoma)

Two types of mixed tumors are recognized in the skin, namely apocrine mixed tumor and eccrine mixed tumor. Chondroid syringoma, also known as benign mixed tumor and pleomorphic adenoma, is a rare tumor of the vulva with only a few cases reported in the literature [138–142]. The rarity of mixed tumors in the vulva is underscored by the absence of a single vulvar case in a series of 244 cases [143]. With exception of a single case of eccrine mixed tumor, reported examples of vulvar mixed tumors were of the apocrine variety [144].

Histologic Features

Histologically, the tumor contains a mixture of epithelial and myoepithelial cells associated with a myxoid or cartilaginous stroma [140] (Fig. 11.19).

Differential Diagnosis

The malignant counterpart or malignant chondroid syringoma is characterized by infiltrative growth, greater cellularity and nuclear pleomorphism, mitotic activity, and necrosis [145]. Malignant chondroid syringoma can recur and metastasize to regional lymph nodes and distant sites. However, if strict diagnostic criteria of malignant mixed tumor are applied (presence of a residuum of benign mixed tumor which also contains an unquestionable malignant component), malignant apocrine or eccrine mixed tumor of the skin is an extremely rare neoplasm with no convincing cases documented in the vulva so far [146].

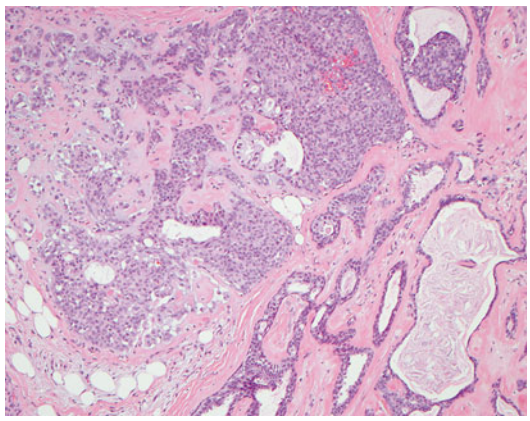


Fig. 11.19 *Chondroid syringoma*. A mixture of epithelial and myoepithelial cells associated with a myxoid or cartilaginous stroma

Summary

Clinical Presentation

- Erythematous to skin-colored nodule

Histologic Features

- A mixture of epithelial and myoepithelial cells associated with a myxoid or cartilaginous stroma

Differential Diagnosis

- Malignant chondroid syringoma

Takeaway Essentials

Clinical Relevant Pearls

- As clinical presentation is nonspecific, the differential diagnosis of an erythematous to skin-colored mass in the vulva needs to include this adnexal tumor.

Pathology Interpretation Pearls

- With exception of a single case of eccrine mixed tumor, reported examples of vulvar mixed tumors were of the apocrine variety.

Metastatic Carcinoma

Metastatic tumors involving the vulva are rare, and the literature is comprised of a few series and case reports [147–150]. In the largest series of 66 cases, approximately equal numbers of the metastases were from genital and extragenital sites [149] (Table 11.2). The most frequent extragynecologic primaries are colorectal in origin [147, 149]. Metastatic tumors usually present as multiple erythematous firm nodules and are associated with postmenopausal women, widespread disease, and poor clinical course [149]. They portend poor prognosis since approximately 90 % of these patients also have multiple simultaneous metastases to other sites [149].

Table 11.2 Genital and extragenital primary sites of 63 metastases in the descending order of frequency

Genital primary	
Cervix	15 (24 %)
Ovary	8 (13 %)
Endometrium	6 (9.5 %)
Vagina	2 (3 %)
Extragenital primary	
Colorectal and anal	12 (19 %)
Breast	4 (6 %)
Skin	4 (6 %)
Lung	3 (5 %)
Urethra	1 (2 %)
Bladder	1 (2 %)
Pancreas	1 (2 %)
Uncertain	6 (9.5 %)

Data from: Neto et al. [149]

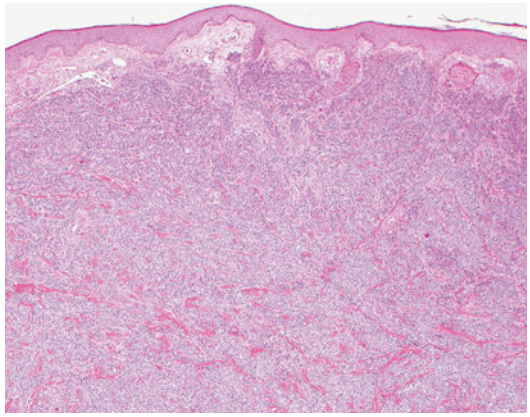


Fig. 11.20 *Metastatic carcinoma from the endometrium.* A nodule of epithelioid neoplastic cells is seen replacing the entire dermis

Histologic Features

The histologic appearance of the metastases would correspond to the primary tumors (Figs. 11.20 and 11.21). A panel of immunohistochemical stains is helpful in distinguishing primary versus metastatic carcinoma to the vulva. CDX2 and CK20 expression would be helpful in ruling a colorectal metastasis; whereas CK7 and ER/PR expression would suggest an ovarian primary.

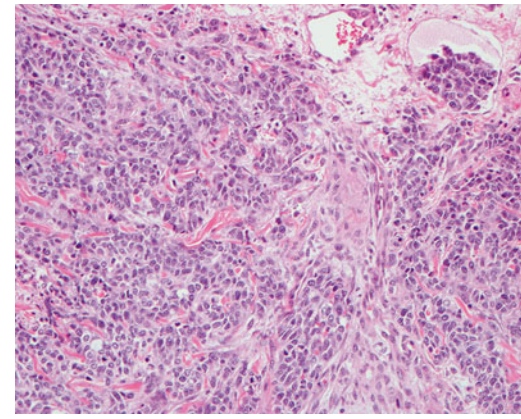


Fig. 11.21 *Metastatic carcinoma from the endometrium.* Lymphovascular invasion is noted at the upper right-hand corner

Summary

Clinical Presentation

- Multiple erythematous firm nodules
- Seen in postmenopausal women, widespread disease, and poor prognosis

Histologic Features

- Histologic appearance of these metastases would correspond to the primary tumors.

Differential Diagnosis

- Broad and depend on the organ of origin

Takeaway Essentials

Clinical Relevant Pearls

- Often with simultaneous metastases to other sites.
- Clinical history is essential to reach a definitive diagnosis.

Pathology Interpretation Pearls

- Prominent lymphovascular invasion is often indicative of a metastasis.

Immunohistochemical/Molecular Findings

- Colonic adenocarcinoma: CDX2+, CK20+, and MUC5AC−
- Ovarian adenocarcinoma: CK7+, ER/PR+, CK20−, and MUC5AC+
- Endometrial adenocarcinoma: CK7+ and vimentin+

Case Vignettes

Vignette 1

Clinical History: A 28-year-old woman presented with multiple, small, pearly white nodules whose sizes ranging from 1 to 4 mm. These nodules were seen on an area measuring 2.0 × 7.0 cm on her left vulva. An excision was performed.

Microscopic Description: The majority of these lesions show well-circumscribed nodules of tumor in the dermis and some extending into subcutaneous tissue. A sharply demarcated border is seen between the deep aspect of the lesion and the subcutaneous tissue (Fig. 11.22). Small ductal structures are seen evenly distributed in a collagenous stroma (Fig. 11.23).

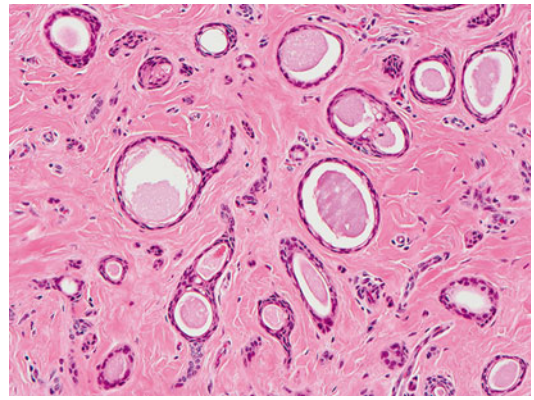


Fig. 11.22 Vignette 1. A circumscribed nodule of tumor in the dermis exhibiting sharply demarcated border between its deep aspect and the subcutaneous tissue

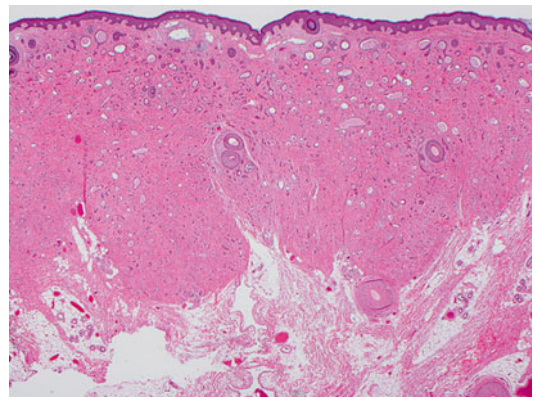


Fig. 11.23 Vignette 1. Small ductal structures are seen evenly distributed in a collagenous stroma

(continued)

Diagnosis: Deep syringoma

Discussion: The deep extension of this syringoma is unusual, and there have been rare cases reported in the literature [95, 96]. Although one "giant" vulvar syringoma has been reported, there has been no mention as to whether there was deep extension of the tumor [151]. The main differential diagnosis of a deep syringoma is a microcystic adnexal carcinoma which is typically a solitary lesion. Syringomas are typically multiple, and solitary presentation would be very unusual [152].

Vignette 2

Clinical History: An 80-year-old female presented with a nodule on her right labium majus. The clinical impression was acrochordon versus neurofibroma versus others.

Microscopic Description: The skin biopsy shows a nodular proliferation of basaloid neoplastic cells exhibiting follicular differentiation, peripheral palisade, and extracellular mucin (Figs. 11.24 and 11.25).

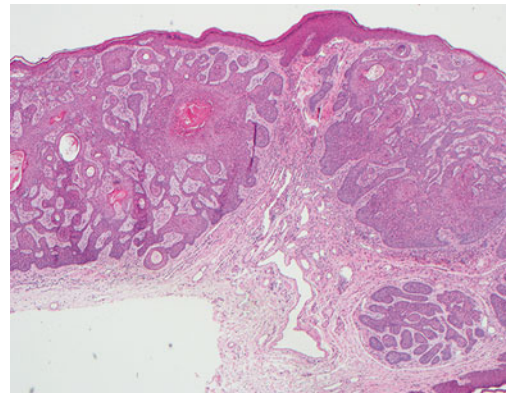


Fig. 11.24 Vignette 2. A nodular proliferation of basaloid neoplastic cells exhibiting follicular differentiation

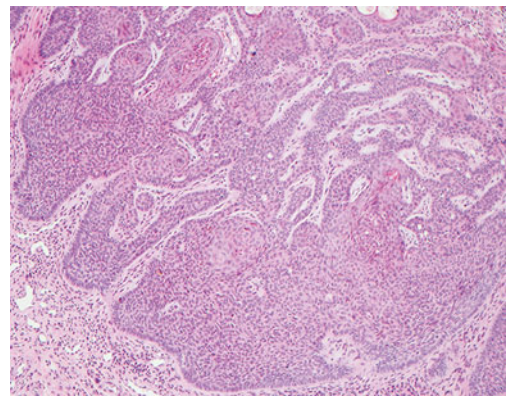


Fig. 11.25 Vignette 2. Peripheral palisade and extracellular mucin are seen

(continued)

Diagnosis: Basal cell carcinoma

Discussion: Basal cell carcinoma (BCC) is the most common cutaneous cancer in the U.S. [153]. However, only approximately 300 cases of anogenital BCC have been reported [153–158], accounting for less than 1 % of all BCC [150] and 3 % of vulvar cancer [155]. The median age appears to be the seventh decade [155, 158]. Only rarely do the patients develop nodal metastases. The occurrence of BCC in non-sun-exposed area raises the possibility of other etiologic agents such as chronic irritation, chronic infection, trauma, irradiation, and arsenical compounds [157]. HPV testing by in situ hybridization for serotypes 6, 11, 16, 18, 31, 33, and 51 was reported negative in five tested cases [158]. This review identified two cases of vulvar BCC (4 % of malignant vulvar adnexal neoplasms), both of which were nodular type, the most common histopathologic subtype [158].

Germ line mutations in the patched or patched 1 (PTCH) gene, a tumor suppressor gene, and the human homolog of the Drosophila patched gene, on chromosome 9q22.3, were demonstrated in BCCs of basal cell nevus syndrome (60–70 %) as well as in sporadic BCCs [159, 160].

Vignette 3

Clinical History: An 82-year-old woman presented with 1-year history of perianal itching that has recently started to spread anteriorly. Physical examination revealed an erythematous plaque extending bilaterally from her anal verge laterally towards both buttocks and anteriorly to the perineal body. Anoscopy showed no obvious intra-anal extension of the lesion.

Microscopic Description: Multiple skin biopsies obtained from her anal as well as right and left labia showed similar histologic findings. There is a proliferation of single and polygonal neoplastic cells within the epidermis (Fig. 11.26). Intracytoplasmic mucin is noted in many of these tumor cells, some with a signet-ring appearance (Fig. 11.27). The tumor cells are strongly positive for cytokeratin 20 (Fig. 11.28), carcinoembryonic antigen, and CDX2 (Fig. 11.29). They are negative for cytokeratin 7 and gross cystic disease fluid protein-15.

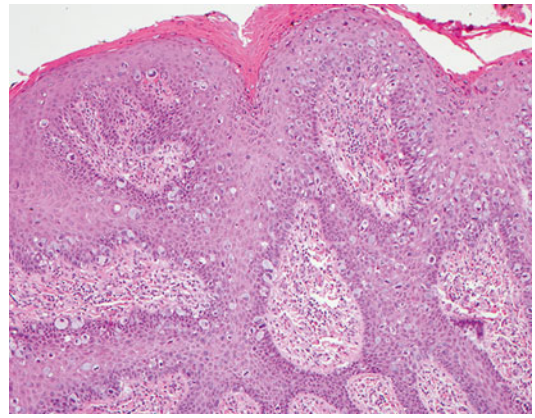


Fig. 11.26 Vignette 3. A proliferation of single polygonal neoplastic cells is seen within the epidermis

(continued)

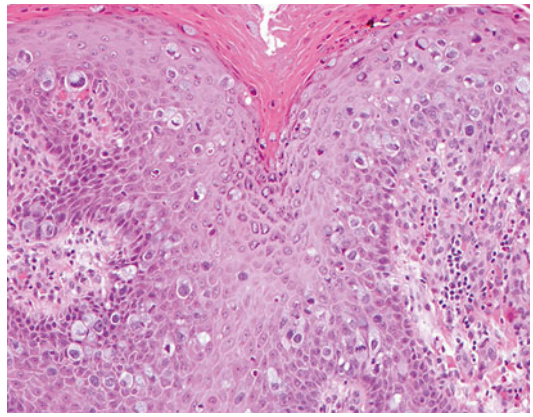


Fig. 11.27 Vignette 3. Intracytoplasmic mucin is noted

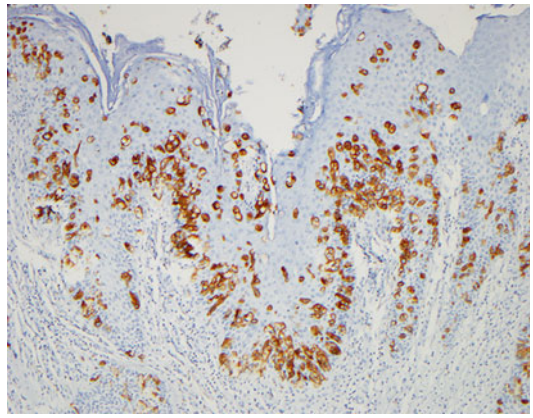


Fig. 11.28 Vignette 3. The tumor cells are positive for cytokeratin 20

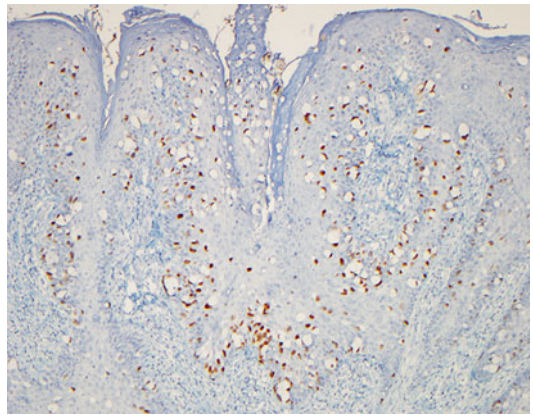


Fig. 11.29 Vignette 3. Nuclear CDX2 expression is noted within the tumor cells

(continued)

Diagnosis: Secondary EMPD from a colorectal primary

Discussion: In a large meta-analysis of 650 reported cases of vulvoperineal EMPD by Preti et al. [161], 10 % of the cases are associated with an underlying visceral malignancy, most commonly colorectal and urothelial carcinomas. Although the definitive diagnosis would depend on clinical pathologic correlation, an immunohistochemical panel would be very helpful in narrowing the differential diagnosis (Table 11.2). Whereas primary EMPD lesions would typically have an immunoprofile of CK7+ CK20- GCDFP-15+ (Fig. 11.7); secondary EMPD lesions would likely be CK7−/+CK20+ GCDFP-15− [58]. As illustrated in this case, the co-expression of CK20 and CDX2 is indicative of secondary EMPD of a colorectal primary [59, 60]. HER-2/neu would be seen in 65 % of primary EMPD in contrast to only 15 % of secondary EMPD, while CDX2 would be seen in 100 % and 33 % of secondary and primary EMPD, respectively [61, 62]. In addition, MUC5AC is commonly expressed in the majority of primary EMPD, whereas MUC2 would be positive in secondary EMPD from a colonic primary [162, 163]. Secondary EMPD of urothelial origin would express Uroplakin III and p63 [63].

References

1. Williams PL, Bannister LH, Berry MM. Gray's anatomy. 38th ed. New York: Churchill Livingstone; 1995.
2. Van der Putte SC. Anogenital "sweat" glands. Histology and pathology of a gland that may mimic mammary glands. Am J Dermatopathol. 1991;13(6):557–67.
3. Van der Putte SC. Mammary-like glands of the vulva and their disorders. Int J Gynecol Pathol. 1994;13(2):150–60.
4. Kazakov DV, Spagnolo DV, Kacerovska D, Michal M. Lesions of anogenital mammary-like glands: an update. Adv Anat Pathol. 2011;18(1):1–28.
5. Toker C. Clear cells of the nipple epidermis. Cancer. 1970;25(3):601–10.
6. Willman JH, Golitz LE, Fitzpatrick JE. Vulvar clear cells of Toker: precursors of extramammary Paget's disease. Am J Dermatopathol. 2005;27(3):185–8.
7. Friedel R. Ein Fibroadenom einer nebenbrustdruese im rechten labium maius. Virchows Arch F Path Anat. 1932;286:62–9.
8. Sington JD, Manek S, Hollowood K. Fibroadenoma of the mammary-like glands of the vulva. Histopathology. 2002;41(6):563–5.
9. Carter JE, Mizell KN, Tucker JA. Mammary-type fibroepithelial neoplasms of the vulva: a case report and review of the literature. J Cutan Pathol. 2008;35(2):246–9.
10. Vella JE, Taibjee SM, Sanders DS, Stellakis M, Carr RA. Fibroadenoma of the anogenital region. J Clin Pathol. 2008;61(7):871–2.
11. Kazakov DV, Spagnolo DV, Stewart CJ, Thompson J, Agaimy A, Magro G, et al. Fibroadenoma and phyllodes tumors of anogenital mammary-like glands: a series of 13 neoplasms in 12 cases, including mammary-type juvenile fibroadenoma, fibroadenoma with lactation changes, and neurofibromatosis-associated pseudoangiomatous stromal hyperplasia with multinucleated giant cells. Am J Surg Pathol. 2010;34(1):95–103.
12. Mannan AA, Kahvic M, Aziz AH. Phyllodes tumor of the vulva: report of a rare case and review of the literature. Am J Dermatopathol. 2010;32(4):384–6.
13. Heffernan TP, Sarode VR, Hoffman B, Lea J. Recurrent phyllodes tumor of the vulva: a case report with review of diagnostic criteria and differential diagnosis. Int J Gynecol Pathol. 2010;29(3):294–7.
14. Audisio T, Crespo-Roca F, Giraudo P, Ramallo R. Fibroadenoma of the vulva – simultaneous with breast fibroadenomas and uterine fibroma. J Low Genit Tract Dis. 2011;15(1):75–9.
15. Hassim AM. Bilateral fibroadenoma in supernumerary breasts of the vulva. J Obstet Gynaecol Br Commonw. 1969;76(3):275–7.
16. Fu L, Lau S, Roy I, Ferenczy A. Phyllodes tumor with malignant stromal morphology of the vulva: a case report and review of the literature. Int J Gynecol Pathol. 2011;30(2):198–202.
17. Kazakov DV, Bisceglia M, Mukensnabl P, Michal M. Pseudoangiomatous stromal hyperplasia in lesions involving anogenital mammary-like glands. Am J Surg Pathol. 2005;29(9):1243–6.
18. Kazakov DV, Nemcova J, Mikyskova I, Belousova IE, Vazmitel M, Michal M. Human papillomavirus in lesions of anogenital mammary-like glands. Int J Gynecol Pathol. 2007;26(4):475–80.
19. Gugliotta P, Fibbi ML, Fessia L, Canevini P, Bussolati G. Lactating supernumerary mammary

gland tissue in the vulva. Appl Pathol. 1983;1(2): 61–5.
20. Lee ES, Kim I. Multiple vulvar lactating adenomas. Obstet Gynecol. 2011;118(2 Pt 2):478–80.
21. O'Hara MF, Page DL. Adenomas of the breast and ectopic breast under lactational influences. Hum Pathol. 1985;16(7):707–12.
22. Lev-Cohain N, Kapur P, Pedrosa I. Vulvar fibroadenoma with lactational changes in ectopic breast tissue. Case Rep Obstet Gynecol. 2013;2013:924902.
23. Woodworth H, Docketty MB, Wilson RB, Pratt JH. Papillary hidradenoma of the vulva: a clinicopathologic study of 69 cases. Am J Obstet Gynecol. 1971;110(4):501–8.
24. Requena L, Kiryu H, Ackerman AB. Neoplasms with apocrine differentiation. Philadelphia: Lippincott-Raven; 1998.
25. Parks A, Branch KD, Metcalf J, Underwood P, Young J. Hidradenoma papilliferum with mixed histopathologic features of syringocystadenoma papilliferum and anogenital mammary-like glands: report of a case and review of the literature. Am J Dermatopathol. 2012;34(1):104–9.
26. Nishie W, Sawamura D, Mayuzumi M, Takahashi S, Shimizu H. Hidradenoma papilliferum with mixed histopathologic features of syringocystadenoma papilliferum and anogenital mammary-like glands. J Cutan Pathol. 2004;31(8):561–4.
27. Scurry J, van der Putte SC, Pyman J, Chetty N, Szabo R. Mammary-like gland adenoma of the vulva: review of 46 cases. Pathology. 2009;41(4): 372–8.
28. Baker GM, Selim MA, Hoang MP. Vulvar adnexal lesions: a 32-year, single-institution review from Massachusetts General Hospital. Arch Pathol Lab Med. 2013;137(9):1237–46.
29. Kazakov DV, Mikyskova I, Kutzner H, Simpson RHW, Hes O, Mukensnabl P, et al. Hidradenoma papilliferum with oxyphilic metaplasia: a clinicopathological study of 18 cases, including detection of human papillomavirus. Am J Dermatopathol. 2005;27(2):102–10.
30. Sington J, Chandrapala R, Manek S, Hollowood K. Mitotic count is not predictive of clinical behavior in hidradenoma papilliferum of the vulva: a clinicopathologic study of 19 cases. Am J Dermatopathol. 2006;28(4):322–6.
31. Plumb SJ, Argenyi ZB, Stone MS, De Young BR. Cytokeratin 5/6 immunostaining in cutaneous adnexal neoplasms and metastatic adenocarcinoma. Am J Dermatopathol. 2004;26(6):447–51.
32. Mahalingam M, Srivastava A, Hoang MP. Expression of stem-cell markers (cytokeratin 15 and nestin) in primary adnexal neoplasms – clues to etiopathogenesis. Am J Dermatopathol. 2010;32(8):774–9.
33. Swanson PE, Mazoujian G, Mills SE, Campbell RJ, Wick MR. Immunoreactivity for estrogen receptor protein in sweat gland tumors. Am J Surg Pathol. 1991;15(9):835–41.
34. Mazoujian G, Margolis R. Immunohistochemistry of gross cystic disease fluid protein (GCDFP-15) in 65 benign sweat gland tumors of the skin. Am J Dermatopathol. 1988;10(1):28–35.
35. Castro CY, Deavers M. Ductal carcinoma in-situ arising in mammary-like glands of the vulva. Int J Gynecol Pathol. 2001;20(3):277–83.
36. Pelosi G, Martignoni G, Bonetti F. Intraductal carcinoma of mammary-type apocrine epithelium arising within a papillary hidradenoma of the vulva. Report of a case and review of the literature. Arch Pathol Lab Med. 1991;115(12):1249–54.
37. Shah SS, Adelson M, Mazur MT. Adenocarcinoma in situ arising in vulvar papillary hidradenoma: report of 2 cases. Int J Gynecol Pathol. 2008;27(3):453–6.
38. Vazmitel M, Spagnolo DV, Nemcova J, Michal M, Kazakov DV. Hidradenoma papilliferum with a ductal carcinoma in situ component: case report and review of the literature. Am J Dermatopathol. 2008;30(4):392–4.
39. Crocker H. Paget's disease affecting the scrotum and penis. Trans Pathol Soc Lond. 1889;40:187–91.
40. Lam C, Funaro D. Extramammary Paget's disease: summary of current knowledge. Dermatol Clin. 2010;28(4):807–26.
41. Belousova IE, Kazakov DV, Michal M, Suster S. Vulvar Toker cells: the long-awaited missing link: a proposal for an origin-based histogenetic classification of extramammary Paget disease. Am J Dermatopathol. 2006;28(1):84–6.
42. Lloyd J, Flanagan AM. Mammary and extramammary Paget's disease. J Clin Pathol. 2000;53(10):742–9.
43. Regauer S. Extramammary Paget's disease – a proliferation of adnexal origin? Histopathology. 2006; 48(6):723–9.
44. Chanda JJ. Extramammary Paget's disease: prognosis and relationship to internal malignancy. J Am Acad Dermatol. 1985;13(6):1009–14.
45. Shepherd V, Davidson EJ, Davies-Humphreys J. Extramammary Paget's disease. BJOG. 2005;112(3): 273–9.
46. Kanitakis J. Mammary and extramammary Paget's disease. J Eur Acad Dermatol Venerol. 2007;21(5): 581–90.
47. Goldblum JR, Hart WR. Perianal Paget's disease: a histologic and immunohistochemical study of 11 cases with and without associated rectal adenocarcinoma. Am J Surg Pathol. 1998;22(2):170–9.
48. De Magnis A, Checcucci V, Catalano C, Corazzesi A, Pieralli A, Taddei G, Frambrini M. Vulvar paget disease: a large single-centre experience on clinical presentation, surgical treatment, and long-term outcomes. J Low Genit Tract Dis. 2013;17(2): 104–10.
49. Cai Y, Sheng W, Xiang L, Wu X, Yang H. Primary extramammary Paget's disease of the vulva: the clinicopathological features and treatment outcomes in a series of 43 patients. Gynecol Oncol. 2013;129(2): 412–6.

50. Shaco-Levy R, Bean SM, Vollmer RT, Papalas JA, Bentley RC, Selim MA, Robboy SJ. Paget disease of the vulva: a histologic study of 56 cases correlating pathologic features and disease course. Int J Gynecol Pathol. 2010;29(1):69–78.
51. Siesling S, Elferink MA, van Dijck JA, Pierie P, Blokx WA. Epidemiology and treatment of extramammary Paget disease in the Netherlands. Eur J Surg Oncol. 2007;33(8):951–5.
52. Kawatsu T, Miki Y. Triple extramammary Paget's disease. Arch Dermatol. 1971;104(3):316–9.
53. Jones ISC, Crandon A, Sanday K. Paget's disease of the vulva: diagnosis and follow-up key to management; a retrospective study of 50 cases from Queensland. Gynecol Oncol. 2011;122(1):42–4.
54. Parker LP, Parker JR, Bodurka-Bevers D, Deavers M, Bevers MW, Shen-Gunther J, Gerhenson DM. Paget's disease of the vulva: pathology, pattern of involvement, and prognosis. Gynecol Oncol. 2000;77(1):183–9.
55. Kodama S, Kanedo T, Saito M, Yoshiya N, Honma S, Tanaka K. A clinicopathologic study of 30 patients with Paget's disease of the vulva. Gynecol Oncol. 1995;56(1):63–70.
56. Karam A, Dorigo O. Increased risk and pattern of secondary malignancies in patients with invasive extramammary Paget disease. Br J Dermatol. 2014;170(3):661–71.
57. Feuer GA, Shevchuk M, Calanog A. Vulvar Paget's disease: the need to exclude an invasive lesion. Gynecol Oncol. 1990;38(1):81–9.
58. Goldblum JR, Hart WR. Vulvar Paget's disease: a clinicopathologic and immunohistochemical study of 19 cases. Am J Surg Pathol. 1997;21(10):1178–87.
59. De Nisi MC, D'Amuri A, Toscano M, Lalinga AV, Pirtoli L, Miracco C. Usefulness of CDX2 in the diagnosis of extramammary Paget disease associated with malignancies of intestinal type. Br J Dermatol. 2005;153(3):677–9.
60. Ohnishi T, Watanabe S. The use of cytokeratins 7 and 20 in the diagnosis of primary and secondary extramammary Paget's disease. Br J Dermatol. 2000;142(2):243–7.
61. Perrotto J, Abbott JJ, Ceilley RI, Ahmed I. The role of immunohistochemistry in discriminating primary from secondary extramammary Paget's disease. Am J Dermatopathol. 2010;32(2):137–43.
62. Plaza JA, Torres-Cabala C, Ivan D, Prieto VG. HER-2/neu expression in extramammary Paget disease: a clinicopathologic and immunohistochemistry study of 47 cases with and without underlying malignancy. J Cutan Pathol. 2009;36(7):729–33.
63. Brown HM, Wilkinson EJ. Uroplakin-III to distinguish primary vulvar Paget disease from Paget disease secondary to urothelial carcinoma. Hum Pathol. 2002;33(5):545–8.
64. Liegl B, Horn HC, Moinfar F. Androgen receptors are frequently expressed in mammary and extramammary Paget's disease. Mod Pathol. 2005;18(10):1283–8.
65. Hikita T, Ohtsuki Y, Maeda T, Furihata M. Immunohistochemical and fluorescence studies on noninvasive and invasive extramammary Paget's disease. Int J Surg Pathol. 2012;20(5):441–8.
66. Kang Z, Xu F, Zhang QA, Lin J, Wu Z, Zhang X, et al. Correlation of DLC1 gene methylation with oncogenic PIK3CA mutations in extramammary Paget's disease. Mod Pathol. 2012;25(8):1160–8.
67. Kang Z, Xu F, Zhang QA, Wu Z, Zhang X, Xu J, et al. Oncogenic mutations in extramammary Paget's disease and their clinical relevance. Int J Cancer. 2013;132(4):824–31.
68. Qian Y, Zhang N, Chen S, Chu S, Feng A, Liu H. PI3K, Rac1 and pPAK1 are overexpressed in extramammary Paget's disease. J Cutan Pathol. 2012;39(11):1010–5.
69. McCluggage WG. Recent developments in vulvovaginal pathology. Histopathology. 2009;54(2):156–73.
70. Abbott JJ, Ahmed I. Adenocarcinoma of mammary-like glands of the vulva: report of a case and review of the literature. Am J Dermatopathol. 2006;28(2):127–33.
71. Wick MR, Ockner DM, Mills SE, Ritter JH, Swanson PS. Homologous carcinomas of the breast, skin, and salivary glands. Am J Clin Pathol. 1998;109(1):75–84.
72. Van der Putte SC, van Gorp LH. Adenocarcinoma of the mammary-like glands of the vulva: a concept unifying sweat gland carcinoma of the vulva, carcinoma of supernumerary mammary glands and extramammary Paget's disease. J Cutan Pathol. 1994;21(2):157–63.
73. Tanaka H, Umekawa T, Nagao K, Ishihara A, Toyoda N. Adenocarcinoma of mammary-like glands in the vulva successfully treated by weekly paclitaxel. Int J Gynecol Cancer. 2005;15(3):568–71.
74. Alsaad KO, Obaidat N, Dube V, Chapman W, Ghazarian D. Vulvar apocrine adenocarcinoma: a case with nodal metastasis and intranodal mucinous differentiation. Pathol Res Pract. 2009;205(2):131–5.
75. Benito V, Arribas S, Martinez D, Medina N, Lubrano A, Arencibia O. Metastatic adenocarcinoma of mammary-like glands of the vulva successfully treated with surgery and hormonal therapy. J Obstet Gynaecol Res. 2013;39(1):450–4.
76. Kazakov DV, Belousova IE, Sima R, Michal M. Mammary type tubulolobular carcinoma of the anogenital area: report of a case of a unique tumor presumably originating in anogenital mammarylike glands. Am J Surg Pathol. 2006;30(9):1193–6.
77. Fernandez-Figueras MT, Michal M, Kazakov DV. Mammary-type tubulolobular carcinoma of anogenital mammary-like glands with prominent stromal elastosis. Am J Surg Pathol. 2010;34(8):1224–6.
78. Carneiro SJ, Gardner HL, Knox JM. Syringoma: three cases with vulvar involvement. Obstet Gynecol. 1972;39(1):95–9.

79. Young Jr AW, Herman EW, Tovell HM. Syringoma of the vulva: incidence, diagnosis, and cause of pruritus. Obstet Gynecol. 1980;55(4):515–8.
80. Huang YH, Chuang YH, Kuo TT, Yang LC, Hong HS. Vulvar syringoma: a clinicopathologic and immunohistologic study of 18 patients and results of treatment. J Am Acad Dermatol. 2003;48(5):735–9.
81. Wallace ML, Smoller BR. Progesterone receptor positivity supports hormonal control of syringomas. J Cutan Pathol. 1995;22(5):442–5.
82. Yorganci A, Kale A, Dunder I, Ensari A, Sertcelik A. Vulvar syringoma showing progesterone receptor positivity. BJOG. 2000;107(2):292–4.
83. Trager JD, Silvers J, Reed JA, Scott RA. Neck and vulvar papules in an 8-year-old girl. Arch Dermatol. 1999;135(2):203. 206.
84. Chandler WM, Bosenberg MW. Autoimmune acrosyringitis with ductal cysts: reclassification of case of eruptive syringoma. J Cutan Pathol. 2009;36(12): 1312–5.
85. Tay YK, Tham SN, Teo R. Localized vulvar syringomas – an unusual cause of pruritus vulvae. Dermatology. 1996;192(1):62–3.
86. Belardi MG, Maglione MA, Vighi S, di Paola GR. Syringoma of the vulva: a case report. J Reprod Med. 1994;39(12):957–9.
87. Zhu WY. Vulvar syringoma associated with epidermal cyst. Int J Dermatol. 1989;28(2):142–3.
88. Furue M, Hori Y, Nakabayashi Y. Clear-cell syringoma. Association with diabetes mellitus. Am J Dermatopathol. 1984;6(2):131–8.
89. Missall TA, Burkemper NM, Jensen SL, Hurley MY. Immunohistochemical differentiation of four benign eccrine tumors. J Cutan Pathol. 2009;36(2):190–6.
90. Eckert F, Nilles M, Schmid U, Altmannsberger M. Distribution of cytokeratin polypeptides in syringomas. An immunohistochemical study on paraffin-embedded material. Am J Dermatopathol. 1992; 14(2):115–21.
91. Ohnishi T, Watanabe S. Immunohistochemical analysis of keratin expression in clear cell syringoma. A comparative study with conventional syringoma. J Cutan Pathol. 1997;24(6):370–6.
92. Kim BC, Park EJ, Kwon IH, Cho HJ, Park HR, Kim KH, Kim KJ. An immunohistochemical study of the origin of the solid strand in syringoma, using carcinoembryonic antigen, epithelial membrane antigen, and cytokeratin 5. Int J Dermatol. 2012;51(7): 817–22.
93. Langbein L, Cribier B, Schirmacher P, Praetzel-Wunder S, Peltre B, Schweizer J. New concepts on the histogenesis of eccrine neoplasia from keratin expression in the normal eccrine gland, syringoma and poroma. Br J Dermatol. 2008;159(3):633–45.
94. Kazakov DV, Bouda Jr J, Kacerovska D, Michal M. Vulvar syringomas with deep extension: a potential histopathologic mimic of microcystic adnexal carcinoma. Int J Gynecol Pathol. 2011;30(1):92–4.
95. Suwattee P, McClelland MC, Huiras EE, Warshaw EM, Lee PK, Kaye VN, et al. Plaque-type syringoma: two cases misdiagnosed as microcystic adnexal carcinoma. J Cutan Pathol. 2008;35(6):570–4.
96. Kersting DW. Clear cell hidradenoma and hidradenocarcinoma. Arch Dermatol. 1963;87:323–33.
97. Bondi R, Ambrosi L. Clear cell hidradenoma (with special reference to the usual localization in the vulva. Arch De Vecchi Anat Patol. 1963;41:201–25.
98. Messing MJ, Richardson MS, Smith MT, King L, Gallup DG. Metastatic clear-cell hidradenocarcinoma of the vulva. Gynecol Oncol. 1993;48(2):264–8.
99. Biedrzycki OJ, Rufford B, Wilcox M, Barton DPJ, Jameson C. Malignant clear cell hidradenoma of the vulva: report of a unique case and review of the literature. Int J Gynecol Pathol. 2008;27(1):142–6.
100. Webb JB, Beswick IP. Eccrine hidradenocarcinoma of the vulva with Paget's disease. Case report with a review of the literature. Br J Obstet Gynaecol. 1993;90(1):90–5.
101. Kazakov DV, Ivan D, Kutzner H, Spagnolo DV, Grossmann P, Vanecek T, et al. Cutaneous hidradenocarcinoma: a clinicopathological, immunohistochemical, and molecular biologic study of 14 cases, including Her2/neu gene expression/amplification, TP53 gene mutation analysis, and t(11;19) translocation. Am J Dermatopathol. 2009;31(3):236–47.
102. Nash JW, Barrett TL, Kies M, Ross MI, Sneige N, Diwan AH, Lazar AJ. Metastatic hidradenocarcinoma with demonstration of Her-2/neu gene amplification by fluorescence in situ hybridization: potential treatment implications. J Cutan Pathol. 2007;34(1): 49–54.
103. Dias-Santagata D, Lam Q, Bergethon K, Baker GM, Iafrate AJ, Rakheja D, Hoang MP. A potential role for targeted therapy in a subset of metastasizing adnexal carcinomas. Mod Pathol. 2011;24(7):974–82.
104. Le LP, Dias-Santagata D, Pawlak AC, Cosper AK, Nguyen AT, Selim MA, et al. Apocrine-eccrine carcinomas: molecular and immunohistochemical analyses. PLoS One. 2012;7(10):e47290.
105. Nazarian RM, Kapur P, Rakheja D, Piris A, Duncan LM, Mihm Jr MC, Hoang MP. Atypical and malignant hidradenomas: a histologic and immunohistochemical study. Mod Pathol. 2009;22(4):600–10.
106. Stefanato CM, Ferrara G, Chaudhry IH, Guevara Pineda C, Waschkowski G, Rose C, Calonje E. Clear cell nodular hidradenoma involving the lymphatic system: a tumor of uncertain malignant potential or a novel example of "metastasizing" benign tumor? Am J Surg Pathol. 2012;36(12):1835–40.
107. Carlson JW, McGlennen RC, Gomez R, Longbella C, Carter J, Carson LF. Sebaceous carcinoma of the vulva: a case report and review of the literature. Gynecol Oncol. 1996;60(3):489–91.
108. Rulon DB, Helwig EB. Cutaneous sebaceous neoplasms. Cancer. 1974;33(1):82–102.
109. Jacobs DM, Sandles LG, Leboit PE. Sebaceous carcinoma arising from Bowen's disease of the vulva. Arch Dermatol. 1986;122(10):1191–3.
110. Escalonilla P, Grilli R, Canamero M, Soriano ML, Farina MC, Manzarbeitia F, et al. Sebaceous

carcinoma of the vulva. Am J Dermatopathol. 1999; 21(5):468–72.
111. Pusiol T, Morichetti D, Zorzi MG. Sebaceous carcinoma of the vulva: critical approach to grading and review of the literature. Pathologica. 2011;103(3): 64–7.
112. Khan Z, Misra G, Fiander AN, Dallimore NS. Sebaceous carcinoma of the vulva. BJOG. 2003; 110(2):227–8.
113. Kawamoto M, Fukuda Y, Kamoi S, Sugisaki Y, Yamanaka N. Sebaceous carcinoma of the vulva. Pathol Int. 1995;45(10):767–73.
114. Mojtahed A, Schrijver I, Ford JM, Longacre TA, Pai RK. A two-antibody mismatch repair protein immunohistochemistry screening approach for colorectal carcinomas, skin sebaceous tumors, and gynecologic tract carcinomas. Mod Pathol. 2011; 24(7):1004–14.
115. Ostler DA, Prieto VG, Reed JA, Deavers MT, Lazar AJ, Ivan D. Adipophilin expression is sebaceous tumors and other cutaneous lesions with clear cell histology: an immunohistochemical study of 117 cases. Mod Pathol. 2010;23(4):567–73.
116. Boussahmain C, Mochel MC, Hoang MP. Perilipin and adipophilin expression in sebaceous carcinoma and mimics. Hum Pathol. 2013;44(9):1811–6.
117. Pinkus H, Rogin JR, Goldman R. Eccrine poroma. Tumors exhibiting features of the epidermal sweat duct unit. AMA Arch Dermatol. 1956;74(5): 511–21.
118. Battistella M, Langbein L, Peltre B, Cribier B. From hidroacanthoma simplex to poroid hidradenoma: clinicopathologic and immunohistochemic study of poroid neoplasms and reappraisal of their histogenesis. Am J Dermatopathol. 2010;32(5):459–68.
119. Mahalingam M, Richards JE, Selim MA, Muzikansky A, Hoang MP. An immunohistochemical comparison of cytokeratin 7, cytokeratin 15, cytokeratin 19, CAM5.2, carcinoembryonic antigen, and nestin in differentiating porocarcinoma from squamous cell carcinoma. Hum Pathol. 2012;43(8): 1265–72.
120. Ichihashi N, Kitajima Y. Loss of heterozygosity of adenomatous polyposis coli gene in cutaneous tumors as determined by using polymerase chain reaction and paraffin section preparations. J Dermatol Sci. 2000;22(2):102–6.
121. Adegboyega PA. Eccrine porocarcinoma of the vulva: a case report and review of literature. Int J Gynecol Pathol. 2011;30(1):95–100.
122. Katsanis WA, Doering DL, Bosscher JR, O'Conner DM. Vulva eccrine porocarcinoma. Gynecol Oncol. 1996;62(3):396–9.
123. Liegl B, Regauer S. Eccrine carcinoma (nodular porocarcinoma) of the vulva. Histopathology. 2005; 47(3):324–6.
124. Stephen MR, Matalka I, Hanretty K. Malignant eccrine poroma of the vulva. Br J Obstet Gynaecol. 1998;105(4):471–2.
125. Crain RC, Helwig EB. Dermal cylindroma (dermal eccrine cylindroma). Am J Clin Pathol. 1961;35: 504–15.
126. Young AL, Kellermayer R, Szigeti R, Teszas A, Azmi S, Celebi JT. CYLD mutations underlie Brooke-Spiegler, familial cylindromatosis, and multiple familial trichoepithelioma syndromes. Clin Genet. 2006;70(3):246–9.
127. Grossmann P, Vanecek T, Steiner P, Kacerovska D, Spagnolo DV, Cribier B, et al. Novel and recurrent germline and somatic mutations in a cohort of 67 patients from 48 families with Brooke-Spiegler syndrome including the phenotypic variant of multiple familial trichoepitheliomas and correlation with the histopathologic findings in 379 biopsy specimens. Am J Dermatopathol. 2013;35(1):34–44.
128. Sayre GP. Cylindroma of the vulva; adenocarcinoma, cylindroma type, of the vulva; report of a case of 27 years' duration. Proc Staff Meet Mayo Clin. 1949;24(9):224–33.
129. Kacerovska D, Szepe P, Vanecek T, Nemcova J, Michal M, Mukensnabl P, Kazakov DV. Spiradenocylindroma-like basaloid carcinoma of the anus and rectum: case report, including HPV studies and analysis of the CYLD gene mutations. Am J Dermatopathol. 2008;30(5):472–6.
130. Nambo NC. Eccrine spiradenoma: clinical and pathologic study of 49 tumors. J Cutan Pathol. 1983;10(5):312–20.
131. Kacerovska D, Kazakov DV, Kutzner H, Michal M. Spiradenoma with marked adenomyoepitheliomatous features. Am J Dermatopathol. 2010;32(7):744–6.
132. Kazakov DV, Magro G, Kutzner H, Spagnolo DV, Yang Y, Zaspa O, Mukensnabl P, Michal M. Spiradenoma and spiradenocylindroma with an adenomatous or atypical adenomatous component: a clinicopathological study of 6 cases. Am J Dermatopathol. 2008;30(5):436–41.
133. Kazakov DV, Zelger B, Rütten A, Vazmitel M, Spagnolo DV, Kacerovska D, et al. Morphologic diversity of malignant neoplasms arising in preexisting spiradenoma, cylindroma, and spiradenocylindroma based on the study of 24 cases, sporadic or occurring in the setting of Brooke-Spiegler syndrome. Am J Surg Pathol. 2009;33(5):705–19.
134. Chen G, Cheuk W, Cheung JS, Chan JK. Carcinosarcoma ex eccrine spiradenoma of the vulva: report of the first case. Int J Gynecol Pathol. 2011;30(3):301–5.
135. Emam EE, Sawan AS, Al-Tamimi SR, Molah RM. Malignant spiradenoma/cylindroma of the vulva. Saudi Med J. 2012;33(11):1229–33.
136. Chase DM, Basu T, Saffari B, Ries S, Berman ML. Malignant eccrine spiradenoma of the vulva: a case report and review of the literature. Int J Gynecol Cancer. 2006;16(3):1465–9.
137. Su A, Apple SK, Moatamed NA. Pleomorphic adenoma of the vulva, clinical reminder of a rare occurrence. Rare Tumors. 2012;4(1):e16.

138. Ordonez NG, Manning JT, Luna MA. Mixed tumor of the vulva: a report of two cases probably arising in Bartholin's gland. Cancer. 1981;48(1):181–6.
139. Dykgraaf RH, van Veen MM, van Bekkum-de Jonge EE, Gerretsen J, de Jong D, Burger CW. Pleomorphic adenoma of the vulva: a review illustrated by a clinical case. Int J Gynecol Cancer. 2006;16(2):920–3.
140. Rorat E, Wallach RC. Mixed tumors of the vulva: clinical outcome and pathology. Int J Gynecol Pathol. 1984;3(3):323–8.
141. Wilson D, Woodger BA. Pleomorphic adenoma of the vulva. J Obstet Gynaecol Br Commonw. 1974;81(12):1000–2.
142. Chome J, Giard R. Case report of an unusual tumor of the vulva: epithelioma of rearranged stroma or so-called mixed tumor. Bull Fed Soc Gynecol Obstet Lang Fr. 1956;8(5):562–5 [Article in French].
143. Kazakov DV, Belousova IE, Bisceglia M, Calonje E, Emberger M, Grayson W, et al. Apocrine mixed tumor of the skin ("mixed tumor of the folliculosebaceous-apocrine complex"). Spectrum of differentiations and metaplastic changes in the epithelial, myoepithelial, and stromal components based on a histopathologic study of 244 cases. J Am Acad Dermatol. 2007;57(3):467–83.
144. Kazakov DV, Kacerovska D, Hantschke M, Zelger B, Kutzner H, Requena L, et al. Cutaneous mixed tumor, eccrine variant: a clinicopathologic and immunohistochemical study of 50 cases, with emphasis on unusual histopathologic features. Am J Dermatopathol. 2011;33(6):557–68.
145. Gemer O, Piura B, Segal S, Inbar IY. Adenocarcinoma arising in a chondroid syringoma of vulva. Int J Gynecol Pathol. 2003;22(4):398–400.
146. Kazakov DV, Michal M, Kacerovska D, McKee PH. Cutaneous adnexal tumors. Philadelphia: William & Wilkins Lippincott; 2012.
147. Mazur MT, Hsueh S, Gersell DJ. Metastases to the female genital tract. Analysis of 325 cases. Cancer. 1984;53(9):1978–84.
148. Dehner LP. Metastatic and secondary tumors of the vulva. Obstet Gynecol. 1973;42(1):47–57.
149. Neto AG, Deavers MT, Silva EG, Malpica A. Metastatic tumors of the vulva: a clinicopathologic study of 66 cases. Am J Surg Pathol. 2003;27:799–804.
150. Thomakos N, Rodolakis A, Akrivos N, Skampardonis N, Sotiropoulou M, Biliatis I, et al. Metastatic medullary carcinoma of the vulva in a patient with MEN IIb syndrome. In Vivo. 2010; 24(5):791–4.
151. Blasdale C, McLelland J. Solitary giant vulval syringoma. Br J Dermatol. 1999;141(2):374–5.
152. Henner MS, Shapiro PE, Ritter JH, Leffell DJ, Wick MR. Solitary syringoma: report of five cases and clinicopathologic comparison with microcystic adnexal carcinoma of the skin. Am J Dermatopathol. 1995;17(5):465–70.
153. Miller SJ. Biology of basal cell carcinoma (part 1). J Am Acad Dermatol. 1991;24(1):1–13.
154. De Giorgi V, Salvini C, Massi D, Raspollini M, Carli P. Vulvar basal cell carcinoma: retrospective study and review of literature. Gynecol Oncol. 2005;97(1): 192–4.
155. Benedet JL, Miller DM, Ehlen TG, Bertrand MA. Basal cell carcinoma of the vulva: clinical features and treatment results in 28 patients. Obstet Gynecol. 1997;90(5):765–8.
156. Betti R, Bruscagin C, Inselvini E, Crosti C. Basal cell carcinomas of covered and unusual sites of the body. Int J Dermatol. 1997;36(7):503–5.
157. Feakins RM, Lowe DG. Basal cell carcinoma of the vulva: clinicopathologic study of 45 cases. Int J Gynecol Pathol. 1997;16(4):319–24.
158. Gibson GE, Ahmed I. Perianal and genital basal cell carcinoma: a clinicopathologic review of 51 cases. J Am Acad Dermatol. 2001;45(1):68–71.
159. Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, et al. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science. 1996;272(5268):1668–71.
160. Hahn H, Wicking C, Zaphiropoulos PG, Gailani MR, Shanley S, Chidambaram A, et al. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell. 1996;85(6):841–51.
161. Preti M, Micheletti L, Massobrio M, Ansai S-I, Wilkinson EJ. Vulvar Paget disease: one century after first reported. J Low Genit Tract Dis. 2003; 7(2):122–35.
162. Kuan SF, Montaq AG, Hart J, Krausz T, Recant W. Differential expression of mucin genes in mammary and extramammary Paget's disease. Am J Surg Pathol. 2001;25(12):1469–77.
163. Yoshii N, Kitajima S, Yonezawa S, Matsukita S, Setoyama M, Kanzaki T. Expression of mucin core proteins in extramammary Paget's disease. Pathol Int. 2002;52(5–6):390–9.

Cysts, Glandular Lesions, and Others

12

Mai P. Hoang, Dmitry V. Kazakov, and Maria Angelica Selim

Introduction

A wide range of endogenous structures, besides cutaneous adnexal structures and anogenital mammary-like glands, can give rise to cystic lesions as well as benign and malignant neoplasms (Table 12.1). They include Bartholin gland (Bartholin gland cyst, hyperplasia, adenoma, and carcinoma), paraurethral (Skene) gland (Skene gland cyst, hyperplasia, and carcinoma), prostatic tissue type of the vulva, canal of Nuck (mesothelial cyst), mesonephric remnant (mesonephric-like or Gartner cyst), endometriosis, and cloacogenic remnants (cloacogenic carcinoma). Miscellaneous lesions of the vulva will also be discussed in this chapter such as mucinous metaplasia of the vulva, vestibular papillomatosis, granular cell tumor, hidradenitis suppurativa, and epidermolytic hyperkeratosis.

Bartholin Gland

In 1677, Caspar Secundus Bartholin, a Dutch anatomist, described the major vestibular glands [1]. The Bartholin gland is a bilateral, round-to-oval gland measuring about 1 cm in diameter (hence the term major vestibular gland compared to small minor vestibular glands) which drains through a 2.5 cm duct that opens into the vestibule, exterior and adjacent to the hymen in a posterolateral location (at the 4:00 and 8:00 of the vulvar vestibule). After the age of 30, the gland gradually involutes [2] (see Chap. 1). Normal histology of the gland is that of acini lined by columnar mucus-secreting cells surrounded by a peripheral layer of myoepithelial cells. The Bartholin duct is lined by transitional-like epithelium in its proximal part, but at the vestibular surface, the lining is nonkeratinized squamous epithelium (Fig. 12.1). Cystic lesions and inflammations are the most common benign causes of swelling of the Bartholin gland. Although rare, benign lesions, such as nodular hyperplasia and adenoma, and malignant lesions, including carcinomas, are the most common tumors arising at the posterolateral aspect of the vestibule [3–11].

Bartholin Gland Cyst and Mucous Cyst

Using magnetic resonance imaging studies of 430 asymptomatic women, the incidence of Bartholin gland cysts has been determined to be approximately 3 % [12]. In a study of 104 Bartholin glands from 103 patients, 84.6 % of cases had retention cysts (blockage of duct with retention of secretions leading to secondary cyst formation), abscesses in 10.6 %, endometriosis in

M.P. Hoang (✉)
Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: mhoang@mgh.harvard.edu

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_12, © Springer Science+Business Media New York 2015

Table 12.1 Potential origin of non-squamous carcinomas of the vulva

Anogenital mammary-like glands[1]
Sweat glands[1]
Hair follicles and sebaceous glands[1]
Bartholin glands
Minor vestibular glands
Paraurethral (Skene) gland
Mesonephric duct remnants
Cloacogenic remnants
Endometriosis

[1]See Chap. 11

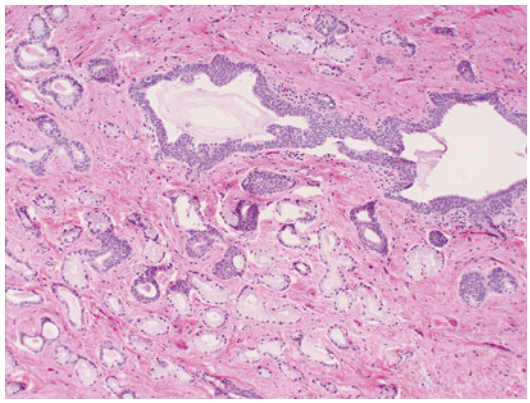


Fig. 12.1 Bartholin gland. Mucous acini and columnar and transitional lining of the duct are seen

2 %, and neoplasms in 3 % [13]. Bartholin gland cyst results from dilatation of the Bartholin duct due to obstruction. The majority of these cysts are seen in women in reproductive age, most frequently in the third decade. The characteristic clinical presentation consists of an asymptomatic unilateral soft cystic or firm nodule of variable size. Large cyst can affect the labia majora and even extend upward and simulate an inguinal hernia or a cyst of the canal of Nuck. Tenderness and discomfort are complains when the lesions are large or infected. A recent meta-analysis of 24 studies, each with 14–200 patients with Bartholin duct cyst or abscess, identified multiple treatment modalities including silver nitrate ablation, cyst excision, carbon dioxide laser, marsupialization, needle aspiration, fistulization, and incision and draining; yet the analysis failed to identify the best treatment form [14].

Histologic Features

Squamous, transitional, ciliated, or mucous epithelium lines the cyst. Any combination of the above epithelial types can be encountered (Fig. 12.2a, b). Areas resembling the fallopian tube epithelium or apocrine-type secretion can rarely be seen. Preexisting Bartholin gland tissue is often detected in the vicinity of the cyst [15, 16]. Nearly half of the retention cysts had vari-

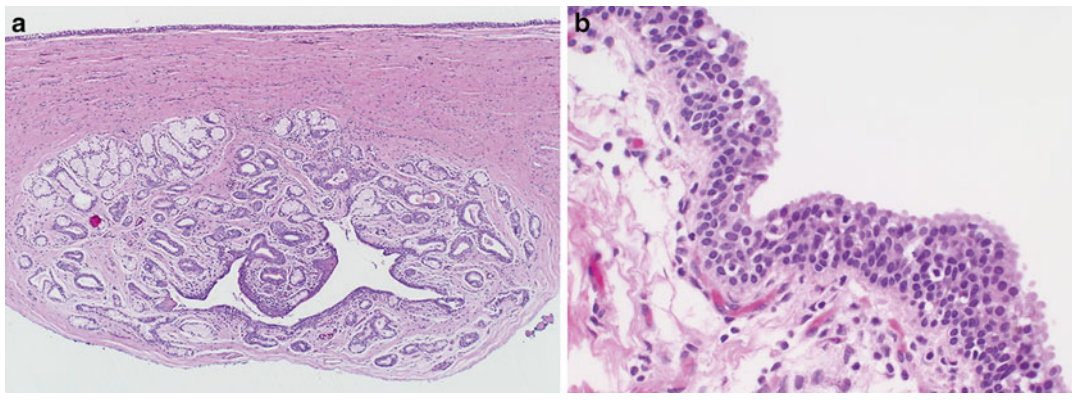


Fig. 12.2 Bartholin gland cyst. (**a**) A combination of squamous, transitional, and mucous epithelium is seen lining the wall of the cyst. (**b**) Transitional epithelium at high magnification

able degrees of inflammation [13]. Co-occurrence with endometriosis has been reported [17]. Association with human papilloma virus (HPV) has been reported even with the development of high-grade squamous intraepithelial neoplasia [18, 19]. Melanin pigment and melanocytes in the cyst wall were documented in an extraordinary case [20].

Differential Diagnosis

Mucous cysts, likely resulting from occlusion of minor vestibular glands, are smaller cysts containing mucinous and squamous cells. It is likely that some of the so-called mucinous and ciliated cysts of the vulva in fact represent the Bartholin gland cyst. Location is the main discriminant of these lesions. Epidermoid cyst, most frequently located in the labia majora, is lined by stratified squamous epithelium and contains white-yellowish grumous content. Mesonephric-like or Gartner cyst can be differentiated from Bartholin gland cyst by its location in the lateral aspect of the vulva and lining by low columnar nonmucinous epithelium. Cyst of the canal of Nuck, also called mesothelial cyst, can be distinguished from Bartholin gland cyst by its position in the superolateral portion of the labia majora and lining by flattened mesothelial cells.

Summary

Clinical Presentation

- Commonly affect women of reproductive age
- A soft and cystic nodule of variable size

Histologic Features

- A cyst lined by squamous, transitional, ciliated, or mucous epithelium

Differential Diagnosis

- Mucinous cyst
- Ciliated cyst
- Epidermoid cyst
- Mesonephric-like or Gartner cyst
- Cyst of the canal of Nuck

Takeaway Essentials

Clinical Relevant Pearls

- Most frequent vulvar cyst.
- Location is the most discriminative diagnostic factor.
- The majority of these cysts are asymptomatic.

Pathology Interpretation Pearls

- Any combination of squamous, transitional, ciliated, or mucous epithelium can be seen lining the cyst.
- Presence of residual mucinous glands around the cysts is an important diagnostic clue.

Nodular Hyperplasia

Nodular hyperplasia is a rare and benign lesion of the Bartholin gland presenting clinically as an asymptomatic or slightly painful nodular lesion measuring up to 4–5 cm [6, 21–25]. The ages of the patients ranged from 19 to 56 years [6, 23]. Nodular hyperplasia might be more common than reported since most are often misdiagnosed as cysts clinically; thus, incision and drainage is done rather than excision [21–23]. For cases with incomplete excision, follow-up has not demonstrated recurrence [23]. Dyspareunia and vulvar vestibulitis are rare associations [24, 26]. Since nodular hyperplasia is frequently associated with inflammation and duct obstruction, some have speculated that these may be the inciting factors for the hyperplastic proliferation.

Histologic Features

Grossly, these lesions are multilobular, nonencapsulated, tan to gray in color, and up to 4–5 cm in greatest dimension [6, 22, 23]. It is a well-circumscribed and lobular lesion with an increased number of secretory acini maintaining a normal duct-acinar relationship (Fig. 12.3). The individual acini are composed of cuboidal or columnar cells, with copious, pale, mucin-filled cytoplasm and bland, basally located nuclei resting on a peripheral layer of attenuated myoepithelial spindled

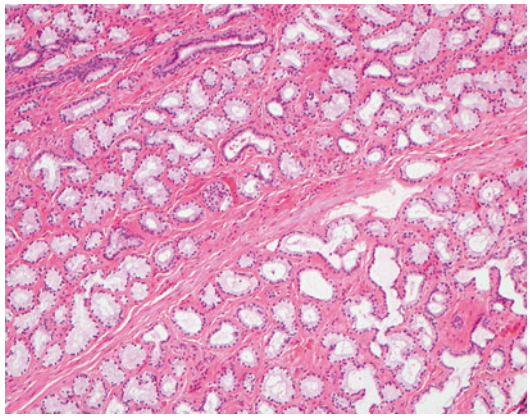


Fig. 12.3 Nodular hyperplasia of Bartholin gland. Although there are an increased number of secretory acini maintaining, a normal duct-acinar relationship is maintained

cells. A sparse lymphohistiocytic infiltrate, squamous metaplasia of the ducts, and extravasated stromal mucin can be seen [6, 22, 23].

By immunohistochemistry, all ten cases of Bartholin gland hyperplasia in Santos et al.'s series (2006) were positive for keratins AE1/AE3, CAM5.2, and high molecular weight epithelial membrane antigen (EMA) and polyclonal carcinoembryonic antigen (CEA). These lesions in Santos and Koenig's series were negative for estrogen receptor (ER) and progesterone receptor (PR), with a low Ki-67 proliferation index [6, 23]. Smooth muscle actin (SMA) highlights the periacinar myoepithelial cells.

Clonality analysis by HUMARA was demonstrated to be clonal in one case, suggesting that these lesions could be neoplastic rather than hyperplastic [22].

Differential Diagnosis

Lesions similar to nodular hyperplasia were reported as "Bartholin gland adenoma" or adenoma of minor vestibular glands resulting in confusion [4, 7, 27]. The proliferation of mucinous acini in nodular hyperplasia would maintain the lobular architecture and duct-acinar relationship and lacks encapsulation with irregular outline, whereas adenoma would be characterized by a sharply circumscribed and encapsulated yet haphazard proliferation of glands and acini without the normal duct-acinar arrangement [4, 6, 7, 27].

Summary

Clinical Presentation

- Solitary nodule

Histologic Features

- Unencapsulated proliferation with irregular borders
- Increased number of secretory acini maintaining a normal duct-acinar relationship and lobular architecture

Differential Diagnosis

- Normal Bartholin gland
- Bartholin gland adenoma

Takeaway Essentials

Clinical Relevant Pearls

- Most Bartholin gland hyperplasia lesions are often misdiagnosed as cysts clinically.

Pathology Interpretation Pearls

- Bartholin gland hyperplasia contains an increased number of acini yet with a preserved duct-to-acinar ratio.

Immunohistochemical/Molecular Findings

- Estrogen receptor negative, progesterone receptor negative, and low Ki-67 proliferation index.
- Smooth muscle actin highlights the periacinar myoepithelial cells.

Bartholin Gland Adenoma and Adenomyoma

Both lesions are extremely rare. There have been approximately ten cases of Bartholin gland adenoma and only one case of adenomyoma reported in the literature [3, 5–7, 28, 29]. These are often well-circumscribed and solid lesions, up to 2.5 cm in greatest dimension [6].

Histologic Features

Bartholin gland adenoma, as defined by Koenig and Tavassoli [6], represents a compact, well-circumscribed, encapsulated lobular proliferation of small bilayered tubuloductular structures with

intraluminal colloid-like secretions contiguous with identifiable Bartholin gland structures. There is a lack of normal duct-acinar arrangement. Bartholin gland adenomyoma is a well-circumscribed tumor composed of tubuloglandular components and a fibromuscular stroma positive for SMA and desmin [6]. In comparison to adenoma, adenomyoma would have less glandular elements and a prominent fibromuscular stroma that is immunoreactive for both smooth muscle actin and desmin.

Differential Diagnosis

The main differential diagnosis would be Bartholin gland nodular hyperplasia which would lack a capsule and yet maintain the duct-acinar relationship [6].

Summary

Clinical Presentation

- Solitary vulvar mass, well circumscribed and solid

Histologic Features

- Adenoma: an encapsulated yet haphazard proliferation of glands and tubules/duct without the normal duct-acinar arrangement
- Adenomyoma: less glandular elements and a prominent fibromuscular stroma

Differential Diagnosis

- Bartholin gland nodular hyperplasia

Takeaway Essentials

Clinical Relevant Pearls

- Both lesions are extremely rare.

Pathology Interpretation Pearls

- For both adenoma and adenomyoma, there is a lack of normal duct-acinar arrangement.

Immunohistochemical/Molecular Findings

- Fibromuscular stroma of adenomyoma would be immunoreactive for smooth muscle actin and desmin.

Bartholin Gland Carcinomas

Bartholin gland carcinomas account for 1 % of all female genital tract malignancies and 2–7 % of vulvar carcinomas [30]. This may be an overestimation if strict criteria are applied [31–33]. These include (1) presence of areas of apparent transition from normal to neoplastic elements, (2) involvement of areas of the Bartholin gland and origin histologically compatible from the gland, and (3) no evidence of a concurrent primary tumor elsewhere [34]. Although the first criterion is the most reliable, it can only be identified in less than half of the tumors in reviewed series of these tumors, especially in large tumors.

In general primary adenocarcinoma of the Bartholin gland is a slow-growing tumor with marked tendency for perineural and local invasion. The main clinical complaint is itching and burning sensation that are caused by pressure from a palpable mass present in the posterior part of the labium majorus. Bartholin gland carcinoma frequently metastasizes to the inguinal-femoral lymph nodes in advanced disease. Radical vulvectomy and wide local excision with inguinal lymph node dissection are the main treatment methods. Currently, chemotherapy and radiation have not been shown to improve survival [35]. The overall 5-year survival has been reported to be 71–84 %; however, the 5-year survival drops to 18 % with multiple lymph node involvement [35]. It appears that the stage of the disease will define the prognosis over the histologic type of tumor [33].

Histologic Features and Differential Diagnosis

The most common histologic subtypes are squamous cell carcinoma and adenocarcinoma occurring with an approximately equal frequency and together accounting for up to 90 % of Bartholin gland carcinoma [36–41] (Table 12.2).

Squamous Cell Carcinoma

The lining of the Bartholin gland duct at the vestibular orifice changes from transitional to squamous epithelium, and from this transition zone, squamous cell carcinoma would arise. A painless mass is the most common clinical presentation [10].

Table 12.2 Histologic types of Bartholin gland carcinomas

Squamous cell carcinoma
Adenocarcinoma
Clear cell adenocarcinoma
Adenoid cystic carcinoma
Polymorphous low-grade adenocarcinoma
Salivary gland-type basal cell adenocarcinoma
Low-grade epithelial-myoepithelial carcinoma
Transitional cell carcinoma
Neuroendocrine/Merkel cell carcinoma
Lymphoepithelioma-like carcinoma

The histology is similar to any squamous cell carcinoma characterized by keratin pearl formation and neoplastic cells with keratinization (Fig. 12.4a, b). The association of squamous cell carcinoma of Bartholin gland and HPV, especially HPV16, has been established [42].

Adenocarcinoma

Histologic sections show a carcinoma with glandular architecture. The tumor cells have abundant cytoplasm and peripheral hyperchromatic nuclei (Fig. 12.5a, b). They are ER and PR positive and

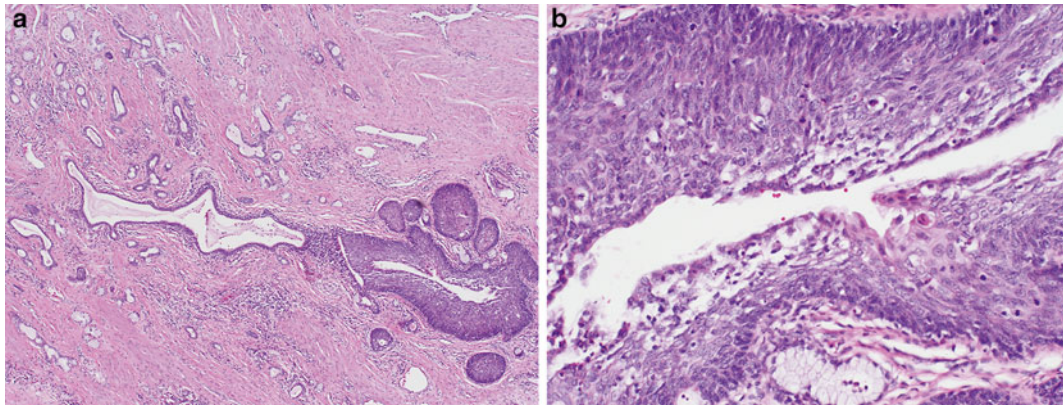


Fig. 12.4 Bartholin gland squamous cell carcinoma. (**a**) An in situ component is seen arising from the Bartholin gland duct. (**b**) Full-thickness dysplasia with keratinization is seen

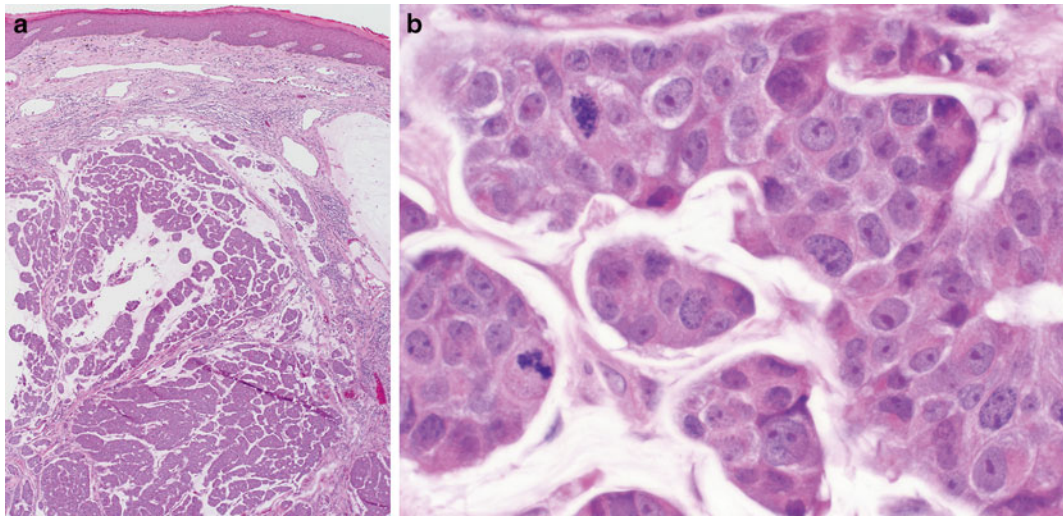


Fig. 12.5 Bartholin gland adenocarcinoma. (**a**) A proliferation of strands and nests of epithelial neoplastic cells are seen associated with a mucinous background. (**b**) The tumor cells are polygonal and with prominent nucleoli

negative for CK20, CK7, CDX2, mammaglobin, and Her2/neu. Rare case of primary adenocarcinoma of the right labium minus has given rise to bone metastasis [43]. Rarely the adenocarcinoma would be comprised of clear neoplastic cells [40]. In this instance, a metastatic clear cell adenocarcinoma from the kidney or ovary needs to be excluded.

Adenoid Cystic Carcinoma

Salivary gland-type carcinomas rarely arise from the Bartholin gland, and they most commonly are adenoid cystic carcinoma [44–56], combined adenoid cystic and squamous cell carcinoma [57], polymorphous low-grade adenocarcinoma [58], salivary gland-type basal cell adenocarcinoma [59], and low-grade epithelial-myoepithelial carcinoma [60].

Adenoid cystic carcinoma of the vulva is a rare tumor [44–56], and one must exclude the possibility of metastasis before rendering the diagnosis of primary adenoid cystic carcinoma of the vulva. Some do consider that adenoid cystic carcinoma can arise from anogenital mammary-like glands [61]. The sizes of the tumor range from 0.5 to 4 cm [50]. The clinical presentation can be nonspecific including pain, burning sensation, palpable mass, or pruritus; and it is frequently misdiagnosed and treated as an infected Bartholin gland or cyst [62]. The treatment often includes local excision and radical vulvectomy, with or without regional lymph node dissection [55]. The overall survival is excellent with the 5-year and 10-year survival rates being 47–83 % and 33–38 %, respectively [50]. The bone and lung are the most common sites of metastasis; and the liver, kidney, and brain are less frequently involved sites [56].

Histopathologically, the growth pattern can be cribriform, tubular, solid, or a combination of these (Fig. 12.6). Perineural invasion is a common histologic feature which accounts for the frequent recurrence. In their series of 17 cases, Milchgrub et al. [56] reported that the histologic pattern, whether classical, tubular, or mixed, did not predict survival. CD117 expression is noted in invariably all adenoid cystic carcinomas [63].

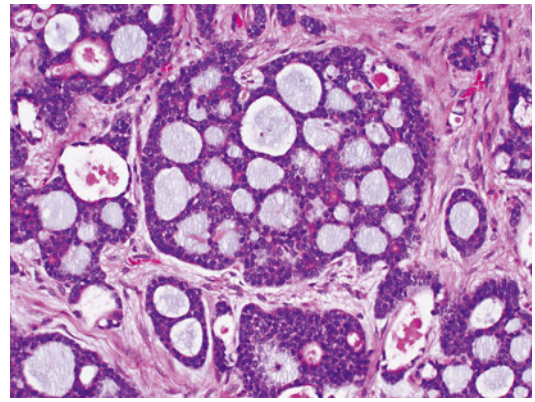


Fig. 12.6 Adenoid cystic carcinoma. A cribriform architecture is noted

MYB immunostain has recently been reported to be specific for adenoid cystic carcinoma although the sensitivity is only 65 % [64] (see Vignette 1 at the end of this chapter).

Other Salivary Gland-Type Carcinomas

Basal cell adenocarcinoma of the salivary gland is a low-grade neoplasm showing myoepithelial differentiation. There has been one rare case with similar histology reported in the vulva [59]. Tumor lobules with peripheral palisade were comprised of two types of cells. The small and basaloid cells at the periphery had a myoepithelial phenotype and were SMA and calponin positive. The inner cells had abundant amphophilic cytoplasm and were positive for keratin CAM5.2 and 34betaE12 [59]. The lack of epidermal connection and the myoepithelial phenotype are helpful distinguishing features from an adenoid basal cell carcinoma. Squamous differentiation, which can be seen in basal cell adenocarcinoma, is typically absent in the solid variant of adenoid cystic carcinoma.

Similarly, in two cases of low-grade epithelial-myoepithelial carcinoma reported by McCluggage et al. [60], normal Bartholin gland tissue was seen at the periphery. Tubular, trabecular, and insular patterns were comprised of outer myoepithelial cells (p63+, calponin+, SMA+) and inner cuboidal epithelial cells (broad-spectrum keratin+, EMA+). Both cases were strongly positive for CD117.

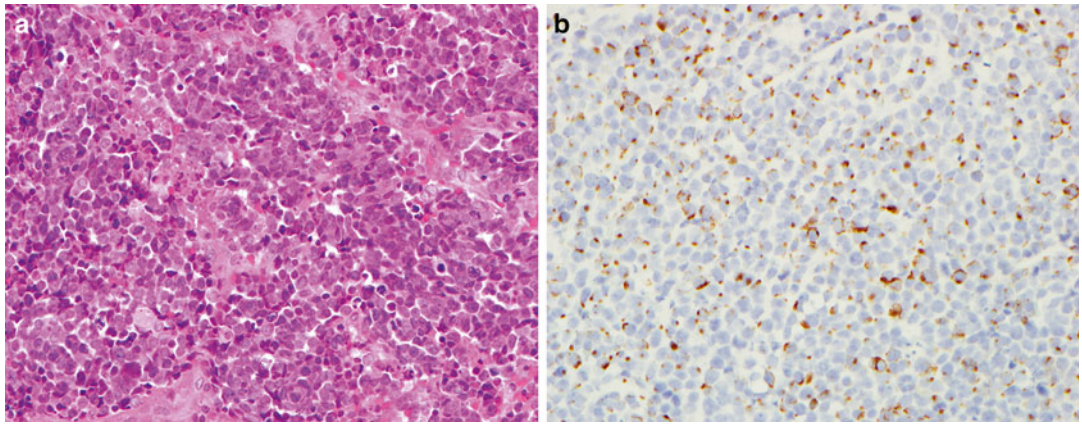


Fig. 12.7 Merkel cell carcinoma. (**a**) The neoplastic cells have scant cytoplasm, granular nuclear chromatin, frequent mitotic figures, and apoptotic bodies. (**b**) Cytokeratin 20 demonstrates dot-like perinuclear positivity

Transitional Cell Carcinoma

Rare cases of transitional cell carcinomas have been reported originating from the duct of Bartholin glands [65–67]. A progression from low-grade dysplasia to invasive transitional cell carcinoma was described in the case report by Fujiwaki [67]. The transitional cell carcinoma of Bartholin gland would have similar histologic features as noted in transitional cell carcinomas of other sites.

Neuroendocrine or Merkel Cell Carcinoma

Neuroendocrine carcinoma or Merkel cell carcinoma can rarely involve the vulvar skin and arise from the Bartholin gland [68–70]. Clinically, it can mimic a Bartholin gland abscess [71]. Histologically, a tumor is composed of neoplastic cells with scant cytoplasm and granular chromatin, frequent mitotic figures, and apoptotic bodies (Fig. 12.7a). The neoplastic cells express cytokeratin 20 in a perinuclear dot-like pattern (Fig. 12.7b) and exhibits positivity for wide-spectrum and low molecular weight cytokeratins, EMA, and neuroendocrine markers including chromogranin, synaptophysin, and neuron-specific enolase [72]. The differential diagnosis would include a metastatic small cell carcinoma from other sites.

Lymphoepithelioma-Like Carcinoma

In the four reported cases of lymphoepithelioma-like carcinoma of the vulva [73–76], syncytial sheets of epithelial cells of variable shapes were infiltrated by a notable number of lymphocytes and plasma cells. The tumor cells had large nuclei, vesicular chromatin, and prominent nucleoli. In one case, a transition from neoplastic cells to Bartholin gland acini was seen [73]. The tumor cells expressed epithelial markers including keratins AE1/AE3 and epithelial membrane antigen [73]. CD3 and CD20 demonstrated a mixed infiltrate of T and B lymphocytes within the associated inflammatory infiltrate [73]. The association with Epstein-Barr virus as in undifferentiated carcinoma of other sites has not been documented for these tumors from the vulva [73–75].

Summary

Clinical Presentation

- A slow-growing tumor with marked tendency for perineural and local invasion.
- The main clinical complaint is itching and burning sensation.

Histologic Features

- The most common histologic subtypes are squamous cell carcinoma and adenocarcinoma.
- Adenoid cystic carcinoma is the most common from the salivary gland-type carcinomas arising in Bartholin gland.

Differential Diagnosis

- Metastatic carcinoma to Bartholin gland

Takeaway Essentials

Clinical Relevant Pearls

- A clinically diagnosed Bartholin gland cyst refractory to treatment needs to be biopsied to rule out a carcinoma, especially in a postmenopausal woman.
- It appears that the stage of the disease will define the prognosis over the histologic type of tumor.

Pathology Interpretation Pearls

- Criteria for diagnosing primary carcinoma of Bartholin gland: (1) presence of areas of apparent transition from normal to neoplastic elements, (2) involvement of areas of the Bartholin gland and origin histologically compatible from the gland, (3) no evidence of a concurrent primary tumor elsewhere

Immunohistochemical/Molecular Findings

- Bartholin gland carcinoma is typically ER+ and PR+.
- Although specific, MYB immunostain is detected in only 65 % of adenoid cystic carcinomas.

Other Lesions of Bartholin Gland

Bartholin gland may rarely be secondarily involved by neoplastic cells in extramammary Paget disease or melanoma, display metaplastic changes (squamous, apocrine, or intestinal metaplasia) in long-standing inflammatory processes of the vulva, and show alterations resembling those seen in salivary glands in necrotizing sialometaplasia.

Paraurethral (Skene) Gland Cyst and Prostatic-Type Tissue of the Vulva

The skene gland is a paired organ located on either side of the urethral meatus, representing the female homologue of the male prostate. Paraurethral gland cyst results from cystic dilatation of the duct of the gland. This 1–2 cm-sized lesion is asymptomatic and involves the upper lateral introitus [77, 78].

There have been three cases of prostatic-type tissue involving the vulva reported in the literature [79, 80]. The ages of these patients ranged from 43 to 54 years [79, 80]. The lesion appeared as intermittent pea-sized bilateral swellings next to or near the urethral meatus which occasionally discharged pus-like material. It has been speculated that these benign lesions are derived from the Skene glands and misplaced during embryologic development [80].

Histologic Features

The skene gland is comprised of two layers of epithelium with the outer being columnar and contains eosinophilic secretion (Fig. 12.8a, b). Skene gland cyst is derived from the duct, and the wall is lined by either transitional or stratified squamous epithelium with rare mucinous cells (Fig. 12.9a, b). In the case of prostatic-type tissue of the vulva, there are small lobular clusters of benign glands within the superficial dermis, focally in continuity with the base of the epidermis, lined by a double-layered epithelium containing PAS-positive bright, eosinophilic cytoplasmic granules in some of the luminal cells, resembling Paneth cell-like change (Fig. 12.10a, b). The glands are positive for prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) [79, 80].

Differential Diagnosis

Skene gland hyperplasia (analogous to benign prostatic hyperplasia in male) (Fig. 12.11a, b) and adenocarcinoma are rare entities [81, 82]. The adenocarcinomas presented as periurethral masses and are associated with elevated preoperative serum PSA level [83]. The tumor cells are also positive for PSA and PAP immunostains [84]. Urethral diverticula are lined also by transitional epithelium originating from the lower segment of the urethra, and only clinical findings and imaging are able to separate these diseases.

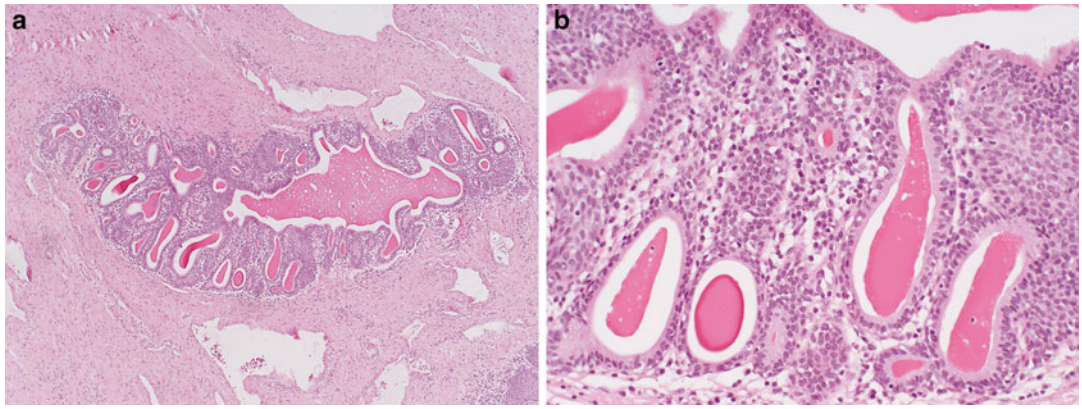


Fig. 12.8 (**a**, **b**) Skene gland. Two layers of epithelium and eosinophilic secretion are seen

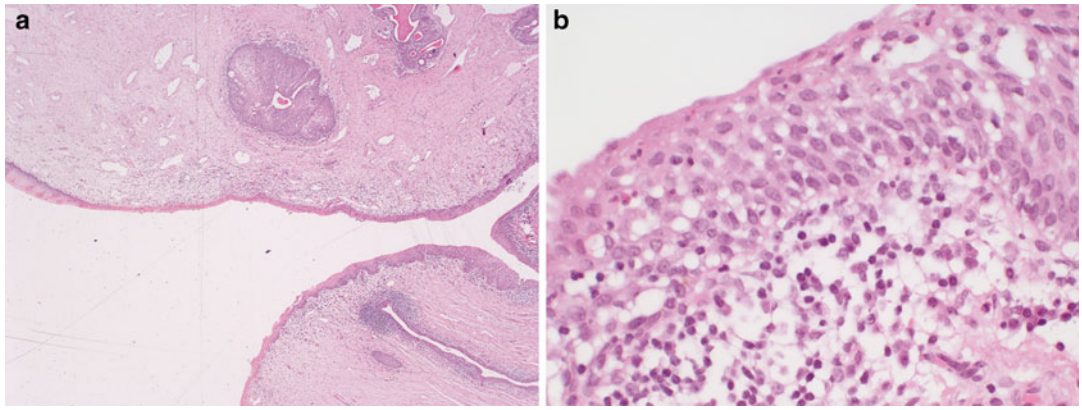


Fig. 12.9 (**a**, **b**) Skene gland cyst. Derived from the duct, the lining of the cyst is comprised of stratified squamous epithelium

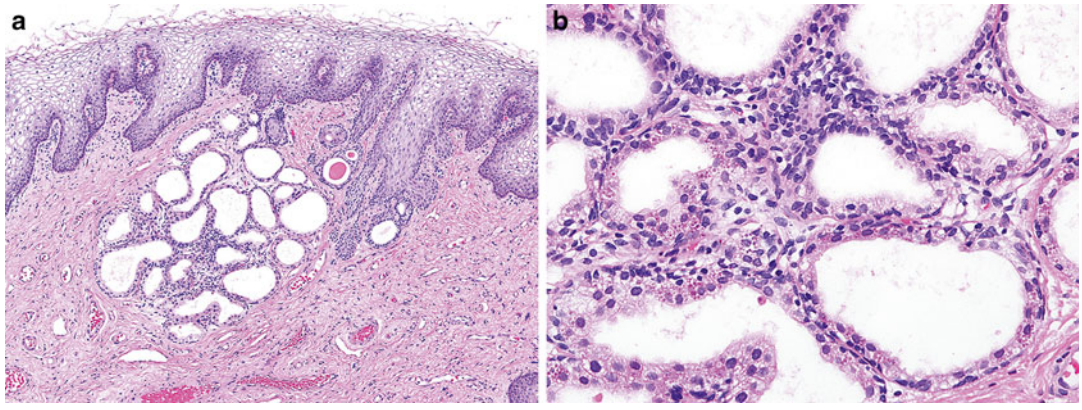


Fig. 12.10 (**a**, **b**) Prostatic-type tissue of the vulva. Lobular clusters of benign glands within the superficial dermis lined by a double-layered epithelium (Courtesy of Colin J.R. Stewart, FRCPA, Perth, Western Australia, Australia)

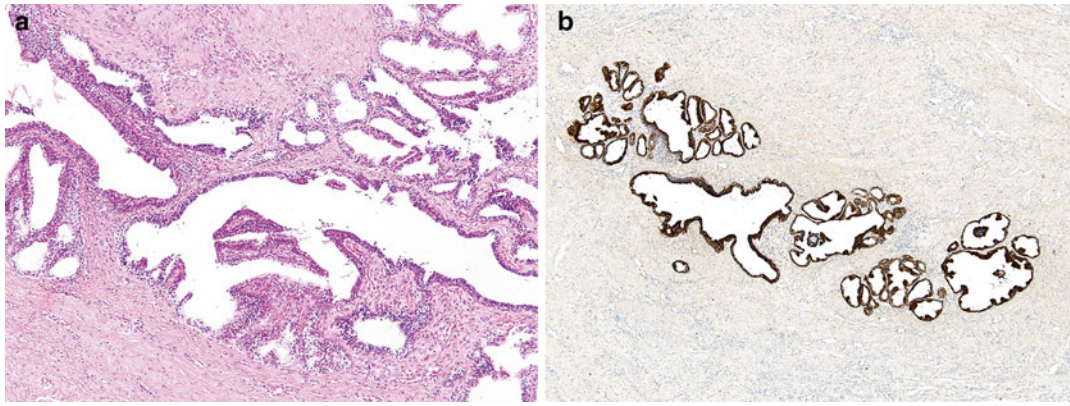


Fig. 12.11 Hyperplasia of paraurethral Skene glands. (**a**) Hyperplasia of the glandular as well as the stromal components is seen similar to prostatic hyperplasia. (**b**) The glandular epithelium expresses prostatic acid phosphatase (×100)

Summary

Clinical Presentation

- Prostatic-type tissue of the vulva: intermittent and bilateral swellings near or at the urethral meatus

Histologic Features

- The wall of Skene gland cyst is comprised of transitional or stratified squamous epithelium with rare mucinous cells.
- Prostatic-type tissue of the vulva: small lobular clusters of benign glands, with double-layered epithelium, within the superficial dermis.

Differential Diagnosis

- Skene gland hyperplasia
- Urethral diverticula

Takeaway Essentials

Clinical Relevant Pearls

- Skene gland represents the female homologue of the male prostate.
- The adenocarcinomas arising from Skene gland are associated with elevated preoperative serum prostatic specific antigen level.

Pathology Interpretation Pearls

- Prostatic-type tissue of the vulva: small clusters of benign glands are lined by a double-layered epithelium containing PAS-positive bright, eosinophilic cytoplasmic granules in some of the luminal cells, resembling Paneth cell-like change.

Immunohistochemical/Molecular Findings

- The ectopic prostatic glands are positive for prostate-specific antigen and prostatic acid phosphatase.

Cyst of the Canal of Nuck (Mesothelial Cyst)

The cyst of the canal of Nuck, also known as hydrocele of the canal of Nuck, was first described in 1691 by the Dutch anatomist Anton Nuck [85]. The canal of Nuck is a cystic remnant of the processus vaginalis peritonei representing a rudimentary sac of peritoneal mesothelium carried down by the round ligament as it passes through the inguinal canal and inserts into the labium majus [86]. Failure in obliteration of this structure during the first year of life results in blockage and cystic dilatation [87].

Three types of cyst of the canal of Nuck have been reported: (1) the type that does not have patency between the cyst and peritoneal cavity, (2) the type with patency, and (3) the "hourglass type" that consisted of two cysts with the proximal one connected to the peritoneal cavity [88].

Patients, usually girls or young women, present with an asymptomatic, nontender swelling (average of 3 cm, up to 7 cm) in the groin or, rarely, with a mass in the labium majus or mons pubis. Clinically, these lesions are commonly mistaken for inguinal hernia [89, 90]. Anecdotal cases of endometriosis and ectopic pregnancy developed in association with the cyst of the canal of Nuck have been reported [91–93]. Aspiration of the cyst causes recurrence; thus, surgical excision and ligation of the neck of processus vaginalis is the standard treatment [88].

Histologic Features

The wall of the cyst is lined by low cuboidal cells and surrounded by loosely cellular fibrous tissue. The epithelium is of mesothelial origin; thus, it is immunoreactive for calretinin and D2-40. Stromal fibrosis and hemosiderin deposition can be seen.

Differential Diagnosis

The clinical differential diagnosis includes round ligament cysts, varicosities of the round ligament, inguinal herniation of the ovary, epidermoid cyst, and abscesses. Definitive diagnosis can be made with ultrasonography or MRI [90, 92, 94].

Summary

Clinical Presentation

- An asymptomatic and nontender mass in the labium majus or mons pubis

Histologic Features

- The wall of the cyst is lined by low cuboidal cells and surrounded by loosely cellular fibrous tissue.

Differential Diagnosis

- Inguinal hernia

Takeaway Essentials

Clinical Relevant Pearls

- Equivalent of the hydrocele in males.
- Definitive diagnosis can be made with ultrasonography or magnetic resonance imaging.
- Aspiration of the cyst causes recurrence; thus, surgical excision is the treatment of choice.

Pathology Interpretation Pearls

- The epithelium is of mesothelial origin.

Immunohistochemical/Molecular Findings

- The epithelial cells are positive for calretinin and D2-40.

Mesonephric-Like Cyst and Ciliated Cyst

Mesonephric-like (mesonephric Gartner) cyst is a rare cystic lesion of presumed mesonephric/Wolffian origin that involves the lateral aspect of the vulva and vagina and manifests itself as a thin-walled, translucent cyst containing clear fluid [95]. Ciliated cyst of the vulva is a rare lesion that has been reported in association with Stevens-Johnson syndrome, chronic inflammation, and 5FU therapy [96].

Histologic Features

The wall of a mesonephric-like or Gartner cyst is lined by cuboidal to columnar non-ciliated epithelium (Fig. 12.12a, b), whereas the ciliated cyst is lined by tubal- or endometrial-type epithelium (Fig. 12.13a, b). The ciliated cyst of the vulva can be distinguished from endometriosis by the lack of endometrial-type stroma.

Other Epithelial Cysts

Cysts derived from the overlying epidermis, hair follicles, and apocrine glands include epidermoid cyst (Fig. 12.14), pilar or trichilemmal cyst, dermoid cyst (Fig. 12.15), and hidrocystoma [97].

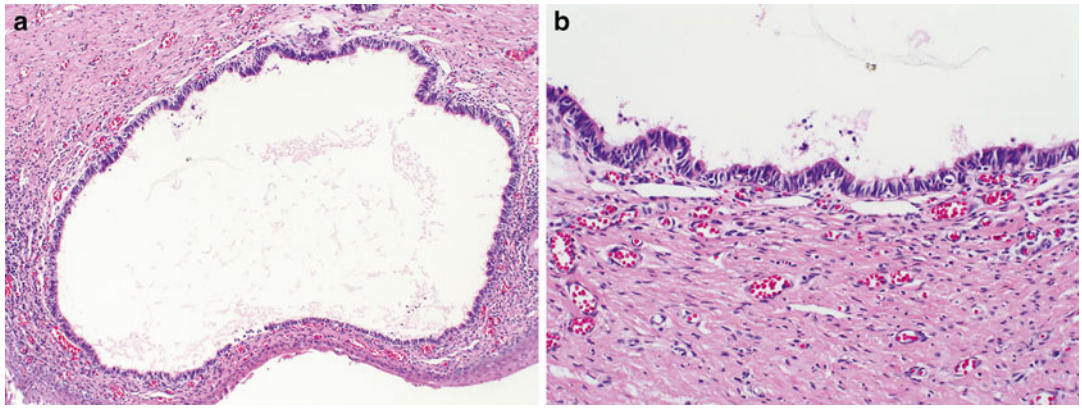


Fig. 12.12 (**a**, **b**) Gartner or mesonephric-like cyst. The wall is comprised of simple cuboidal or low columnar epithelium

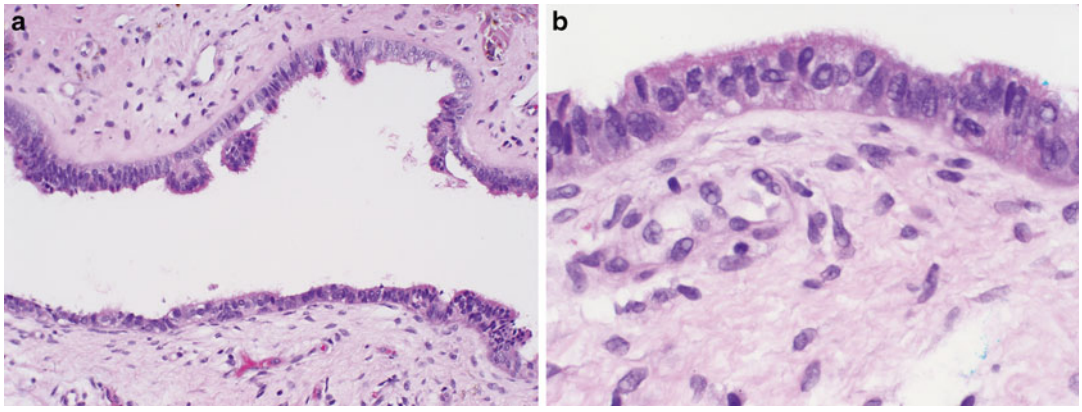


Fig. 12.13 (**a**, **b**) Ciliated cyst. The cyst is lined by ciliated columnar epithelium

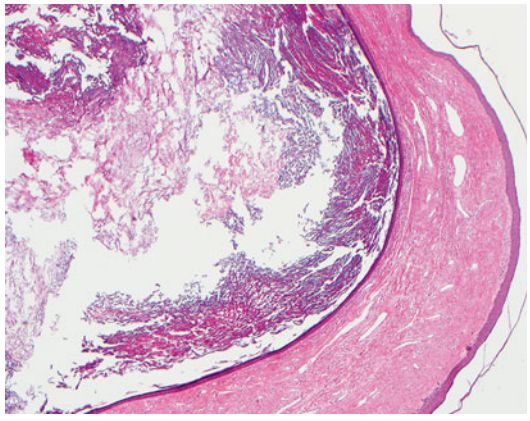


Fig. 12.14 Epidermoid cyst. The cyst wall is comprised of squamous epithelium containing a granular layer

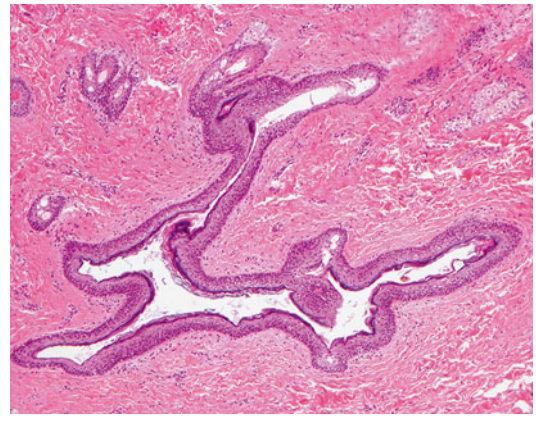


Fig. 12.15 Dermoid cyst. Hair follicles are seen inserting into the wall of the cyst

Epidermoid cysts are often located in the labium majus and clitoris and occur as a result of trauma [98]. These cysts could develop many years after the initial injury [99, 100]. A rare presentation of clitoromegaly has been reported [101]. Less common entities include steatocystoma and cysts of anogenital mammary-like glands (previously reported as milk cysts of supernumerary mammary glands) [102]. Chuang et al. [103] described a case of multiple pigmented follicular cysts of the vulva, characterized by a stratified squamous epithelium with epidermoid keratinization containing multiple pigmented hair shafts and laminated keratin. Rare examples of apocrine cystadenoma and pigmented hidrocystoma derived from vulvar eccrine secretory coils have been reported by Glusac [104] and Kamishima [105], respectively.

Miscellaneous Lesions

Mucinous Metaplasia of the Vulva

First reported by Coghill et al. [106], mucinous metaplasia is an extremely rare condition occurring in conjunction with an inflammatory process, strongly suggesting that it represents a secondary metaplastic phenomenon. There are distinctive clinical features. In the reported cases, mucinous metaplasia was found in females 60 years old and older in association with Zoon vulvitis and lichen sclerosus [106–108].

Histologic Features

Columnar mucinous cells focally replacing the stratified squamous epithelium are present accompanied by features of Zoon vulvitis and lichen sclerosus [107, 108] (Fig. 12.16). The mucinous cells are decorated by mucin stains (e.g., Alcian blue) and periodic acid-Schiff with diastase digestion and are immunoreactive for CK7, CEA, EMA, estrogen receptors, and CAM5.2 [109].

Differential Diagnosis

Toker cells can be found in the vulva; they can be differentiated from mucinous metaplasia by the absence of an associated inflammatory process. The most important differential diagnosis is extramammary Paget disease (EMPD) (see Chap. 11). The absence of nuclear atypia, adnexal involvement, preservation of nuclear polarity, confinement of mucin containing cells to the epidermis, and predominant location of the cells in the upper layers of the epithelium distinguish mucinous metaplasia from EMPD [109]. Mucinous metaplastic epithelium would exhibit a similar immunoprofile to EMPD – express CK7, CEA, and EMA; however, a band-like pattern of mucinous metaplastic epithelium is different from the scattered pattern within a preserved squamous epithelium of EMPD. In addition, nuclear pleomorphism and Ki-67-positive mucinous cells in the superficial epithelial layers are features of EMPD. In contrast to vulval intraepithelial neoplasia with mucinous differentiation, mucinous metaplasia of the vulva has not been shown to be associated with HPV or exhibit cytologic atypia [109].

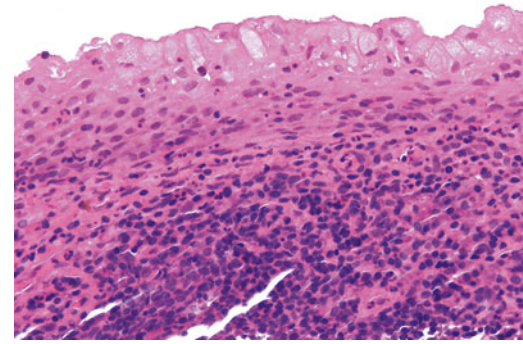


Fig. 12.16 Mucinous metaplasia. Columnar mucinous cells are seen instead of squamous epithelium (Courtesy of Dr. Maria Teresa Fernandez Figueras, Department of Anatomic Pathology, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain)

Summary

Clinical Presentation

- In postmenopausal females

Histologic Features

- Mucinous cells replacing squamous epithelial cells, especially in the upper part

Differential Diagnosis

- Vulvar Toker cells
- Extramammary Paget disease
- Vulval intraepithelial neoplasia with mucinous differentiation

Takeaway Essentials

Clinical Relevant Pearls

- Incidental finding in the setting of Zoon vulvitis or lichen sclerosus

Pathology Interpretation Pearls

- A band-like pattern of mucinous metaplastic epithelium is different from the scattered pattern within a preserved squamous epithelium of EMPD.
- Nuclear pleomorphism would be a feature of EMPD.

Immunohistochemical/Molecular Findings

- The mucinous cells stain positive for mucin (e.g., Alcian blue) and periodic acid-Schiff with diastase digestion stains.
- Express CK7, CEA, and EMA – a similar immunoprofile to EMPD.
- Ki-67 positive mucinous cells in the superficial epithelial layers are seen in EMPD.

Endometriosis

Endometriosis can be seen in the vulva often at sites of trauma [110]. These lesions can become hemorrhagic nodules due to the cyclical bleeding. The histologic features are similar to endometriosis at other sites of the body. These include endometrial glands and stroma with hemosiderin-laden macrophages (Fig. 12.17a, b). Decidualization can be observed in women under progesterone treatment and pregnancy. The lack of cytologic atypia and the presence of endometrial-type stroma surrounding these glands would be distinguishing features from an adenocarcinoma, although rarely an adenocarcinoma can arise in the setting of endometriosis [111]. This endometrioid adenocarcinoma is indistinguishable from primary endometrial and ovarian endometrioid carcinomas; therefore, metastatic disease from the uterus or the ovary should always be excluded by clinical work-up.

Vestibular Papillomatosis

Vestibular papillomatosis is likely a normal anatomic variation of the vulva with no known significant associations. It has been postulated that vestibular papillomatosis represents the female equivalent of pearly penile papules in men [112–114]. This condition has been found in 1–33 % of healthy young women. The lesions are mainly found in the vestibule and represent as soft, small (1–3 mm), asymmetrically distributed papules, having the same color as the adjacent mucosa. Larger lesions can be seen in pregnancy [112, 115]. The role of HPV in these lesions remains unclear. While DNA was not detected in these lesions by Southern blot hybridization in one study [116], a recent study using polymerase chain reaction demonstrated the presence of HPV16 DNA in 39 % of studied cases [112].

Histologic Features

There is a loose connective fibrovascular tissue covered by unremarkable squamous epithelium (Fig. 12.18a). Viral cytopathic effect is not noted within the squamous epithelium (Fig. 12.18b).

Differential Diagnosis

From a clinical perspective, unlike warts, the bases of individual papules are broad, and the papules remain unchanged upon application of 5 % acetic acid.

Summary

Clinical Presentation

- Soft, small (1–3 mm) papules in the vestibule with the same color as the adjacent mucosa

Histologic Features

- Loose connective fibrovascular tissue covered by unremarkable squamous epithelium

Differential Diagnosis

- Condyloma or genital warts

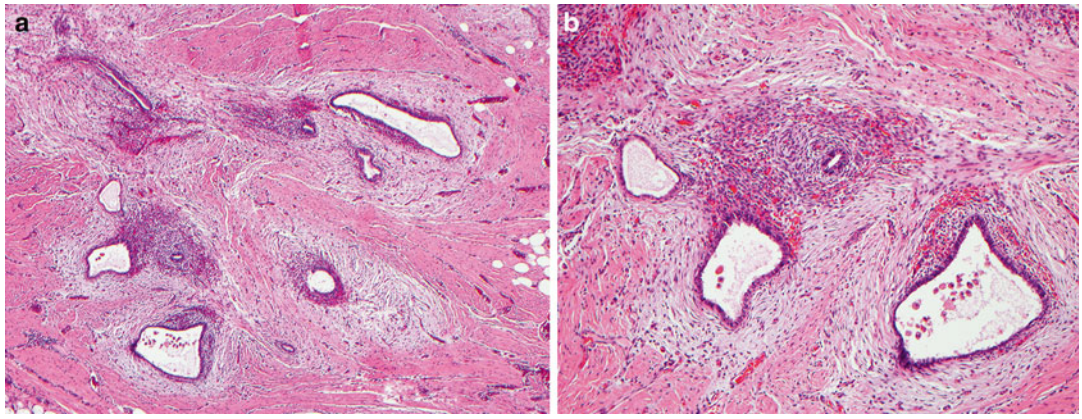


Fig. 12.17 (**a**, **b**) Endometriosis. Endometrial glands surrounded by a stroma containing hemosiderin-laden macrophages

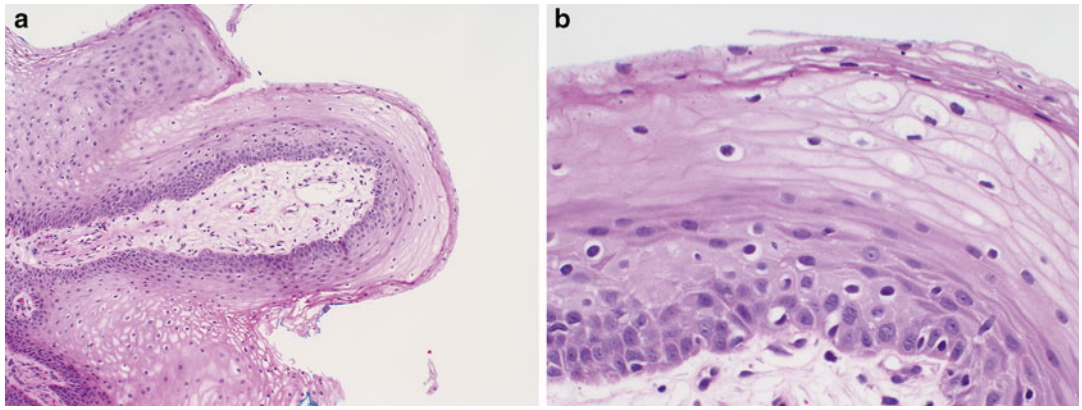


Fig. 12.18 Vestibular papillomatosis. (**a**) A polypoid squamous mucosa is seen. (**b**) There is no evidence of human papilloma virus cytopathic effect within the squamous epithelium

Takeaway Essentials

Clinical Relevant Pearls

- Likely a normal anatomic variation of the vulva
- The female equivalent of pearly penile papules in men

Pathology Interpretation Pearls

- The papilla is formed by normal elements of the vestibule; this supports the position that it is an anatomic variation of the normal vulva.

Immunohistochemical/Molecular Findings

- The role of HPV in these lesions remains unclear.

Carcinoma of Cloacogenic Remnants

Novak and Woodruff were the first to suggest that cloacogenic remnants can be found in the vulva [117]. Tiltman and Knutzen [118] subsequently proposed that villoglandular adenocarcinomas can rarely develop from these embryonic rests and they are characterized by villoglandular pattern as well as an enteric phenotype. There have been approximately 10 cases of vulvar cloacogenic carcinoma reported in the literature [119–126]. The ages of these patients ranged from 43 to 80 years (median 57 years). The clinical course appears to be indolent after radical vulvectomy or wide local excision. Inguinal

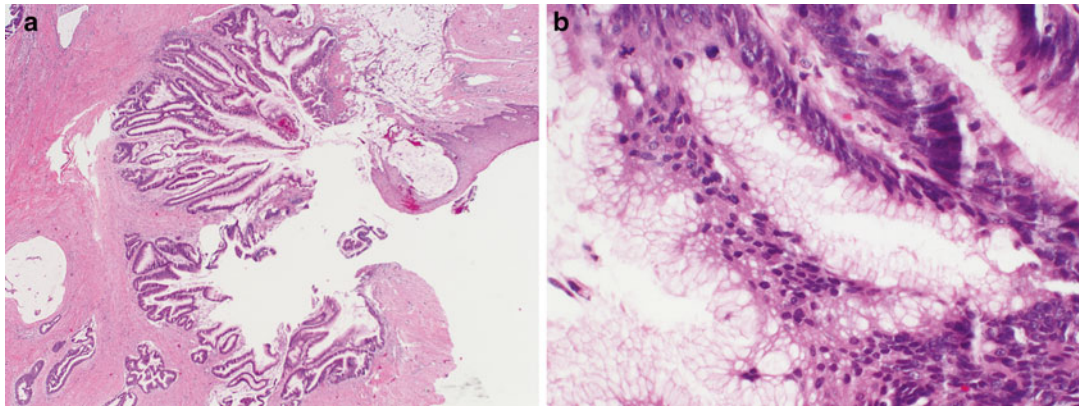


Fig. 12.19 Cloacogenic carcinoma. (**a**) An adenocarcinoma with associated extracellular mucin is seen in the dermis and connects to the epidermis. (**b**) The neoplastic glands are lined by columnar epithelium containing goblet cells

lymph node dissection was done in five reported cases [118, 120–122], and ipsilateral metastasis was noted in only one case [118].

Histologic Features

Cloacogenic remnant is characterized by columnar cells with prominent brush border and with admixed goblet cells and endocrine cells. The associated adenocarcinoma tends to be superficial with direct continuity with the epidermis [119, 120, 122] (Fig. 12.19a). Glands lined by columnar epithelium containing goblet cells and with intraluminal neutrophils are seen (Fig. 12.19b). Endocrine cells are highlighted by chromogranin [119]. The tumor cells are typically positive for CAM5.2, CK7, and CK20 and focally for polyclonal CEA and negative for ER and PR [119, 120].

Differential Diagnosis

The differential diagnosis includes a metastatic carcinoma from a colonic primary. Since the primary and metastatic tumors would exhibit a similar immunoprofile, clinical work-up is necessary.

Summary

Clinical Presentation

- The clinical course of these cases appears to be indolent after radical vulvectomy or wide local excision.

Histologic Features

- Glands lined by columnar epithelium containing goblet cells and with intraluminal neutrophils are seen.

Differential Diagnosis

- Metastatic colonic carcinoma

Takeaway Essentials

Clinical Relevant Pearls

- Clinical work-up is necessary to distinguish cloacogenic carcinoma of the vulva from a metastatic colonic adenocarcinoma.

Pathology Interpretation Pearls

- Cloacogenic carcinoma of the vulva tends to be superficial with direct continuity with the epidermis.

Immunohistochemical/Molecular Findings

- The tumor exhibits a colonic phenotype: CK7+, CK20+, CDX2+.

Granular Cell Tumor

Originally reported in 1926 by Abrikossoff as a smooth muscle tumor, i.e., granular cell myoblastoma, granular cell tumor is now known as a Schwann cell-related neoplasm [127, 128]. Approximately 10 % of GCTs involve the vulva

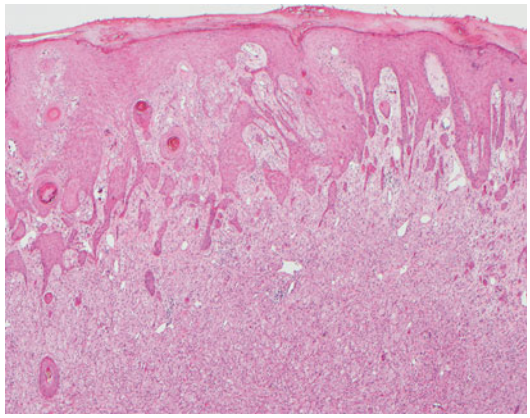


Fig. 12.20 Granular cell tumor. Pseudoepitheliomatous hyperplasia of the overlying epidermis can pose a diagnostic pitfall

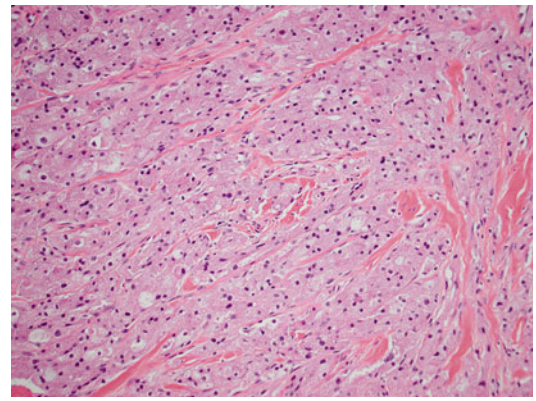


Fig. 12.21 Granular cell tumor. Polygonal neoplastic cells with abundant cytoplasm and central nuclei

[129]. Granular cell tumors often present as small, slow-growing, poorly circumscribed, solitary, and painless nodules associated with pruritus and overlying cutaneous hyperpigmentation. Multicentric tumors can be seen in 10–15 % of cases [130, 131], and they can occur at the same time or over many years. Familial cases of granular cell tumor have been rarely reported [132]. In a series of 17 vulvar GCTs, the average age at presentation was 46 years, and 84 % of the patients were African-American [131]. The most common location was the labia majora with an average tumor size of 2.1 cm [131]. The general surgical approach for vulvar GCT is wide local excision with a low risk of recurrences [129].

Histologic Features

Gross examination of these tumors reveals nonencapsulated and well-defined white to yellow dermal/submucosal nodule with extension to the subcutis. The overlying epidermis can exhibit marked pseudoepitheliomatous hyperplasia and can be misdiagnosed as squamous cell carcinoma [133] (Fig. 12.20). Sheets of large, polygonal-to-elongated tumor cells with abundant, granular eosinophilic cytoplasm and small central hyperchromatic nuclei are seen in the dermis, either in a pushing, nodular, or infiltrative fashion (Fig. 12.21). The cytoplasmic granules, representing phagolysosomes, are typically positive for periodic acid-Schiff (PAS) stain and diastase resistant. The entrapment of adnexal structures and perineural involvement can be identified. The tumor cells express S100, neuron-specific enolase, laminin, and CD68; yet they are negative for neurofilament and glial fibrillary acidic protein [134].

Differential Diagnosis

The differential diagnosis would include an atypical granular cell tumor and a malignant granular cell tumor. Two cases of atypical GCTs have been reported in the vulva [135]. The presence of metastasis is the only reliable criterion for malignancy, and there have been rare cases of vulvar malignant granular cell tumor with widespread metastases in one case and regional lymph node metastases in the other [136, 137]. Some have proposed to diagnose a granular cell tumor as benign in the absence of the following histologic features: spindling of the tumor cells, high nuclear-to-cytoplasmic ratio, diffuse nuclear pleomorphism, prominent nucleoli, necrosis, and mitotic activity greater than 2 per 10 high-power fields at 200× [138]. It has been suggested that a GCT be classified as atypical when one or two of these features are present and as malignant when three or more are seen [138]. Although both alveolar soft part sarcoma and GCTs express TFE3, only GCTs express S100, Sox10, inhibin, nestin, and calretinin [139].

Summary

Clinical Presentation

- Small, slow-growing, solitary, and painless nodule in the dermis and/or subcutaneous tissue

Histologic Features

- A proliferation of large, polygonal-to-elongated tumor cells with abundant, granular eosinophilic cytoplasm and small central hyperchromatic nuclei

Differential Diagnosis

- Atypical and malignant granular cell tumor
- Alveolar soft part sarcoma

Takeaway Essentials

Clinical Relevant Pearls

- Multicentric tumors can be seen in 10–15 % of cases.
- Multiple tumors can appear at the same time or over many years.

Pathology Interpretation Pearls

- Of the following histologic features: spindling of the tumor cells, high nuclear-to-cytoplasmic ratio, diffuse nuclear pleomorphism, prominent nucleoli, necrosis, and mitotic activity greater than 2 per 10 high-power fields at 200×:
  Benign: none
  Atypical: 1–2 features
  Malignant: 3 or more features
- The overlying epidermis can exhibit marked pseudoepitheliomatous hyperplasia and can be misdiagnosed as squamous cell carcinoma.

Immunohistochemical/Molecular Findings

- Although 91 % of alveolar soft part sarcoma and GCTs express TFE3, only GCTs express S100, Sox10, inhibin, and nestin.

Hidradenitis Suppurativa

Hidradenitis suppurativa (HS) is a recurrent and chronic disease that preferentially involves sites rich in apocrine or mammary-like glands including the axillary, mammary, inframammary, inguinal, pubic, perineal, and perianal regions [140]. Its prevalence has been estimated to be 1–4 % [141, 142]. There is an association of HS with other follicular occlusion disorders (acne conglobata, dissecting cellulitis of the scalp, and pilonidal sinus) forming the so-called follicular occlusion tetrad [143], Crohn disease, smoking, and obesity [140]. In a large retrospective series of over 2,000 patients, there is a 50% increased risk for malignancy and a 4.6-fold increase in developing SCC; however, smoking might play a role in this [144, 145]. The lesions are initially tender subcutaneous nodules that progress to painful abscesses with draining sinuses and then heal, resulting in dermal fibrosis and contractures. The diagnosis can typically be made based on clinical criteria including chronicity of disease, unresponsiveness to antibiotic treatment, double comedones, and sinus and scarring formation. The treatment for HS can be either medical or surgical ones [140].

Histologic Features

Studies involving careful histologic examination have concluded that HS is likely a disease of follicular origin [146, 147]. Acute folliculitis and perifolliculitis are seen in the early lesions (Fig. 12.22a). A dense and mixed inflammatory infiltrate and often abscess formation are present in established lesions (Fig. 12.22b). Secondary involvement of apocrine glands can be seen in 12 % of cases (Fig. 12.22c) and eccrine glands in 25 % [146]. Sinus tracts and associated inflamed granulation tissue and fibrosis are chronic histologic features (Fig. 12.23a, b).

Differential Diagnosis

Hidradenitis suppurativa can be distinguished from other entities based on the clinical presentation of lesions, characteristic locations, treatment unresponsiveness to antibiotics, and recovery of multiple bacterial species rather than one on culture. An inflamed and ruptured Bartholin cyst would be a localized lesion in contrast to the multifocal nature of HS. Crohn disease would have accompanied gastrointestinal symptoms. Microbiology cultures would be helpful in separating HS from various types of infections.

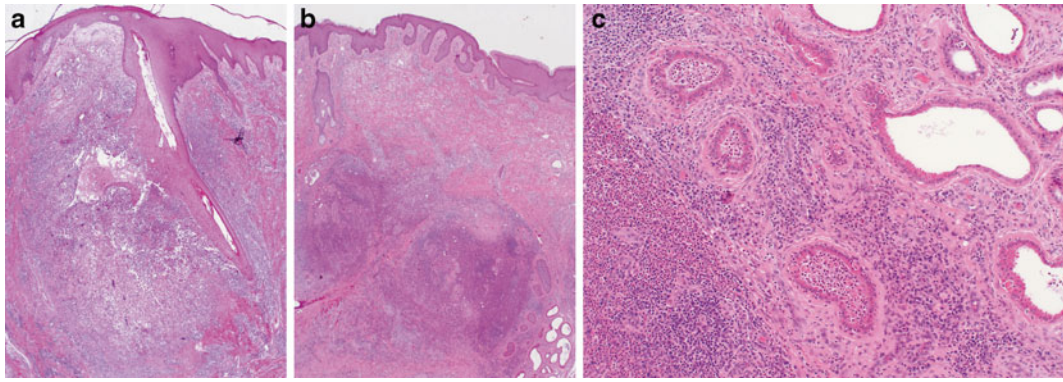


Fig. 12.22 Hidradenitis suppurativa. (**a**) An early lesion is typically an acute folliculitis. (**b**) An established lesion is characterized by dense and mixed inflammatory infiltrate with abscess formation. (**c**) Suppurative inflammation is seen involving the apocrine glands

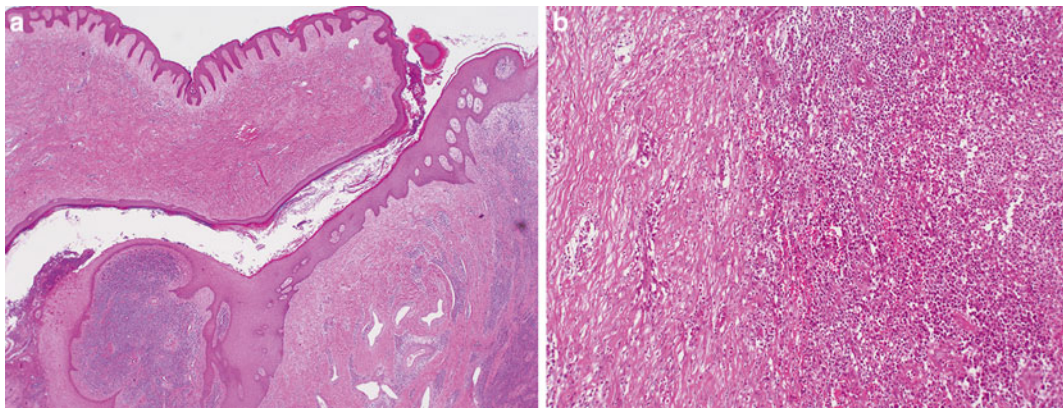


Fig. 12.23 (**a**, **b**) Hidradenitis suppurativa. Chronic changes include sinus tract formation, inflamed granulation tissue, and fibrosis

Summary

Clinical Presentation

- Women in the second or third decades of life.
- Axilla is the most commonly affected site.

Histologic Features

- Early: acute folliculitis and perifolliculitis
- Established: a dense and mixed inflammatory infiltrate and often with abscess formation
- Chronic: sinus tracts and fibrosis

Differential Diagnosis

- Inflamed and ruptured Bartholin cyst
- Crohn disease
- Infections

Takeaway Essentials

Clinical Relevant Pearls

- Affects sites rich in apocrine or mammary-like glands including the axillary, mammary, inframammary, inguinal, pubic, perineal, and perianal regions.
- Double comedones are typical of the disease.
- Follicular occlusion is the likely etiology.
- Bacteria are colonizers and not the etiologic agents.

Case Vignettes

Vignette 1

Clinical History: A 56-year-old woman presented with a deeply situated left vulvar lesion and 3-month history of dyspareunia. An excision was performed which revealed a 2 × 1.5 × 1 cm rubbery nodule. The cut surface was lobulated, firm, and tan brown. Subsequent left pelvic and external iliac lymph node dissection demonstrated no evidence of metastatic carcinoma.

Microscopic Description: Histologically, a cribriform pattern composed of nests of cells arranged concentrically around gland-like spaces containing eosinophilic materials and/or mucin is seen (Fig. 12.24). Perineural invasion is identified. The tumor is diffusely positive for CD117 and focally for MYB (Fig. 12.25).

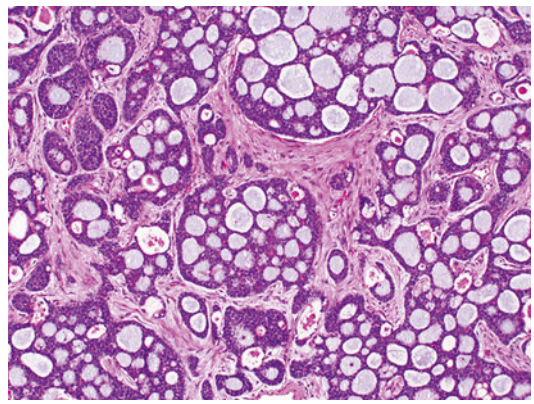


Fig. 12.24 Vignette 1. A tumor with a cribriform architecture is present in the deep dermis and subcutaneous tissue

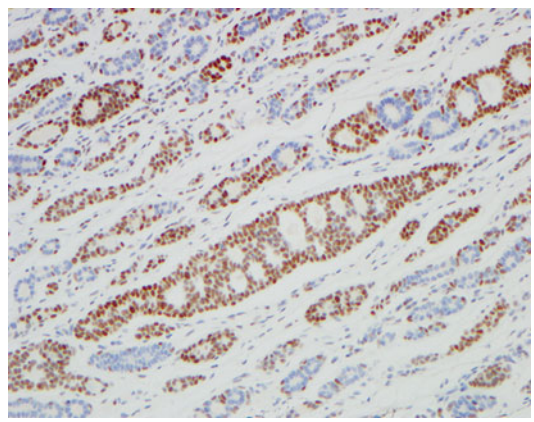


Fig. 12.25 Vignette 1. Nuclear MYB staining in the tumor cells

(continued)

Diagnosis: Adenoid cystic carcinoma

Discussion: Despite CD117 expression in invariably all adenoid cystic carcinoma, *KIT* mutation has not been documented in ACC of various anatomical sites [63, 148]. Recently Persson and colleagues [149] have shown a characteristic translocation between *MYB* and *NFIB* genes in adenoid cystic carcinomas. This fusion is between *MYB* or myeloblastosis viral oncogene homologue and *NFIB* or nuclear factor IB gene. This fusion occurs in adenoid cystic carcinomas from various anatomic locations including the salivary gland, sinonasal cavity, tracheobronchial tree, larynx, breast, and vulva [64]. Although specific, MYB immunostain is detected in only 65 % of adenoid cystic carcinomas [64].

Vignette 2

Clinical History: A 49-year-old female presented with a 2.5 cm nodule on her right labia.

Microscopic Description: An excisional biopsy showed a nodule proliferation of polygonal neoplastic cells with abundant and granular cytoplasm (Figs. 12.26 and 12.27). Nuclear pleomorphism and prominent nucleoli are noted (Fig. 12.28). The tumor cells were positive for S100 and negative for CD68, keratins AE1/AE3, and desmin.

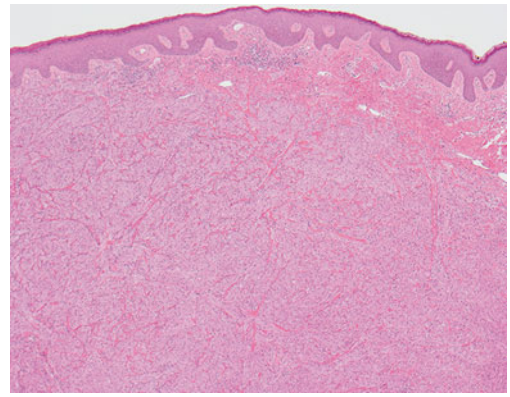


Fig. 12.26 Vignette 2. An expansile and nodular tumor is seen in the dermis

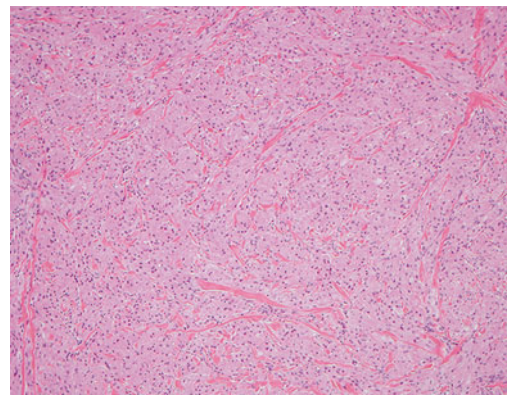


Fig. 12.27 Vignette 2. The tumor is comprised of polygonal neoplastic cells with granular cytoplasm

Diagnosis: Atypical granular cell tumor

Discussion: When a granular cell tumor has one or two of the following histologic features, it has been suggested to diagnose it as an atypical granular cell tumor: spindling of the tumor cells, high nuclear-to-cytoplasmic ratio, diffuse nuclear pleomorphism, prominent nucleoli, necrosis, and mitotic activity greater than 2 per 10 high-power fields at 200× [138]. When three or more of these features are present, the diagnosis of a malignant granular cell tumor should be raised, even though the presence of metastasis is the only reliable criterion for malignancy [138].

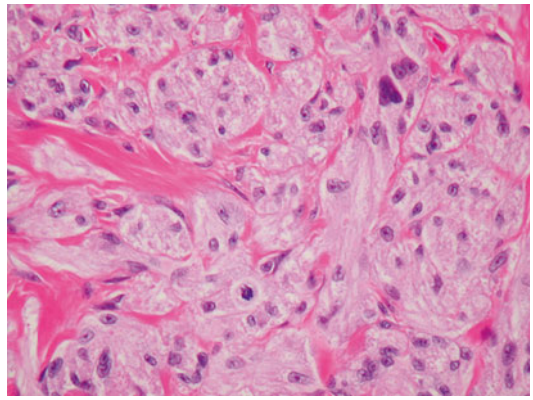


Fig. 12.28 Vignette 2. Nuclear pleomorphism and prominent nucleoli are noted

Vignette 3

Clinical History: A 69-year-old woman presented with white plaques on right vulva. They were thought to be molluscum contagiosum clinically.

Microscopic Description: A skin biopsy showed a cup-shaped invagination of acanthotic epidermis with overlying hyperkeratosis (Fig. 12.29). Granular keratohyaline clumping and dyskeratosis are seen (Fig. 12.30).

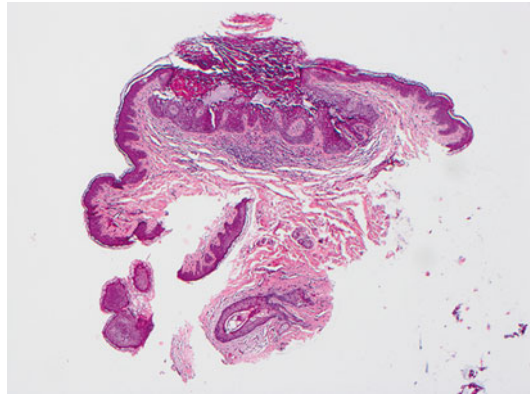


Fig. 12.29 Vignette 3. A cup-shaped invagination of acanthotic epidermis with overlying hyperkeratosis is seen

(continued)

Diagnosis: Epidermolytic hyperkeratosis

Discussion: Epidermolytic hyperkeratosis is rare with only few cases being reported in the literature of epidermolytic hyperkeratosis affecting the female genital tract [150–155]. Epidermolytic hyperkeratosis can be seen not only as part of diffuse involvement in congenital ichthyosiform erythroderma but also identified in a localized pattern. These patients suffer from white hyperkeratotic papules or small plaques. The cutaneous manifestations of generalized or localized epidermolytic hyperkeratosis are caused by mutation in the genes encoding keratins 1 and 10.

Epidermolytic hyperkeratosis is characterized by epidermal acanthosis, dissolution of the suprabasilar epithelium resulting in perinuclear clear zones, granular keratohyaline clumping, hypergranulosis, dyskeratosis, and intracellular eosinophilic globules ("cell within a cell") [154]. These lesions are frequently confused with HPV lesions such as verruca vulgaris, verruca plana, and condyloma acuminatum resulting in concern of sexually transmitted disease for the patient and unnecessary treatments.

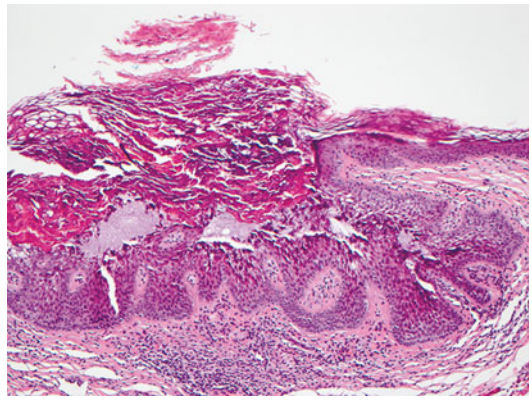


Fig. 12.30 Vignette 3. There are granular keratohyaline clumping and dyskeratosis

References

1. Marzano DA, Haefner HK. The bartholin gland cyst: past, present, and future. J Low Genit Tract Dis. 2004;8(3):195–204.
2. Rorat E, Ferenczy A, Richart RM. Human Bartholin gland, duct, and duct cyst. Arch Pathol. 1975;99(7):1367–74.
3. Honore LH, O'Hara KE. Adenoma of the Bartholin gland: report of three cases. Eur J Obstet Gynecol Reprod Biol. 1978;8:335–40.
4. Axe S, Parmley T, Woodruff JD, Hlopak B. Adenomas in minor vestibular glands. Obstet Gynecol. 1986;68(1):16–8.
5. Chapman GW, Hassan N, Page D, Mostoufi-Zadeh M, Leyman D. Mucinous cystadenoma of Bartholin's gland: a case report. J Reprod Med. 1987;32(12):939–41.
6. Koenig C, Tavassoli FA. Nodular hyperplasia, adenoma, and adenomyoma of Bartholin's gland. Int J Gynecol Pathol. 1998;17(4):289–94.
7. Foushee JH, Reeves WJ, McCool JA. Benign masses of Bartholin's gland. Solid adenomas, adenomas with cyst, and Bartholin's gland with varices and thrombosis or cavernous hemangioma. Obstet Gynecol. 1968;31(5):695–701.

8. Padmanabhan V, Cooper K. Concomitant adenoma and hybrid carcinoma of salivary gland type arising in Bartholin's gland. Int J Gynecol Pathol. 2000;19(4):377–80.
9. Leuchter RS, Hacker NF, Voet RL, Berek JS, Townsend DE, Lagasse LD. Primary carcinoma of the Bartholin gland: a report of 14 cases and review of the literature. Obstet Gynecol. 1982;60(3):361–8.
10. Wheelock JB, Goplerud DR, Dunn LJ, Oates 3rd JF. Primary carcinoma of the Bartholin gland: a report of ten cases. Obstet Gynecol. 1984;63(6): 820–4.
11. Cardosi RJ, Speights A, Fiorica JV, Grendys Jr EC, Hakam A, Hoffman MS. Bartholin's gland carcinoma: a 15-year experience. Gynecol Oncol. 2001;82(2):247–51.
12. Berger MB, Betschart C, Khandwala N, DeLancey JO, Haefner HK. Incidental Bartholin gland cysts identified on pelvic magnetic resonance imaging. Obstet Gynecol. 2012;120(4):798–802.
13. Sosnik H, Sosnik K, Halon A. The pathomorphology of Bartholin's gland. Analysis of surgical data. Pol J Pathol. 2007;58(2):99–103.
14. Wechter ME, Wu JM, Marzano D, Haefner H. Management of Bartholin duct cysts and abscesses: a systematic review. Obstet Gynecol Surv. 2009;64(6):395–404.
15. Kurman R. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer; 2002.
16. Robboy SJ, Ross JS, Prat J, Keh PC, Welch WR. Urogenital sinus origin of mucinous and ciliated cysts of the vulva. Obstet Gynecol. 1978;51(3): 347–51.
17. Gocmen A, Inaloz HS, Sari I, Inaloz SS. Endometriosis in the Bartholin gland. Eur J Obstet Gynecol Reprod Biol. 2004;114(1):110–1.
18. Sheard JD, Vijayanand R, Herrington CS, Giannoudis A, Shaw G. High-grade squamous intraepithelial neoplasia in a Bartholin's gland cyst associated with HPV 16 infection. Histopathology. 2000;37(1):87–8.
19. Enghardt M, Valente PT, Day DH. Papilloma of Bartholin's gland duct cyst: first report of a case. Int J Gynecol Pathol. 1993;12(1):86–92.
20. Ishida M, Iwai M, Yoshida K, Kagotani A, Okabe H. The first reported case of pigmented Bartholin duct cyst. Int J Clin Exp Pathol. 2013;6(9):1961–3.
21. Ben-Harosh S, Cohen I, Bornstein J. Bartholin's gland hyperplasia in a young woman. Gynecol Obstet Invest. 2008;65(1):18–20.
22. Kazakov DV, Curik R, Vanecek T, Mukensnabl P, Michal M. Nodular hyperplasia of the Bartholin gland: a clinicopathological study of Two cases, including detection of clonality by HUMARA. Am J Dermatopathol. 2007;29(4):385–7.
23. Santos LD, Kennerson AR, Killingsworth MC. Nodular hyperplasia of Bartholin's gland. Pathology. 2006;38(3):223–8.
24. Fiori E, Ferraro D, Borrini F, De Cesare A, Leone G, Crocetti A, Schillaci A. Bartholin's gland hyperplasia. Case report and a review of literature. Ann Ital Chir. 2013;84(ePub). pii: S2239253X13021774.
25. Argenta PA, Bell K, Reynolds C, Weinstein R. Bartholin's gland hyperplasia in a postmenopausal woman. Obstet Gynecol. 1997;90(4 Pt 2):695–7.
26. Prayson RA, Stoler MH, Hart WR. Vulvar vestibulitis. A histopathologic study of 36 cases, including human papillomavirus in situ hybridization analysis. Am J Surg Pathol. 1995;19(2):154–60.
27. Tavassoli FA, Devilee P, editors. World Health Organization Classification of tumours. Pathology and genetics of tumours of the breast and female genital organs. Lyons: IARC; 2003.
28. Punia RP, Bal A, Jain P, Mohan H. Minor vestibular gland adenoma: a case report. Aust N Z J Obstet Gynaecol. 2003;43(4):322–3.
29. Mandsager NT, Young TW. Pain during sexual response due to bilateral Bartholin's gland adenomas. A case report. J Reprod Med. 1992;37(12):983–5.
30. Dodson MG, O'Leary JA, Averette HE. Primary carcinoma of Bartholin gland. Obstet Gynecol. 1970; 35(4):578–84.
31. Leuchter RS, Hacker NF, Voet RL, Berek JS, Townsend DE, Lagasse LD. Primary carcinoma of the Bartholin gland: a report of 14 cases and review of the literature. Obstet Gynecol. 1982;60(3):361–8.
32. Wheelock JB, Goplerud DR, Dunn LJ, Oates 3rd JF. Primary carcinoma of the Bartholin gland: a report of ten cases. Obstet Gynecol. 1984;63(6):820–4.
33. Cardosi RJ, Speights A, Fiorica JV, Grendys Jr EC, Hakam A, Hoffman MS. Bartholin's gland carcinoma: a 15-year experience. Gynecol Oncol. 2001; 82(2):247–51.
34. Chamlian DL, Taylor HB. Primary carcinoma of Bartholin's gland. A report of 24 patients. Obstet Gynecol. 1972;39(4):489–94.
35. Ouldamer L, Chraibi Z, Arbion F, Barillot I, Body G. Bartholin's gland carcinoma: epidemiology and therapeutic management. Surg Oncol. 2013;22(2): 117–22.
36. Addison A. Adenocarcinoma of Bartholin gland in a 14-year-old girl: report of a case. Am J Obstet Gynecol. 1977;127(2):214–5.
37. Purola E, Widholm O. Primary carcinoma of Bartholin gland. Acta Obstet Gynecol Scand. 1966; 45(2):205–10.
38. Dennefors B, Bergman B. Primary carcinoma of the Bartholin gland. Acta Obstet Gynecol Scand. 1980;59(1):95–6.
39. Johnson CA. Bartholin gland cancer. Am Fam Physician. 1989;39(4):195–7.
40. Lim KC, Thompson IW, Wiener JJ. A case of primary clear cell adenocarcinoma of Bartholin gland. BJOG. 2002;109(11):1305–7.
41. Gharoro EP, Okonkwo CA, Onafowokan O. Adenocarcinoma of the Bartholin gland in a 34 years old multipara. Acta Obstet Gynecol Scand. 2001;80(3):279–80.
42. Felix JC, Cote RJ, Kramer EE, Saigo P, Goldman GH. Carcinomas of Bartholin's gland. Histogenesis

and the etiological role of human papillomavirus. Am J Pathol. 1993;142(3):925–33.
43. Ferrandina G, Testa AC, Zannoni GF, Poerio A, Scambia G. Skull metastasis in primary vulvar adenocarcinoma of the Bartholin's gland: a case report. Gynecol Oncol. 2005;98(2):322–4.
44. Paraskevaidis E, Zioga C, Chouliara S, Koliopoulos G, Tzioras S, Lolis D. Adenoid cystic carcinoma of Bartholin gland: a case report. Clin Exp Obstet Gynecol. 2001;28(2):109–10.
45. Rosenberg P, Simonsen E, Risberg B. Adenoid cystic carcinoma of Bartholin gland: a report of five new cases treated with surgery and radiotherapy. Gynecol Oncol. 1989;34(2):145–7.
46. Addison A, Parker RT. Adenoid cystic carcinoma of Bartholin gland: a review of the literature and report of a patient. Gynecol Oncol. 1977;5(2):196–201.
47. Dunn S. Adenoid cystic carcinoma of Bartholin gland – a review of the literature and report of a patient. Acta Obstet Gynecol Scand. 1995;74(1): 78–80.
48. Krasevic M, Haller H, Iternicka Z, Valstelic I, Matejcic N. Adenoid cystic carcinoma of Bartholin gland: a case report. Eur J Gynaecol Oncol. 2001;22(3):213–4.
49. Morita Y, Hikage S, Ogino M. Adenoid cystic carcinoma of Bartholin gland. Int J Gynaecol Obstet. 1996;54(3):279–80.
50. Lelle RJ, Davis KP, Roberts JA. Adenoid cystic carcinoma of the Bartholin gland: the University of Michigan experience. Int J Gynecol Cancer. 1994;4(3):145–9.
51. DePasquale SE, McGuinness TB, Mangan CE, Husson M, Woodland MB. Adenoid cystic carcinoma of Bartholin gland: a review of the literature and report of a patient. Gynecol Oncol. 1996;61(1):122–5.
52. Yamagiwa S, Niwa K, Yokoyama Y, Tanaka T, Murase T, Shimonaka E, et al. Primary adenoid cystic carcinoma of Bartholin gland. A case report. Acta Cytol. 1994;38(1):79–82.
53. Abrao FS, Marques AF, Marziona F, Abrao MS, Uchoa Junqueira LC, Torloni H. Adenoid cystic carcinoma of Bartholin gland: review of the literature and report of two cases. J Surg Oncol. 1985;30(2):132–7.
54. Bernstein SG, Voet RL, Lifshitz S, Buchsbaum HJ. Adenoid cystic carcinoma of Bartholin gland. Case report and review of the literature. Am J Obstet Gynecol. 1983;147(4):385–90.
55. Anaf V, Buxant F, Rodesch F, Simon P, van de Stadt J, Noel JC, van Geertruyden J. Adenoid cystic carcinoma of Bartholin gland: what is the optimal approach? Eur J Surg Oncol. 1999;25(4):406–9.
56. Milchgrub S, Wiley EL, Vuitch F, Albores-Saavedra J. The tubular variant of adenoid cystic carcinoma of the Bartholin's gland. Am J Clin Pathol. 1994;101(2):204–8.
57. Webb JB, Lott M, O'Sullivan JC, Azzopardi JG. Combined adenoid cystic and squamous carcinoma of Bartholin gland. Case report. Br J Obstet Gynaecol. 1984;91(3):291–5.
58. Young S, Leon M, Talerman A, Teresi M, Emmadi R. Polymorphous low-grade adenocarcinoma of the vulva and vagina: a tumor resembling adenoid cystic carcinoma. Int J Surg Pathol. 2003;11(1):43–9.
59. Felix A, Moura Nunes JF, Soares J. Salivary gland-type basal cell adenocarcinoma of presumed Bartholin's gland origin: a case report. Int J Gynecol Pathol. 2002;21(2):194–7.
60. McCluggage WG, Aydin NE, Wong NA, Cooper K. Low-grade epithelial-myoepithelial carcinoma of Bartholin gland: report of 2 cases of a distinctive neoplasm arising in the vulvovaginal region. Int J Gynecol Pathol. 2009;28(3):286–91.
61. Kazakov DV, Spagnolo DV, Kacerovska D, Michal M. Lesions of anogenital mammary-like glands: an update. Adv Anat Pathol. 2011;18(1):1–28.
62. Woida FM, Ribeiro-Silva A. Adenoid cystic carcinoma of the Bartholin gland: an overview. Arch Pathol Lab Med. 2007;131(5):796–8.
63. Mino M, Pilch BZ, Faquin WC. Expression of KIT (CD117) in neoplasms of the head and neck: an ancillary marker for adenoid cystic carcinoma. Mod Pathol. 2003;16(12):1224–31.
64. West RB, Kong C, Clarke N, Gilks T, Lipskick JS, Cao H, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol. 2011;35(1):92–9.
65. Wahlstrom T, Vesterinen E, Saksela E. Primary carcinoma of Bartholin glands: a morphological and clinical study of six cases including a transitional cell carcinoma. Gyn Oncol. 1978;6(4):354–62.
66. Haswgawa K, Minegishi K, Sugihara K, Toyoshima K, Itoh K, Nishino R, et al. A case of primary transitional cell carcinoma of the Bartholin gland with human papillomavirus type 18 infection (abstract). Nippon Sanka Fujinka Gakkai Zasshi Acta Obstet Gynaecol Japonica. 1995;47:1385–8.
67. Fujiwaki R, Takahashi K, Nishiki Y, Ryuko K, Kitao M. Rare case of transitional cell carcinoma originating in Bartholin's gland duct. Gynecol Obstet Invest. 1995;40(4):278–80.
68. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol. 2010;37(1):20–7.
69. Khoury-Collado F, Elliott KS, Lee YC, Chen PC, Abulafia O. Merkel cell carcinoma of the Bartholin's gland. Gynecol Oncol. 2005;97(3):928–31.
70. Jones MA, Mann EW, Caldwell CL, Tarraza HM, Dickersin GR, Young RH. Small cell neuroendocrine carcinoma of Bartholin's gland. Am J Clin Pathol. 1990;94(4):439–42.
71. Pawar R, Vijayalakshmy AR, Khan S, al Lawati FA. Primary neuroendocrine carcinoma (Merkel's cell carcinoma) of the vulva mimicking as a

Bartholin's gland abscess. Ann Saudi Med. 2005;25(2):161–4.
72. Hierro I, Blanes A, Matilla A, Munoz S, Vicioso L, Nogales FF. Merkel cell (neuroendocrine) carcinoma of the vulva. A case report with immunohistochemical and ultrastructural findings and review of the literature. Pathol Res Pract. 2000;196(7):503–9.
73. Kacerovska D, Nemcova J, Petrik R, Michal M, Kazakov DV. Lymphoepithelioma-like carcinoma of the Bartholin gland. Am J Dermatopathol. 2008;30(6):586–9.
74. Niu W, Heller DS, D'Cruz C. Lymphoepithelioma-like carcinoma of the vulva. J Low Genit Tract Dis. 2003;7(3):184–6.
75. Slukvin II, Schink JC, Warner TF. Lymphoepithelioma-like carcinoma of the vulva: a case report. J Low Genit Tract Dis. 2003;7(2):136–9.
76. Axelsen SM, Stamp IM. Lymphoepithelioma-like carcinoma of the vulvar region. Histopathology. 1995;27(3):281–3.
77. Fathi K, Pinter A. Paraurethral cysts in female neonates. Case reports. Acta Paediatr. 2003;92(6):758–9.
78. Kimbrough Jr HM, Vaughan Jr ED. Skene's duct cyst in a newborn: case report and review of the literature. J Urol. 1977;117(3):387–8.
79. Kazakov DV, Stewart CJ, Kacerovska D, Leake R, Kreuzberg B, Chudacek Z, et al. Prostatic-type tissue in the lower female genital tract: a morphologic spectrum, including vaginal tubulosquamous polyp, adenomyomatous hyperplasia of paraurethral Skene glands (female prostate), and ectopic lesion in the vulva. Am J Surg Pathol. 2010;34(7):950–5.
80. Kelly P, McBride HA, Kennedy K, Connolly LE, McCluggage WG. Misplaced Skene's glands: glandular elements in the lower female genital tract that are variably immunoreactive with prostate markers and that encompass vaginal tubulosquamous polyp and cervical ectopic prostatic tissue. Int J Gynecol Pathol. 2011;30(6):605–12.
81. Korytko TP, Lowe GJ, Jimenez RE, Pohar KS, Martin DD. Prostate-specific antigen response after definitive radiotherapy for Skene's gland adenocarcinoma resembling prostate adenocarcinoma. Urol Oncol. 2012;30(5):602–6.
82. Pongtippan A, Malpica A, Levenback C, Deavers MT, Silva EG. Skene's gland adenocarcinoma resembling prostatic adenocarcinoma. Int J Gynecol Pathol. 2004;23(1):71–4.
83. Dodson MK, Cliby WA, Keeney GL, Peterson MF, Podratz KC. Skene's gland adenocarcinoma with increased serum level of prostate-specific antigen. Gynecol Oncol. 1994;55(2):304–7.
84. Zaviacic M, Sidlo J, Borovsky M. Prostate specific antigen and prostate specific acid phosphatase in adenocarcinoma of Skene's paraurethral glands and ducts. Virchows Arch A Pathol Anat Histopathol. 1993;423(6):503–5.
85. Block RE. Hydrocele of the canal of nuck. A report of five cases. Obstet Gynecol. 1975;45(4):464–6.
86. Qureshi NJ, Lakshman K. Laparoscopic excision of cyst of canal of Nuck. J Minim Access Surg. 2014;10(2):87–9.
87. Schneider CA, Festa S, Spillert CR, Bruce CJ, Lazaro EJ. Hydrocele of the canal of Nuck. N J Med. 1994;91(1):37–8.
88. Akkoyun I, Kucukosmanoglu I, Yalinkilinc E. Cyst of the canal of nuck in pediatric patients. N Am J Med Sci. 2013;5(6):353–6.
89. Choi YM, Lee GM, Yi JB, Yoon KL, Shim KS, Bae CW, et al. Two cases of female hydrocele of the canal of nuck. Korean J Pediatr. 2012;55(4):143–6.
90. Jagdale R, Agrawal S, Chhabra S, Jewan SY. Hydrocele of the canal of Nuck: value of radiological diagnosis. J Radiol Case Rep. 2012;6(6):18–22.
91. Bagul A, Jones S, Dundas S, et al. Endometriosis in the canal of Nuck hydrocele: an unusual presentation. Int J Surg Case Rep. 2011;2(8):288–9.
92. Gaeta M, Minutoli F, Mileto A, Racchiusa S, Donato R, Bottari A, Blandino A. Nuck canal endometriosis: MR findings and clinical features. Abdom Imaging. 2010;35(6):737–41.
93. Noguchi D, Matsumoto N, Kamata S, Kaneko K. Ectopic pregnancy developing in a cyst of the canal of Nuck. Obstet Gynecol. 2014;123(2 Pt 2 Suppl 2):472–6.
94. Anderson CC, Broadie TA, Mackey JE, Kopecky KK. Hydrocele of the canal of Nuck: ultrasound appearance. Am Surg. 1995;61(11):959–61.
95. Junaid TA, Thomas SM. Cysts of the vulva and vagina: a comparative study. Int J Gynaecol Obstet. 1981;19(3):239–43.
96. Hamada M, Kiryu H, Ohta T, Furue M. Ciliated cyst of the vulva. Eur J Dermatol. 2004;14(5):347–9.
97. Baker GM, Selim MA, Hoang MP. Vulvar adnexal lesions: a 32-year, single-institution review from Massachusetts General Hospital. Arch Pathol Lab Med. 2013;137(9):1237–46.
98. Celik N, Yalcin S, Gucer S, Karnak I. Clitoral epidermoid cyst secondary to blunt trauma in a 9-year-old child. Turk J Pediatr. 2011;53(1):108–10.
99. Hamoudi A, Shier M. Late complications of childhood female genital mutilation. J Obstet Gynaecol Can. 2010;32(6):587–9.
100. Asante A, Omurtag K, Roberts C. Epidermal inclusion cyst of the clitoris 30 years after female genital mutilation. Fertil Steril. 2010;94(3):1097. e1–3.
101. Mueller BE, Laudenschlager MD, Hansen KA. Epidermoid cyst of the clitoris: an unusual cause of clitoromegaly in a patient without history of previous female circumcision. J Pediatr Adolesc Gynecol. 2009;22(5):e130–2.
102. Van der Putte SCJ, van Gorp LH. Cysts of mammary-like glands in the vulva. Int J Gynecol Pathol. 1995;14(2):184–8.
103. Chuang YH, Hong HS, Kuo TT. Multiple pigmented follicular cysts of the vulva successfully treated with CO_2 laser: case report and literature review. Dermatol Surg. 2004;30(9):1261–4.

104. Glusac EJ, Hendrickson MS, Smoller BR. Apocrine cystadenoma of the vulva. J Am Acad Dermatol. 1994;31(3 Pt 1):498–9.
105. Kamishima T, Igarashi S, Takeuchi Y, Ito M, Fukuda T. Pigmented hidrocystoma of the eccrine secretory coil in the vulva: clinicopathologic, immunohistochemical and ultrastructural studies. J Cutan Pathol. 1999;26(3):145–9.
106. Coghill SB, Tyler X, Shaxted EJ. Benign mucinous metaplasia of the vulva. Histopathology. 1990;17(4):373–5.
107. Rakha E, Mayne C, Brown L. Mucinous metaplasia of the vulva in a case of lichen sclerosus. A case report. J Clin Pathol. 2005;58(11):1217–8.
108. Thomson MA, Carr RA, Ganesan R, Humphreys F. Extensive mucinous metaplasia of the vulva arising within Zoon's vulvitis. Br J Dermatol. 2007; 156(4):750–2.
109. Boer-Auer A, August C, Falk TM, Jung JE, Kohl K, Metze D. Benign mucinous metaplasia of the genital mucosa: histomorphological and immunohistochemical features and criteria for differentiation from extramammary Paget disease. Br J Dermatol. 2011;165(6):1263–72.
110. Stern RC, Dash R, Bentley RC, Snyder J, Haney AF, Robboy SJ. Malignancy in endometriosis: frequency and comparison of ovarian and extraovarian types. Int J Gynecol Pathol. 2001;20(2):133–9.
111. Mesko JD, Gates H, McDonald TW, Youmans R, Lewis J. Clear cell ("mesonephroid") adenocarcinoma of the vulva arising in endometriosis: a case report. Gynecol Oncol. 1988;29(3):385–91.
112. Welch JM, Nayagam M, Parry G, Das R, Campbell M, Whatley J, Bradbeer C. What is vestibular papillomatosis? A study of its prevalence, aetiology and natural history. Br J Obstet Gynaecol. 1993; 100(10):939–42.
113. Wollina U, Verma S. Vulvar vestibular papillomatosis. Indian J Dermatol Venereol Leprol. 2010;76(3):270–2.
114. Sangueza JM, Saenz ML. Challenge. Vestibular papilloma of the vulva. Am J Dermatopathol. 2007;29(2):210. 219-20.
115. van Beurden M, van der Vange N, de Craen AJ, Tjong-A-Hung SP, ten Kate FJ, ter Scheqqet J, Lammes FB. Normal findings in vulvar examination and vulvoscopy. Br J Obstet Gynaecol. 1997; 104(3):320–4.
116. Moyal-Barracco M, Leibowitch M, Orth G. Vestibular papillae of the vulva. Lack of evidence for human papillomavirus etiology. Arch Dermatol. 1990;126(12):1594–8.
117. Novak ER, Woodruff JD. Gynecologic and obstetric pathology with clinical and endocrine relations. 7th ed. Philadelphia: Saunders; 1974. p. 26.
118. Tiltman AJ, Knutzen VK. Primary adenocarcinoma of the vulva originating in misplaced cloacal tissue. Obstet Gynecol. 1978;51 Suppl 1:30s–3s.
119. Willen R, Bekassy Z, Carlen B, Bozoky B, Cajander S. Cloacogenic adenocarcinoma of the vulva. Gynecol Oncol. 1999;74(2):298–301.
120. Dube V, Veilleux C, Plante M, Tetu B. Primary villoglandular adenocarcinoma of cloacogenic origin of the vulva. Hum Pathol. 2004;35(3):377–9.
121. Ghamande SA, Kaszinca J, Griffiths T, Finkler NJ, Hamid AM. Mucinous adenocarcinoma of the vulva. Gynecol Oncol. 1995;57(1):117–20.
122. Kennedy JC, Majmudar B. Primary adenocarcinoma of the vulva, possibly cloacogenic. A report of two cases. J Reprod Med. 1993;38(2):113–6.
123. Liu SH, Ho CM, Huang SH, Shih B, Lee FK. Cloacogenic adenocarcinoma of the vulva presenting as recurrent Bartholin's gland infection. J Formos Med Assoc. 2003;102(1):49–51.
124. Lee KC, Su WP, Muller SA. Multicentric cloacogenic carcinoma: report of a case with anogenital pruritus at presentation. J Am Acad Dermatol. 1990;23(5 Pt 2):1005–8.
125. Zaidi SN, Conner MG. Primary vulvar adenocarcinoma of cloacogenic origin. South Med J. 2001; 94(7):744–6.
126. Vulcan P, Dumitriu E, Grigore M. Heterotopic cloacogenic carcinoma of the lower lip. Ann Dermatol Venereol. 1994;121(2):156–8.
127. Abrikossoff A. Uber Myome, ausgehen von der quergestreiften wilulkrlichen Muskulatur. Virchows Arch Pathol Anat Physiol. 1926;260:215–33.
128. Fisher ER, Wechsler H. Granular cell myoblastoma – a misnomer: electron microscopic and histochemical evidence concerning its Schwann cell derivation and nature (granular cell Schwannoma). Cancer. 1962;15:936–54.
129. Horowitz IR, Copas P, Majmudar B. Granular cell tumors of the vulva. Am J Obstet Gynecol. 1995;173(6):1710–4.
130. Sargenti-Neto S, Brazao-Silva MT, do Nascimento Souza KC, de Faria PR, Durighetto-Junior AF, Loyola AM, Cardoso SV. Multicentric granular cell tumor: report of a patient with oral and cutaneous lesions. Br J Oral Maxillofac Surg. 2009;47(1): 62–4.
131. Papalas JA, Shaco-Levy R, Robboy SJ, Selim MA. Isolated and synchronous vulvar granular cell tumors: a clinicopathologic study of 17 cases in 13 patients. Int J Gynecol Pathol. 2010;29(2):173–80.
132. Kardhashi A, Assunta Deliso M, Renna A, Trojano G, Zito FA, Trojano V. Benign granular cell tumor of the vulva: first report of multiple cases in a family. Gynecol Obstet Invest. 2012;73(4):341–8.
133. Wolber RA, Talerman A, Wilkinson EJ, Clement PB. Vulvar granular cell tumors with pseudocarcinomatous hyperplasia: a comparative analysis with well-differentiated squamous carcinoma. Int J Gynecol Pathol. 1991;10(1):59–66.
134. Filie AC, Lage JM, Azumi N. Immunoreactivity of S100 protein, alpha-1-antitrypsin, and CD68 in adult

and congenital granular cell tumors. Mod Pathol. 1996;9(9):888–92.
135. Kapur P, Rakheja D, Balani JP, Roy LC, Amirkhan RH, Hoang MP. Phosphorylated histone H3, Ki-67, p21, fatty acid synthase, and cleaved caspase-3 expression in benign and atypical granular cell tumors. Arch Pathol Lab Med. 2007;131(1):57–64.
136. Schmidt O, Fleckenstein GH, Gunawan B, Fuzesi L, Emons G. Recurrence and rapid metastasis formation of a granular cell tumor of the vulva. Eur J Obstet Gynecol Reprod Biol. 2003;106(2):219–21.
137. Robertson AJ, McIntosh W, Lamont P, Guthrie W. Malignant granular cell tumour (myoblastoma) of the vulva: report of a case and review of the literature. Histopathology. 1981;5(1):69–79.
138. Fanburg-Smith JC, Meis-Kindblom JM, Fante R, Kindblom LG. Malignant granular cell tumor of soft tissue: diagnostic criteria and clinicopathologic correlation. Am J Surg Pathol. 1998;22(7):779–94.
139. Chamberlain BK, McClain CM, Gonzalez RS, Coffin CM, Cates JM. Alveolar soft part sarcoma and granular cell tumor: an immunohistochemical comparison study. Hum Pathol. 2014;45(5):1039–44.
140. Alikhan A, Lynch PJ, Eisen DB. Hidradenitis suppurativa: a comprehensive review. J Am Acad Dermatol. 2009;60(4):539–61.
141. Revus JE, Canoui-Poitrine F, Wolkenstein P, Viallette C, Gabison G, Pouget F, et al. Prevalence and factors associated with hidradenitis suppurativa: results from two case-control studies. J Am Acad Dermatol. 2008;59(4):596–601.
142. Jemec GB, Heidenheim M, Nielsen NH. The prevalence of hidradenitis suppurativa and its potential precursor lesions. J Am Acad Dermatol. 1996;35(2 Pt 1):191–4.
143. Chicarilli ZN. Follicular occlusion triad: hidradenitis suppurativa, acne conglobata, and dissecting cellulitis of the scalp. Ann Plast Surg. 1987;18(3):230–7.
144. Lapins J, Ye W, Nyren O, Emtestam L. Incidence of cancer among patients with hidradenitis suppurativa. Arch Dermatol. 2001;137(6):730–4.
145. Short KA, Kalu G, Mortimert PS, Higgins EM. Vulval squamous cell carcinoma arising in chronic hidradenitis suppurativa. Clin Exp Dermatol. 2005;30(5):481–3.
146. Jemec GB, Hansen U. Histology of hidradenitis suppurativa. J Am Acad Dermatol. 1996;34(6):994–9.
147. Yu CC, Cook MG. Hidradenitis suppurativa: a disease of follicular epithelium, rather than apocrine glands. Br J Dermatol. 1990;122(6):763–9.
148. Wetterskog D, Wilkerson PM, Rodriques DN, Lambros MB, Fritchie K, Andersson MK, et al. Mutation profiling of adenoid cystic carcinomas from multiple anatomical sites identifies mutations in the RAS pathway, but no KIT mutations. Histopathology. 2013;62(4):543–50.
149. Persson M, Andren Y, Mark J, Horling HM, Persson F, Stenmann G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci U S A. 2009;106(44):18740–4.
150. Russell P, Valmadre S, Howard V. Localized epidermolytic hyperkeratosis of the vulva: a case of mistaken identity. Pathology. 2010;42(5):483–5.
151. Kudreja T, Krunic A. Multiple epidermolytic acanthomas must not be confused with genital human papillomavirus infection. Acta Derm Venereol. 2009;89(2):169–72.
152. Reguiai Z, Cribier B, Derancourt C, Perceau G, Bernard P. Multiple spreading epidermolytic acanthomas of the genital and perigenital skin. Dermatology. 2005;211(2):152–4.
153. Swann MH, Pujals JS, Pillow J, Collier SL, Hiatt K, Smoller BR. Localized epidermolytic hyperkeratosis of the female external genitalia. J Cutan Pathol. 2003;30(6):379–81.
154. Quinn TR, Young RH. Epidermolytic hyperkeratosis in the lower female genital tract: an uncommon stimulant of mucocutaneous papillomavirus infection – a report of two cases. Int J Gynecol Pathol. 1997;16(2):163–8.
155. Thomas M, George R, Thomas M. Linear epidermolytic acanthosis of vulva: an unusual presentation. Indian J Dermatol Venereol Leprol. 2010;76(1):49–51.

Part VI

Mesenchymal Proliferations of the Vulva

Fibrous/Myofibroblastic Proliferations of the Vulva

13

Cesar A. Llanos and Andrew E. Rosenberg

Introduction

Fibrous and myofibroblastic vulvar proliferations are some of the most common mesenchymal tumors of the vulva (Table 13.1). They are usually benign and composed of spindle and stellate-shaped cells with a stroma that contains collagen and a supportive vascular tree. Many of these tumors tend to be restricted to the lower gynecological anatomic region and are less frequently identified in analogous regions in men. As they are uncommon and have distinctive and often overlapping features, their accurate diagnosis can be challenging to the surgical pathologist. Ancillary studies such as immunohistochemistry may be helpful in their recognition; however, they are usually not pathognomonic because of similar antigen expression profiles by fibroblasts and myofibroblasts. Accordingly, morphology is the mainstay for diagnosis.

A.E. Rosenberg, M.D. (✉)
Department of Pathology, University of Miami Hospital, Miami, FL, USA
e-mail: arosenberg@med.miami.edu

Fibroepithelial Stromal Polyp

Clinical Features

Fibroepithelial stromal polyps often present as an incidental lesions that arise in the vulvovaginal region. Clinically, they may produce a mass sensation, bleeding, or discharge [1, 2].

They occur in premenopausal women and are hormone related; therefore, their development in postmenopausal women has been shown to be associated with hormone replacement therapy [3, 4]. The lesions are pedunculated, variable in size, and usually do not exceed 5 cm; rare examples of tumors as large as 20 cm have been described [5]. Fibroepithelial polyps are usually solitary lesions; however, multiple lesions can be seen during pregnancy [6, 7]. Treatment is simple excision and the risk of local recurrence is very low.

Histopathology

The gross appearance is that of a flesh-colored soft solid mass that is covered by squamous mucosa or skin (Fig. 13.1). The lesion has a fibrovascular core that contains spindled fibroblasts and scattered multinucleated stellate stromal cells that are often distributed close to the overlying epithelium without a grenz zone (Figs. 13.2 and 13.3). The stroma is usually hypocellular and edematous, but there can be areas of hypercellularity and limited nuclear pleomorphism. Importantly, a minority of

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_13, © Springer Science+Business Media New York 2015

Table 13.1 Fibrous/myofibroblastic lesions

	Age	Location	Clinical presentation	Gross	Microscopic features	Ancillary tests
Fibroepithelial stromal polyp	Reproductive age	Vulvovaginal region	Single mass Multiple in pregnancy	Flesh-colored mass covered with skin	Fibrous tissue and blood vessels Scattered multinucleated giant cells	Positive immunohistochemistry for desmin, ER, PR
Massive vulvar edema	Mean age: 46.5	Vulva, penis or scrotum	Usually in morbidly obese generalized genital enlargement present from 6 months to 6 years	Massive pedunculated masses	Edematous fibrous tissue with dilated lymphatics Verrucous hyperplasia or papillomatosis of epidermis	
Prepubertal vulvar fibroma	Prepubertal	Labia majora	Unilateral submucosal or subcutaneous mass	Tan-gray and ill delineated	Hypocellular fibrous tissue with bland fibroblast Thick and wavy collagen fibers in stroma	Positive immunohistochemistry for CD34
Cellular angiofibroma	Mean age: 54	Labia majora and perineal region	Small, painless mass in the superficial soft tissues	Yellow to white firm mass	Well-circumscribed hypercellular spindle cell lesion Hyalinized branching blood vessels	Positive immunohistochemistry for CD34, ER, PR
Angiomyofibroblastoma	Middle-aged women	Vulvovaginal region/labia majora	Painful mass that is usually misdiagnosed as Bartholin gland cyst or inguinal hernia	Gray to white, soft to rubbery, well-circumscribed mass	Well demarcated with hypo- and hypercellular areas Wavy collagen fibers with spindle or stellate neoplastic cells	Positive immunohistochemistry for desmin
Aggressive angiomyxoma	Mean age: 40	Pelvis, perineum or vulva	Large asymptomatic lesion sometimes confused with a lipoma or leiomyoma	Tan-gray with rubbery consistence and gelatinous cut surface	Paucicellular tumor with myxoid stroma Bland spindle and stellate cells Medium-sized blood vessels	Positive immunohistochemistry for desmin, SMA, ER, PR, and HMGIC t(8;12) (p12:q15)
Superficial myofibroblastoma	Three to nine decades	Vulvovaginal region	Solitary nodular lesion<3 cm	Tan-pink to white firm lesion covered by vulvar skin	Vague fascicles with lacelike collagen fibers Bland spindle to ovoid cells	Positive immunohistochemistry for desmin, CD34, CD99 Variable ER, PR
Reactive fibroblastic and myofibroblastic proliferation of the vulva	55	Labia majora	Painful nodule perineal nodule exacerbated with physical activity (cycling, horseback riding)	Firm and fibrous with overlying skin	Adipose tissue with mildly cellular hyalinized stroma Haphazardly arranged vessels and nerves	Positive immunohistochemistry for ER, SMA
Postoperative spindle cell nodule	Middle age	Lower genitourinary tract	Three to seven weeks after a surgical procedure	Poorly defined polypoid nodule with reddish-gray appearance	Plump spindle cells in intersecting fascicles Numerous mitosis	Positive immunohistochemistry for SMA, focally desmin Trisomy 7

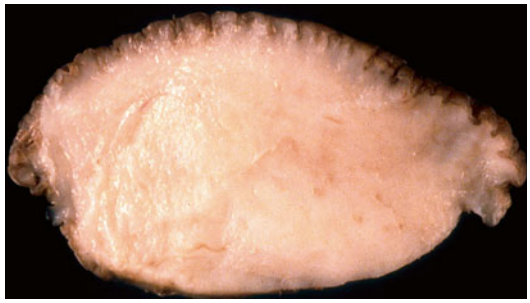


Fig. 13.1 Fibroepithelial polyp is solid, unencapsulated, and tan-white

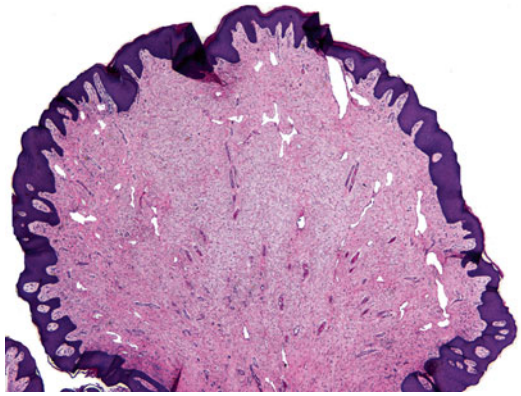


Fig. 13.2 Core of fibroepithelial polyp is composed of fibrous tissue and blood vessels

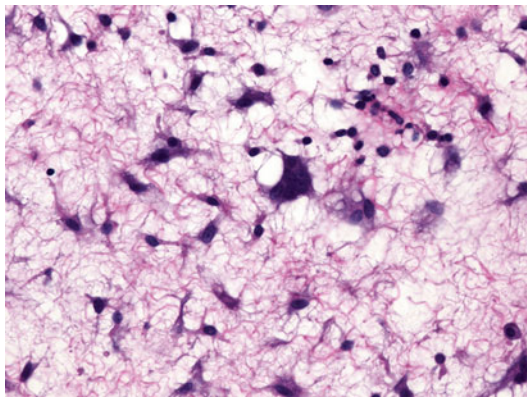


Fig. 13.3 The fibroepithelial polyp contains scattered multinucleated giant cells

polyps during pregnancy may show increased stromal cellularity, nuclear atypia, and numerous mitotic figures (Fig. 13.4) [8]. The overlying epithelium may demonstrate varying degrees of hyperplasia [4, 9, 10]. The stromal cells are often

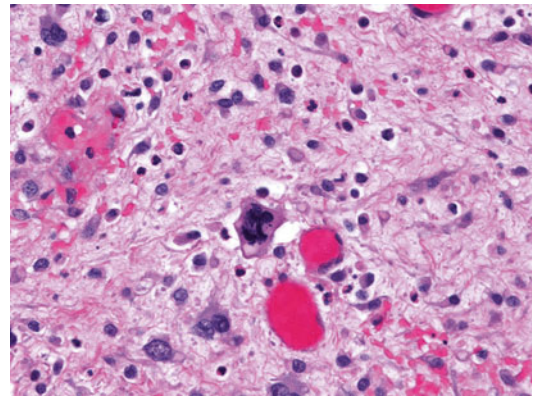


Fig. 13.4 Pseudosarcomatous fibroepithelial polyp is hypercellular and contains cells with enlarged pleomorphic nuclei that are mitotically active

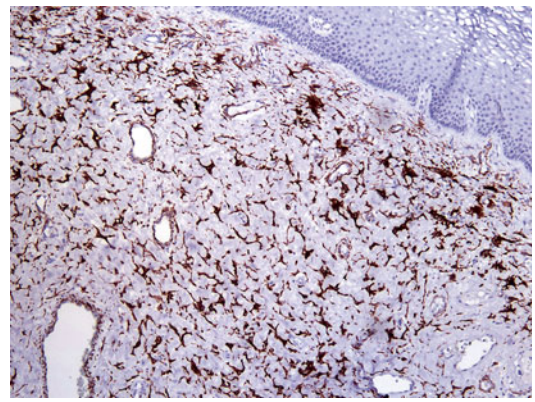


Fig. 13.5 The spindle and stellate cells of fibroepithelial polyps express desmin

positive for desmin, estrogen receptor (ER), and progesterone receptor (PR), and in some cases they express CD34 and smooth muscle actin (SMA) (Fig. 13.5). The lesional cells are negative for keratin, S100, myogenin, and MyoD-1 [8].

Differential Diagnosis

The degree of hypercellularity and nuclear pleomorphism may mimic a malignant process, particularly embryonal rhabdomyosarcoma, and this explains why some lesions have been termed pseudosarcoma botryoides. In distinction from the botryoides variant of rhabdomyosarcoma, a cellular fibroepithelial polyp does

not have a distinct cambium layer, nor does it demonstrate skeletal muscle differentiation by light microscopy or immunohistochemically [3]. (See Vignette 3 at the end of this chapter) The epithelial hyperplasia and fingerlike projections can mimic a condyloma acuminatum. The absence of viral changes such as koilocytes supports the diagnosis of fibroepithelial stromal polyp rather than human papillomavirus (HPV)-derived infection.

The tip of deep-seated aggressive angiomyxoma may present as a polypoid mass; however, it can be distinguished from fibroepithelial stromal polyp by virtue of its infiltrative growth pattern, deep location, presence of a prominent undulating vascular tree, and the hypocellular and myxoid nature of the tumor.

Summary

Clinical Presentation

- Present in premenopausal women
- Hormone related
- Single mass, may be multiple in pregnancy

Histopathologic Features

- Solid, unencapsulated, and tan-white.
- Core composed of fibrous tissue and blood vessels.
- Scattered multinucleated giant cells are characteristic.

Differential Diagnosis

- Embryonal rhabdomyosarcoma
- Condyloma acuminatum

Takeaway Essentials

Clinical Relevant Pearls

- Lesions are hormone related and have been associated with hormone replacement therapy.
- Should be considered in the differential diagnosis of vulvar polyps in premenopausal women.

Pathology Interpretation Pearls

- Hypocellular and edematous stroma without a grenz zone, but can be hypercellular and with nuclear pleomorphism in pregnancy.
- Squamous mucosa may demonstrate reactive hyperplasia that should not be confused with squamous cell carcinoma or viral changes.

Massive Vulvar Edema

Clinical Features

The lesion presents as enlargement of the vulva, most often bilateral, that may be massive and appear as confluent verrucous lesions. It usually affects morbidly obese individuals, and some patients may have a history of immobility, pregnancy, and hypothyroidism [11–13]. The mean age at presentation is 46.5 years [14]. The enlargement is slowly progressive and the overlying skin may ulcerate.

Histopathology

Grossly the lesion is tan-gray and poorly defined and ranges from a few to greater than 20 cm in dimension (Fig. 13.6). The epidermis may show verrucous hyperplasia or papillomatosis [14]. The accompanying adipose tissue shows thickening of the connective tissue septae that contain increased amounts of fibroblasts, collagen, myxoid stroma, lymphatics, and small- to medium-sized blood vessels (Figs. 13.7 and 13.8). The fibroblasts are spindle and stellate shaped and contain eosinophilic cytoplasm and nuclei that have fine chromatin. The fat may show foci of necrosis associated with macrophages, and the blood vessels can have a perivascular chronic inflammatory infiltrate.

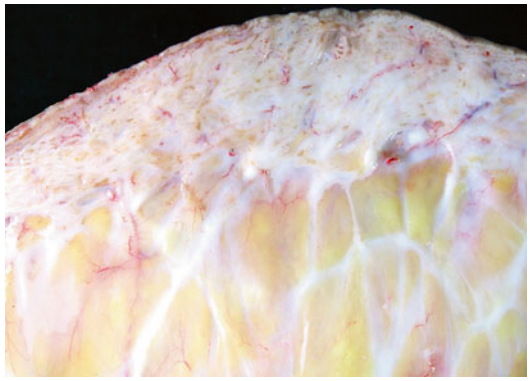


Fig. 13.6 The dermis and fat septae are expanded by *tan-gray* tissue in massive lymphedema

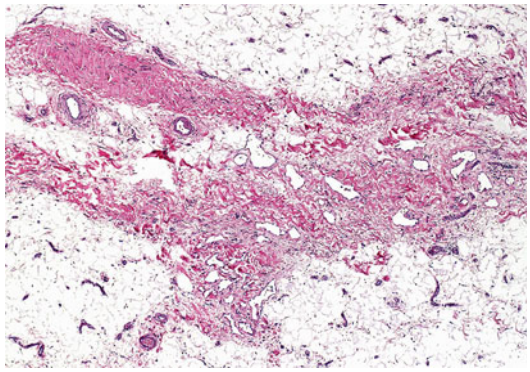


Fig. 13.7 The septae in the fat in massive lymphedema are expanded by edematous fibrous tissue that contains increased numbers of dilated lymphatics

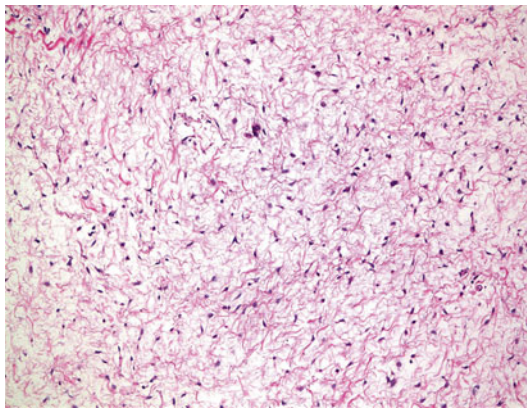


Fig. 13.8 In massive lymph edema, the subcutaneous tissue is replaced by bland fibro-myxoid tissue

Differential Diagnosis

The main lesion in the differential diagnosis is aggressive angiomyxoma. Distinguishing features are the presence of the tortuous and dilated lymphatics present in massive vulvar edema and the absence of broad fibromyxoid tissue. Because of the secondary changes that occur in the fat liposarcoma may be a consideration; however, lipoblasts and cells with enlarged hyperchromatic nuclei characteristic of liposarcoma are absent.

Summary

Clinical Presentation

- Bilateral vulvar enlargement, may be massive
- Slowly progressing, can lead to skin ulceration

Histopathologic Features

- The septae of the subcutis are expanded by fibrous tissue.
- Edematous fibrous tissue contains increased numbers of dilated lymphatics.

Differential Diagnosis

- Aggressive angiomyxoma

Takeaway Essentials

Clinical Relevant Pearls

- Bilateral lesion usually associated with morbid obesity and/or immobility, hyperthyroidism, and pregnancy

Pathology Interpretation Pearls

- Pathologic findings are centered in the subcutis, an unusual location for liposarcoma.
- Dilated lymphatics should not be confused with a primary lymphatic tumor.

Prepubertal Vulvar Fibroma

Clinical Features

The lesion presents as painless progressive swelling that causes enlargement of the labia majora in prepubertal females. It usually appears as a unilateral submucosal or subcutaneous mass with ill-defined borders. Treatment is complete excision as the lesion can recur locally.

Histopathology

Grossly the lesion is tan-gray and poorly delineated from the surrounding tissues. Microscopically, it is hypocellular and composed of scattered spindle cells that are cytologically bland; there is no pleomorphism and mitoses are rare (Fig. 13.9). The stroma consists of collagen fibers that are thick and wavy and contains medium-sized blood vessels (Fig. 13.10). Histologically the lesion is poorly demarcated and infiltrates into the surrounding tissues. The spindle cells are strongly positive for CD34.

Differential Diagnosis

The infiltrative borders and hypocellularity can suggest the possibility of aggressive angiomyxoma; however, the collagenous stroma in aggressive angiomyxoma is composed of delicate fibrils, different from the thick, wavy fibers present in this entity, and the vessels in aggressive angiomyxoma are larger, more numerous, and undulating. Immunohistochemistry can also be helpful in the distinction in that the tumor cells in angiomyxoma express desmin, whereas the cells in prepubertal vulvar fibroma are typically negative for this antigen. Cytogenetics is also useful in separating these tumors from one another; however, the histological and immunohistochemical findings should allow for accurate identification [15].

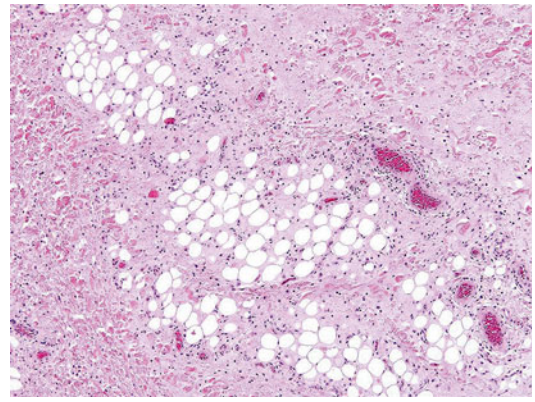


Fig. 13.9 Hypocellular fibrous tissue replacing fat in prepubertal fibroma

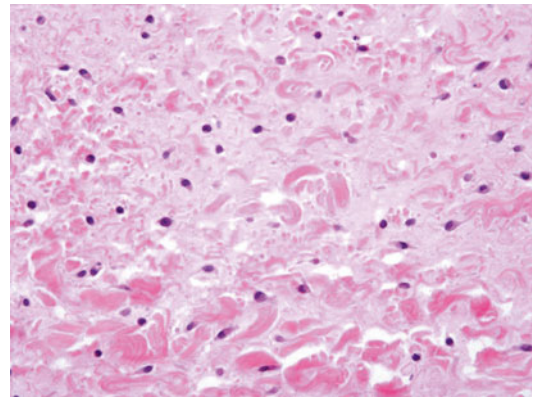


Fig. 13.10 The fibroblasts in prepubertal fibroma have bland nuclei and are associated with varying amounts of collagen

Angiomyofibroblastoma should also be distinguished from prepubertal vulvar fibroma; however, the former is usually well circumscribed and shows a greater degree of cellularity. Additionally, the tumor cells are desmin positive. Fibroepithelial polyps can be included in the differential, as well, but they protrude from the surface and are more cytologically heterogeneous with multinucleation, nuclear pleomorphism, and mitotic activity [16].

Summary

Clinical Presentation

- Occurs in prepubertal females
- Unilateral submucosal or subcutaneous mass in labia majora

Histopathologic Features

- Hypocellular fibrous tissue with bland fibroblasts replacing fat.
- Stroma has thick and wavy collagen fibers.

Differential Diagnosis

- Aggressive angiomyxoma
- Angiomyofibroblastoma
- Fibroepithelial stromal polyp

Takeaway Essentials

Clinical Relevant Pearls

- Mass in labia majora of prepubertal females with painless progressive swelling
- Can cause emotional distress and should be addressed promptly

Pathology Interpretation Pearls

- Bland fibroblasts admixed with variable amounts of thick and wavy collagen are good clues of the benign nature of the lesion.
- Infiltrative growth into the surrounding tissues can cause confusion with more aggressive soft tissue tumors.
- CD34 reactivity can be also seen in vulvar prepubertal fibromas and cellular angiofibroma; however, vulvar fibroma lacks hyalinized vessels and is not well circumscribed.

Cellular Angiofibroma

Clinical Features

The tumor arises in the vulva, particularly the labia majora and perineal regions of women who are middle aged (mean age, 54 years). Cellular angiofibroma is painless, relatively small (mean, 3.7 cm),

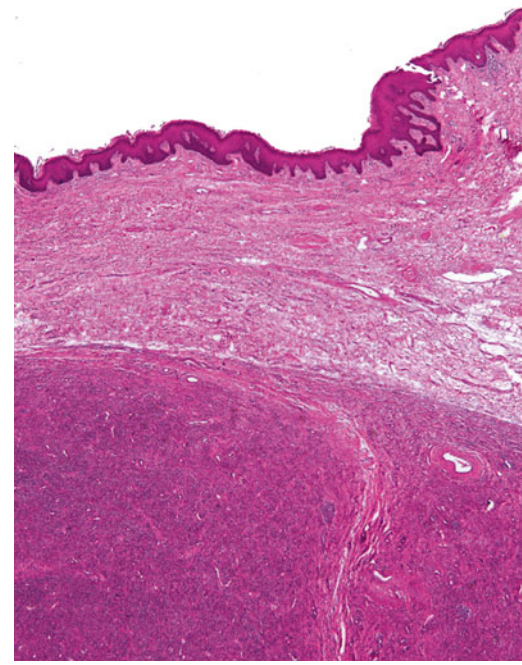


Fig. 13.11 Cellular angiofibroma has well-delineated margins

and sometimes exophytic, that is, well circumscribed, and is centered in the superficial soft tissues [17–19]. Biologically, it is benign, rarely undergoes malignant transformation, and treatment is complete excision; recurrence rates are very low [20]. It harbors a monoallelic deletion of *RB1* and *FOXO1* located on 13q14 that also occurs in spindle cell lipoma and myofibroblastoma, and this finding suggests that these are very closely related to one another [21].

Histopathology

Grossly, cellular angiofibromas are yellow to white firm masses that are sometimes polypoid and infrequently gelatinous or cystic. They tend to be partially encapsulated but can have focally infiltrative borders [21] (Fig. 13.11).

Histologically the tumor is hypercellular and composed of haphazardly arranged spindle cells with fusiform or ovoid bland nuclei and pale indistinct eosinophilic cytoplasm. Interspersed in the tumor there are multiple small- to medium-sized blood vessels that usually have hyalinized

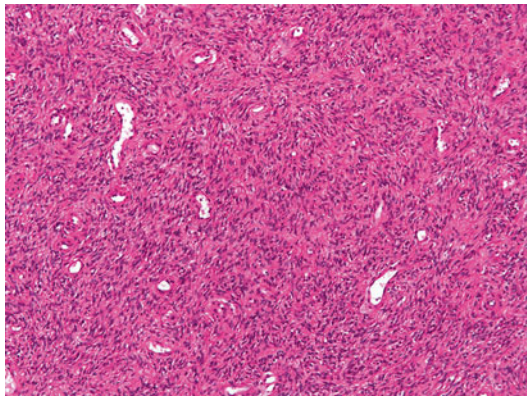


Fig. 13.12 Cellular angiofibroma is hypercellular and contains a staghorn-like vascular tree

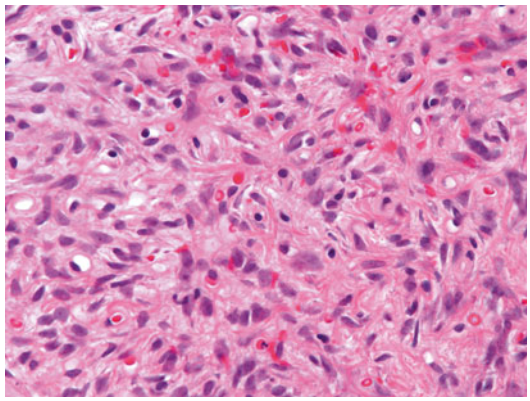


Fig. 13.13 The tumor cells in cellular angiofibroma are spindled with bland nuclei. The stroma contains wirelike bundles of collagen

walls and are arranged in a staghorn-like branching pattern (Fig. 13.12). The stroma contains wirelike collagen fibers, and scattered adipocytes and mast cells may be present (Fig. 13.13). The lesional cells are positive for CD34 and exhibit nuclear positivity staining for estrogen and androgen receptor and are often negative for all smooth muscle markers including SMA, desmin and h-caldesmon [22].

In a minority of cases the tumor may contain scattered cells with enlarged hyperchromatic nuclei that are reminiscent of those in symplastic leiomyoma and ancient schwannoma [19]. The mitotic rate for these tumors is usually low (<1 mitosis per 10 high-power fields). Another very uncommon variant are cellular angiofibromas that have discrete areas of sarcomatous transformation. The sarcomatous component may exhibit the features of pleomorphic liposarcoma, atypical lipomatous tumor, or an undifferentiated pleomorphic spindle cell sarcoma. Immunohistochemistry has shown that the malignant component expresses p16, whereas the benign component is negative for this antigen. Despite the sarcomatous components, the affected patients have done well with no recurrences or metastases [19].

Differential Diagnosis

There are morphological and genetic similarities between cellular angiofibroma, spindle cell lipoma and myofibroblastoma, and these tumors likely represent a family of related lesions [21]. Myofibroblastoma has a fascicular arrangement of the tumor cells in contrast to the haphazard arrangement of cells in angiofibroma. Spindle cell lipoma has characteristic thick ropey collagen fibers that are typically absent in angiofibroma, and it also usually has a more prominent fatty component. Additionally, the hyalinized vessels are distinctive of cellular angiofibroma and not a feature of spindle cell lipoma [21].

Summary

Clinical Presentation

- Arises in labia majora of the vulva and perineum
- Unilateral subcutaneous mass

Histopathologic Features

- Well-circumscribed hypercellular spindle cell lesion with fusiform or ovoid nuclei
- Multiple hyalinized branching blood vessels
- Wirelike collagen fibers in the stroma
- Positive for CD34, nuclear positivity for ER and PR
- Negative for muscle markers

Differential Diagnosis

- Spindle cell lipoma
- Myofibroblastoma

Takeaway Essentials

Clinical Relevant Pearls

- Painless unilateral vulvar and perineal lesion, relatively small
- Monoallelic deletion of *RB1* and *FOXO1* on 13q14-same derangement as spindle cell lipoma and myofibroblastoma

Pathology Interpretation Pearls

- Well-delineated margins support benign nature of the tumor.
- Hypercellularity can cause confusion with more aggressive neoplasms.

Angiomyofibroblastoma

Clinical Features

This is an uncommon lesion that arises in the vulvovaginal area of middle-aged women, particularly in the labia majora [23]. It is often misdiagnosed clinically as a Bartholin gland cyst or inguinal hernia. Sometimes it presents as a pedunculated or painful mass. The treatment of choice is complete excision and local recurrence is infrequent [17, 18].

Histopathology

Angiomyofibroblastoma is a well-circumscribed tumor that has a soft to rubbery and gray-white to yellowish appearance (Fig. 13.14). Sometimes it can be shiny and gelatinous and rarely is cystic and hemorrhagic. They are usually small as they are usually less than 5 cm in greatest dimension.

Microscopically, angiomyofibroblastoma is well demarcated and has hypo- and hypercellular areas (Fig. 13.15). The stroma is edematous and contains wavy collagen fibers. The neoplastic cells are variable in appearance with spindle, stellate, plasmacytoid, or epithelioid shapes. They are randomly arranged and can be isolated or oriented in cords or nests around small- to medium-sized blood vessels (Figs. 13.16 and 13.17).

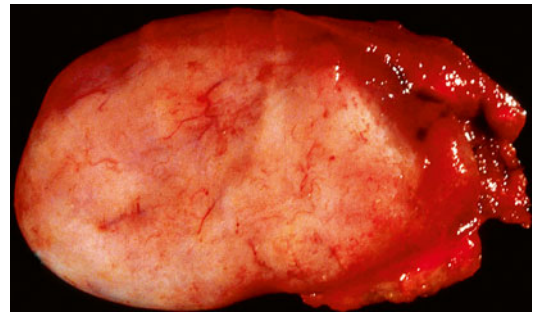


Fig. 13.14 Angiomyofibroblastoma is *tan*, *pale yellow* and well circumscribed

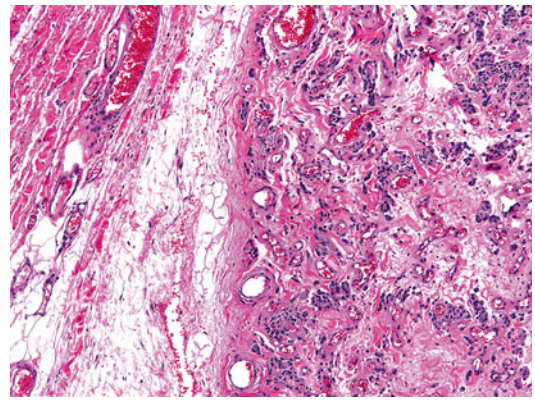


Fig. 13.15 Angiomyofibroblastoma has well-demarcated margins from the adjacent soft tissue

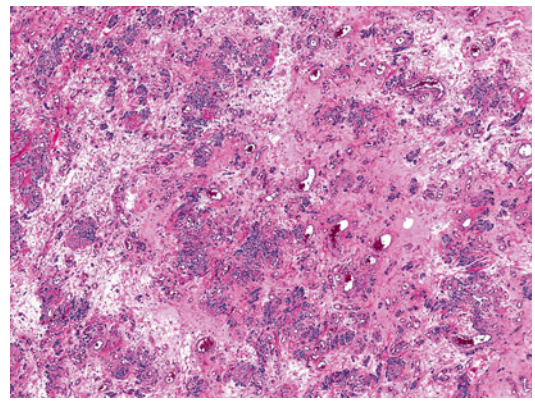


Fig. 13.16 Angiomyofibroblastoma contains scattered blood vessels with tumor cells growing in cords around blood vessels

Mitotic activity is limited (0–7 mitoses per 50 high-power fields). Adipocytes may be scattered throughout the mass. Immunohistochemically, the cells express desmin and are typically

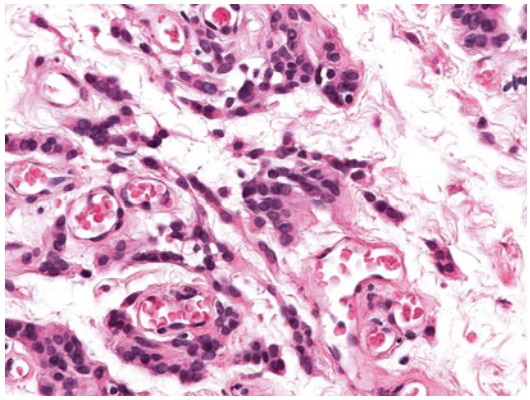


Fig. 13.17 The tumor cells in angiomyofibroblastoma have round bland nuclei and eosinophilic cytoplasm

negative for CD34 [17, 23, 24]. Extremely rare examples have been reported in which a typical angiomyofibroblastoma merged with sarcomatous areas that have the appearance of myxofibrosarcoma [23].

Differential Diagnosis

Aggressive angiomyxoma is an entity that should be considered in the differential diagnosis, as both contain spindle cells that are desmin positive. However, aggressive angiomyxoma is infiltrative, has larger undulating vessels, and lacks the hypercellularity [25].

Summary

Clinical Presentation

- Vulvovaginal lesion of middle-aged women
- Presents as painful mass

Histopathologic Features

- Well-demarcated lesion with hypo- and hypercellular areas
- Edematous stroma with wavy collagen
- Positive for desmin and negative for CD34

Differential Diagnosis

- Aggressive angiomyxoma

Takeaway Essentials

Clinical Relevant Pearls

- Painful mass that can be mistaken for a Bartholin gland cyst

Pathology Interpretation Pearls

- Clear demarcation from the surrounding tissue
- Tumor cells growing in cords around scattered blood vessels

Aggressive Angiomyxoma

Clinical Features

Aggressive angiomyxoma is a locally aggressive neoplasm that arises in the pelvis, perineum, or vulva region of middle-aged women (mean age, 40 years) and rarely in older men (mean age, 50–60) [26–29] (Fig. 13.18). The tumors can be asymptomatic but tend to be large (>10 cm) and are clinically confused with a Bartholin cyst, vaginal cyst, leiomyoma, or lipoma [30, 31]. This attests to the fact that their true size is often underestimated by clinical examination alone. Treatment is a wide (1 cm margin) excision; however, this may be difficult to attain which explains why the local recurrence rate is as high as 47 % [32, 33]. Rare cases of metastases have been reported [34]. Molecular analyses of a limited number of cases have revealed structural abnormalities of 12q13–15 that result in aberrant expression of the *HMGA2* gene. The structural aberrations include t(8;12)(p12;q15), and the expression of *HMGA2* can be detected by immunohistochemistry and used as a marker of microscopic residual disease [35]. Expression of HMGA2 is not limited to aggressive angiomyxoma as it is also present in other benign mesenchymal neoplasms including lipomas, leiomyomas, and pulmonary hamartomas [15, 36, 37].

Histopathology

The neoplasm is grossly circumscribed but histologically has infiltrative margins and invades the surrounding soft tissues. It is tan-gray to pink in

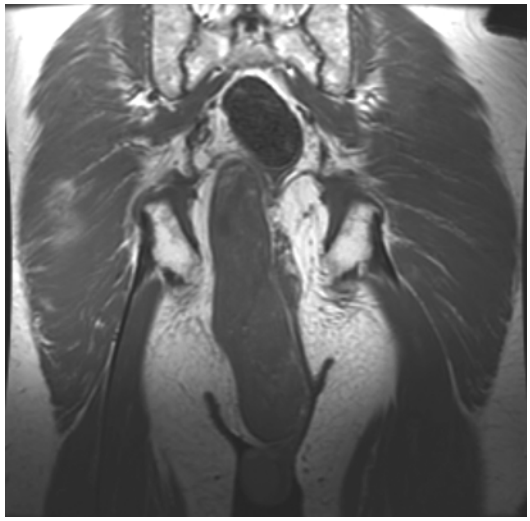


Fig. 13.18 Coronal T1-weighted MR of aggressive angiomyxoma shows a vertically oriented oblong dark mass in the pelvis

appearance, has a rubbery consistency, and has a gelatinous cut surface with foci of hemorrhage [27] (Fig. 13.19). Histologically, the tumor is paucicellular with the tumor cells enmeshed in a pale myxoid stroma that contains haphazardly arranged, linear, medium- to large-sized blood vessels that may have hyalinized walls (Fig. 13.20). The tumor cells are round to spindle and stellate with bland nuclei and ill-defined eosinophilic cytoplasm (Figs. 13.21 and 13.22). Delicate collagen fibrils and smooth muscle cells are associated with the blood vessels [8]. Immunohistochemically, the tumor cells express desmin, smooth muscle actin, and estrogen and progesterone receptors [31, 38].

Differential Diagnosis

Tumors that can be confused with aggressive angiomyxoma include angiomyofibroblastoma, superficial angiomyxoma, fibroepithelial stromal polyp, and fibromatosis. Findings supporting aggressive angiomyxoma are the classic infiltrative borders, involvement of deep tissues, paucicellularity, and the distinctive vasculature [32]. Fibromatosis can be deep seated, large, and infiltrative; however, the uniform neoplastic fibroblasts are arranged in broad sweeping fascicles, and the tumor does not contain the large blood vessels characteristic of aggressive angiomyxoma. (See Vignette 1 at the end of this chapter.) Superficial angiomyxoma may involve the vulva and attain a large size. However, this lesion is limited to the superficial soft tissues; the stroma is more myxoid and lacks large blood vessels, and the tumor cells do not express ER, PR, and desmin [39].

Summary

Clinical Presentation

- Neoplasm of middle-aged women in the pelvis, perineum, and vulva
- Usually large and has a high rate of local recurrence (up to 47 %)

Histopathologic Features

- Paucicellular with myxoid stroma and entrapped fat
- Bland spindle and stellate cells with medium to large vessels
- Expresses desmin, SMA, ER, and PR

Differential Diagnosis

- Angiomyofibroblastoma
- Superficial angiomyxoma
- Fibroepithelial stromal polyp
- Fibromatosis
- Superficial angiomyxoma

Takeaway Essentials

Clinical Relevant Pearls

- Asymptomatic tumor that is often greater than 10 cm in size.
- Deep location, infiltrative, and requires wide excision to prevent recurrence.
- Long-term follow-up is recommended due to the presence of late recurrences.

Pathology Interpretation Pearls

- Hypocellular myxocollagenous stroma with large vessels that infiltrate surrounding tissue
- Deceptively bland spindle and stellate cells that can lead to underdiagnosis
- Expression of HMGA2 can be detected by immunohistochemistry and used as a marker of microscopic residual disease

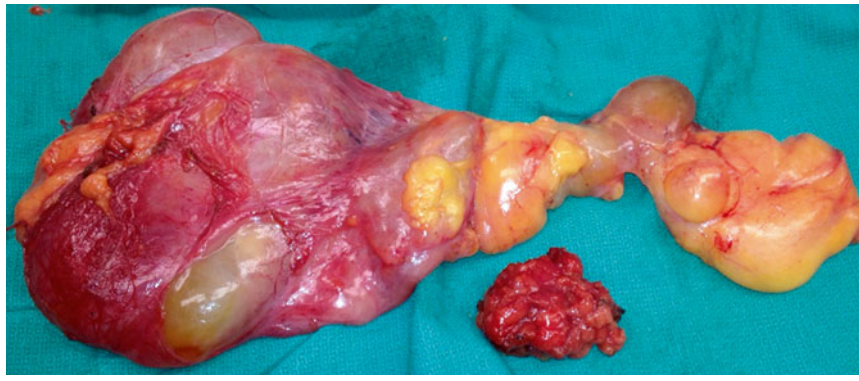


Fig. 13.19 Large polypoid *tan*, *red*, *gray* aggressive angiomyxoma with attached fat

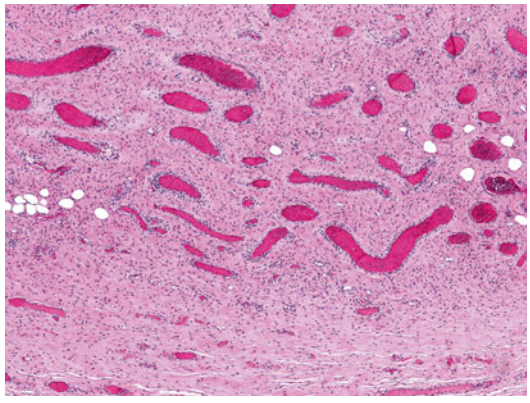


Fig. 13.20 Large undulating vessels with entrapped fat in aggressive angiomyxoma

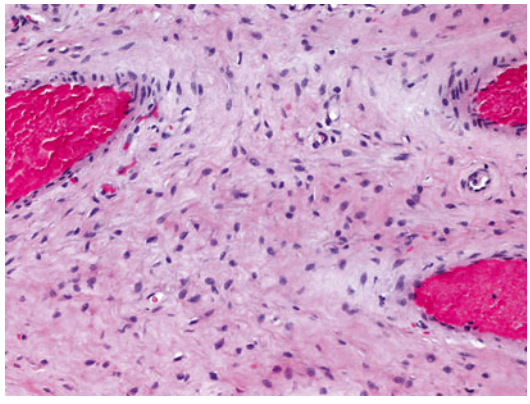


Fig. 13.21 Aggressive angiomyxoma with large vessels surrounded by spindle and stellate cells and the stroma is myxocollagenous

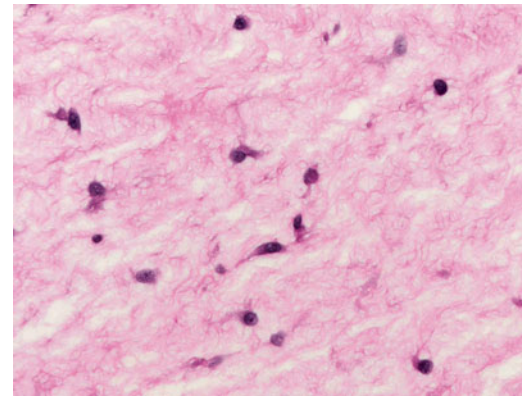


Fig. 13.22 The tumor cells in aggressive angiomyxoma have fine chromatin and indistinct cytoplasm

Superficial Myofibroblastoma

Clinical Features

As the name implies, the lesion is a superficial vulvovaginal polypoid or nodular mass that arises in females. The patients range in age from the third to the ninth decade (mean, 55 years) and presents as a solitary lesion that is usually less than 3 cm [40]. There is no proven hormonal influence although some reports have associated the lesion with tamoxifen therapy [41]. Adequate management is simple local excision; the tumor rarely recurs [42]. This lesion and spindle cell lipoma and cellular angiofibroma share alterations in the 13q14 region with loss of *RB1*.

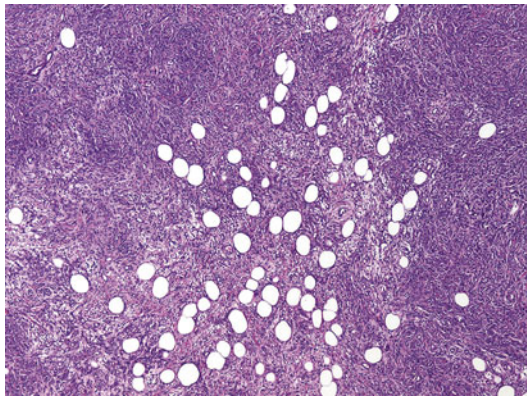


Fig. 13.23 Superficial myofibroblastoma is hypercellular and may contain fat

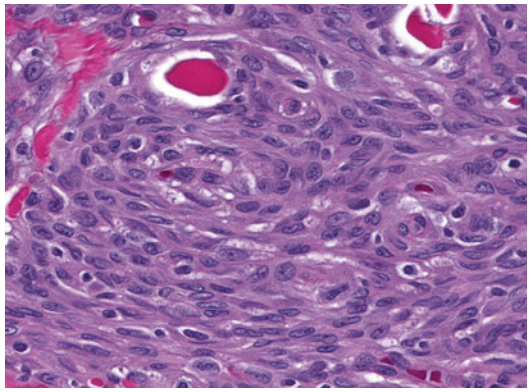


Fig. 13.24 The tumor cells in superficial myofibroblastoma have fine chromatin, and the stroma contains a wire-like collagen

Histopathology

The mass is well circumscribed, is firm and white to tan-pink, and has a smooth glistening to fleshy cut surface [41, 43].

Histologically, the tumor is composed of bland spindle to ovoid or stellate cells that have scant eosinophilic cytoplasm (Fig. 13.23). A minority of cells is multinucleated, and entrapped adipocytes are often present. The tumor cells are arranged in a variety of architectural patterns including vague fascicles with dense collagen fibers (Fig. 13.24) and a lacelike or sievelike pattern with myxoid stroma. Commonly, there is a grenz zone between the tumor and the overlying epithelium [8, 40, 41, 43].

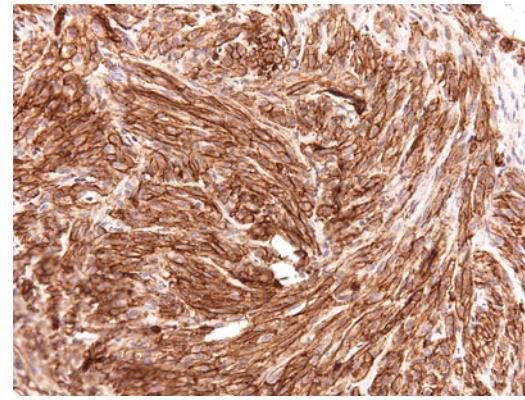


Fig. 13.25 The tumor cells in superficial myofibroblastoma are strongly positive for CD34

Immunohistochemically, the tumor cells are diffusely positive for desmin, CD34, CD99 and variably express estrogen and androgen receptors, and Bcl-2 (Fig. 13.25).

Differential Diagnosis

The differential diagnosis includes angiomyofibroblastoma and aggressive angiomyxoma. Angiomyofibroblastoma contains round to epithelioid cells with a perivascular arrangement, a characteristic that is not present in superficial myofibroblastoma. Aggressive angiomyxoma is a deep, poorly circumscribed infiltrative lesion, which helps in making the correct diagnosis [40].

Summary

Clinical Presentation

- Polypoid superficial vulvovaginal mass
- Solitary lesion usually less than 3 cm
- Rarely recurs

Histopathologic Features

- Bland spindle to ovoid cells
- Vague fascicles with dense collagen fibers or lacelike with myxoid stroma
- Positive for CD34 and CD99 and variable expression of ER, PR, and Bcl-2

Differential Diagnosis

- Angiomyofibroblastoma
- Aggressive angiomyxoma

Takeaway Essentials

Clinical Relevant Pearls

- Reports associating it with tamoxifen therapy
- Shares the loss of *RB1*-like spindle cell lipoma and cellular angiofibroma

Pathology Interpretation Pearls

- Well circumscribed with grenz zone
- Hypercellular with wirelike collagen fibers

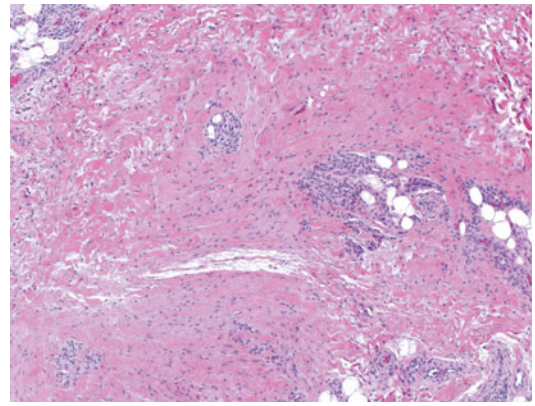


Fig. 13.26 Reactive fibroblastic and myofibroblastic lesion containing hyalinized fibrous tissue associated with blood vessels

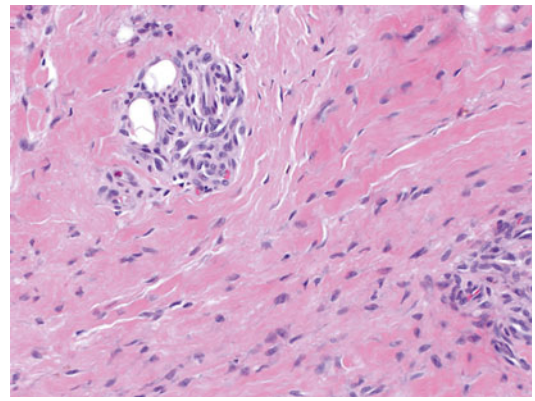


Fig. 13.27 The fibroblasts in reactive fibroblastic and myofibroblastic lesion have spindle nuclei with fine chromatin

Reactive Fibroblastic and Myofibroblastic Proliferation of the Vulva

Clinical Features

Also described as cyclist's nodule, it presents as a superficial nodule arising in the labia majora in competitive female cyclers, and similar lesions have been described in a horseback rider and a male cyclist [44, 45]. It is likely due to repeated microtrauma with a reparative response of fibroblasts and myofibroblasts. It measures from 1 to 4 cm. Although a benign nonneoplastic lesion, some cases have recurred, requiring re-excision.

Histopathology

Grossly the lesion is firm and fibrous, with no clear circumscription from the surrounding tissues. The overlying skin may show mild acanthosis or be unremarkable.

Histologically, the lesion is composed of adipose tissue admixed with mildly cellular hyalinized stroma composed of bland fibroblasts with small- to medium-sized vessels and nerves arranged haphazardly throughout the lesion (Figs. 13.26 and 13.27). Some of the lesional cells are ganglion-like, epithelioid, or plasmacytoid in appearance.

Immunohistochemistry is nonspecific, with the cells expressing ER and smooth muscle actin, and is negative for desmin, S100, and CD34.

Differential Diagnosis

It is limited, but the presence of fat may suggest infiltration as seen in aggressive angiomyxoma, but this lesion is not myxoid and cytologically different. The presence of fibrous tissue, adipose tissue, nerves, and blood vessels is similar to a prepubertal fibroma, but the age and specific association with repetitive trauma as well as the ganglion-like cells help to differentiate these two lesions [46].

Summary

Clinical Presentation

- Described as cyclist nodule
- Present as small nodule in labia majora of competitive female cyclists
- Rarely recurs

Histopathologic Features

- Adipose tissue with mildly cellular hyalinized stroma
- Haphazardly arranged small to medium vessels and nerves

Differential Diagnosis

- Aggressive angiomyxoma
- Prepubertal fibroma

Takeaway Essentials

Clinical Relevant Pearls

- A thorough clinical history is necessary to elicit the association of this tumor with repetitive trauma like in professional cyclists.

Pathology Interpretation Pearls

- Hyalinized fibrous tissue may be prominent in these lesions.
- These lesions show a wide range of proliferative cells, from spindled to ganglion-like, epithelioid or plasmacytoid in appearance.

Postoperative Spindle Cell Nodule

Clinical Features

The lesion develops within the lower genitourinary tract during a period of 3–7 weeks following a surgical procedure. Specific sites of involvement include the vulva, vagina, urethra, endometrium, bladder, and oral cavity [47]. It has benign behavior and rarely recurs after surgical excision [48].

Histopathology

The lesion is characterized as a poorly defined polypoid nodule with a reddish-gray appearance

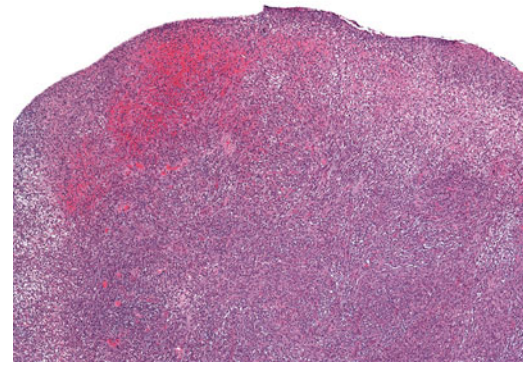


Fig. 13.28 Surface of postoperative spindle cell nodule that has ulcerated the overlying mucosa

that usually measures 2–3 cm (Fig. 13.28). Sometimes the nodule causes ulceration of the overlying mucosa.

Microscopically, the tumor is composed of intersecting fascicles of plump, spindle-shaped cells with moderate amounts of eosinophilic cytoplasm, and the nuclei have fine chromatin and small nucleoli. Mitotic figures are frequent and no atypical forms are present. The stroma is inconspicuous and contains an intervening network of small caliber blood vessels and scattered chronic inflammatory cells (Fig. 13.29a, b). The lesion can exhibit focal irregular infiltration into the adjacent soft tissues.

Immunohistochemistry shows that the spindle cells are positive for smooth muscle actin and focally for desmin. S100 and keratin are negative [48]. Cytogenetic abnormalities include trisomy 7 [49], although this is a controversial aberration in cancer cytogenetics because it has been described in a variety of neoplasms, nonneoplastic lesions, and normal tissues.

Differential Diagnosis

The differential diagnosis includes spindle cell sarcomas, spindle cell carcinoma, and benign entities such as nodular fasciitis. The latter shows more edema, the arrangement of cells is less compact, and a myxoid stroma is more prominent. (See Vignette 2 at the end of the chapter.) The spindle cell sarcomas, particularly leiomyosarcoma, are the most important in the differential

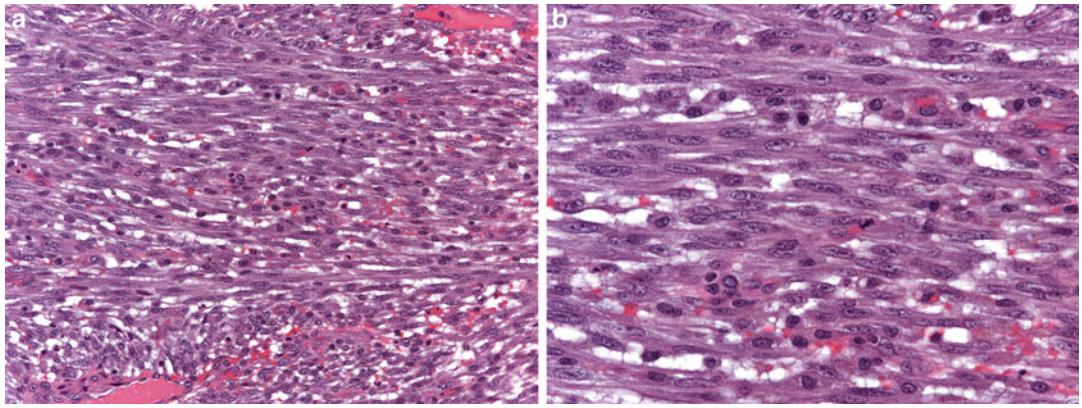


Fig. 13.29 (**a**, **b**) The densely cellular postoperative spindle cell nodule is composed of spindle cells that are mitotically active with scattered inflammatory cells

diagnosis, and useful distinguishing features include the benign-appearing nuclei of the postoperative spindle cell nodule, the contrasting atypia manifest in sarcomas, and the presence of atypical mitoses. Keratin immunohistochemistry and cytological features can help differentiate this entity from spindle cell carcinoma [48].

Summary

Clinical Presentation

- Lesion associated with recent (3–7 weeks) surgical procedure.
- Rapid growth with benign behavior.
- Rare recurrences have been reported.

Histopathologic Features

- Hypercellular, composed of plump spindle cells (fibroblasts and myofibroblasts) arranged in intersecting fascicles.
- Numerous mitoses, with no atypical forms.
- Cells express SMA and desmin (focal), and they are negative for S100 and keratin.

Differential Diagnosis

- Leiomyosarcoma
- Spindle cell carcinoma
- Nodular fasciitis

Takeaway Essentials

Clinical Relevant Pearls

- The rapid growth of the lesion is not associated with aggressive behavior.
- Has trisomy of chromosome 7, although this has been reported in benign lesions.

Pathology Interpretation Pearls

- Awareness of its increased mitotic activity should help avoid overdiagnosing this benign lesion.

Case Vignettes

Vignette 1

Clinical history: A 31-year-old woman noted a slowly enlarging mass in the vulvar region. On examination the lesion is firm, measures 6 cm on palpation, and is painless.

Microscopic description: The excised mass is leathery and tan-white in appearance and has poorly defined margins (Fig. 13.30). The tumor is moderately cellular and composed of a uniform population of fibroblasts arranged in broad sweeping fascicles. (Fig. 13.31) The tumor cells are spindle shaped, with an undulating configuration and have fine chromatin and small nucleoli (Fig. 13.32). Immunohistochemistry shows that the tumor cells are negative for CD 34 and desmin, but the nuclei express beta-catenin (Fig. 13.33).

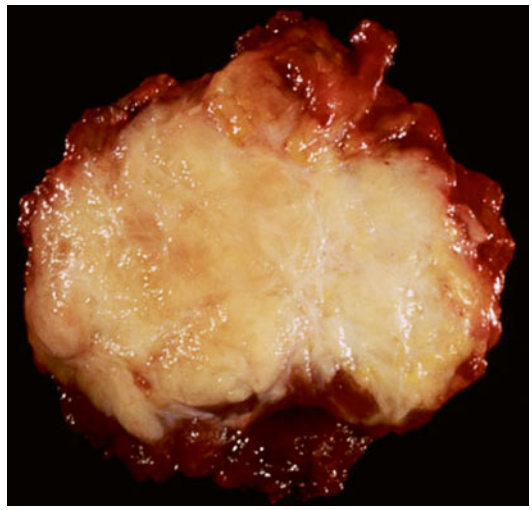


Fig. 13.30 Vignette 1. leathery, *tan-white*, and poorly defined mass

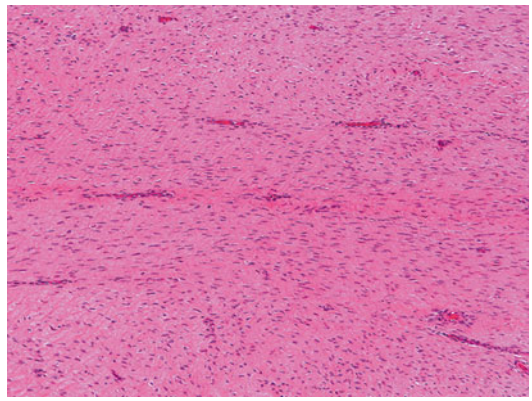


Fig. 13.31 Vignette 1. The tumor cells are arranged in broad sweeping fascicles

(continued)

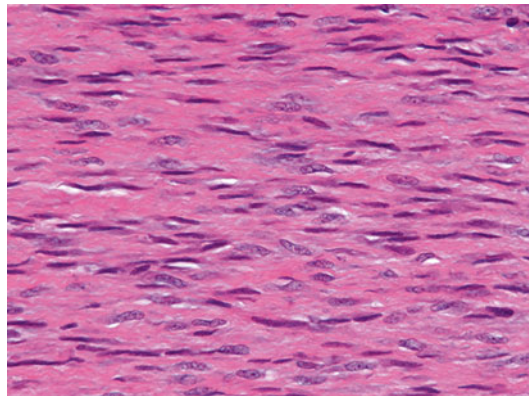


Fig. 13.32 Vignette 1. The tumor cells are spindle shaped and have undulating nuclei that follow the contours of the neighboring collagen fibers

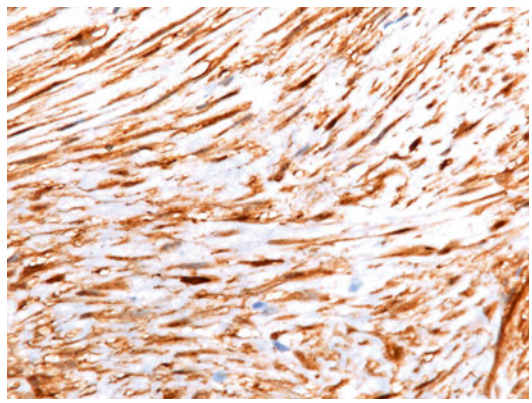


Fig. 13.33 Vignette 1. Beta-catenin stains the nuclei of scattered tumor cells. The cytoplasm also shows staining

Diagnosis: Musculoaponeurotic fibromatosis (desmoid tumor)

Discussion: Musculoaponeurotic fibromatosis is a benign but locally aggressive neoplasm that usually arises in the deep soft tissues [50]. The neoplastic cells are fibroblastic in phenotype and are arranged in broad sweeping fascicles that infiltrate the surrounding soft tissues. A minority of cases occur in the setting of syndromes including Gardner syndrome and adenomatous polyposis syndrome. Sporadic tumors are associated with mutations in the beta-catenin or *APC* genes. Treatment is medical therapy or surgical excision; resected tumors have a high rate of local recurrence.

Vignette 2

Clinical history: A 19-year-old woman presents to her physician with a rapidly growing mass on the left labia majora.

Microscopic description: The tumor is richly cellular and consists of plump, immature-appearing fibroblasts arranged in irregular short fascicles (Fig. 13.34). The cells vary in size and shape (spindle to stellate) and have discrete nucleoli and abundant mitotic figures which are not abnormal (Fig. 13.35). Aggregates of extravasated red blood cells and scattered mononuclear inflammatory cells are common.

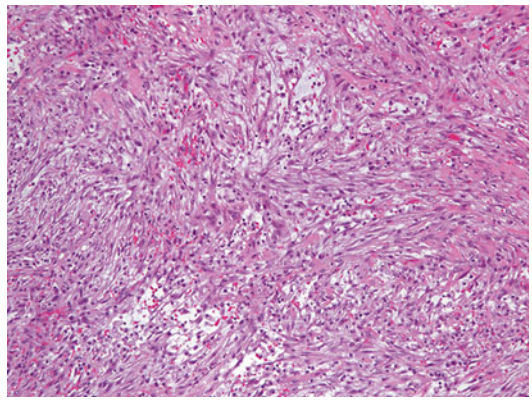


Fig. 13.34 Vignette 2. Sections show at the beginning of the phrase intersecting fascicles of spindle cells which are enmeshed in a myxocollagenous stroma

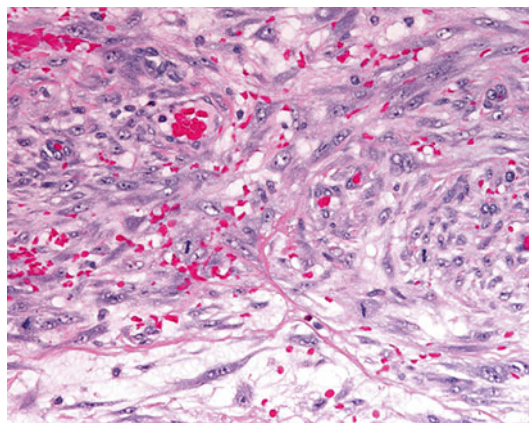


Fig. 13.35 Vignette 2. The plump spindle cells have vesicular nuclei and prominent nucleoli and foci of scattered extravasated red blood cells

Diagnosis: Nodular fasciitis

Discussion: Nodular fasciitis is a relatively common benign lesion that is associated with a consistent translocation involving *MYH9* and *USP6* [51]. Most patients present complaining of a several-week history of a solitary, rapidly growing, and sometimes painful mass. The patients range from infants

(continued)

to the elderly with the majority within the third and sixth decades of life. The lesions are most commonly located in the soft tissues of the volar aspect of the forearm followed in frequency by the chest and back. Most cases arise in the subcutis, but they may also originate in the dermis, deep fascia, and skeletal muscle. Treatment is simple excision and the recurrence rate is very low.

Vignette 3

Clinical history: A 10-year-old girl presents with a rapidly growing polypoid mass protruding from the vulva (Fig. 13.36).

Microscopic description: The tumor is cellular with a hypercellular zone beneath the squamous epithelium (Fig. 13.37). The tumor cells are composed of cytologically atypical round and spindle cells. Some of the spindle cells have abundant eccentric cytoplasm that contains fibrillar cytoplasm with cross striations evident (Fig. 13.38). Immunohistochemistry shows that the tumor cells are strongly positive for desmin and myogenin (Fig. 13.39).

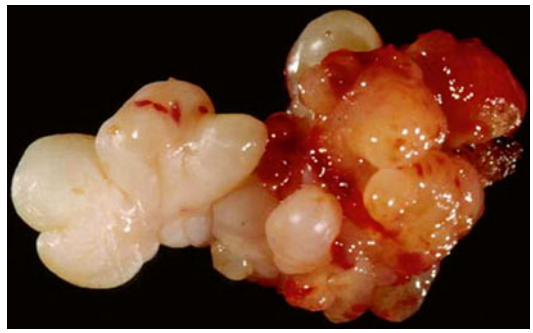


Fig. 13.36 Vignette 3. Glistening polypoid mass composed of multiple grapelike nodules of tumor

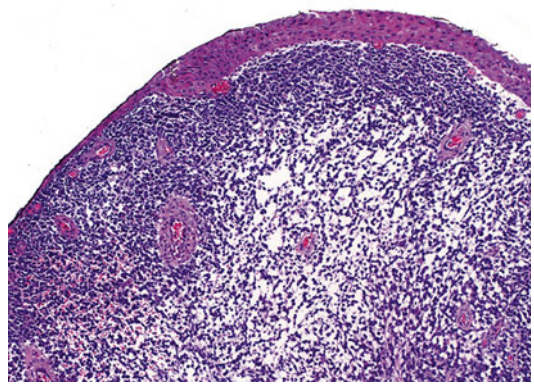


Fig. 13.37 Vignette 3. Hypercellular region beneath the squamous epithelium

(continued)

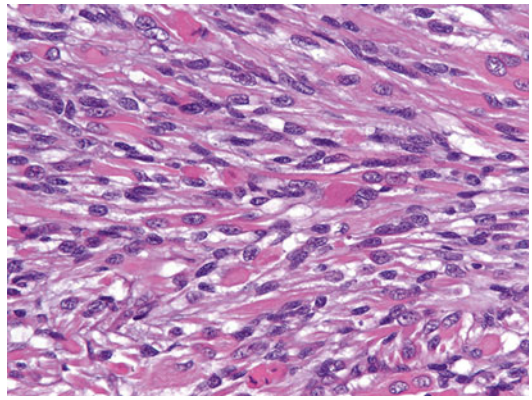


Fig. 13.38 Vignette 3. Some of the tumor cells have eccentric fibrillar eosinophilic cytoplasm and represent rhabdomyoblasts

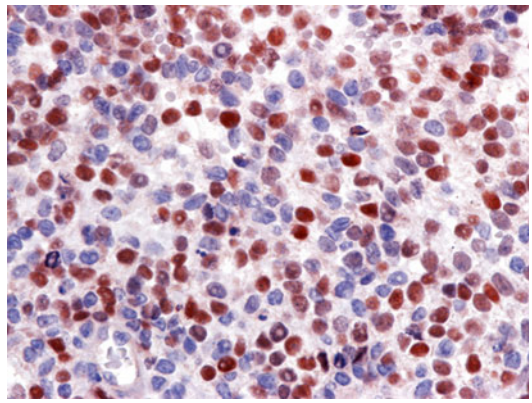


Fig. 13.39 Vignette 3. Scattered neoplastic cells show nuclear expression of myogenin

Diagnosis: Embryonal rhabdomyosarcoma

Discussion: Embryonal rhabdomyosarcoma often arises in the genital region of children and can present as a polypoid tumor that can be confused with benign lesions such as a fibroepithelial polyp. Histologically the tumor is cytologically malignant which helps distinguish it from the benign fibroblastic and myofibroblastic tumor of the vulva.

References

1. Chirayil SJ, Tobon H. Polyps of the vagina: a clinicopathologic study of 18 cases. Cancer. 1981;47(12): 2904–7.
2. Norris HJ, Taylor HB. Polyps of the vagina. A benign lesion resembling sarcoma botryoides. Cancer. 1966;19(2):227–32.
3. Hartmann CA, Sperling M, Stein H. So-called fibroepithelial polyps of the vagina exhibiting an unusual but uniform antigen profile characterized by expression of desmin and steroid hormone receptors but no muscle-specific actin or macrophage markers. Am J Clin Pathol. 1990;93(5):604–8.
4. Mucitelli DR, Charles EZ, Kraus FT. Vulvovaginal polyps. Histologic appearance, ultrastructure, immunocytochemical characteristics, and clinicopathologic correlations. Int J Gynecol Pathol. 1990;9(1): 20–40.
5. Chan MM, Yong TT, Sittampalam K. Giant labial fibroepithelial stromal polyp. Malays J Pathol. 2013; 35(1):91–4.
6. Elliott GB, Reynolds HA, Fidler HK. Pseudo-sarcoma botryoides of cervix and vagina in pregnancy. J Obstet Gynaecol Br Commonw. 1967;74(5):728–33.
7. O'Quinn AG, Edwards CL, Gallager HS. Pseudosarcoma botryoides of the vagina in pregnancy. Gynecol Oncol. 1982;13(2):237–41.
8. McCluggage WG. A review and update of morphologically bland vulvovaginal mesenchymal lesions. Int J Gynecol Pathol. 2005;24(1):26–38.
9. Burt RL, Prichard RW, Kim BS. Fibroepithelial polyp of the vagina. A report of five cases. Obstet Gynecol. 1976;47(1):52S–4.
10. Ostör AG, Fortune DW, Riley CB. Fibroepithelial polyps with atypical stromal cells (pseudosarcoma botryoides) of vulva and vagina. A report of 13 cases. Int J Gynecol Pathol. 1988;7(4):351–60.
11. McCluggage WG, Nielsen GP, Young RH. Massive vulval edema secondary to obesity and immobilization: a potential mimic of aggressive angiomyxoma. Int J Gynecol Pathol. 2008;27(3):447–52.
12. Nucci MR, Young RH, Fletcher CD. Cellular pseudosarcomatous fibroepithelial stromal polyps of the lower female genital tract: an underrecognized lesion often misdiagnosed as sarcoma. Am J Surg Pathol. 2000;24(2):231–40.
13. Vang R, Connelly JH, Hammill HA, Shannon RL. Vulvar hypertrophy with lymphedema. A mimicker of aggressive angiomyxoma. Arch Pathol Lab Med. 2000;124(11):1697–9.
14. Plaza JA, Requena L, Kazakov DV, Vega E, Kacerovska D, Reyes G, et al. Verrucous localized lymphedema of genital areas: clinicopathologic report of 18 cases of this rare entity. J Am Acad Dermatol. 2014;71(2):320–6.
15. Kazmierczak B, Wanschura S, Meyer-Bolte K, Caselitz J, Meister P, Bartnitzke S, et al. Cytogenic and molecular analysis of an aggressive angiomyxoma. Am J Pathol. 1995;147(3):580–5.
16. Iwasa Y, Fletcher CDM. Distinctive prepubertal vulval fibroma: a hitherto unrecognized mesenchymal tumor of prepubertal girls: analysis of 11 cases. Am J Surg Pathol. 2004;28(12):1601–8.
17. Nucci MR, Granter SR, Fletcher CD. Cellular angiofibroma: a benign neoplasm distinct from angiomyofibroblastoma and spindle cell lipoma. Am J Surg Pathol. 1997;21(6):636–44.
18. Laskin WB, Fetsch JF, Mostofi FK. Angiomyofibroblastomalike tumor of the male genital tract: analysis of 11 cases with comparison to female angiomyofibroblastoma and spindle cell lipoma. Am J Surg Pathol. 1998;22(1):6–16.
19. McCluggage WG, Perenyei M, Irwin ST. Recurrent cellular angiofibroma of the vulva. J Clin Pathol. 2002;55(6):477–9.
20. Chen E, Fletcher CDM. Cellular angiofibroma with atypia or sarcomatous transformation: clinicopathologic analysis of 13 cases. Am J Surg Pathol. 2010; 34(5):707–14.
21. Flucke U, van Krieken JHJM, Mentzel T. Cellular angiofibroma: analysis of 25 cases emphasizing its relationship to spindle cell lipoma and mammary-type myofibroblastoma. Mod Pathol. 2011;24(1):82–9.
22. Iwasa Y, Fletcher CDM. Cellular angiofibroma: clinicopathologic and immunohistochemical analysis of 51 cases. Am J Surg Pathol. 2004;28(11): 1426–35.
23. Sims SM, Stinson K, McLean FW, Davis JD, Wilkinson EJ. Angiomyofibroblastoma of the vulva: a case report of a pedunculated variant and review of the literature. J Low Genit Tract Dis. 2012;16(2):149–54.
24. Nielsen GP, Rosenberg AE, Young RH, Dickersin GR, Clement PB, Scully RE. Angiomyofibroblastoma of the vulva and vagina. Mod Pathol. 1996;9(3):284–91.
25. Kairi-Vassilatou E, Dastamani C, Vouza E, Mavrigiannaki P, Hasiakos D, Kondi-Pafiti A. Angiomyofibroblastoma of the vulva: a clinicopathological and immunohistochemical analysis of a rare benign mesenchymal tumor. Eur J Gynaecol Oncol. 2011;32(3):353–5.
26. Begin LR, Clement PB, Kirk ME, Jothy S, McCaughey WT, Ferenczy A. Aggressive angiomyxoma of pelvic soft parts: a clinicopathologic study of nine cases. Hum Pathol. 1985;16(6):621–8.
27. Steeper TA, Rosai J. Aggressive angiomyxoma of the female pelvis and perineum. Report of nine cases of a distinctive type of gynecologic soft-tissue neoplasm. Am J Surg Pathol. 1983;7(5):463–75.
28. Iezzoni JC, Fechner RE, Wong LS, Rosai J. Aggressive angiomyxoma in males. A report of four cases. Am J Clin Pathol. 1995;104(4):391–6.
29. Tsang WY, Chan JK, Lee KC, Fisher C, Fletcher CD. Aggressive angiomyxoma. A report of four cases occurring in men. Am J Surg Pathol. 1992;16(11):1059–65.
30. Fetsch JF, Laskin WB, Lefkowitz M, Kindblom LG, Meis-Kindblom JM. Aggressive angiomyxoma: a

clinicopathologic study of 29 female patients. Cancer. 1996;78(1):79–90.
31. Granter SR, Nucci MR, Fletcher CD. Aggressive angiomyxoma: reappraisal of its relationship to angiomyofibroblastoma in a series of 16 cases. Histopathology. 1997;30(1):3–10.
32. Sutton BJ, Laudadio J. Aggressive angiomyxoma. Arch Pathol Lab Med. 2012;136(2):217–21.
33. Haldar K, Martinek IE, Kehoe S. Aggressive angiomyxoma: a case series and literature review. Eur J Surg Oncol. 2010;36(4):335–9.
34. Blandamura S, Cruz J, Faure Vergara L, Machado Puerto I, Ninfo V. Aggressive angiomyxoma: a second case of metastasis with patient's death. Hum Pathol. 2003;34(10):1072–4.
35. Nucci MR, Fletcher CD. Vulvovaginal soft tissue tumours: update and review. Histopathology. 2000; 36(2):97–108.
36. Nucci MR, Weremowicz S, Neskey DM, Sornberger K, Tallini G, Morton CC, et al. Chromosomal translocation t(8;12) induces aberrant HMGIC expression in aggressive angiomyxoma of the vulva. Genes Chromosomes Cancer. 2001;32(2):172–6.
37. Rabban JT, Dal Cin P, Oliva E. HMGA2 rearrangement in a case of vulvar aggressive angiomyxoma. Int J Gynecol Pathol. 2006;25(4):403–7.
38. McCluggage WG, Patterson A, Maxwell P. Aggressive angiomyxoma of pelvic parts exhibits oestrogen and progesterone receptor positivity. J Clin Pathol. 2000;53(8):603–5.
39. Kim HS, Kim GY, Lim SJ, Ki KD, Kim HC. Giant superficial angiomyxoma of the vulva: a case report and review of the literature. J Cutan Pathol. 2010; 37(6):672–7.
40. Magro G, Caltabiano R, Kacerovska D, Vecchio GM, Kazakov D, Michal M. Vulvovaginal myofibroblastoma: expanding the morphological and immunohistochemical spectrum. A clinicopathologic study of 10 cases. Hum Pathol. 2012;43(2):243–53.
41. Laskin WB, Fetsch JF, Tavassoli FA. Superficial cervicovaginal myofibroblastoma: fourteen cases of a distinctive mesenchymal tumor arising from the specialized subepithelial stroma of the lower female genital tract. Hum Pathol. 2001;32(7):715–25.
42. Stewart CJ, Amanuel B, Brennan BA, Jain S, Rajakaruna R, Wallace S. Superficial cervico-vaginal myofibroblastoma: a report of five cases. Pathology. 2005;37(2):144–8.
43. Ganesan R, McCluggage WG, Hirschowitz L, Rollason TP. Superficial myofibroblastoma of the lower female genital tract: report of a series including tumours with a vulval location. Histopathology. 2005;46(2):137–43.
44. de Saint Aubain Somerhausen N, Geurde B, Couvreur Y. Perineal nodular induration: the 'third testicle of the cyclist', an under-recognized pseudotumour. Histopathology. 2003;42(6):615–6.
45. Devers KG, Heckman SR, Muller C, Joste NE. Perineal nodular induration: a trauma-induced mass in a female equestrian. Int J Gynecol Pathol. 2010; 29(4):398–401.
46. McCluggage WG, Smith JH. Reactive fibroblastic and myofibroblastic proliferation of the vulva (Cyclist's Nodule): a hitherto poorly described vulval lesion occurring in cyclists. Am J Surg Pathol. 2011;35(1):110–4.
47. Proppe KH, Scully RE, Rosai J. Postoperative spindle cell nodules of genitourinary tract resembling sarcomas. A report of eight cases. Am J Surg Pathol. 1984;8(2):101–8.
48. Manson CM, Hirsch PJ, Coyne JD. Post-operative spindle cell nodule of the vulva. Histopathology. 1995;26(6):571–4.
49. Micci F, Haugom L, Abeler VM, Bjerkehagen B, Heim S. Trisomy 7 in postoperative spindle cell nodules. Cancer Genet Cytogenet. 2007;174(2):147–50.
50. Devata S, Chugh R. Desmoid tumors: a comprehensive review of the evolving biology, unpredictable behavior, and myriad of management options. Hematol Oncol Clin North Am. 2013;27(5):989–1005.
51. Erickson-Johnson MR, Chou MM, Evers BR, Roth CW, Seys AR, Jin L, et al. Nodular fasciitis: a novel model of transient neoplasia induced by MYH9-USP6 gene fusion. Lab Invest. 2011;91(10):1427–33.

Vascular Lesions of the Vulva

14

Mai P. Hoang and Omar P. Sangueza

Introduction

Some soft tissue lesions, which have a predilection for the vulva, namely, angiomyofibroblastoma and aggressive angiomyxoma, have a significant vascular component, even though they are not considered tumors originating from vascular endothelium. On the other hand, vascular lesions such as malformations, dilatations, hyperplasias, and true vascular neoplasms, although not commonly affecting the vulva, do not always histopathologically resemble their non-genital counterparts. As a consequence, classifying vulvar vascular lesions is challenging due to both the wide variety of clinical presentations that may be encountered and their histopathologic heterogeneity. Vascular proliferations can be divided into different categories such as hamartomas, malformations, dilatation of preexisting vessels, hyperplasias, and benign and malignant tumors, which is useful [1] (Table 14.1). Hamartomas and malformations are rarely seen in the vulva and will not be considered in this review.

M.P. Hoang, M.D. (✉)
Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: mhoang@mgh.harvard.edu

Dilatation of Preexisting Vessels

Dilatation of preexisting vessels includes the following range of lesions: angiokeratomas, lymphangiectasis, venous lake, vulvar varices, and capillary aneurysm. From this group of disorder, angiokeratomas and lymphangiectasis are the lesions most frequently seen in the vulva.

Lymphangiectasis

Lymphangiectasis, also known as acquired lymphangioma, occurs on the vulva and involves the lymphatic channels in the dermis. Young et al. [2] reported only 1 patient with this condition among 1,000 patients seen in their vulval-cutaneous clinic over 7 years. Similarly Stewart et al. [3] identified only eight cases over 15-year period.

Lymphangiectases are the result of permanent dilatation of lymphatic capillaries and develop in areas of the skin affected by obstruction or destruction of lymphatic drainage [3, 4]. The lymphatic plexus of the superficial dermis drains a fixed skin area through the vertical collecting lymphatics to the deep plexus. Damage of the deep lymphatic vessels leads to dilatation of the superficial dermal lymphatics [5]. Causes include surgery, lymphadenectomy, pregnancy, radio-

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_14,

Table 14.1 Summary of classification of vulvar vascular lesions

Dilatation of preexisting vessels
Acquired lymphangiectasis
Vulvar varices (venous lake)
Angiokeratoma
Vascular hyperplasias
Pyogenic granuloma (lobular capillary hemangioma)
Intravascular papillary endothelial hyperplasia (Masson's tumor)
Benign neoplasms
Hemangioma
Infantile hemangioma
Congenital hemangioma
Lymphangioma
Glomus tumor
Malignant neoplasms
Kaposi's sarcoma
Epithelioid hemangioendothelioma
Angiosarcoma
Lymphangiosarcoma

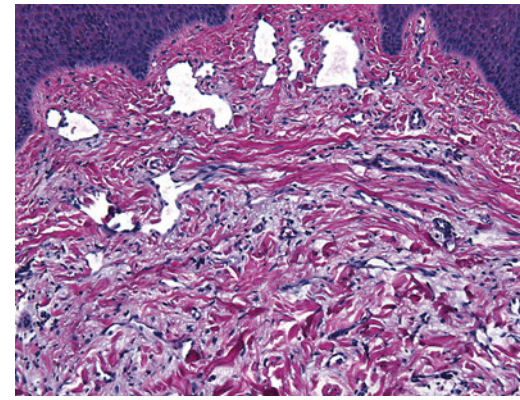


Fig. 14.1 *Lymphangiectasia*. Dilated lymphatic vessels are seen within the superficial and mid-dermis

therapy, Crohn's disease, scleroderma, neoplasia, or infections such as tuberculosis, lymphogranuloma venereum, and filariasis [3, 4, 6–11].

The age of affected women ranges from 22 to 75 years with a mean of 48 years [5]. The patients can present with pruritus progressing to pain, vulvar wetness, edema, or no symptoms. These lesions typically develop more than 10 years following damage to the lymphatic drainage and can be recurrent. Clinically, lymphangiectases are localized lesions often on the labia majora. The cobblestones appearance is due to thin-walled translucent vesicles filled with clear fluid [3, 4]. On rare occasions, they are hyperkeratotic, pedunculated, and polypoid lesions and may mimic genital warts, herpes infection, or molluscum contagiosum [12]. Some cases are complicated by secondary infection. The goal of treatment is to reduce the underlying lymphedema and to control infection. Excisional surgery and carbon dioxide laser are two major forms of treatment [13, 14].

A special form of lymphangiectasis, localized lymphedema, can be seen on the vulva of morbidly obese women [15]. Also known as massive vulval edema, vulvar lymphedematous pseudotumor, and vulvar hypertrophy with lymphedema, localized lymphedema (elephantiasis) typically present with papillomatous plaques, vulvar enlargement, or massive pedunculated masses (see Chap. 13). The duration of these lesions ranges from 3 months to 36 years, and their sizes range from 0.6 to 45 cm [15].

Histopathology

Lymphangiectases are characterized by the presence of dilated lymphatic vessels within superficial and mid dermis (Fig. 14.1). The overlying epidermis may exhibit hyperkeratosis and acanthosis. There may be red blood cells and lymphocytes within these lymphatic channels. Stromal edema is a histologic feature that is consistently seen in all examined patients with localized massive lymphedema [15]. In addition, multinucleated giant cells, lymphangiectasia, dermal chronic inflammation, and fibrosis are seen in subset of cases [15].

By immunohistochemistry, the lymphatic endothelial cells are positive for D2-40, Prox-1, and CD31, yet they are negative for CD34 [3] (Table 14.2). D2-40 or podoplanin is a relatively specific immunostain for lymphatic endothelium [16]. Prox-1 is a homeobox-containing nuclear transcription factor that has recently been shown to exhibit better specificity for lymphatic endothelium than D2-40 [17].

Table 14.2 Summary of vascular markers in blood vessels and lymphatics

Immunostain	Blood vessels	Lymphatics
D2-40	–	+
Prox-1	–	++
CD31	++	+
CD34	++	–

Data from Ref. [17]

Differential Diagnosis

Acquired lymphangiectasis has similar clinical and histologic features to lymphangioma circumscriptum, a developmental defect of the deep dermal and subcutaneous lymphatics. Clinical history is essential to differentiate the two since lymphangiomas are present since birth or early childhood, while acquired lymphangiectasis is associated with a number of causes [4, 8]. (See Vignette 3 at the end of this chapter.) While acquired lymphangiectasis affects the superficial and mid lymphatics, lymphangioma circumscriptum affects those within the deep dermis and subcutaneous tissue. The lack of CD34 expression is helpful in distinguishing acquired lymphangiectasia from other vulvar vascular lesions. Angiokeratomas are small and not associated with damage to lymphatic drainage [18]. In addition, angiokeratomas do not recur and do not typically require additional treatment. Vulvar varices, also known as venous lake, would have erythrocytes within a dilated lumen. (See Vignette 1 at the end of this chapter.)

Takeaway Essentials

Clinical Relevant Pearls

- Lymphangiectases are the result of permanent dilatation of lymphatic capillaries and develop in areas of the skin affected by obstruction or destruction of lymphatic drainage.
- Vulvar lymphangiectasis can be misdiagnosed clinically as warts and thus are nonresponsive to treatment.

Pathology Interpretation Pearls

- While acquired lymphangiectasis affects superficial and mid-lymphatics, lymphangioma circumscriptum or congenital lymphangioma affects those within the deep dermis and subcutaneous tissue.
- Stromal edema is a histologic feature that is consistently seen in all examined cases of localized massive lymphedema.

Immunohistochemical Findings

- The lymphatic endothelial cells are positive for D2-40, Prox-1, and CD31; yet they are negative for CD34.
- The lack of CD34 expression is helpful in distinguishing acquired lymphangiectasia from other vulvar vascular lesions.

Summary

Clinical Presentation

- These lesions typically develop more than 10 years following damage to the lymphatic drainage and can be recurrent.

Histologic Features

- Dilated lymphatic vessels are seen within the superficial and mid-dermis.

Differential Diagnosis

- Congenital lymphangioma
- Angiokeratomas
- Vulvar varices or venous lake

Angiokeratoma

Angiokeratoma is a group of several unrelated conditions, whose common denominator is the presence of dilated blood vessels in association with epidermal hyperplasia. Four clinical variants of angiokeratomas have been recognized: solitary, Fordyce's angiokeratoma, Mibelli's angiokeratoma, and angiokeratoma corporis diffusum. The latter has been associated with different diseases of which Fabry's disease is the most common.

Angiokeratomas of the vulva are rare and present as keratotic, red to brown papules in patients of 20–40 years of age [19–21], but they

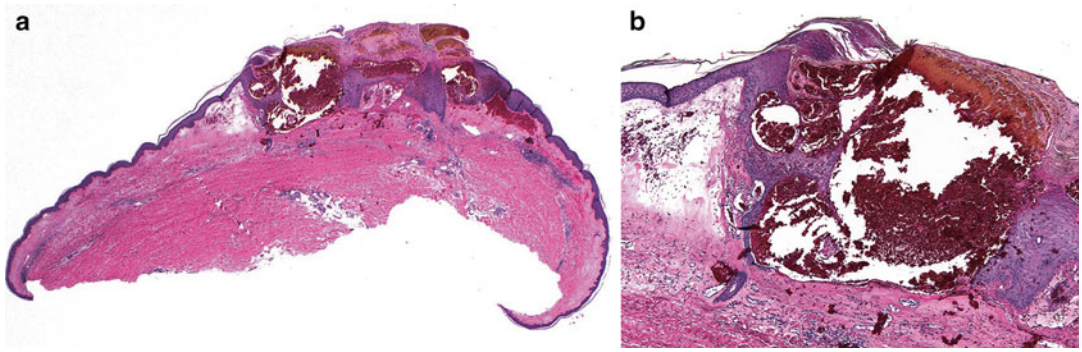


Fig. 14.2 (**a**, **b**) *Angiokeratoma*. Dilated blood vessels lined by a layer of endothelial cells are seen associated with marked epidermal hyperplasia

can be seen in younger individuals [22]. The lesions are typically 2–5 mm, unilateral, multiple, and asymptomatic [19, 20, 23]. Rare case of clitoral involvement has been reported [24]. It is thought that increased local venous pressure results in subepithelial vascular dilation; thus, increased parity, excess body weight, hemorrhoids, pelvic inflammatory disease, prior hysterectomy, varicose veins, and vulvar varicosity are risk factors [19, 20, 25]. These lesions can be treated by surgical excision or physical and chemical cauterization [20].

Histopathology

All variants of angiokeratomas share similar histologic features including the presence of dilated thin-walled blood vessels, lined by a layer of endothelial cells, in the papillary dermis and a variable degree of hyperkeratosis (Fig. 14.2a). The overlying epidermis often exhibits irregular acanthosis with elongated rete ridges partially surrounding the vascular channels (Fig. 14.2b). There is minimal dermal inflammation. Vacuolation of smooth muscle of arterioles and arteries and pilar muscles is seen in lesions associated with Fabry's disease. Electron-dense lipid bodies can be demonstrated ultrastructurally in the cytoplasm of endothelial cells, pericytes, smooth muscle cells, and fibroblasts [26].

Differential Diagnosis

Clinically, angiokeratomas of the vulva may resemble benign as well as malignant melanocytic lesions, seborrheic keratosis, condyloma acuminatum, vulvar intraepithelial neoplasia, pyogenic granuloma, and lymphangioma [18, 19]. The main histologic differential diagnosis would be a hemangioma, which would not have associated epidermal acanthosis that is seen in angiokeratoma. Although a verrucous angioma would have marked epidermal hyperplasia, it has a deep vascular component that is not typically present in angiokeratoma.

Summary

Clinical Presentation

- Rare lesions in individuals 20–40 years of age
- Keratotic, red to brown papules
- 2–5 mm, unilateral, multiple, and asymptomatic

Histologic Features

- Dilated thin-walled blood vessels in the papillary dermis that lined by a layer of endothelial cells and associated with epidermal hyperplasia

Differential Diagnosis

- Hemangioma

Takeaway Essentials

Clinical Relevant Pearls

- Four clinical variants of angiokeratomas have been recognized: solitary, Fordyce's angiokeratoma, Mibelli's

angiokeratoma, and angiokeratoma corporis diffusum (Fabry's disease).
- Vulvar lesions are thought to be equivalent to the Fordyce type.

Pathology Interpretation Pearls
- In contrast to hemangioma, there are associated epidermal hyperplasia with angiokeratoma and lack of a deep vascular component.

Hyperplasias

Hyperplasia is defined as an abnormal increase in the absolute number of normal cells, in an appropriately arranged tissue. Inherent in this terminology is the premise that hyperplasia ceases when the initiating stimulus has been removed; thereafter the tissue may or may not completely revert to its normal state. In the vulva the most common hyperplasia is pyogenic granuloma. Other hyperplasias such as intravascular papillary endothelial hyperplasia and reactive angioendotheliomatosis can be seen, but they are not very common in the vulva.

Pyogenic Granuloma

Lobular capillary hemangioma or pyogenic granuloma often presents as a solitary, pedunculated, or sessile growth [27]. Although rare, pyogenic granuloma presents with multiple lesions in the vulva [28–30]. Labia majora is commonly involved. In all three reports, the lesions did not recur status post excision [28–30].

Pyogenic granuloma has been thought to represent a reactive vascular proliferation in response to a variety of stimuli rather than a true hemangioma [27]. Increased production of tumor angiogenesis factor from trauma or underlying cutaneous disease such as inflammatory dermatoses, viral infection, arthropod bite, and port-wine stain may play a role in the pathogenesis of pyogenic granuloma [31, 32]. Since there is a female predominance, the role of estrogen and progesterone receptors in the development of these lesions has been postulated. However, estrogen and progesterone receptors were found to be negative in a series of 21 cutaneous pyogenic granulomas by Nichols et al. [33].

Histopathology

Pyogenic granuloma, at least in its initial phase, is an exuberant proliferation of vessels of different sizes and shapes, lined by prominent endothelium embedded in an edematous stroma (Fig. 14.3a, b). The overlying epidermis is often ulcerated; thus, the lesion has associated acute as well as chronic inflammatory infiltrate, similar to granulation tissue.

Differential Diagnosis

The clinical differential diagnosis of pyogenic granuloma would include other polypoid lesions including condylomas, angiomas, and verrucous

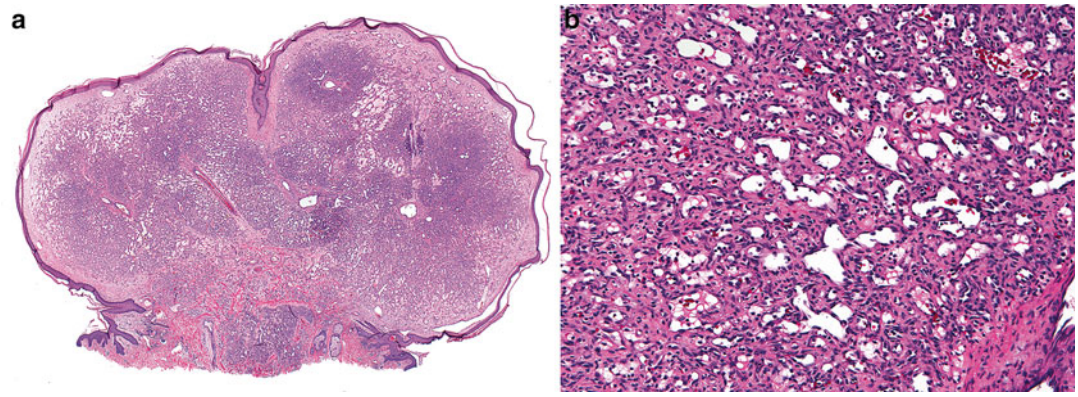


Fig. 14.3 (**a**, **b**) *Pyogenic granuloma*. An exophytic and lobular proliferation of blood vessels is seen in the dermis

carcinoma. The main histologic differential diagnosis would be granulation tissue and a hemangioma. Some do consider pyogenic granuloma is a subtype of hemangioma. Clinical history is needed to distinguish pyogenic granuloma from granulation tissue.

Summary

Clinical Presentation

- Rarely present as multiple lesions in the vulva

Histologic Features

- Proliferation of vessels of different sizes and shapes, lined by prominent endothelium embedded in an edematous stroma

Differential Diagnosis

- Granulation tissue
- Hemangioma

Takeaway Essentials

Clinical Relevant Pearls

- These lesions appear not to recur after excision.
- The presence of multiple pyogenic granulomas is not associated with syndrome or aggressive behavior.

Pathology Interpretation Pearls

- The presence of mitoses and prominent endothelial cells is part of the spectrum of reactive changes.

Immunohistochemical Findings

- Kaposi sarcoma can simulate pyogenic granuloma histologically, and HHV8 immunostain can be helpful in setting apart these lesions.

Intravascular Papillary Endothelial Hyperplasia

Only two cases of intravascular papillary endothelial hyperplasia or Masson's tumor have been reported in the vulva [34, 35]. One case of Masson's tumor in the vulva was in a patient undergoing radiation therapy for vulvar cancer [35]. The predisposing factors for Masson's tumor are not known, but irradiation has been reported to induce similar papillary endothelial proliferation in experimental animals [36]. The lesion commonly presents as a small firm mass in the deep dermis or subcutaneous tissue.

Histopathology

The proliferation of endothelial cells is present within one or more vascular lumina that have been occluded by a thrombus (Fig. 14.1a). In fully developed lesions, numerous papillary fronds lined by a single layer of plump endothelial cells extend from the wall of the vessel into the lumina. The histologic features of intravascular papillary endothelial hyperplasia are not specific, but can be found in multiple vascular proliferations (Fig. 14.4b).

Differential Diagnosis

The differential diagnosis includes pyogenic granuloma, Kaposi's sarcoma, and angiosarcoma. Pyogenic granuloma is a proliferation of blood vessels of variable sizes and shapes. An infiltrative architecture as well as cytologic atypia would be seen in Kaposi's sarcoma and angiosarcoma, whereas Masson's tumor is a circumscribed lesion with a central thrombotic zone.

Summary

Clinical Presentation

- Small firm mass in the deep dermis or subcutaneous tissue

Histologic Features

- Proliferation of endothelial cells is present within one or more vascular lumina that have been occluded by a thrombus.

Differential Diagnosis

- Pyogenic granuloma
- Kaposi's sarcoma
- Angiosarcoma

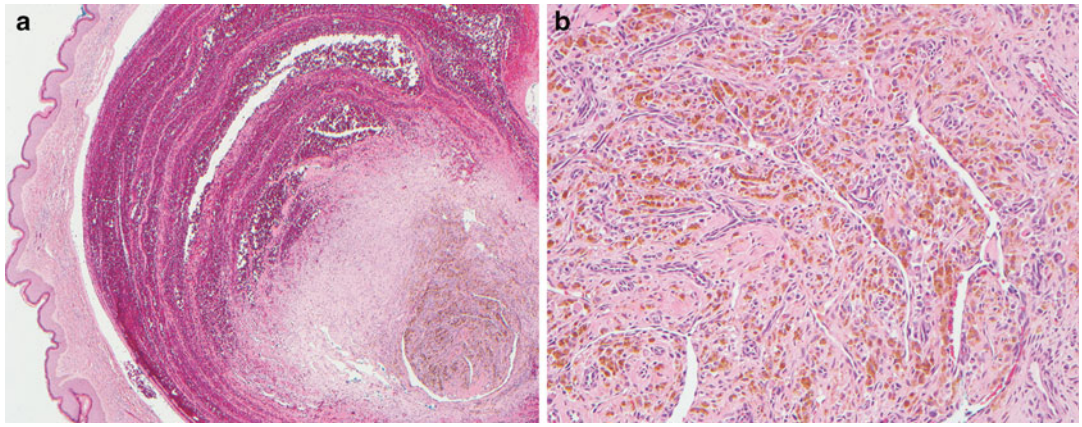


Fig. 14.4 *Masson's lesion*. (**a**) A well-circumscribed lesion with central thrombosis is seen in the dermis. (**b**) Irregular vascular spaces lined by plump endothelial cells and with associated hemosiderin deposition

Takeaway Essentials

Pathology Interpretation Pearls

- The histologic features of intravascular papillary endothelial hyperplasia are not specific, but can be found in multiple vascular proliferations.

Benign Neoplasms

Hemangioma

Cavernous hemangioma appears to be the most common form reported in the vulva [37–39]. Massive clitoral enlargement due to a cavernous hemangioma was reported in a young woman [37].

Histopathology

These lesions are comprised of large, dilated, blood-filled vessels lined by flattened endothelium. These vessels can be either in a lobular arrangement or a haphazard fashion. Arteriovenous hemangioma consists of a well-circumscribed proliferation of thick-walled muscle-containing blood vessels, lined by a single layer of endothelial cells involving the upper and mid-reticular dermis (Fig. 14.5a, b).

Differential Diagnosis

Cavernous hemangioma can be mistaken clinically as vulvar varices (see Vignette 1 at the end of this chapter) as reported in a case of vulvar hemangioma [40]. Lymphangioma would not have blood-filled vessels, and these endothelial cells would be positive for lymphatic markers such as D2-40 and Prox-1.

Summary

Clinical Presentation

- Cavernous hemangioma appears to be the most common form of hemangioma in the vulva.

Histologic Features

- Large, dilated, blood-filled vessels lined by flattened endothelium

Differential Diagnosis

- Vulvar varices
- Lymphangioma

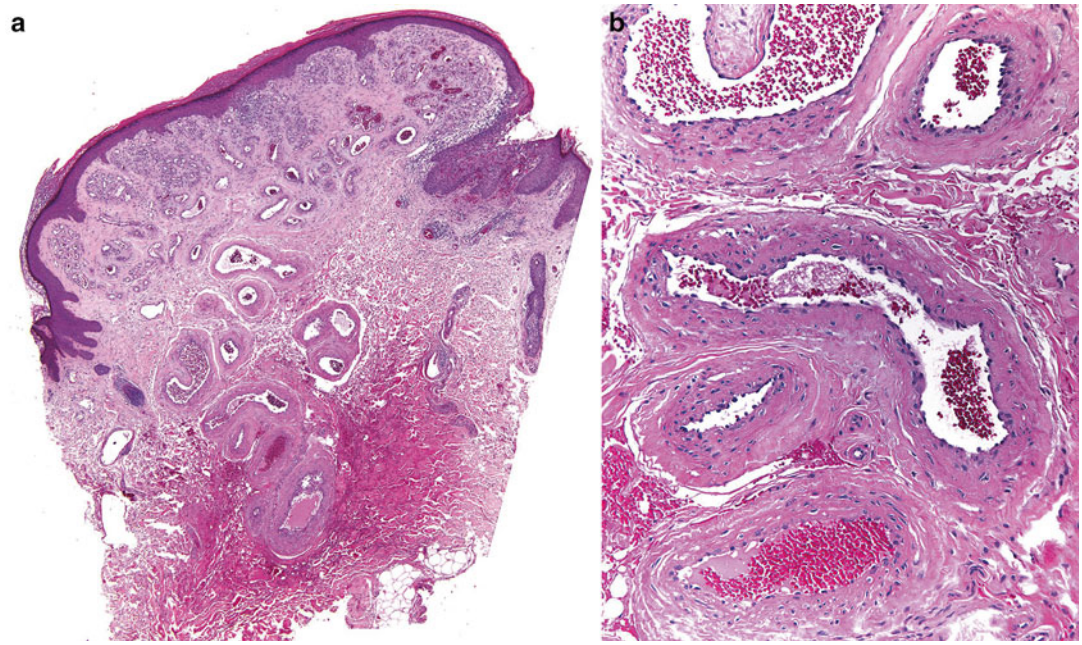


Fig. 14.5 (**a**, **b**) *Arteriovenous hemangioma*. A well-circumscribed proliferation of thick-walled muscle-containing blood vessels is seen in the superficial and mid-dermis

Takeaway Essentials

Clinical Relevant Pearls

- Vulvar hemangiomas are multifocal in only 3 % of cases, while vulvar lymphangioma circumscriptum is bilateral or multifocal in 40 % of cases.

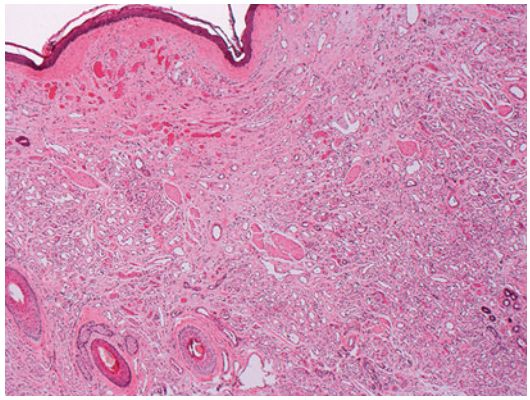
Immunohistochemical Findings

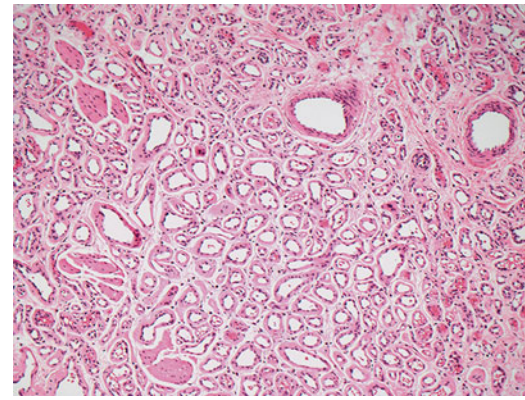
- Hemangioma would be positive for CD34, while negative for D2-40 and Prox-1.

Infantile Hemangioma

There has been only a single description of an infantile hemangioma on the vulva of an 11-week female infant which was successfully treated with topical propranolol [41]. Infantile hemangiomas (IH) are benign tumors of the vascular endothelium with a unique natural history characterized by proliferative and involution phases. At birth, lesions appear as faint erythematous macules or areas of pallor with telangiectasia. Starting in the first weeks of life, lesions enlarge and become raised and bright red in color, usually reaching maximum size by 3–6 months. A brief plateau period ensues. Total or partial involution follows by 5–7 years of age, even in the absence of therapy [42]. The involution phase does not result in normal-appearing skin with about one half of lesions showing residual changes such as scarring, atrophy, redundant skin, discoloration, and telangiectasias.

The majority of infantile hemangiomas is self-limited and may be treated with "watchful waiting." Five to twenty-one percent of lesions ulcerate and require active treatment due to a risk of bleeding, infection, and scarring. In the last few years, oral propranolol, a nonselective beta-blocker, has emerged as a potential first-line therapy with impressive efficacy and improved tolerance when compared with the significant side effects associated with steroids and chemotherapeutic agents such as vincristine and interferon [43]. Several case series and larger observational studies have shown oral propranolol to be an effective treatment for all types of IH, including periocular, airway, and cutaneous lesions [44].





Figs. 14.6 and 14.7 *Infantile hemangioma*. A cellular vascular proliferation characterized by plump endothelial cells

Histopathology

The histopathologic composition of infantile hemangiomas varies with the age of the lesion. Early hemangiomas are highly cellular and are characterized by plump endothelial cells aligned to vascular spaces with small inconspicuous lumina (Figs. 14.6 and 14.7). As the lesions mature, blood flow increases, endothelium flattens, and the lumina of the vessels enlarge and become more obvious. During this interval the vessels convey a "cavernous" appearance that can be misinterpreted as a venous malformation. Regression is portrayed as progressive interstitial fibrosis and adipose metaplasia, a process without known stimulus.

Immunohistochemical studies have shown a complex expression profile including markers for endothelial cells (CD31 positive), pericytes (SMA positive), dendritic cells (factor XIIIa positive), and mast cells [42]. Of particular clinical importance, IH express high levels of erythrocyte-type glucose transporter protein, isotope 1 (GLUT-1), which is not expressed in the vasculature of normal skin nor in other tumors or vascular malformations, making it a highly selective and diagnostically useful marker (Fig. 14.8) [45]. GLUT-1 is also expressed in normal endothelium at sites of blood-tissue barriers, including the brain, eye, nerve, and placenta. North et al. [46] showed that of these tissues, IH's expression profile is closest to that of fetal placental microvasculature, suggesting a possible origin of these tumors.

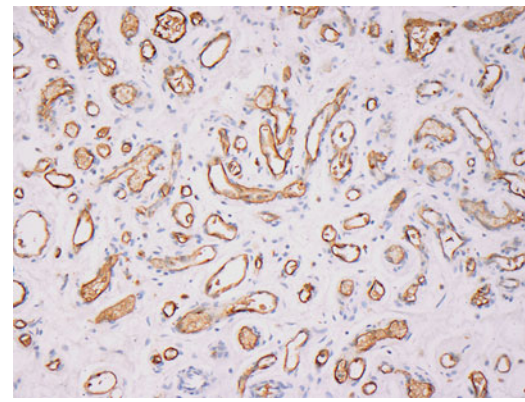


Fig. 14.8 *Infantile hemangioma*. The vascular proliferation is positive for GLUT-1 immunostain

Differential Diagnosis

The differential diagnosis of IH includes vascular malformations and congenital hemangiomas. Fortunately, vascular malformations and congenital hemangiomas that affect the infant population usually do not stain for GLUT-1 (Table 14.3). Congenital hemangiomas differ from IH in that they are fully formed in utero. They can be detected by ultrasound as early as 12 weeks' gestation [47]. Two types have been recognized based on their natural history: rapidly involuting congenital hemangioma (RICH) and non-involuting congenital hemangioma (NICH) [48, 49].

RICHs are fully developed at birth, may be large and exophytic, and, as their name implies,

Table 14.3 Summary of GLUT-1 and D2-40 immunoreactivity in vascular tumors

	GLUT-1	D2-40
Infantile hemangioma	+	–
Congenital hemangioma	–	–/+
Non-involuting congenital hemangioma		
Rapidly involuting congenital hemangioma		
Kaposiform hemangioendothelioma	–	+
Tufted angioma	–	+/–
Pyogenic granuloma	–	–
Spindle cell hemangioma	–	–

involute rapidly during the first month of life. RICHs also rarely require treatment, although they may elicit clinical concern due to associated transient abnormalities (thrombocytopenia, low fibrinogen, and elevated fibrin degradation products). Histopathologically, RICHs are highly cellular with multiple well-defined lobules of proliferating capillaries that anastomose with each other to form ribbons within the dermis or subcutaneous tissue.

NICH grow proportionately with the child. Their vessel walls are often thicker than in IH, and their endothelium may be hobnail in appearance [50]. NICH do not regress as do the other infantile hemangiomas, while RICHs involute in a short period of time. NICH are characterized histopathologically by lobular collections of small, thin-walled vessels with large, often stellate, central lumina, separated by variable amounts of fibrous tissue richly supplied with normal and abnormal veins and arteries.

Summary

Clinical Presentation

- Has a unique natural history characterized by proliferative and involution phases

Histologic Features

- Proliferative stage: highly cellular and characterized by plump endothelial cells with small inconspicuous lumina
- Involution stage: progressive interstitial fibrosis and adipose metaplasia

Differential Diagnosis

- Congenital hemangioma including noninvoluting congenital hemangioma (NICH) and rapidly involuting congenital hemangioma (RICH)

Takeaway Essentials

Clinical Relevant Pearls

- Oral propranolol, a nonselective beta-blocker, has emerged as a potential first-line therapy.

Pathology Interpretation Pearls

- Between the proliferative and involution phases, the vessels can have a "cavernous" appearance that can be misinterpreted as a venous malformation.

Immunohistochemical Findings

- Expresses high levels of erythrocyte-type glucose transporter protein, isotope 1 (GLUT-1)

Lymphangioma

Lymphangioma circumscriptum can uncommonly present in the vulva [51–54]. Congenital lymphangioma circumscriptum of the vulva typically affects females from 9 to 36 years [51]. Cutaneous lymphangiomas can be either superficial or deep. The superficial form or lymphangioma circumscriptum presents as multiple small vesicles over a localized skin area. Complications of lymphangioma circumscriptum include vulvar swelling, pain, and cellulitis [51]. Rarely lymphangiosarcoma can arise from a preexisting lymphangioma circumscriptum [51].

Treatment can include sclerotherapy, liquid nitrogen therapy, carbon dioxide laser therapy, radiotherapy, and surgical excision [52, 53].

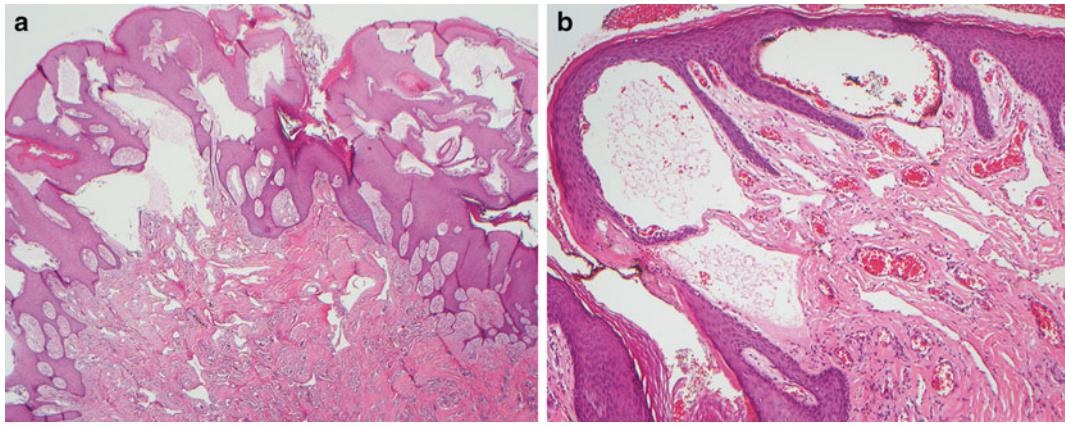


Fig. 14.9 (**a**, **b**) *Lymphangioma*. Dilated lymphatic vessels are seen in the superficial dermis associated with epidermal hyperplasia and also in the deep dermis

Propranolol has been shown to be safe in infants and children with congenital lymphangiomatosis [55].

Histopathology

Dilated lymphatic channels are seen at the junction of papillary and reticular dermis in the superficial form or lymphangioma circumscriptum (Figs. 14.9a, b). Both the superficial and deep lymphatic channels are involved in the deep type.

Differential Diagnosis

The clinical differential diagnosis of lymphangioma circumscriptum of the vulva includes molluscum contagiosum and genital warts [56]. The histologic differential diagnosis includes acquired lymphangiectasis, vulvar varices (venous lake), and angiokeratoma. Clinical history is essential to distinguish primary from acquired lymphangiomas, since lymphangiomas are present since birth or early childhood, while acquired lymphangiectasis is a secondary change associated with a number of causes.

Summary

Clinical Presentation

- Lymphangioma circumscriptum presents as multiple small vesicles over a localized skin area.

Histologic Features

- Dilated lymphatic channels are seen at the junction of papillary and reticular dermis.

Differential Diagnosis

- Acquired lymphangiectasis
- Vulvar varices (venous lake)
- Angiokeratoma

Takeaway Essentials

Clinical Relevant Pearls

- Lymphangiomas are present since birth or early childhood, while acquired lymphangiectasis is associated with a number of causes.

Pathology Interpretation Pearls

- While acquired lymphangiectasis affects superficial and mid-lymphatics, lymphangioma circumscriptum or congenital lymphangioma affects those within the deep dermis and subcutaneous tissue.

Immunohistochemical Findings

- The lymphatic endothelial cells are positive for D2-40, Prox-1, and CD31; yet they are negative for CD34.

Glomus Tumor

Glomus tumors are benign vascular neoplasms that are associated with intense pain localized to the tumor nodule. There have been five cases of glomus tumor reported on the vulva [57–60]. Two of these five tumors are clitoral in location [57, 59]. Glomus tumors often present as small blue-red nodules in the deep dermis or subcutaneous tissue. Treatment is often simple excision.

Histopathology

Glomus tumor is comprised of glomus cells and vascular structures. The glomus cells are with round or polygonal shape and eosinophilic cytoplasm (Fig. 14.10). The vascular component is more prominent in glomangioma. They are invariably positive for smooth muscle actin [61], and variably for desmin [62]. Depending on the cellularity of the lesion, glomus tumor can resemble a hemangioma or an epithelial tumor.

Differential Diagnosis

Although the histologic features of glomus tumor are very distinctive, glomus tumor can occasionally be mistaken for adnexal tumors or intradermal nevi. However, one can often appreciate the presence of a vascular component on high magnification in glomus tumor, and these tumor cells would express smooth muscle actin.

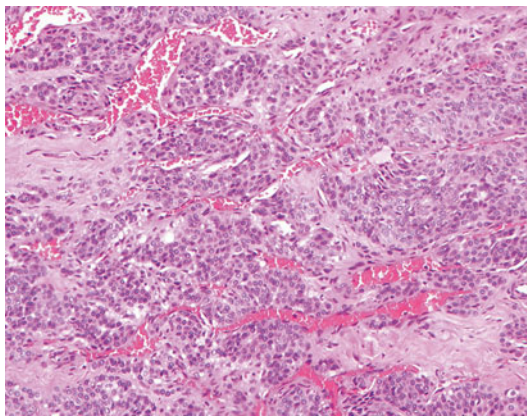


Fig. 14.10 *Glomus tumor*. Solid proliferation of uniform cells with intervened blood vessels

Summary

Clinical Presentation

- Small blue-red nodule

Histologic Features

- Comprised of glomus cells and vascular structures
- Glomus cells: round or polygonal shape and eosinophilic cytoplasm

Differential Diagnosis

- Intradermal nevi

Takeaway Essentials

Clinical Relevant Pearls

- Glomus tumor is often associated with intense pain.

Pathology Interpretation Pearls

- The vascular component is more prominent in glomangioma.

Immunohistochemical Findings

- Invariably positive for smooth muscle actin

Malignant Neoplasms

Malignant vascular neoplasms of vulva are rare with case reports of Kaposi's sarcoma [63, 64], epithelioid hemangioendothelioma [65, 66], radiation-induced angiosarcoma [67, 68], and conventional angiosarcoma. Lymphangiosarcoma have been documented in settings of radiation therapy [69] and long-standing lymphedema status post bilateral lymphadenectomy and postoperative radiotherapy [70].

Kaposi's Sarcoma

The clinical features and the biological behavior of Kaposi's sarcoma are different and depend on the epidemiological type. There are four types: the classical type of Kaposi's sarcoma which affects mainly elderly patients, the African-endemic variant, Kaposi's sarcoma associated

with immunosuppressive drugs (iatrogenic), and AIDS-associated Kaposi's sarcoma. Human herpesvirus 8 (HHV8) is the etiologic agent of all subtypes [71].

Vulvar involvement by Kaposi's sarcoma is rare and seen in patients with acquired immunodeficiency syndrome (AIDS) [63, 64]. In a 5-year retrospective study in a teaching hospital in Nigeria, unusual sites including the vulva, oropharynx, conjunctiva, and rectum were documented [72].

Histopathology

The earliest lesions of Kaposi's sarcoma known as the patch stage are characterized by inconspicuous changes and may produce the erroneous impression of an inflammatory condition. At scanning magnification these lesions show sparse, superficial, and deep perivascular mononuclear cell infiltrates in conjunction with an increased number of irregular, jagged, vascular spaces lined by thin endothelial cells (Fig. 14.11a). The vessels are mainly found in the upper part of the dermis. The neoplastic vessels of Kaposi's sarcoma show a tendency to be present around preexisting normal adnexal structures and blood vessels producing the so-called promontory sign (Fig. 14.11b). In other areas, the blood vessels infiltrate collagen bundles of the dermis giving the appearance that they are "dissecting" the stroma. The inflammatory cells present are predominantly lymphocytes and plasma cells. The identification of plasma cells around newly formed irregular blood vessels is a helpful clue in the histopathologic diagnosis of the patch stage of Kaposi's sarcoma (Fig. 14.11c). Plaque lesions of Kaposi's sarcoma tend to involve the entire dermis and even the upper part of the subcutaneous fat. At this stage, there is an increased number of spindle cells arranged in short fascicles between collagen bundles centered around proliferating vascular channels. The spindle cells line irregularly shaped, slit-like vascular spaces that contain isolated erythrocytes. They display minimal or no atypia, with few or no mitotic figures. When the number of spindle cells increases, lesions of Kaposi's sarcoma become nodular (Fig. 14.12a). Then, the spindle cells are arranged in interwoven fascicles with erythrocytes scattered in the interstices (Fig. 14.12b). Nuclear atypia, pleomorphism, and mitotic figures may be seen, but are usually not very prominent. In rare instances, however, especially in the African variant, a significant number of mitotic figures and atypical cells may be seen in lesions of Kaposi's

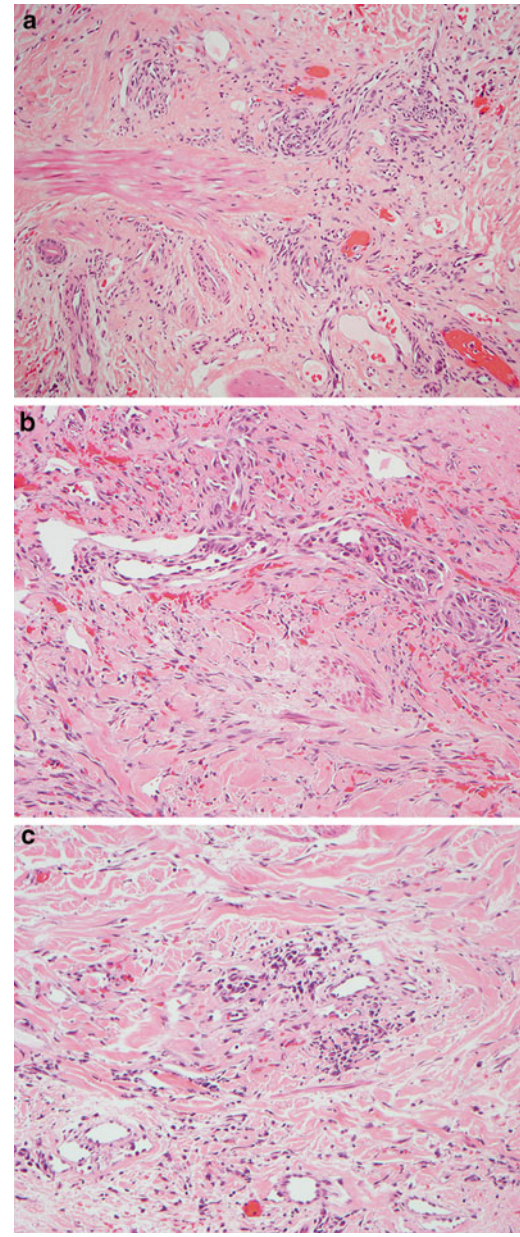


Fig. 14.11 *Kaposi's sarcoma, patch stage*. (**a**) Early changes in the dermis can resemble an inflammatory process. At higher magnification, slit-like vascular channels are seen. (**b**) The neoplastic vessels surround preexisting normal adnexae and blood vessels producing the so-called promontory sign. (**c**) The presence of plasma cells and hemosiderin deposition are helpful diagnostic clues

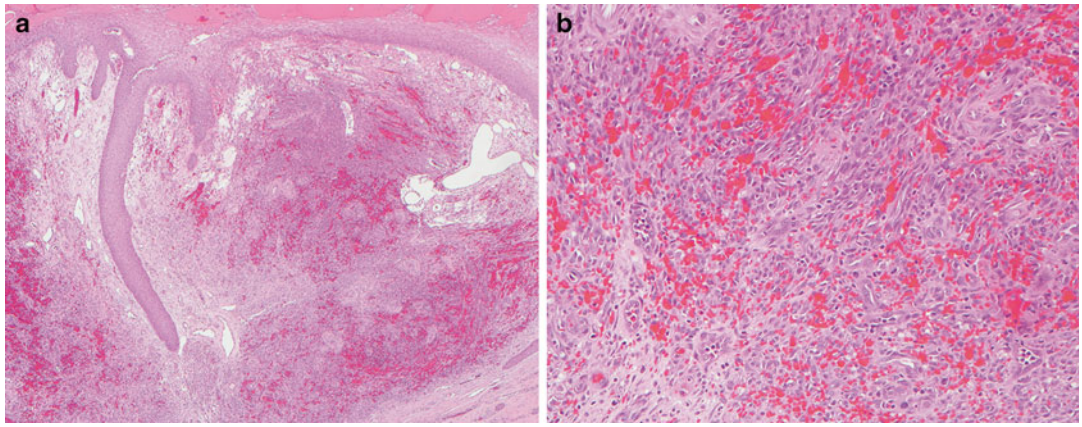


Fig. 14.12 (**a**, **b**) *Kaposi's sarcoma, tumor stage*. A cellular proliferation of spindle neoplastic cells exhibiting slit-like vascular channels with associated extravasated erythrocytes

sarcoma. A rather characteristic, but probably not specific, histopathologic finding is the presence of the so-called hyaline globules. Although these are most common in the plaque and nodular lesions of Kaposi's sarcoma, they can be present at any stage of the disease. These globules are located both intra- and extracellularly and are periodic acid-Schiff (PAS) positive and diastase resistant and consist of eosinophilic spherules measuring between 1 and 10 μm. Most likely these hyaline globules represent degenerated erythrocytes that are phagocytized and confined to the phagolysosomes of the neoplastic cells.

The tumor cells of Kaposi's sarcoma are immunoreactive with endothelial markers (CD31, CD34, and factor 8-related antigen) as well as lymphatic specific markers (D2-40, LYVE-1, VEGFR-3, and Prox-1) [73–75]. Stronger CD34 expression is seen in advanced-stage lesions. The expression of HHV8 immunostain is helpful in distinguishing Kaposi's sarcoma from histologic mimics [76] (Fig. 14.13); however, HHV8 expression has been reported in some angiosarcomas, hemangiomas, and dermatofibromas [77].

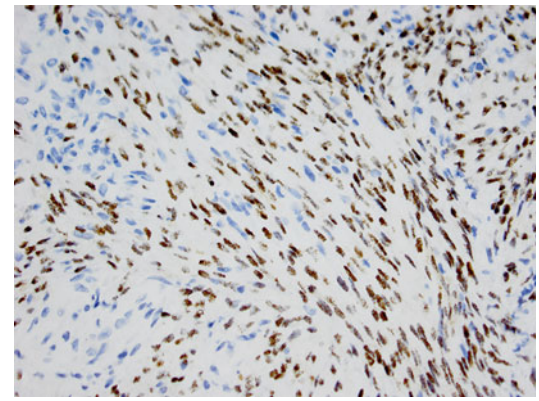


Fig. 14.13 *Kaposi's sarcoma*. The tumor cells exhibit strong nuclear staining with HHV8 immunostain

Differential Diagnosis

The histologic differential diagnosis would include targetoid hemosiderotic hemangioma for patch-stage KS and tufted angioma, bacillary angiomatosis, and angiomatoid fibrous histiocytoma for nodular KS. Targetoid hemosiderotic hemangioma or hobnail hemangioma is composed of dilated vessels lined by hobnail, CD31+, and D2-40+ endothelial cells superficially. Tufted angioma and kaposiform hemangioendothelioma are now considered to be a different spectrum of the same disease. These lesions most commonly affect children and young adults, but both congenital and very late-onset cases have been described. The lesions have a predilection for the perineal area among other anatomic sites. These neoplasms grow slowly and insidiously and may eventually cover a large area of the skin.

Histopathologically, tufted angioma presents with multiple individual vascular lobules within the dermal and subcutaneous fat. These aggregations are more prominent in the middle and lower part of the dermis. Each lobule is composed of

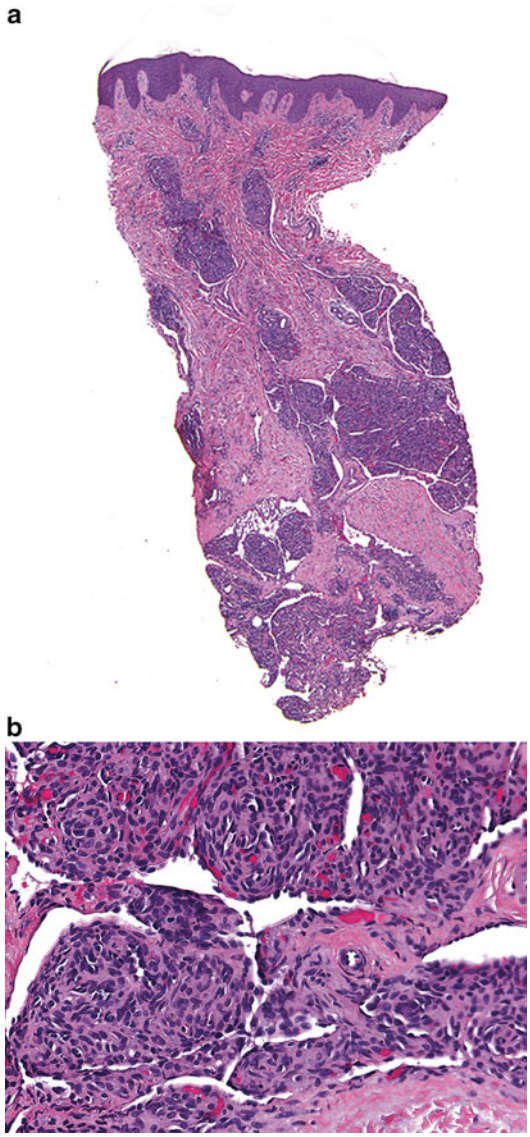


Fig. 14.14 *Tufted angioma*. (**a**) Multiple individual vascular lobules are seen within the dermal and subcutaneous fat. (**b**) Each lobule is comprised of aggregates of endothelial cells that form a concentric whorl around a preexisting vascular plexus

aggregates of endothelial cells that form a concentric whorl around a preexisting vascular plexus. Some lobules bulge into the walls of dilated thin-walled vascular structures, giving these vessels a slit-like or semilunar appearance (Fig. 14.14a, b). Microscopic hemorrhage, irregular cystic spaces, and dense peripheral chronic inflammation would be seen in an angiomatoid fibrous histiocytoma. The main clinical differential diagnosis is bacillary angiomatosis [78] (see Chap. 6). Lobular epithelioid vascular proliferation and clumps of bacteria, highlighted by Warthin-Starry stain, would be present in bacillary angiomatosis.

Summary

Clinical Presentation

- Vulvar involvement by Kaposi's sarcoma is rare and seen in patients with acquired immunodeficiency syndrome.

Histologic Features

- Spindle cells arrange in short fascicles between collagen bundles centered around proliferating vascular channels.
- The spindle cells line irregularly shaped, slit-like vascular spaces that contain isolated erythrocytes.

Differential Diagnosis

- Targetoid hemosiderotic hemangioma
- Tufted angioma
- Bacillary angiomatosis
- Angiomatoid fibrous histiocytoma

Takeaway Essentials

Clinical Relevant Pearls

- There are four types: the classical type of Kaposi's sarcoma which affects mainly elderly patients, the African-endemic variant, Kaposi's sarcoma associated with immunosuppressive drugs (iatrogenic), and AIDS-associated Kaposi's sarcoma.
- Human herpesvirus 8 (HHV8) is the etiologic agent of all subtypes.

Pathology Interpretation Pearls

- Promontory sign—neoplastic vessels present around preexisting normal adnexal structures and blood vessels.
- Helpful clue in diagnosing patch stage—presence of plasma cells around newly formed irregular blood vessels.

Immunohistochemical Findings

- The expression of HHV8 immunostain is helpful in distinguishing Kaposi's sarcoma from histologic mimics.

Epithelioid Hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) was first reported by Weiss and Enzinger [79]. EHE of soft tissue is classified as classic or malignant based on mitotic activity and tumor size [80]. EHE shares many similarities, both clinically and histologically, with retiform hemangioendothelioma and Dabska's tumor; thus, they are best considered as part of a histologic spectrum.

Epithelioid hemangioendothelioma of the vulva is rare, and there have been only two cases reported in the literature, one involving the labia majora [65] and another involving the clitoris [66]. Clinically, they usually appear as solitary, slightly painful tumors, which in some cases may ulcerate. Surgical excision is the treatment of choice in these two cases [65, 66].

Histopathology

The lesions of EHE present as circumscribed dermal or subcutaneous nodules. EHE often has a propensity for angiocentric growth, expanding the vessel wall and obliterating the lumen as they extend from the vascular lumen to the adjacent soft tissue. (See Vignette 2 at the end of this chapter.) The neoplasm is composed of cords, strands, and nests of plump, epithelioid cells embedded in a fibromyxoid or sclerotic stroma (Fig. 14.15a, b). Many of the neoplastic cells contain vacuoles in their cytoplasm as a sign of primitive vascular differentiation. Slight nuclear pleomorphism and occasional mitotic figures may be seen.

The tumor cells of epithelioid hemangioendothelioma express CD31 and CD34. Approximately one fourth of epithelioid hemangioendothelioma expresses cytokeratin, but the staining is usually focal [81]. Epithelioid hemangioendothelioma expresses cytokeratin (CK) 18, CK7, and CK8 in 100 %, 50 %, and 10 % of cases, respectively [82]. They are negative for CK14 and CK19 [82].

Dabska's tumor is composed of interconnecting vascular channels lined by atypical endothelial cells. The vascular spaces vary in size and shape ranging from narrow channels to large vascular structures. The most characteristic histopathologic feature consists of papillary plugs of atypical endothelium, with a central sclerotic core of connective tissue, projecting into the lumina and producing a glomeruloid appearance. The endothelial cells are round to polyhedral with an atypical, hyperchromatic, and eccentrically placed nuclei, located in the luminal border of the cell, producing a surface bulge, accounting for the term "hobnail" or "matchstick." Retiform hemangioendothelioma consists of elongated, arborizing blood vessels involving the dermis, arranged in an architectural pattern reminiscent to that of the normal rete testis. Monomorphic hobnail endothelial cells line the vessels composing the neoplasm. Cytologic atypia is minimal in the hobnail cells of retiform hemangioendothelioma and few or no mitotic figures are seen. In some areas, the retiform pattern is obscured by the presence of a dense inflammatory infiltrate of mature lymphocytes.

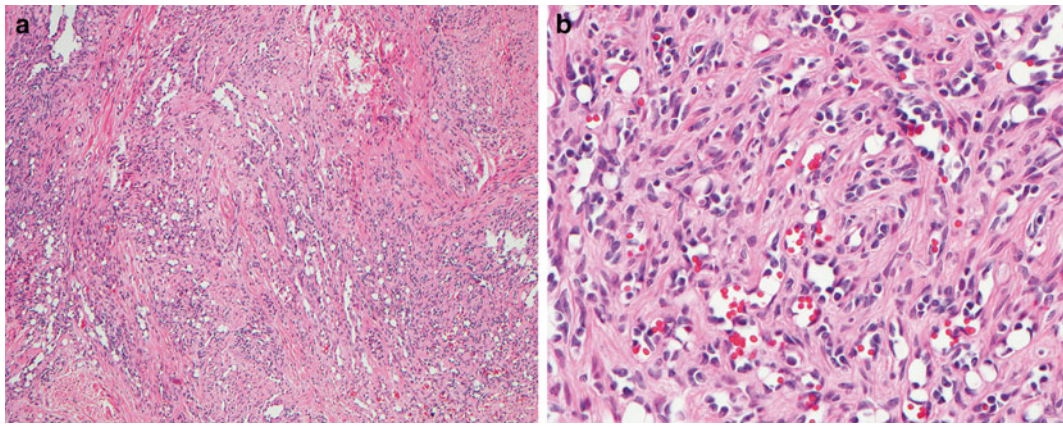


Fig. 14.15 *Epithelioid hemangioendothelioma*. Cords of neoplastic cells with intracytoplasmic vacuoles are seen

A t(1;3)(p36.3;q25) translocation was initially reported in two cases of EHE by Mendlick et al. in 2001 [83]. A decade later, *WWTR1* (or *TAZ*) on 3q25, a gene involved in transcriptional factor activation, and *CAMTA1*, on 1p36, belongs to a family of calmodulin-binding transcription activators, were identified [84, 85]. Recently, a *YAP1-TFE3* gene fusion has been reported in a subset of EHE, and these tumors are characterized by strong TFE3 immunoreactivity [86].

Differential Diagnosis

The differential diagnosis includes metastatic carcinoma, melanoma, epithelioid angiosarcoma, and epithelioid sarcoma. A panel of immunohistochemical stains including broad spectrum keratins, S100, and MelanA, in addition to CD31 and CD34, would help either excluding or ruling in carcinoma and melanoma. Since epithelioid hemangioendothelioma also expresses keratin marker, using a broad panel of immunohistochemical markers is important [81]. Epithelioid angiosarcoma would be comprised of solid proliferation of atypical and mitotically active epithelioid endothelial cells. Epithelioid sarcoma would express CD34 and subsets of keratins; however, it would be negative for CD31.

The *WWTR1-CAMTA1* fusion, a feature of EHE, has not been detected in epithelioid hemangioma, pseudomyogenic hemangioendothelioma (epithelioid sarcoma-like hemangioendothelioma), or epithelioid angiosarcoma; thus, it can be an ancillary molecular test in difficult cases [83–85].

Summary

Clinical Presentation

- Solitary and slightly painful tumors

Histologic Features

- Circumscribed dermal or subcutaneous nodules
- Cords, strands, and nests of plump, epithelioid cells embedded in a fibromyxoid or sclerotic stroma
- Minimal nuclear pleomorphism and only occasional mitotic figures

Differential Diagnosis

- Epithelioid sarcoma
- Epithelioid angiosarcoma
- Metastatic carcinoma
- Melanoma

Takeaway Essentials

Clinical Relevant Pearls

- Epithelioid hemangioendothelioma shares many similarities, both clinically and histologically, with retiform hemangioendothelioma and Dabska's tumor; thus, they are best considered as part of a spectrum.

Pathology Interpretation Pearls

- Propensity for angiocentric growth with 50 % of cases arises from vessels.
- Cytoplasmic vacuoles containing erythrocytes can be seen in many cases as a sign of primitive vascular differentiation.

Immunohistochemical and Molecular Findings

- The tumor cells of epithelioid hemangioendothelioma express CD31 and CD34.
- Approximately one-fourth of epithelioid hemangioendothelioma focally expresses cytokeratin.
- *WWTR1-CAMTA1* fusion is a feature of epithelioid hemangioendothelioma.

Angiosarcoma

Angiosarcomas are highly aggressive neoplasms that can present in different areas of the body, including the vulva. Angiosarcomas can be induced by sun exposure, radiation, and chronic lymphedema. However, sometimes, they are idiopathic. Clinically, the lesions appear as ill-defined bruise-like areas that simulate a hematoma. More

advanced lesions present as indurate plaques with raised, nodular, and occasionally ulcerated components accompanied by smaller satellite lesions in the same vicinity. Some of the cases affecting the vulva are associated to chronic lymphedema. Postradiation cutaneous angiosarcoma is a rare condition that has been described following the use of radiotherapy for the treatment of diverse conditions, both benign and malignant [67, 68].

Postradiation angiosarcoma has been reported in the right groin 25 years status post radiotherapy for vulvar squamous cell carcinoma [68]. In order to associate radiotherapy with the sarcoma development, several criteria must be present: (1) site of sarcoma must be within field of radiotherapy, (2) there must be at least 3–5 years interval, and (3) the induced sarcoma has a different histology than the primary cancer [68].

Histopathology

Regardless of the clinical variant, angiosarcomas are histopathologically similar. Well-differentiated angiosarcomas appear as irregular, dilated vascular channels lined by flattened endothelial cells with an innocuous appearance, which may lead one to confuse them with a hemangioma, lymphangioma, or an inflammatory process (Fig. 14.16). However, careful observation of these lesions reveals the presence of irregular vascular channels dissecting the dermis. These channels tend to communicate with each other, forming an anastomosing network. Furthermore, some of the endothelial cells appear large, hyperchromatic, and pleomorphic, protruding within vascular lumina, forming small papillations. Poorly differentiated angiosarcomas demonstrate solid proliferations of polygonal or spindle-shaped pleomorphic endothelial cells, with prominent mitotic activity and poorly formed vascular spaces, which sometimes makes it difficult to distinguish them from carcinoma, melanoma, or a high-grade fibrosarcoma (Fig. 14.17a, b).

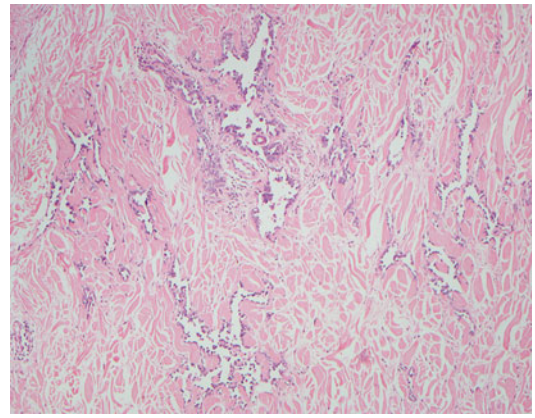


Fig. 14.16 *Well-differentiated angiosarcoma*. Irregular vascular channels lined by bland endothelial cells are seen infiltrating the dermis

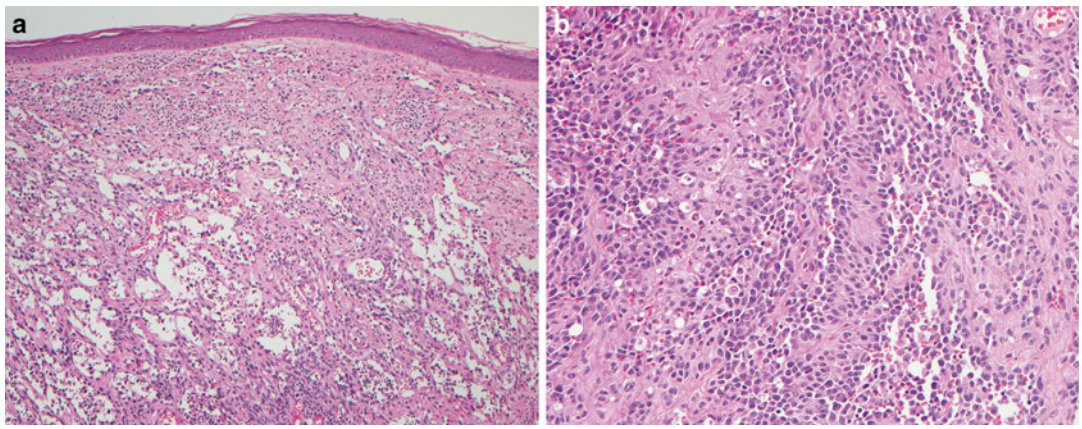


Fig. 14.17 *Angiosarcoma*. (**a**) Pleomorphic tumor cells are seen dissecting the dermis. (**b**) Irregular slit-like vessels are lined by hyperchromatic and polygonal neoplastic cells mimicking a carcinoma

Of considerable value for the diagnosis of these cases is the presence of cytoplasmic vacuoles within the neoplastic cells. Patchy lymphoid infiltrates are also a common finding. The number of erythrocytes that are present within the vascular spaces varies from a few to none in the poorly differentiated areas. Preexisting adnexal, neural, and vascular structures of the dermis are frequently involved and destroyed by the tumor.

Epithelioid angiosarcomas express CK8 and CK18 in approximately 50 % of cases, epithelial membrane antigen (EMA) in 25 %, and only rarely for CK7 and CK19 [82]. Secondary angiosarcomas, such as radiation induced, are characterized by high frequency of high-level *MYC* amplification [87]; thus, *MYC* amplification has been shown to be helpful in the distinction of postradiation cutaneous angiosarcoma from atypical vascular lesions status postradiation therapy [88]. MYC nuclear expression and Prox-1 staining were seen in cutaneous postradiation angiosarcoma, whereas these markers were negative in atypical vascular lesions status postradiation therapy [88]. Of interest, *MYC* amplification and overexpression has recently been documented in a series of cutaneous primary angiosarcomas [89].

Differential Diagnosis

High-level *MYC* amplification is a feature of radiation-induced and lymphedema-associated angiosarcoma [87]. *FLT4* co-amplification is seen in 25 % of secondary angiosarcoma [90]. Both of these gene abnormalities are not typically seen in radiation-induced atypical vascular lesions and can serve as distinguishing feature. Lymphangiosarcoma or lymphedema-associated angiosarcoma would also be in the differential diagnosis and clinical history, and immunohistochemical studies would be needed in order to distinguish it from conventional angiosarcoma [69, 70]. A high-grade squamous cell carcinoma can exhibit pseudovascular changes that can be mistaken for angiosarcoma [91, 92]; however, these tumors would be positive for p63 and keratins and negative for vascular markers including CD31, Fli-1, and Erg.

Summary

Clinical Presentation

- Bruise-like areas or violaceous nodules superimposed on the brown non-pitting edema

Histologic Features

- Well-differentiated angiosarcomas: irregular, dilated vascular channels lined by flattened endothelial cells with an innocuous appearance
- Poorly differentiated angiosarcomas: solid proliferations of polygonal or spindle-shaped pleomorphic endothelial cells, with prominent mitotic activity and poorly formed vascular spaces

Differential Diagnosis

- *Radiation-induced* atypical lesions
- Lymphangiosarcoma
- Pseudoangiosarcomatous carcinoma

Takeaway Essentials

Clinical Relevant Pearls

- Clinically, the lesions can simulate a hematoma.

Pathology Interpretation Pearls

- The presence of cytoplasmic vacuoles within the neoplastic cells is a helpful histologic clue.
- Preexisting adnexal, neural, and vascular structures of the dermis are frequently involved and destroyed by the tumor.

Immunohistochemical and Molecular Findings

- Epithelioid angiosarcomas express CK8 and CK18 in approximately 50 % of cases.
- High-level *MYC* amplification is a feature of radiation-induced and lymphedema-associated angiosarcoma.
- MYC nuclear expression and Prox-1 staining are seen in cutaneous postradiation angiosarcoma, whereas these markers are negative in atypical vascular lesions status postradiation therapy.

Case Vignettes

Vignette 1

Clinical History: A 44-year-old woman presented with a one-month history of a raised and very painful lesion on her left labia. She noticed this raised area to be discolored and purple in the shower. The patient had tried to use a needle in order to "pop" it.

Microscopic Description: A skin biopsy shows a thin-walled venule beneath the epidermis filled with erythrocytes (Fig. 14.18). Thrombosis with reorganization is noted at one side of the lesion.

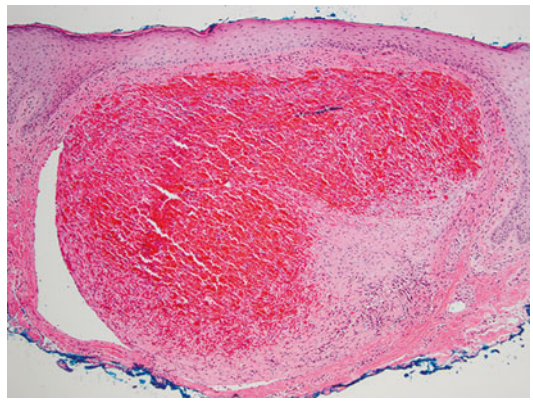


Fig. 14.18 Vignette 1. A solitary vascular space filled with erythrocytes seen in the dermis

Diagnosis: Venous lake (vulvar varices)

Discussion: Vulvar varices, also known as venous lake or capillary aneurysm, are seen in 4 % of women and most commonly associated with pregnancy [93]. These lesions can present as small protrusions or sudden development of a growing dark papule on labia majora or large masses on the vulva and perivulvar region [93, 94]. Pain and swelling of the vulva are noted around menstruation time [95]. When present in nonpregnant women, vulvar varices are associated with leg varices or venous malformations of the labia and clitoral areas with or without arteriovenous malformations on the limbs or trunk (Klippel-Trenaunay-Weber syndrome and Parkes Weber syndrome) [96]. They can be seen in pelvic congestion syndrome —pelvic pain, dyspareunia, dysmenorrhea, dysuria, and vulvar and perivulvar varices [97]. Histologically, vulvar varices or capillary aneurysms present as a thin-walled venule beneath the epidermis. When the thrombus dissolves the lesion acquires the appearance of a venous lake. The treatment is conservative, and sclerotherapy can be used after delivery [98].

Vignette 2

Clinical History: A 45-year-old woman presented with an enlarging clitoral mass. An excision biopsy was performed. The gross specimen of the tumor shows a tumor that resembles an organizing thrombus (Fig. 14.19).

Microscopic Description: A well-circumscribed tumor is seen in the soft tissue (Fig. 14.20). Intracytoplasmic lumina are seen (Fig. 14.21). The tumor appears to arise in the lumen of a vascular channel (Fig. 14.22). The tumor cells are positive for CD31 (Fig. 14.23) and also for keratin 7 (Fig. 14.24).

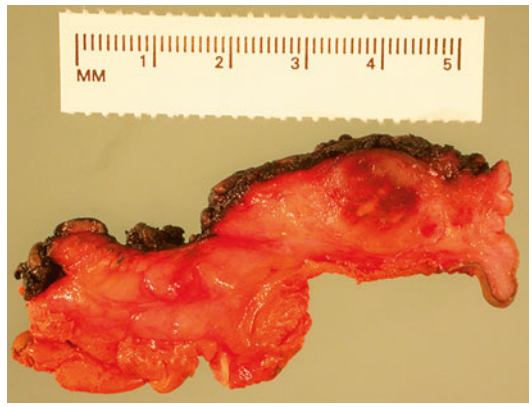


Fig. 14.19 Vignette 2. A 1.5 cm tumor is seen grossly resembling a thrombosed vessel

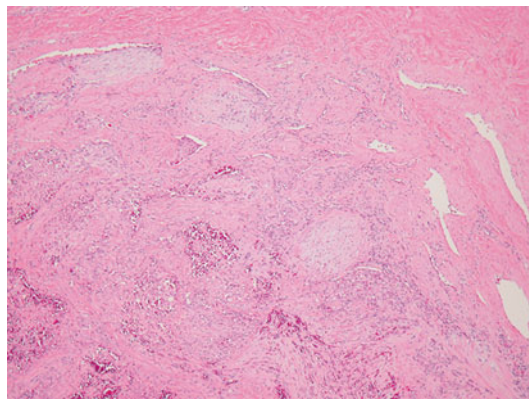


Fig. 14.20 Vignette 2. A well-circumscribed vascular tumor is seen

(continued)

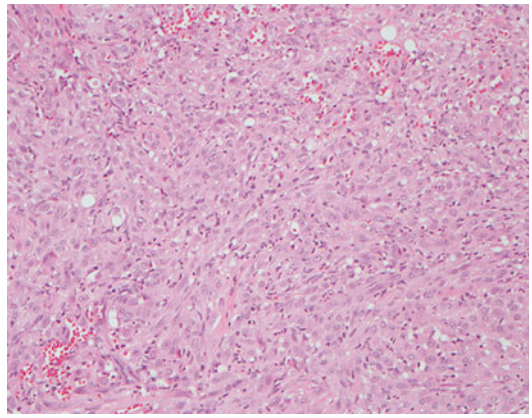


Fig. 14.21 Vignette 2. Intracytoplasmic lumina are seen

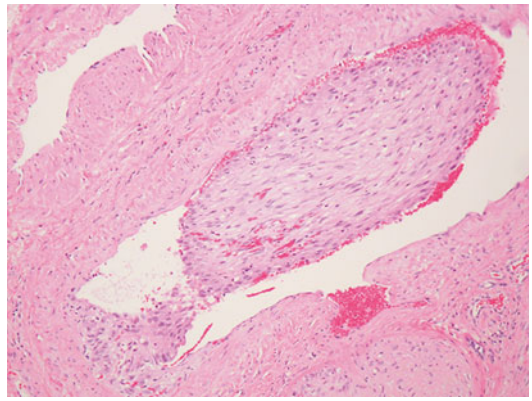


Fig. 14.22 Vignette 2. The tumor is seen within vascular lumen

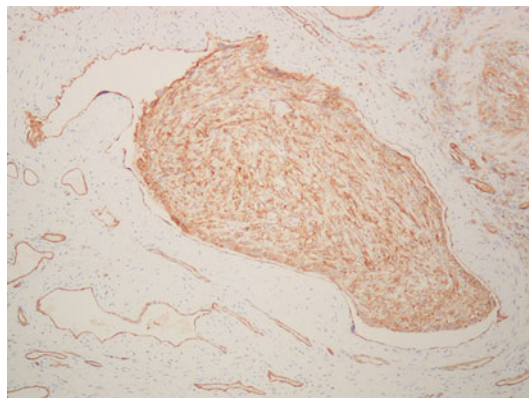


Fig. 14.23 Vignette 2. The tumor is positive for CD31

(continued)

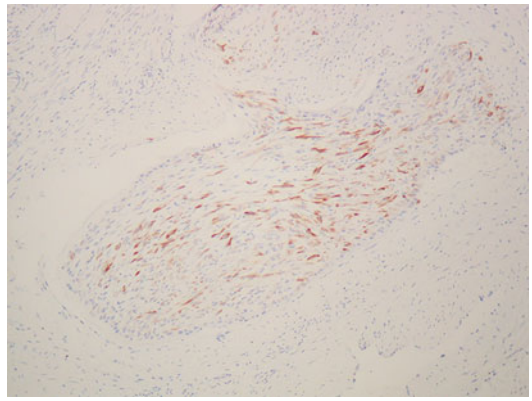


Fig. 14.24 Vignette 2. The tumor is focally positive for keratin 7

Diagnosis: Epithelioid hemangioendothe-lioma

Discussion: Epithelioid hemangioendothelioma is often associated with a vessel in approximately half of the cases as seen in this case. A tumor is seen in the lumen (Fig. 14.22). Small intracytoplasmic lumens can be confused with the mucin vacuoles of adenocarcinoma. One histologic clue is the presence of erythrocytes in these lumens. The tumor also appears bland in most cases with minimal mitotic activity. Expression of vascular markers including CD31 and CD34 together with an epithelial marker such as CK7 is helpful in making the diagnosis.

Vignette 3

Clinical History: A 25-year-old female presented with "vesicles" in the labia majora for a few weeks. A biopsy was performed, and the clinical differential diagnosis included infection and a blistering disorder.

Microscopic Description: Dilated lymphatic channels are seen at the junction of the papillary and reticular dermis (Fig. 14.25a, b). They are lined by bland endothelial cells, D2-40 positive (Fig. 14.26), and with minimal mitotic activity.

(continued)

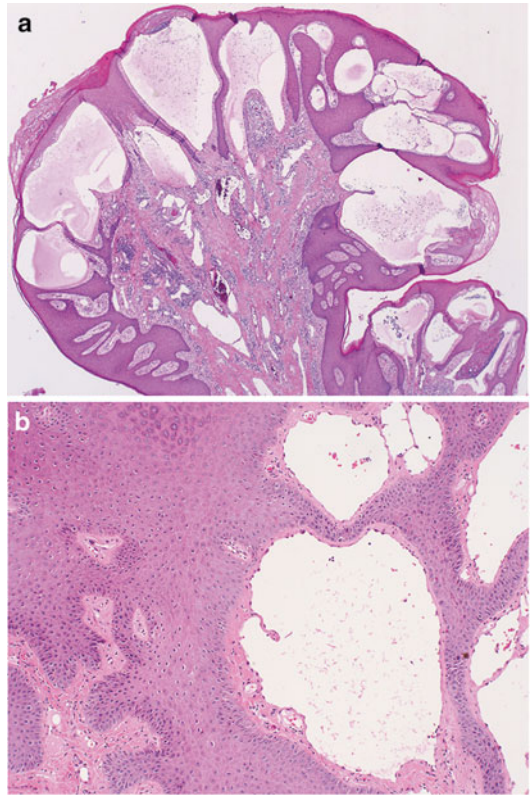


Fig. 14.25 (**a**, **b**) Vignette 3. Dilated lymphatic channels are seen (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

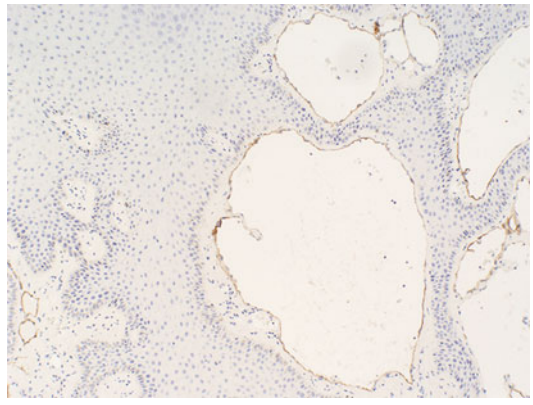


Fig. 14.26 Vignette 3. The endothelial cells are positive for the lymphatic marker D2-40 (Courtesy of Dr. M. Angelica Selim, Department of Pathology, Duke University Medical Center, Durham, NC)

(continued)

Diagnosis: Lymphangioma associated with Crohn's disease

Discussion: Lymphangioma, when occurring in the vulva, can be either congenital or acquired [51]. For the acquired form, a variety of causes have been implicated including surgery, lymphadenectomy, pregnancy, radiotherapy, Crohn's disease, scleroderma, neoplasia, or infections such as tuberculosis, lymphogranuloma venereum, and filariasis [3, 4, 6–11].

There have been 7 cases of acquired lymphangiomas associated with Crohn's disease reported in the literature [11, 99, 100]. In a series of 12 patients with acquired lymphangioma over a 26-year period, Crohn's-associated lymphangioma was seen in 33 % of cases in comparison to 58 % associated with radiation therapy [11]. In comparison with patients with prior radiation, the patients with Crohn's-associated lymphangioma had larger lesions, presented on the average 14 years later, had less associated comorbidities, and did not require additional surgical intervention [11]. One of these patients had granulomatous inflammation associated with the lymphangioma with granulomas seen within and adjacent to the lymphatic channels [11]. The most likely mechanism is direct obstruction caused by fibrosis associated with fistula tract formation which resulted in expansion of the regional lymphatics—similar to that observed after extensive surgery [101, 102].

References

1. Papalas JA, Sangueza OP, Puri PK, Robboy SJ, Selim MA. Vulvar vascular tumors: a clinicopathologic study of 85 patients. Am J Dermatopathol. 2013;35(1):1–7.
2. Young AW, Wind RM, Tovell HMM. Lymphangioma of vulva. Acquired following treatment for cervical cancer. N Y State J Med. 1980;80(6):987–9.
3. Stewart CJR, Chan T, Platten M. Acquired lymphangiectasia ('lymphangioma circumscriptum') of the vulva: a report of 8 cases. Pathology. 2009;41(5):448–53.
4. Haneef NS, Ramachandra S, Metta AK, Haritha K. Lymphangiectasias of vulva. Indian Dermatol Online J. 2011;2(1):40–2. doi:10.4103/2229-5178.79854.
5. Horn LC, Kuhndel K, Pawlowitsch T, Leo C, Einenkel J. Acquired lymphangioma circumscriptum of the vulva mimicking genital warts. Eur J Obstet Gynecol Reprod Biol. 2005;123(1):118–20.
6. Verma S. Pregnancy-induced lymphangiectasias of the vulva. Int J STD AIDS. 2008;19(3):211–2.
7. Mendiratta V, Harjai B, Sardana K. Tubercular lymphadenitis with lymphangiectases of the vulva. J Eur Acad Dermatol Venereol. 2005;19(2):264–5.
8. Bhat RM, Saldanha CS, Kambil SM, Dandakeri S. Cutaneous lymphangiectasia of the vulva secondary to tuberculosis. Indian J Sex Transm Dis. 2012;33(1):35–7.
9. Kennedy CTC. Lymphangiectasia of the vulva following hysterectomy and radiotherapy. Br J Dermatol. 1990;123 Suppl 37:92–3.
10. Buckley DA, Barnes L. Vulvar lymphangiectasia due to recurrent cellulitis. Clin Exp Dermatol. 1996;21(3):215–6.
11. Papalas JA, Robboy SJ, Burchette JL, Foo WC, Selim MA. Acquired vulvar lymphangioma circumscriptum: a comparison of 12 cases with Crohn's associated lesions on radiation therapy induced tumors. J Cutan Pathol. 2010;37(9):958–65.
12. Sharma R, Tomar S, Chandra M. Acquired vulval lymphangiectases mimicking genital warts. Indian J Dermatol Venereol Leprol. 2002;68(3):166–7.
13. Hamida MD, Baccouche D, El Fekih N, Fazaa B, Kamoun R. Lymphangiectasia of the vulva, treatment with CO_2 laser. Indian J Dermatol Venereol Leprol. 2012;78(1):122.
14. Landthaler M, Hohenleutner U, Braun-Falco O. Acquired lymphangioma of the vulva: palliative treatment by means of laser vaporization carbon dioxide. Arch Dermatol. 1990;126(7):967–8.
15. Fadare O, Brannan SM, Arin-Silasi D, Parkash V. Localized lymphedema of the vulva: a clinicopathologic study of 2 cases and a review of the literature. Int J Gynecol Pathol. 2011;30(3):306–13.
16. Fukunaga M. Expression of D2-40 in lymphatic endothelium of normal tissues and in vascular tumours. Histopathology. 2005;46(4):396–402.
17. Castro EC, Galambos C. Prox-1 and VEGFR3 antibodies are superior to D2-40 in identifying endothelial cells of lymphatic malformations – a proposal of a new immunohistochemical panel to differentiate lymphatic from other vascular malformations. Pediatr Dev Pathol. 2009;12(3):187–94.
18. Kontogianni-Katsaros K, Kairi-Vassilatoy E, Grapsa D, Papadias K, Hasiakos D, Kondi-Pafitis A. Angiokeratoma of the vulva: a rare benign tumor mimicking malignancy – case reports. Eur J Gynaecol Oncol. 2006;27(6):632–3.

19. Cohen PR, Young Jr AW, Tovell HM. Angiokeratoma of the vulva: diagnosis and review of the literature. Obstet Gynecol Surv. 1989;44(5):339–46.
20. Fogagnolo L, Cintra ML, Velho PE. Angiokeratoma of the vulva. An Bras Dermatol. 2011;86(2):333–5.
21. Terzakis E, Androutsopoulos G, Zygouris D, Grigoriadis C, Derdelis G, Arnogiannaki N. Angiokeratoma of the vulva. Eur J Gynaecol Oncol. 2011;32(5):597–8.
22. Yigiter M, Arda IS, Tosun E, Celik M, Hicsonmez A. Angiokeratoma of clitoris: a rare lesion in an adolescent girl. Urology. 2008;71(4):604–6.
23. Buljan M, Poduje S, Situm M, Bulat V, Bolanca Z, Tomas D. Multiple angiokeratomas of the vulva: case report and literature review. Acta Dermatovenerol Croat. 2010;18(4):271–5.
24. McNeely TB. Angiokeratomas of the clitoris. Arch Pathol Lab Med. 1992;116(8):880–1.
25. Ulker V, Cakir E, Gedikbasi A, Akyol A, Numanoglu C, Gulkilik A. Angiokeratoma of the clitoris with evident vulvar varicosity. J Obstet Gynaecol Res. 2010;36(6):1249–51.
26. Nakamura T, Kaneko H, Nishino I. Angiokeratoma corporis diffusum (Fabry disease): ultrastructural studies of the skin. Act Derm Venereol. 1981; 61(1):37–41.
27. Requena L, Sangueza OP. Cutaneous vascular proliferation. Part II. Hyperplasias and benign neoplasms. J Am Acad Dermatol. 1997;37(6):887–919.
28. Gupta S, Radotra BD, Kumar B. Multiple, genital lobular capillary haemangioma (pyogenic granuloma) in a young woman: a diagnostic puzzle. Sex Transm Infect. 2000;76(1):51–2.
29. Arikan DC, Kiran G, Savar H, Kostu B, Coskun A, Kiran H. Vulvar pyogenic granuloma in a postmenopausal woman: case report and review of the literature. Case Rep Med. 2011;2011:201901.
30. Kaur T, Gupta S, Kumar B. Multiple pyogenic granuloma involving female genitalia: a rare entity? Pediatr Dermatol. 2004;21(5):614–5.
31. Patrice SJ, Wiss K, Mulliken JB. Pyogenic granuloma (lobular capillary hemangioma): a clinicopathologic study of 178 cases. Pediatr Dermatol. 1991; 8(4):267–76.
32. LeBoit PE. Lobular capillary proliferation: the underlying process in diverse benign cutaneous vascular neoplasms and reactive conditions. Semin Dermatol. 1989;8(4):298–310.
33. Nichols GE, Gaffey MJ, Mills SE, Weiss LM. Lobular capillary hemangioma: an immunohistochemical study including steroid hormone receptor status. Am J Clin Pathol. 1992;97(6):770–5.
34. Kim TH, Lee HH, Koh ES. Intravascular papillary endothelial hyperplasia (Masson's tumour) in the vulva. Eur J Obstet Gynecol Reprod Biol. 2013; 169(2):413–4.
35. Maghari A, Lambert WC, Sherman N, Heller DS. Intravascular papillary endothelial hyperplasia (Masson tumor) manifesting as a cystic vulvar lesion. J Low Genit Tract Dis. 2009;13:260–3.
36. Amerigo J, Berry CL. Intravascular papillary endothelial hyperplasia in the skin and subcutaneous tissue. Virchows Arch A Pathol Anat Histol. 1980;387(1):81–90.
37. Bruni V, Pontella V, Dei M, Alessandrini M, Li Marzi V, Nicita G. Hemangioma of the clitoris presenting as clitoromegaly: a case report. J Pediatr Adolesc Gynecol. 2009;22(5):e137–8.
38. Kondi-Pafiti A, Kairi-Vassilatou E, Spanidou-Carvouni H, Kontogianni K, Dimopoulou K, Goula K. Vascular tumors of the female genital tract: a clinicopathological study of nine cases. Eur J Gynaecol Oncol. 2003;24(1):48–50.
39. Djunic I, Elezovic I, Ljubic A, Markovic O, Tomin D, Tadic J. Diffuse cavernous hemangioma of the left leg, vulva, uterus, and placenta of a pregnant woman. Int J Gynaecol Obstet. 2009;107(3):250–1.
40. Cebesoy FB, Kutlar I, Aydin A. A rare mass formation of the vulva: giant cavernous hemangioma. J Low Genit Tract Dis. 2008;12(1):35–7.
41. Mouhari-Toure A, Azoumah KD, Tchamdja K, Saka B, Kombate K, Tchangai-Walla K, Pitche P. Rapid regression of infantile haemangioma with 2 % propranolol treatment. Ann Dermatol Venereol. 2013;140(6–7):462–4.
42. Frieden IJ, Haggstrom AN, Drolet BA, Mancini AJ, Friedlander SF, Boon L, et al. Infantile hemangiomas: current knowledge, future directions. Proceedings of a research workshop on infantile hemangiomas, April 7–9, 2005, Bethesda, Maryland. Pediatr Dermatol. 2005;22(5):383–406.
43. Hermans DJ, Bauland CG, Zweegers J, van Beynum IM, van der Vleuten CJ. Propanolol in a case series of 174 patients with complicated infantile haemangioma: indications, safety and future directions. Br J Dermatol. 2013;168(4):837–43.
44. Menezes MD, McCarter R, Greene EA, Bauman NM. Status of propranolol for treatment of infantile hemangioma and description of a randomized clinical trial. Ann Otol Rhinol Laryngol. 2011; 120(10):686–95.
45. North PE, Waner M, Mizeracki A, Mihm Jr MC. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. Hum Pathol. 2000;31(1):11–22.
46. North PE, Waner M, Mizeracki A, Mark RE, Micholas R, Kincannon J, et al. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. Arch Dermatol. 2001; 137(5):559–70.
47. Boon LM, Enjolras O, Mulliken JB. Congenital hemangiomas: evidence for accelerated involution. J Pediatr. 1996;128(3):329–35.
48. Berenguer B, Mulliken JB, Enjolras O, Boon LM, Wassef M, Josset P, et al. Rapidly involuting congenital hemangioma: clinical and histopathologic features. Pediatr Dev Pathol. 2003;6(6):495–510.
49. Enjolras O, Mulliken JB, Boon LM, Wassef M, Kozakewich HP, Burrows PE. Noninvoluting congenital hemangioma: a rare cutaneous vascular

anomaly. Plast Reconstr Surg. 2001;107(7): 1647–54.
50. Goh SG, Calonje E. Cutaneous vascular tumours: an update. Histopathology. 2008;52(6):661–73.
51. Vlastos AT, Malpica A, Follen M. Lymphangioma circumscriptum of the vulva: a review of the literature. Obstet Gynecol. 2003;101(5 Pt 1):946–54.
52. Roy KK, Agarwal R, Agarwal S, Kumar S, Malhotra N, Gopendru N. Recurrent vulval congenital lymphangioma circumscriptum – a case report and literature review. Int J Gynecol Cancer. 2006;16(2):930–4.
53. Yildiz F, Atahan IL, Ozyar E, Karcaltincaba M, Cengiz M, Ozyigit G, et al. Radiotherapy in congenital vulvar lymphangioma circumscriptum. Int J Gynecol Cancer. 2008;18(3):556–9.
54. Aggarwal K, Gupta S, Jain VK, Marwah N. Congenital lymphangioma circumscriptum of the vulva. Indian Pediatr. 2009;46(5):428–9.
55. Ozeki M, Fukao T, Kondo N. Propranolol for intractable diffuse lymphangiomatosis. N Engl J Med. 2011;364(14):1380–2.
56. Darmstadt GL. Perianal lymphangioma circumscriptum mistaken for genital warts. Pediatrics. 1996;98(3 Pt 1):461–3.
57. Sonobe H, Ro JY, Ramos M, Diaz I, Mackay B, Ordonez NG, Ayala AG. Glomus tumor of the female external genitalia: a report of two cases. Int J Gynecol Pathol. 1994;13(4):359–64.
58. Katz VL, Askin FB, Bosch BD. Glomus tumor of the vulva: a case report. Obstet Gynecol. 1986;67(3 Suppl):43S–5S.
59. Jagadha V, Srinivasan K, Panchacharam P. Glomus tumor of the clitoris. N Y State J Med. 1985; 85(10):611.
60. Kohorn EI, Merino MJ, Goldenhersh M. Vulvar pain and dyspareunia due to glomus tumor. Obstet Gynecol. 1986;67(3 Suppl):41S–2S.
61. Dervan PA, Tobbia IN, Casey M, O'Loughlin J, O'Brien M. Glomus tumours: an immunohistochemical profile of 11 cases. Histopathology. 1989;14(5): 483–91.
62. Brooks JJ, Miettinen M, Virtanen I. Desmin immunoreactivity in glomus tumors. Am J Clin Pathol. 1987;87(2):292.
63. Hall DJ, Burns JC, Goplerud DR. Kaposi's sarcoma of the vulva: a case report and brief review. Obstet Gynecol. 1979;54(4):478–83.
64. Laartz BW, Cooper C, Degryse A, Sinnott JT. Wolf in sheep's clothing: advanced Kaposi sarcoma mimicking vulvar abscess. South Med J. 2005;98(4): 475–7.
65. Da Silva BB, Lopes-Costa PV, Furtado-Veloso AM, Borges RS. Vulvar epithelioid hemangioendothelioma. Gynecol Oncol. 2007;105(2):539–41.
66. Strayer SA, Yum MN, Sutton GP. Epithelioid hemangioendothelioma of the clitoris: a case report with immunohistochemical and ultrastructural findings. Int J Gynecol Pathol. 1992;11(3):234–9.
67. Guirquis A, Kanbour-Shakir A, Kelley J. Epithelioid angiosarcoma of the mons after chemoradiation for vulvar cancer. Int J Gynecol Pathol. 2007;26(3): 265–8.
68. Sanz C, Moreno F, Armas A, Casado A, Castillo MC. Groin angiosarcoma following radiotherapy for vulvar cancer. Gynecol Oncol. 2005;97(2):677–80.
69. Huey GR, Stehman FB, Roth LM, Ehrlich CE. Lymphangiosarcoma of the edematous thigh after radiation therapy for carcinoma of the vulva. Gynecol Oncol. 1985;20(3):394–401.
70. Leborgne F, Falconi LM. Lymphangiosarcoma of the anterior abdominal wall: a case report. Cancer. 1980;46(5):1228–30.
71. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science. 1994;266(5192): 1865–9.
72. Mohammed AZ, Nwana EJ, Manasseh AN. Changing patterns of Kaposi's sarcoma in Nigerians. Trop Doct. 2005;35(3):168–9.
73. Russell Jones R, Orchard G, Zelger B, Wilson JE. Immunostaining for CD31 and CD34 in Kaposi sarcoma. J Clin Pathol. 1995;48(11):1011–16.
74. Kahn HJ, Bailey D, Marks A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. Mod Pathol. 2002;15(4):434–40.
75. Reis RM, Reis-Filho JS, Longatto Filho A, Tomarev S, Silva P, Lopes JM. Differential Prox-1 and CD31 expression in mucousae, cutaneous and soft tissue vascular lesions and tumors. Pathol Res Pract. 2005;201(12):771–6.
76. Cheuk W, Wong KO, Wong CS, Dinkel JE, Ben-Dor D, Chan JK. Immunostaining for human herpesvirus 8 latent nuclear antigen-1 helps distinguish Kaposi sarcoma from its mimickers. Am J Clin Pathol. 2004;121(3):335–42.
77. Pantanowitz L, Pinkus GS, Dezube BJ, Tahan SR. HHV8 is not limited to Kaposi's sarcoma. Mod Pathol. 2005;18(8):1148–50.
78. Long SR, Whitfeld MJ, Eades C, Koehler JE, Korn AP, Zaloudek CJ. Bacillary angiomatosis of the cervix and vulva in a patient with AIDS. Obstet Gynecol. 1996;88(4 Pt 2):709–11.
79. Weiss SW, Enzinger FM. Epithelioid hemangioendothelioma: a vascular tumor often mistaken for a carcinoma. Cancer. 1982;50(5):970–81.
80. Deyrup AT, Tighiouart M, Montag AG, Weiss SW. Epithelioid hemangioendothelioma of soft tissue: a proposal for risk stratification based on 49 cases. Am J Surg Pathol. 2008;32(6):924–7.
81. Gray MH, Rosenberg AE, Dickersin GR, Bhan AK. Cytokeratin expression in epithelioid vascular neoplasms. Hum Pathol. 1990;21(2):212–7.
82. Miettinen M, Fetsch JF. Distribution of keratins in normal endothelial cells and a spectrum of vascular tumors: implications in tumor diagnosis. Hum Pathol. 2000;31(9):1062–7.
83. Mendlick MR, Nelson M, Pickering D, Johansson SL, Seemayer TA, Neff JR, et al. Translocation t(1;3)(p36.3;q25) is a nonrandom aberration in

epithelioid hemangioendothelioma. Am J Surg Pathol. 2001;25(5):684–7.
84. Tanas MR, Sboner A, Oliveira AM, Erickson-Johnson MR, Hespelt J, Hanwright PJ, et al. Identification of a disease-defining gene fusion in epithelioid hemangioendothelioma. Sci Transl Med. 2011;3(98):98ra82.
85. Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, et al. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. Genes Chromosomes Cancer. 2011;50(8):644–53.
86. Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Genes Chromosomes Cancer. 2013;52(8):775–84.
87. Manner J, Radlwimmer B, Hohenberger P, Mossinger K, Kuffer S, Sauer C, et al. MYC high-level gene amplification in a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. Am J Pathol. 2010;176(1):34–9.
88. Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. Mod Pathol. 2012;25(1):75–85.
89. Shon W, Sukov WR, Jenkins SM, Folpe AL. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. Mod Pathol. 2014;27(4):509–15.
90. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. Genes Chromosomes Cancer. 2011;50(1):25–33.
91. Santos LD, Krivanek MJ, Chan F, Killingsworth M. Pseudoangiosarcomatous squamous cell carcinoma of the vulva. Pathology. 2006;38(6):581–4.
92. Horn LC, Liebert UG, Edelmann J, Hockel M, Einenkel J. Adenoid squamous carcinoma (pseudoangiosarcomatous carcinoma) of the vulva: a rare but highly aggressive variant of squamous cell carcinoma – report of a case and review of the literature. Int J Gynecol Pathol. 2008;27(2):288–91.
93. Bell D, Kane PB, Liang S, Conway C, Tornos C. Vulvar varices: an uncommon entity in surgical pathology. Int J Gynecol Pathol. 2007;26(1):99–101.
94. Cohen-Sacher B, Berger MB, Fenner DE, Burney RE, Haefner HK. Vulvar varicosities mimicking a hernia: case report. J Low Genit Tract Dis. 2012;16(4):464–7.
95. Vin F. Vulvar varices. J Mal Vasc. 1990;15(4):409–9.
96. Leung SW, Leung PL, Yuen PM, Rogers MS. Isolated vulvar varicosity in the non-pregnant state: a case report with review of treatment options. Aust N Z J Obstet Gynaecol. 2005;45(3):254–6.
97. Scultetus AH, Villavicencio JL, Gillespie DL, Kao TC, Rich NM. The pelvic venous syndromes: analysis of our experience with 57 patients. J Vasc Surg. 2002;36(5):881–8.
98. Ninia JG, Goldberg TL. Treatment of vulvar varicosities by injection-compression sclerotherapy and pelvic supporter. Obstet Gynecol. 1996;87(5 Pt 1):786–8.
99. Handfield-Jones SE, Prendiville WJ, Norman S. Vulval lymphangiectasia. Genitourin Med. 1989;65(5):335–7.
100. Mu XC, Tran TA, Dupree M, Carlson JA. Acquired vulvar lymphangioma mimicking genital warts. A case report and review of the literature. J Cutan Pathol. 1999;26(3):150–4.
101. Pedica F, Ligorio C, Tonelli P, Bartolini S, Baccarini P. Lymphangiogenesis in Crohn's disease: an immunohistochemical study using monoclonal antibody D2-40. Virchows Arch. 2008;452(1):57–63.
102. Heatley RV, Bolton PM, Hughes EL, Owen EW. Mesenteric lymphatic obstruction in Crohn's disease. Digestion. 1980;20(5):307–13.

15 Tumors of Smooth Muscle, of Skeletal Muscle, and of Unknown Origin and Tumor-Like Conditions of the Vulva

Kristen M. Paral and Christopher R. Shea

Introduction

Reviews on vulvovaginal mesenchymal tumors [1–5] devote little (if any) attention to the wide range of soft tissue lesions that may occur aside from the vulvar-specific mesenchymal neoplasms. Therefore, a pathologist facing a mesenchymal lesion might inadvertently narrow the scope of diagnostic possibilities. Contributing further to the diagnostic challenge is that many of the entities discussed herein present with nonspecific clinical findings. For instance, the sarcomas often present as seemingly benign, cystic lesions of the labia. Yet, the microscopic picture engenders a malignant differential, often complicated by frequent morphologic and immunophenotypic overlap among the different entities. Fortunately, for many of these sarcomas, molecular testing has emerged as a powerful diagnostic tool. The results of molecular and other ancillary studies must always be interpreted within the context of the histopathologic impression, which requires knowledge of both the classic and variant histologies. The ensuing text delves into these and other facets of selected vulvar lesions, which include malignant, benign, and non-neoplastic entities.

C.R. Shea, M.D. (✉)
Department of Medicine, The University of Chicago Medicine, Chicago, IL, USA
e-mail: cshea@medicine.bsd.uchicago.edu

Tumors of Smooth Muscle

Leiomyoma and Leiomyosarcoma

Clinical Features

Among smooth muscle tumors of the gynecological tract, the number of reported vulvar cases pales in comparison to the number of uterine cases [6–9]. Despite a common origin within the gynecological tract, vulvar and uterine smooth muscle tumors fall into separate categories. In fact, smooth muscle tumors of the external genitalia (vulva and scrotum) have been traditionally considered part of the cutaneous group (which includes arrector pili tumors), although mounting efforts have pushed for separation of genital tumors owing to clinicopathologic disparities [4, 7, 8, 10]. The true incidence of these tumors is unknown, but reports of vulvar leiomyoma outnumber those of vulvar leiomyosarcoma by nearly 3:1 [3]. Vulvar smooth muscle tumors usually present in the third through fifth decades as a painless mass of the labia majora, less often involving the labia minora, vaginal introitus, perineum, or clitoris [7–9, 11–16]. Mild pain or dyspareunia may accompany the mass [7–10, 17–19], which rarely ulcerates [9, 20]. A number of women report enlargement of the mass during pregnancy [9, 19, 21]. Patients report an onset ranging from 5 weeks to 5 years [7, 11, 17, 22]. As with other mesenchymal neoplasms of the vulva, the most commonly rendered clinical

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2_15, © Springer Science+Business Media New York 2015

diagnosis is a Bartholin cyst [7, 18, 19, 23]; less commonly, acquired clitoromegaly is diagnosed [20].

Rarely, the patient presents with a diffuse or multinodular vulvar proliferation rather than a discrete mass, in which case the term *leiomyomatosis* is applied [24]. Owing to the frequency of coexisting esophagogastric leiomyomatosis in these patients together with an occasional familial component, consideration of *esophagovulvar syndrome* seems reasonable [24, 25]. However, these patients may harbor smooth muscle proliferations in other organs as well, such as the uterus [25], bladder [26], and tracheobronchial tree [27]. Thus, the findings in these patients might more appropriately fall under the umbrella of *diffuse leiomyomatosis,* which has been linked to Alport syndrome and its associated mutations in the genes encoding alpha chains of type IV collagen [28].

Prognosis or Course

Unlike in the uterus, where the large number of cases permits systematic study of features predicting tumor behavior [6, 29, 30], the paucity of data on vulvar smooth muscle tumors hinders the ability to formulate robust criteria to distinguish leiomyoma from leiomyosarcoma [4, 7–10]. Over 30 years ago, Tavassoli et al. [9] laid foundations based on follow-up data on 28 patients, which included four recurrences. Three of these recurrences exhibited at least one of the following criteria: size greater than 5 cm, 5 or more mitotic figures per 10 high-power fields, and infiltrating margins. One recurrence lacked all criteria, leading to the conclusion that no case can be guaranteed freedom from recurrence. A subsequent study of 25 vulvar cases by Nielsen et al. [8] largely validated the Tavassoli criteria, with the addition of moderate-to-severe cytologic atypia. The Nielsen group additionally noted coagulative tumor cell necrosis—an established worrisome feature in uterine tumors [30]—as a feature present in two overtly malignant tumors. Later works accepted coagulative tumor cell necrosis as a worrisome feature and approved the proposed criteria for categorizing vulvar smooth muscle tumors as shown in Table 15.1 [3, 5, 10]. The follow-up data from series on vulvar smooth muscle tumors showed that of 58 patients with variable follow-up periods, 8 experienced recurrence and 1 developed metastasis leading to death [7–9]. Among case reports of leiomyosarcoma resulting in death, the typical disease progression pursues a course of one or more local recurrences followed by metastasis and eventual death [8]. Authors agree that leiomyosarcomas should be excised widely, but no consensus has been reached on the proper width. Some authors promote 1–2 cm, while others promote more than 2 cm (which may result in radical vulvectomy) [12]. For atypical leiomyomas, a 1-cm margin has been recommended [3, 4]. Leiomyomas can be excised conservatively with a rim of surrounding normal tissue [4, 8].

Table 15.1 Recommended classification scheme for vulvar smooth muscle tumors

Criteria	
• Size > 5 cm	
• ≥ 5 mitotic figures per 10 high-power fields	
• Infiltrative edges	
• Moderate or severe cytologic atypia	
• Coagulative necrosis[a]	
Number of criteria met	*Diagnosis*
0–1[b]	Leiomyoma
2	Atypical Leiomyoma
> 3	Leiomyosarcoma

[a]Although the initial studies did not include coagulative necrosis as a worrisome feature, subsequent works recommended its inclusion (see text)
[b]Recurrence potential still exists in this category, leading some to suggest a diagnosis of atypical leiomyoma if any criterion is met (regardless of size) [4, 10]

Etiology/Pathogenesis

Vulvar smooth muscle tumors may arise from the tunica dartos labialis within the dermis of the labia majora, the muscular walls of vessels within the crura forming the clitoris, and the erectile tissues within the labia minora [31]. As the pathobiology underlying uterine and soft tissue smooth muscle tumors continues to unravel [6, 30, 32, 33], similar progress in the vulva lags behind. Nonetheless, knowledge of hormone receptor

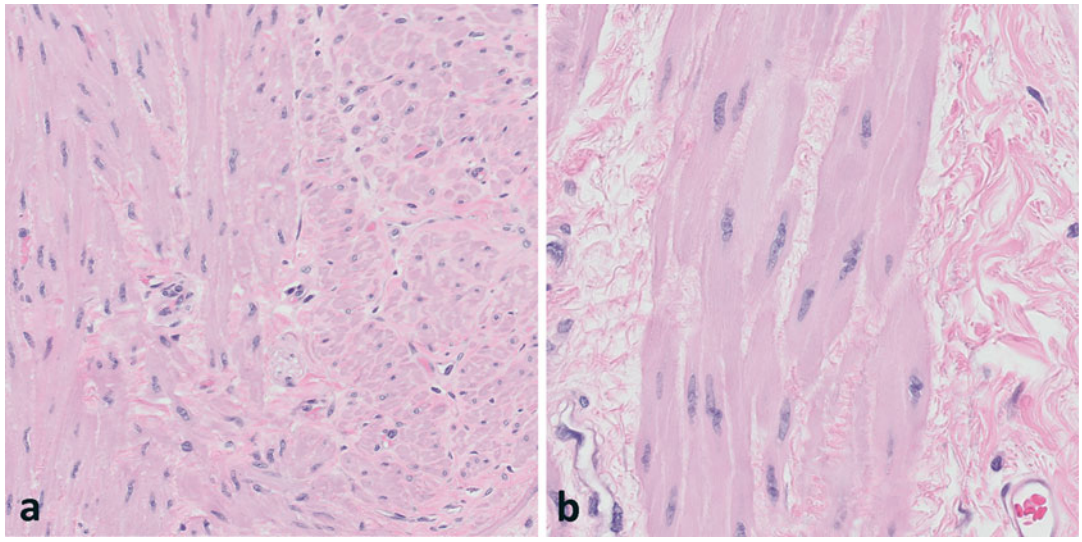


Fig. 15.1 Leiomyoma, spindle cell pattern. (**a**) Fascicles often intersect perpendicularly. (**b**) The spindle cells demonstrate eosinophilic cytoplasm. This field shows both cigar-shaped and corkscrew nuclei

expression in uterine tumors drove analogous vulvar studies [8], which found that most vulvar smooth muscle tumors express estrogen receptor (ER) and progesterone receptor (PR) [8]. These findings, together with ties to pregnancy and hormone therapy [9, 21, 34], support a pathogenic role for hormones and indicate potential for treatment with hormone manipulation [5], which has demonstrated success in uterine tumors [6]. Notably, cutaneous smooth muscle tumors lack ER/PR expression [35], supporting their nosological separation from genital tumors. A potential limiting factor in separating genital and cutaneous tumors is that part of the vulva (the mons pubis and labia majora) is covered by skin. The authors of the studies on vulvar tumors [7–9] do not clarify how cutaneous tumors were excluded from their material.

Histopathology

Macroscopic inspection reveals a gray or tan, rubbery, usually circumscribed tumor averaging 5 cm [8]. Microscopic examination discloses interlacing fascicles of fusiform cells with eosinophilic cytoplasm and cigar-shaped (sometimes corkscrew) nuclei that may have adjacent cytoplasmic vacuoles (Fig. 15.1a, b). The spindled morphology predominates among vulvar tumors, but the cells occasionally assume an epithelioid appearance, with abundant eosinophilic to clear cytoplasm and round cell borders (Fig. 15.2a). Usually, these epithelioid cells intermingle with the typical spindled cells. Vulvar tumors often demonstrate a myxohyaline extracellular matrix that percolates among the fascicles of cells, imparting a plexiform or lacy appearance (Fig. 15.2b). This feature is noted in a higher proportion of vulvar than uterine tumors. Mucin pools may be observed [4, 5, 7, 8, 10]. The histopathologic features of malignancy are reviewed in Table 15.1 (see also Figs. 15.3a–c and 15.4).

Immunohistochemical Features

Pan-muscle markers (desmin and muscle-specific actin) highlight both smooth and striated muscle. For unknown reasons, desmin expression can be absent from smooth muscle tumors arising from vessel walls, making smooth muscle actin (SMA) a better screening marker for smooth muscle tumors [36]. SMA and calponin expression is seen in smooth muscle and myofibroblasts. H-caldesmon and smooth muscle myosin heavy chain demonstrate specific (but variably sensitive) expression with respect to smooth muscle [37, 38]. Generally, expression of the above

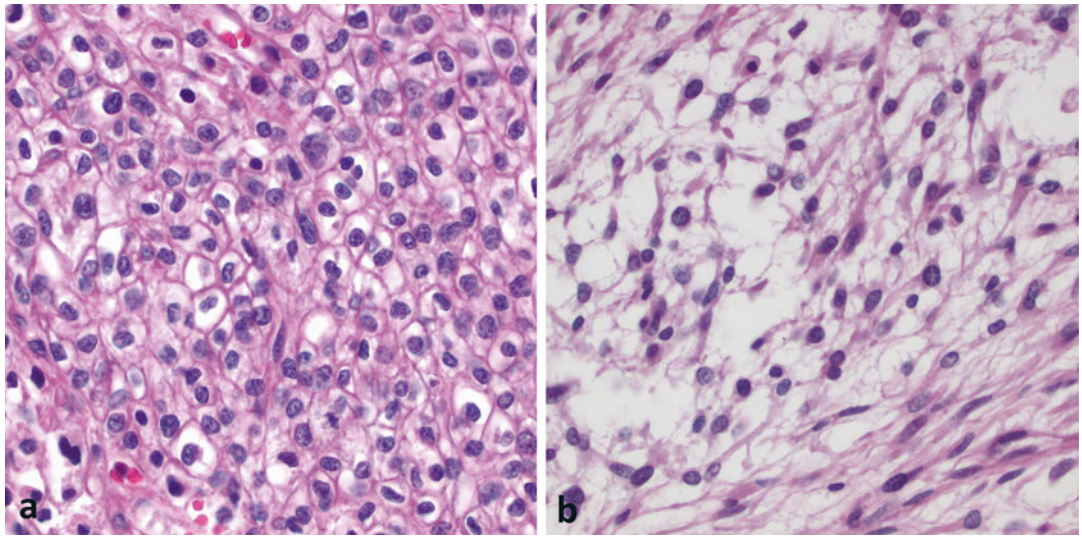


Fig. 15.2 Histomorphologic patterns in smooth muscle tumors. (**a**) Epithelioid morphology with well-defined cell borders and abundant, pale to clear cytoplasm. (**b**) Myxohyaline extracellular matrix imparts a lacy configuration (Images by authors, from case of Dr. Thomas Krausz)

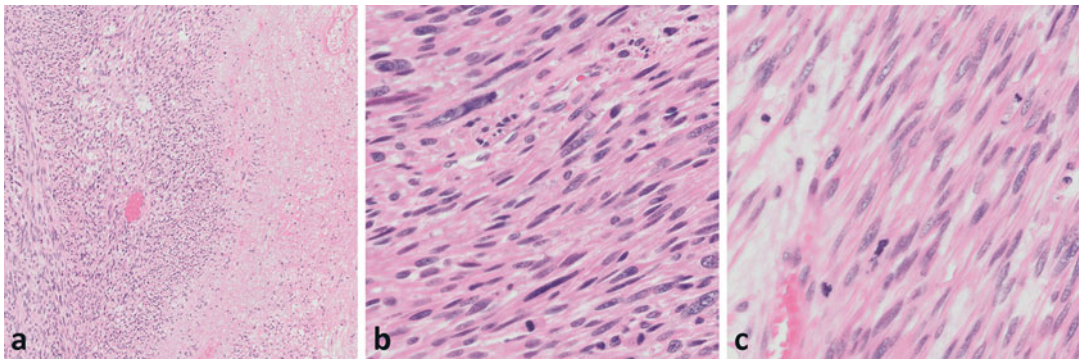


Fig. 15.3 Features of malignancy in smooth muscle tumors. (**a**) Coagulative necrosis, as seen on the right side of the field, should be viewed with concern. (**b**) Cytologic atypia is seen as pleomorphic, hyperchromatic nuclei with occasional bizarre forms. (**c**) Numerous mitotic figures are apparent

markers declines with dedifferentiation and pleomorphic transformation [39]. As noted above, most vulvar smooth muscle tumors express ER and PR [8].

Genetics and Molecular Findings

Numerous aberrations in genetic material and molecular signaling pathways have emerged from studies on smooth muscle tumors in the uterus and soft tissues [6, 30, 32, 33], indicating heterogeneous and complex underpinnings. In the vulva, a single cytogenetic study of a leiomyoma revealed clonal rearrangement of 8q11, a finding detected in uterine leiomyomas [22], but further studies are indicated.

Differential Diagnosis

In the vulva, cellular angiofibroma may enter the differential owing to its intersecting, spindle-shaped cells in a fibrous stroma. In this tumor, mitotic figures can be numerous without clinical consequence, whereas in smooth muscle tumors, they constitute a criterion for malignancy [1, 3–5]. Myxoid components of

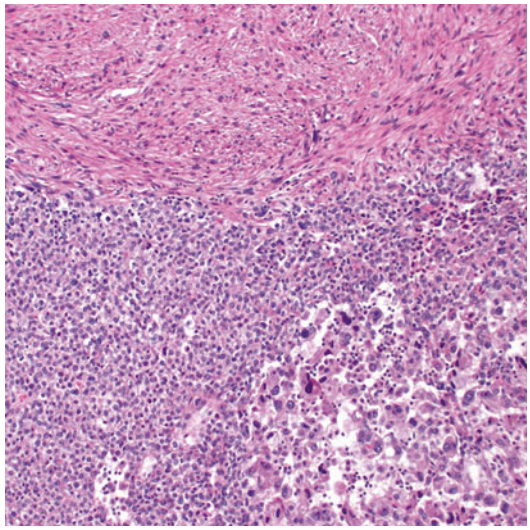


Fig. 15.4 Leiomyosarcoma. This unusual case demonstrates the convergence of three morphologic patterns: spindled (*top*), epithelioid (*left* and *center*), and pleomorphic transformation (*bottom right*) (Images by authors, from case of Dr. Thomas Krausz)

smooth muscle tumors have been confused with the characteristically paucicellular aggressive angiomyxoma [18]. Distinction between the two may require ancillary studies in cases with sampling issues. Table 15.2 aims to resolve this differential. Overtly malignant smooth muscle neoplasms evoke another set of possible diagnoses, including other spindle cell sarcomas, spindle cell carcinoma, and spindle cell melanoma. When low-power patterns and nuclear features fail to provide the answer, immunohistochemistry is required. A recommended panel includes epithelial markers (broad-spectrum cytokeratins and epithelial membrane antigen), S100, SMA, and desmin. Potential immunohistochemical pitfalls include rare SMA expression in spindle cell carcinomas [45], occasional cytokeratin expression in leiomyosarcoma [46], and rare desmin expression in melanoma [36].

Table 15.2 The differential diagnosis of vulvar smooth muscle tumors

	Smooth muscle tumors	Cellular angiofibroma[a]	Aggressive angiomyxoma
Clinical features	• Wide age range of adult women • Usually circumscribed • Benign > malignant	• Wide age range of adult women • Usually circumscribed • Benign	• Women of reproductive age • Poorly circumscribed, infiltrative, displaces pelvic structures
Histopathology	• Intersecting fascicles of spindle cells (sometimes epithelioid) with cigar-shaped nuclei • Myxohyaline extracellular matrix • Regard mitotic figures with concern	• Short fascicles of spindle cells with scant, fibrillary cytoplasm and fusiform nuclei (resemble spindle cells of spindle cell lipoma) • Fibrous stroma, wispy collagen • Numerous thick-walled, hyalinized blood vessels • Mitotic figures inconsequential	• Paucicellular proliferation of small, bland, ovoid, or stellate-shaped cells • Myxoid background • Variably sized vessels from which bundles of smooth muscle radiate • No mitotic figures or atypia
Ancillary studies	• SMA + • Desmin + • H-caldesmon + • Usually ER/PR + • HMGA2 +/– (up to 43 % of vulvar leiomyomas are +) • Rare, focal CD34 expression reported in leiomyosarcoma	• Most SMA – • Most desmin – • Most h-caldesmon – • CD34 + in up to 60 % • Usually ER/PR + • Loss of RB1 and FOXO1 (13q14 region)	• Desmin +/– • SMA +/– • CD34 +/– • ER/PR + • HMGA2 + (most)

Data from: Refs. [1, 4, 5, 8, 40–43]

[a]Leiomyomatous nodules have been reported within cellular angiofibromas [44]

Summary

Clinical Presentation

- Patients are usually adults with a mass of the labia majora, but any vulvar site can be involved.
- Leiomyomatosis presents as a diffuse or multinodular proliferation.
- The patient may report enlargement during pregnancy or hormone therapy.

Histologic Features

- Intersecting fascicles of spindled, sometimes epithelioid cells with eosinophilic cytoplasm
- Cigar-shaped (sometimes corkscrew) nuclei, with occasional adjacent paranuclear vacuoles
- Myxohyaline extracellular matrix separates fascicles, imparting a plexiform appearance

Differential Diagnosis

- Vulvar-specific neoplasms cellular angiofibroma and aggressive angiomyxoma are often considered.
- Leiomyosarcoma can mimic other spindle cell sarcomas, spindle cell carcinoma, and spindle cell melanoma.

Takeaway Essentials

Clinically Relevant Pearls

- These lesions must be excised widely for thorough microscopic evaluation to exclude malignancy and to reduce the risk of recurrence.
- Any mass can recur, regardless of histologic features.

Pathology Interpretation Pearls

- Although smooth muscle neoplasms at all body sites are united by common morphologic characteristics of their constituent cells, each anatomic location must be considered separately owing to different criteria for predicting behavior.
- Worrisome features in smooth muscle tumors of the vulva:
  - Size > 5 cm
  - ≥ 5 mitotic figures per 10 high-power fields
  - Infiltrative edges
  - Moderate or severe cytologic atypia
  - Coagulative necrosis

Immunohistochemical/Molecular Findings

- A supportive immunohistochemical panel includes desmin, smooth muscle actin, and h-caldesmon.
- Smooth muscle tumors of the vulva express ER and PR, unlike their skin and soft tissue counterparts.

Tumors of Skeletal Muscle

Rhabdomyoma

Clinical Features

Rhabdomyomas are divided into two major clinicopathologic categories: cardiac or extracardiac. The extracardiac group is further subdivided into adult, fetal, and genital types, with the genital type being the rarest [47–51]. Adult and fetal rhabdomyomas tend to involve the head and neck region [50, 52]. Genital rhabdomyomas involve the paratesticular region in males and the vulvovaginal region in females. Over 30 cases of genital rhabdomyoma have been reported in females, of which the vast majority arise from the vagina [47, 51]. A few reported cases involve the vulva [53], cervix [54, 55], urethra [56], and even ovary [57]. The average patient presents in the fourth decade [47, 58] with a polypoid vulvovaginal mass that produces a foreign body sensation [58] or dyspareunia [59]. Alternatively, the lesion is discovered incidentally [47, 55, 56, 60, 61]. Physical examination reveals a 1–3 cm, rubbery-to-firm polyp, rarely cystic [61], often pedunculated, with a smooth, intact surface, similar to a fibroepithelial polyp [47, 52, 55, 56, 58–60, 62].

Prognosis or Course

Genital rhabdomyomas manifest benign behavior, with no reported metastases [47] and only a single reported recurrence following excision [63]. Simple excision is the treatment of choice [47, 59, 60, 62].

Etiology/Pathogenesis

The pathogenesis of rhabdomyoma remains elusive. Some authors favor a hamartomatous rather than neoplastic origin [48, 62], but the distinction between the two is obscured by the finding that both entities can be clonal [64]. Given the definition of a hamartoma as a disorganized mass of cells indigenous to a particular site [64], this theory can only explain those developing near the vaginal orifice [48], which contains skeletal muscle as a component of the striated urogenital sphincter complex [65]. Moreover, hamartomas typically occur in young and old alike, whereas genital rhabdomyomas show a predilection for middle-aged women [47, 48, 60].

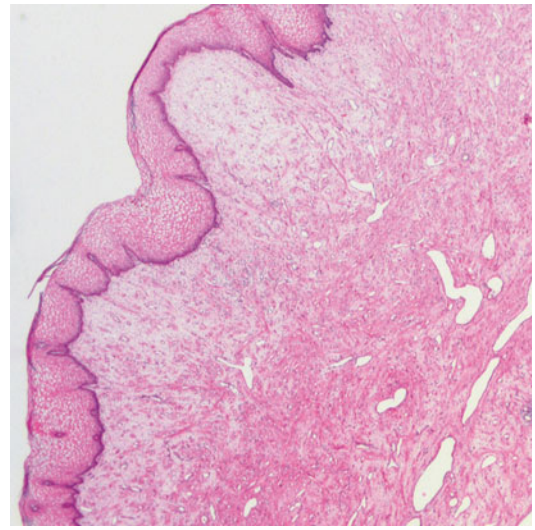


Fig. 15.5 Genital rhabdomyoma. Low-power examination shows unremarkable, glycogenated, non-keratinizing, squamous epithelium overlying stroma containing loosely interweaving, brightly eosinophilic, polymorphous cells (Images by authors, from case of Dr. Thomas Krausz)

Histopathology

Histologic examination reveals unremarkable squamous epithelium overlying loose connective tissue stroma bearing loosely interweaving, polymorphous cells with abundant, brightly eosinophilic cytoplasm, ranging in shape from fusiform to polygonal to strap-like, with variably conspicuous cross-striations (Figs. 15.5 and 15.6a, b). Nuclei are centrally or peripherally located, are round to oval in shape, and display occasional prominent nucleoli [48, 49, 52, 56, 58]. Cells may contain multiple nuclei [52, 55]. Nuclear atypia and mitotic figures are universally absent [47, 48, 52, 56, 58, 60, 62].

Immunohistochemical Features

Immunohistochemical studies confirm skeletal muscle differentiation, with expression of myogenin and alpha-sarcomeric actin. Pan-muscle markers desmin and muscle-specific actin are also expressed [56, 66]. Masson trichrome [67] and phosphotungstic acid-hematoxylin [48, 62, 67] stains were historically applied to highlight cross-striations. However, the characteristic cell morphology together with cross-striations visible by light microscopy usually obviates the need for such ancillary studies.

Genetics and Molecular Findings

Little is known about aberrations in molecular pathways in genital rhabdomyoma. In contrast, fetal rhabdomyoma is associated with alterations in sonic hedgehog signaling, with or without concomitant basal cell nevus syndrome [51]. Emerging studies support a role for activated hedgehog signaling in the pathobiology of fetal rhabdomyoma [68]. Assessment of hedgehog signaling in genital rhabdomyomas would be of interest.

Differential Diagnosis

The two entities that most commonly enter the differential for rhabdomyoma are embryonal rhabdomyosarcoma and fibroepithelial polyp [47–49, 62]. The significant distinguishing features are summarized in Table 15.3. Consideration may also be given to rhabdomyomatous mesenchymal hamartoma, which is exceedingly rare in the vulvovaginal region [70]. This lesion consists of a disordered mixture of skeletal muscle fibers, mature adipose tissue, nerve bundles, and adnexal structures [71].

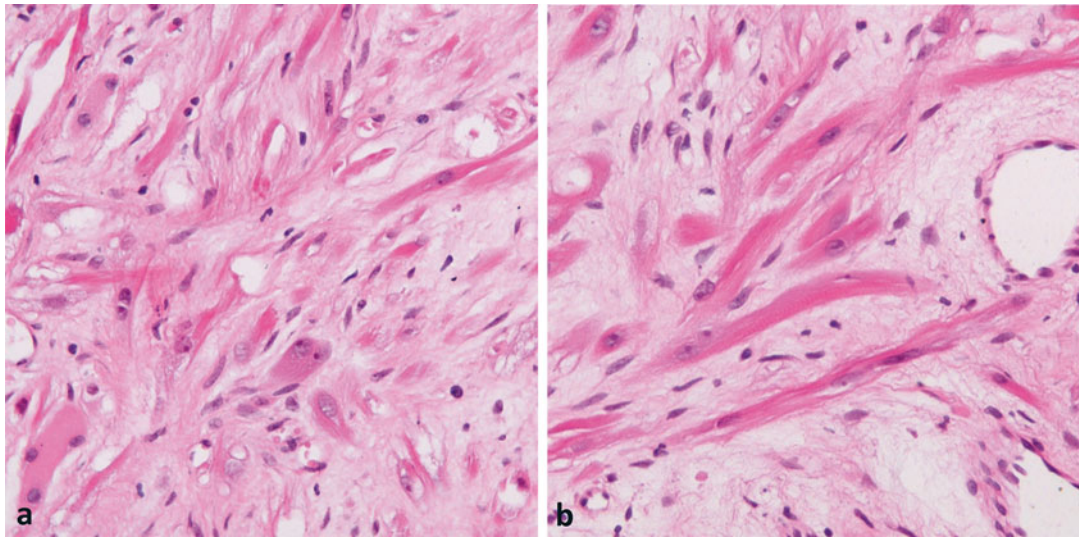


Fig. 15.6 Genital rhabdomyoma. (**a**) Polygonal and strap-like cells are present. Some cells have multiple, peripheral nuclei. (**b**) Cross-striations can be seen (Images by authors, from case of Dr. Thomas Krausz)

Table 15.3 The differential diagnosis of genital rhabdomyoma

	Genital rhabdomyoma	Rhabdomyosarcoma (embryonal/botryoid)	Fibroepithelial polyp
Clinical features	• Adult with a polypoid lesion • Non-ulcerated mucosal covering	• Young female with a mass • Rapid growth • Ulceration of overlying mucosa	• Adult with a polypoid lesion • Non-ulcerated mucosal covering • May be multiple and large
Histopathologic features	• Differentiated, eosinophilic cells, ranging from polygonal to fusiform to strap-like • Cross-striations often present • No cambium layer • No nuclear atypia • No mitotic figures	• Mostly primitive mesenchymal cells • Limited myogenic differentiation, with occasional eosinophilic cells with cross-striations • Cambium layer (condensation of neoplastic cells below epithelium) • Nuclear atypia present • Mitotic figures present	• Scattered fibroblastic cells • May contain markedly atypical stromal cells, with increased mitotic rate in pregnancy (termed cellular pseudosarcomatous fibroepithelial polyps or pseudosarcoma botryoides)
Ancillary studies	• Desmin + • Myogenin +	• Desmin + • Myogenin + • t(1;13), t(2;13)	• ER/PR + (almost always) • Pitfall: rarely focally positive for desmin, myogenin

Data from: Refs. [47–49, 55, 62, 66, 69]

Summary

Clinical Presentation

- Polypoid vulvovaginal mass in women in their 4th decade, either asymptomatic or with symptoms relating to mass effect

Histologic Features

- A spectrum of differentiated skeletal muscle cells appears as interweaving, polymorphous cells with abundant, brightly eosinophilic cytoplasm.
- Cells can be polygonal, fusiform, or strap-like, with or without cross-striations.
- Nuclear atypia and mitotic figures are always absent.

Differential Diagnosis

- Consider embryonal rhabdomyosarcoma and fibroepithelial polyp.

Takeaway Essentials

Clinically Relevant Pearls

- This is a benign lesion with no metastatic potential.

Pathology Interpretation Pearls

- Recognition of a range of muscle cells in later stages of differentiation is key.
- Any atypia, mitotic figures, or primitive mesenchymal cells should raise suspicion for embryonal rhabdomyosarcoma.

Immunohistochemical/Molecular Findings

- Immunohistochemistry does not distinguish rhabdomyoma from rhabdomyosarcoma, but recognition of the mature striated muscle elements usually obviates the need for ancillary studies.
- An immunohistochemical pitfall is presented by focal myogenin and desmin expression in fibroepithelial polyp.

Rhabdomyosarcoma

Clinical Features

Since its emergence in the literature in 1946 [72], rhabdomyosarcoma (RMS) has unveiled several faces, consisting of embryonal (including botryoid), alveolar, and pleomorphic types. Currently, the World Health Organization provisionally lists a separate spindle cell/sclerosing type, but, until recently, it was generally included with embryonal cases [51]. RMS is known as a childhood sarcoma, constituting the largest proportion of soft tissue sarcomas in the pediatric population [73]. Although relatively rare in adults, RMS can affect any age, with age predilections corresponding to subtype [74, 75]. Embryonal RMS is the most common type, most commonly seen in children under age 10, with up to one-half of cases involving the genitourinary tract [73–76]. Second to embryonal in frequency is the alveolar type, which affects a slightly older cohort than embryonal RMS and most often arises in the extremities [73–75, 77]. A small fraction of both embryonal and alveolar types occur in the setting of genetic syndromes involving germ line mutations, such as Beckwith-Wiedemann syndrome [78–80]. The rare pleomorphic type preferentially affects the extremities of older adults [81, 82].

Among cases arising within the gynecological tract, the typical patient is a child or adolescent with a vaginal, uterine, or cervical mass. However, a range of ages from infancy to elderly have been affected, with involvement of the ovaries, fallopian tubes, labia, clitoris, and perineum [83–89]. Patients may present with vaginal bleeding or abdominal pain when the mass arises internally [83, 84]. Masses involving the external genitalia may present as a painless or painful mass [88, 90, 91] that can overgrow and efface the vulvar anatomy [86] or, if in a child, may produce asymmetry mimicking childhood asymmetric labium majus enlargement (Fig. 15.7) [89].

Prognosis or Course

Notoriously an aggressive neoplasm, RMS overall carries 5-year survival rates of 27 % and 61 % for adults and children, respectively [92]. Similar rates are seen in the genital tract [83, 85]. Embryonal

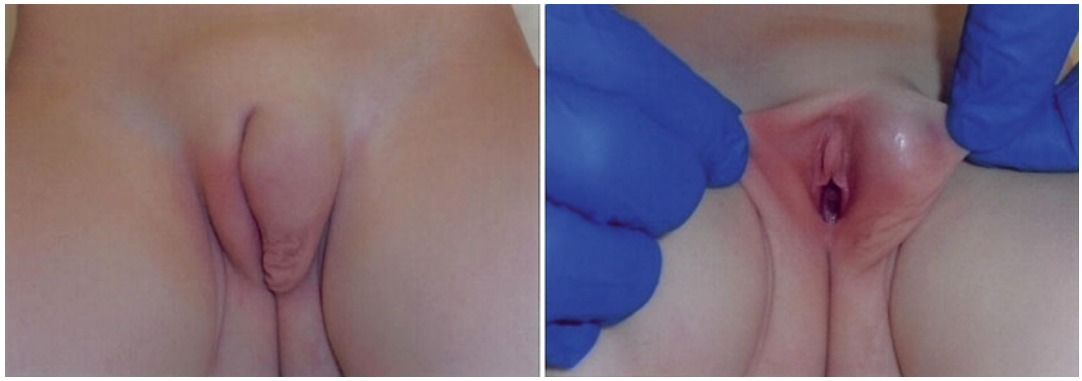


Fig. 15.7 This case of vulvar rhabdomyosarcoma resembled childhood asymmetric labium majus enlargement (Used with permission from Youngstrom EA, Bartkowski DP et al. Vulvar embryonal rhabdomyosarcoma: A case report. J Pediatr Urol. 2013;9(4):e144–6)

RMS manifests more favorable behavior than the other types [82, 84, 93]. All age groups enjoy a better outcome with localized disease, including pediatric gynecological tumors [85]. Data on adult genital RMS are suggestive but do not reach statistical significance [83]. Current data support multimodal treatment for both children and adults, incorporating a combination of surgery, chemotherapy, and radiotherapy [75, 76, 83–85].

Etiology/Pathogenesis

The progenitor cell remains unknown but is postulated to be a mesenchymal stem cell transformed by myogenic and oncogenic events [94]. Genetic aberrations in RMS, whether sporadic or in association with germ line mutation syndromes, underlie changes in cell growth and apoptosis, myogenic differentiation, cell motility, and tumor suppression [94–96].

Histopathology

Other than the botryoid variant of embryonal RMS, the typical RMS specimen consists of a pale-tan, fleshy mass [51]. Embryonal morphology accounts for most genital RMS in general, but alveolar morphology more commonly accounts for vulvar lesions [83, 85–87, 91, 97]. Only one reported case of embryonal RMS occurred in the vulva [89]. Tumors with mixed alveolar-embryonal histology can occur [95]. Both types demonstrate a proliferation of primitive mesenchymal cells with scant cytoplasm and round nuclei. In embryonal RMS, these primitive cells assume a stellate shape and are suspended in a matrix of loose, myxoid material alternating with zones of cellular condensation (Fig. 15.8a–c). A cellular condensation beneath an epithelium (cambium layer) defines the botryoid variant of embryonal RMS [93]. The primitive, stellate cells often exhibit varying degrees of myogenic differentiation, characterized by the acquisition of dense, eosinophilic cytoplasm, peripheral displacement of the nucleus, and cellular elongation, eventually evolving into polymorphous shapes described as strap-like, spidery, and tennis-racket-like (Fig. 15.9a–c). With terminal differentiation, the rhabdomyoblasts fuse into multinucleated cells and develop cross-striations, a feature present in a minority of cases [93, 96].

Compared with the embryonal type, the alveolar type shows less obvious myogenic differentiation, often restricted to a thin rim of eosinophilic cytoplasm around the nucleus [74, 93]. Also, whereas the primitive cells in embryonal RMS assume a stellate shape, the cells of alveolar RMS are monomorphous, small, round cells, hence the alternative designation, "monomorphous round cell" RMS [74]. Alveolar RMS consists of fibrovascular septa forming pseudoalveolar spaces that compartmentalize the neoplastic cells (Fig. 15.10a). A layer of tumor cells is aligned along the septa (Fig. 15.10b), with dishesive clusters in the centers of the pseudoalveolar spaces. Multinucleated giant cells with a wreath-like nuclear configuration can

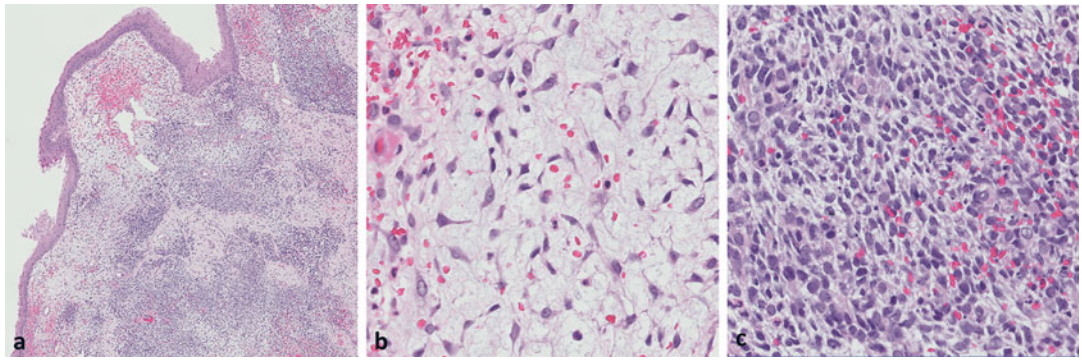


Fig. 15.8 Embryonal RMS. (**a**) Low-power view shows alternating zones of cellularity. Note the absence of a cambium layer. (**b**) High-power view of the hypocellular regions reveals the stellate-shaped, primitive mesenchymal cells. (**c**) High-power view of a cellular condensation

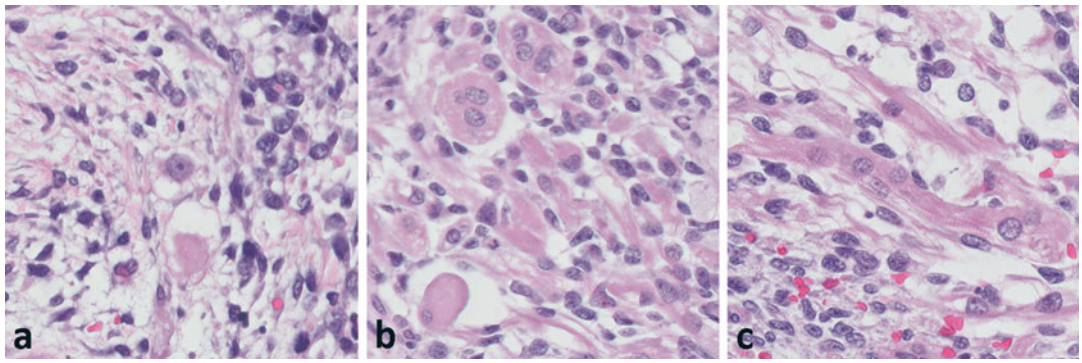


Fig. 15.9 Spectrum of myogenic differentiation. (**a**) Spidery cells. (**b**) Polygonal and strap-like cells. (**c**) Strap-like cells with cross-striations (*center*)

be seen (Fig. 15.10d) [74]. Occasionally, the neoplastic cells grow in sheets without clear septal architecture, in which case the term "solid variant" is applied (Fig. 15.10d). Some cases may show clear-cell change [74, 93] or anaplasia [98, 99].

The pleomorphic type constitutes a small fraction of RMS cases in the gynecological tract, mainly affecting the uterus [81–83, 100]. The neoplastic cells are spindled or polygonal, with large, pleomorphic nuclei [81]. Tumors with the spindle cell/sclerosing morphology display fascicles of fusiform cells intermixed with variable amounts of collagen [93, 101].

Immunohistochemical Features

Since skeletal muscle cells have a unique phenotype, a small panel of immunostains can establish the diagnosis [96]. Numerous studies have demonstrated high sensitivity and specificity of nuclear markers myogenin and MyoD1, which identify cells committed to skeletal muscle differentiation in their early stages [102–105]. Pan-muscle markers desmin and muscle-specific actin offer considerably less specificity [102], although desmin helps to assign cells to a general phenotypic group [96]. At present, myogenin and MyoD1 are the best available markers for confirming a histologic impression of RMS [102]. By far, most tumors entering the differential, including small round cell tumors and spindle cell tumors, lack expression of these markers [102–106]. However, the frequent background cytoplasmic staining for MyoD1 limits interpretation of this marker [102–105]. Notably, entrapped nonneoplastic skeletal muscle may express either marker [102, 104, 105]. Some studies demonstrated differences in staining

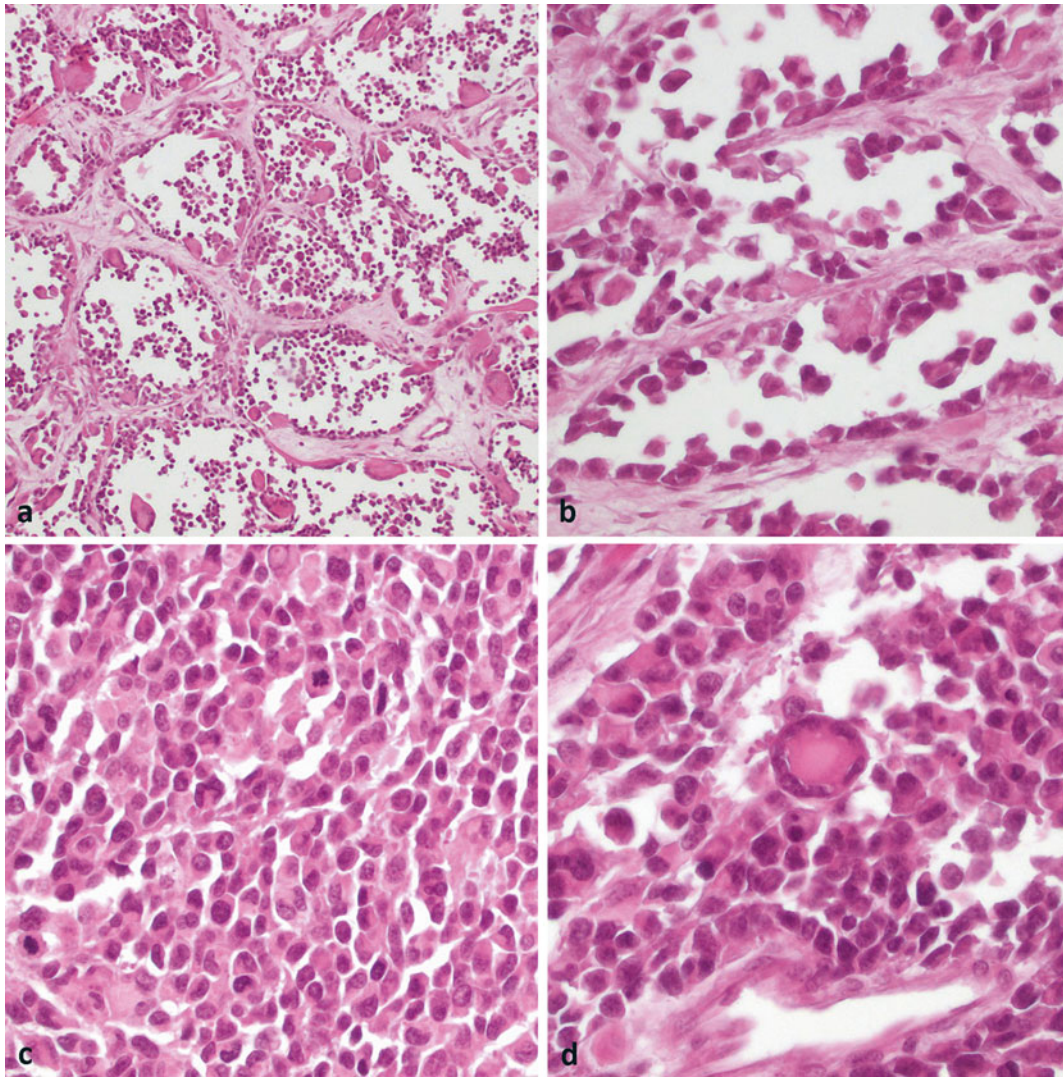


Fig. 15.10 Alveolar RMS. (**a**) Fibrous septa compartmentalize the cells. (**b**) The neoplastic cells align along the fibrous septa. (**c**) The solid variant consists of sheets of neoplastic cells. (**d**) A multinucleated giant cell with a wreath-like nuclear configuration is seen (Images by authors, from case of Dr. Thomas Krausz)

patterns between alveolar and embryonal types, with alveolar tending to stain more diffusely than embryonal [102–104]. Data are limited on the pleomorphic subtype, but expression of nonspecific muscle markers and at least one skeletal-muscle-specific marker is expected [81].

Genetics and Molecular Findings

Initially described by Seidal and colleagues [107], recurrent translocation t(2;13) characterizes most alveolar RMS, with t(1;13) being found in a minority [108, 109]. These translocations generate chimeric genes encoding PAX3- and PAX7-FOXO1 fusion proteins, which enhance transcriptional activity and ultimately affect cell-cycle regulation, apoptosis, and myogenesis [95, 96]. On the other hand, embryonal RMS lacks recurrent translocations, instead bearing multiple gains and losses of chromosome material. Notably, the region most affected by allelic loss (11p15.5) corresponds to

the locus associated with Beckwith-Wiedemann syndrome [95]. Interestingly, embryonal RMS demonstrates an RNA expression profile similar to that of translocation-negative alveolar RMS and has similar favorable clinical behavior relative to fusion-positive alveolar RMS [95, 110–112], supporting the use of fusion status to assess risk and to guide therapy.

Differential Diagnosis

Morphologic and immunophenotypic overlap exists between RMS and primitive ectomesenchymoma, a sarcoma with both myogenic and neural differentiation. This entity behaves similarly to RMS [113]. Alveolar RMS bears morphologic semblance to various small round cell tumors. Immunohistochemistry usually resolves the differential, but overlap can be seen with desmin and CD99 (see Table 15.9) [96]. Notably, neuroendocrine markers may be expressed in 30–40 % of alveolar RMS, emphasizing the importance of applying a panel of markers to include desmin and myogenin or MyoD1 in the diagnosis of small round cell tumors [116]. Embryonal RMS often engenders a deceptively benign impression at first pass owing to its loose, myxoid background and varying degrees of large, eosinophilic cells. It resembles rhabdomyoma or fibroepithelial polyp with atypical stromal cells (pseudosarcoma botryoides) [69]. Importantly, myogenic marker expression in the latter poses a diagnostic pitfall [66, 96]. RMS with spindle cell morphology can mimic smooth muscle tumors and be SMA positive [96, 101], thus increasing the utility of myogenin and MyoD1. Table 15.4 reviews the key features of rhabdomyosarcoma. See also Vignette 2 at the end of this chapter.

Table 15.4 Review of rhabdomyosarcoma of the gynecological tract

	Embryonal	Alveolar	Other
Clinical features	• Children < 10 years	• Children and adolescents	• Pleomorphic type arises in the extremities of adults
	• Only one case report describes a vulvar origin (the vulva is more likely to be indirectly involved)	• More common than embryonal in the vulva	• Spindle cell/sclerosing variant is not clearly a subtype
	• Botryoid: bunch of grapes		
Behavior	• Relatively favorable	• Relatively aggressive	• Pleomorphic → aggressive
Histopathology	• Primitive mesenchymal cells with stellate shape	• Primitive mesenchymal cells with a round shape	• Pleomorphic type has large cells with pleomorphic nuclei
	• Range of myogenesis (gain of eosinophilic nuclei, peripheral nucleus, strap-like cells)	• Restricted range of myogenic differentiation	• Spindle cell/sclerosing morphology has fascicles of fusiform cells intermixed with variable amounts of collagen
	• Myxoid background, variable cellular condensations	• Fibrovascular septa compartmentalizes dishesive cells	
	• Botryoid: cambium layer (condensation of neoplastic cells below epithelium)	• ±Wreath-like multinucleated giant cells • Solid variant: sheets of cells	
Differential diagnosis	• Rhabdomyoma, fibroepithelial polyp	• Small round cell tumors	• Spindled morphology → smooth muscle neoplasms
	• Small round cell tumors		
Ancillary studies	• Lack recurrent abnormalities	• Often more diffuse myogenin expression than embryonal	• Spindled can be SMA +
	• Chromosome gains and losses	• Recurrent translocations t(2;13), t(1;13)	• Lack recurring abnormalities

Takeaway Essentials

Clinically Relevant Pearls

- Consider RMS as a rare cause of a vulvovaginal mass, vulvar asymmetry, or vaginal bleeding.
- Although vulvar RMS is most commonly alveolar, the embryonal type can protrude into the vulva from more proximal sources.
- This aggressive neoplasm requires prompt institution of multimodal therapy.

Pathology Interpretation Pearls

- Histologic classification carries prognostic value, with a relatively favorable outcome for embryonal RMS compared with the aggressive alveolar RMS.
- The solid variant of alveolar RMS is particularly susceptible to morphologic overlap with other neoplasms, requiring ancillary studies to confirm the diagnosis.

Immunohistochemical/Molecular Findings

- Because embryonal RMS lacks recurrent cytogenetic abnormalities, molecular testing is less useful as a diagnostic aid than in alveolar RMS.
- Demonstration of recurrent translocation t(2;13) or t(1;13) in alveolar RMS corresponds to more aggressive behavior, whereas cases lacking a recurrent translocation behave more favorably, similar to embryonal RMS.

Tumorlike Conditions

Traumatic Neuroma

Clinical Features

Traumatic neuroma is a nonneoplastic proliferation of neural tissue occurring in response to transection of a nerve following surgery or other trauma [117], most commonly occurring on the lower extremities after amputation (hence, the alternative designation "amputation neuroma"), followed by the head and neck, especially after tooth extraction [118]. Only a handful of cases have been reported in the vulva, seen in association with episiotomy scar [119, 120], traumatic fall [121], and female genital mutilation [122, 123]. Given the estimated 125 million girls and women across the world who have undergone female genital mutilation [124] together with the relatively high frequency of neuromas developing after limb amputation at 12–26 % [118, 125], vulvar neuromas are perhaps underreported [122] or underdiagnosed [123]. Patients may not present for many years following the initial injury [119]. Clinical examination discloses a tender clitoral nodule (Fig. 15.11) [122, 123], an irregular scar-like thickening of the labia minora [119], or vaguely defined labial pain without a palpable abnormality [121]. Patients report a spectrum of symptoms, including dyspareunia [119, 122, 123] or even pain with simply sitting or wearing tightly fitting clothing [123]. In one case, the patient required analgesics to permit coitus [121]. Surgical excision successfully resolves the symptoms [119–123].

Etiology/Pathogenesis

Normally, after a nerve is injured or severed, the distal segment degenerates while the proximal segment sends regenerating axons distally. The regenerating axons extend toward and into the endoneurial tubes of the degenerating distal segment under the guidance of bands of Büngner, which are columns of proliferating Schwann cells [126]. The outcome of the regenerative attempt hinges on several factors, such as the length of the path from the axons to their final destination as well as the presence of any obstruction within this path (e.g., fibrosis) [127]. If the regeneration path is obstructed by scar tissue, as in traumatic neuroma, then the axons may turn backward onto themselves, leading to a tumultuous knot of nerve fibers [128, 129]. Within this altered environment, the tangled, intact axons become hypersensitive and spontaneously active, generating abnormal pain signals

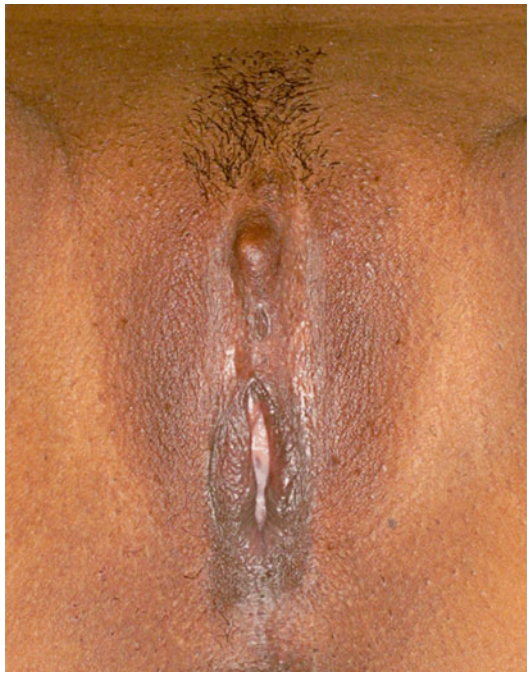


Fig. 15.11 Traumatic neuroma. This painful clitoral nodule developed after female genital mutilation (Used with permission from Abdulcadir J. et al. Clitoral neuroma after female genital mutilation/cutting: a rare but possible event. J Sex Med. 2012;9(4):1220–5)

out of proportion to the stimulus [130, 131], producing the constellation of pain symptoms described above.

Histopathology

The histology reflects the failed attempt at regeneration, demonstrating variably sized, haphazardly arranged nerve fascicles embedded within a collagenous matrix, often in association with a connecting nerve (Fig. 15.12a, b) [128, 132]. The fibrotic matrix may demonstrate myxoid change, corresponding to acidic mucopolysaccharides [128]. The nerve fascicles themselves are normal [132], each ensheathed by perineurial cells, rendering an appearance of multiple separate nerves [128, 133].

Immunohistochemistry/Special Techniques

The perineurial cells encasing the nerve fascicles demonstrate epithelial membrane antigen and GLUT-1 expression (Fig. 15.13) [134, 135]. D2-40 (podoplanin) and claudin-1 immunostaining also highlights the perineurium [136, 137]. Numerous axons within each

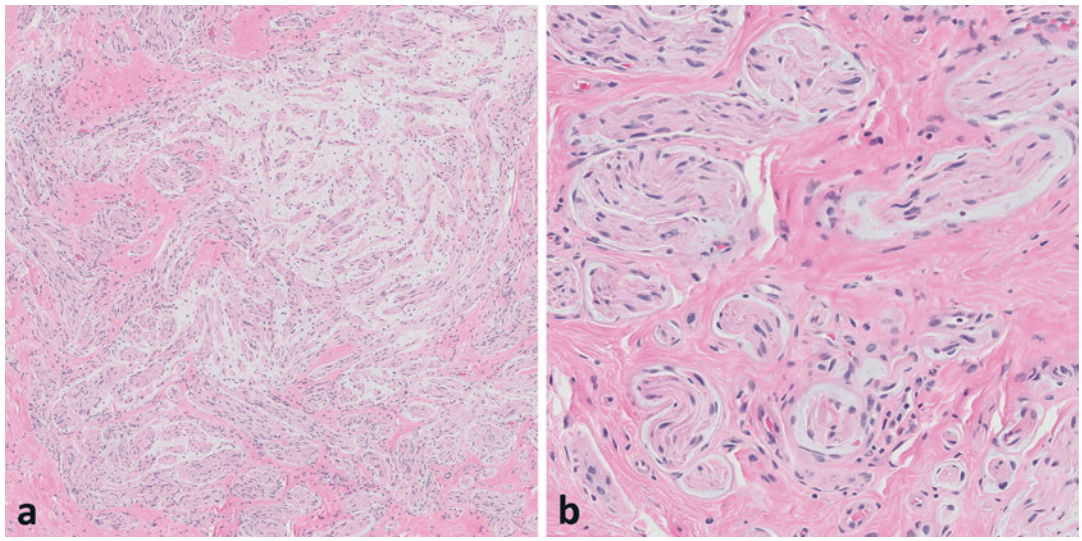


Fig. 15.12 Traumatic neuroma. (**a**) A disrupted nerve segment (*upper right*) gives rise to haphazardly arranged nerve fascicles. (**b**) The nerve fascicles are embedded within a fibrotic stroma

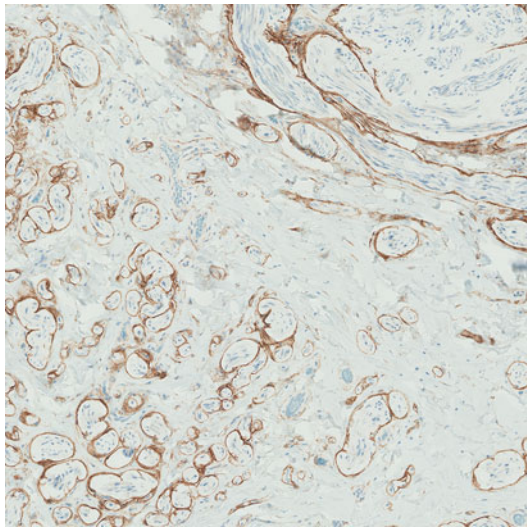


Fig. 15.13 Epithelial membrane antigen immunohistochemistry highlights the perineurium that surrounds each nerve fascicle in traumatic neuroma (×10)

fascicle can be highlighted either by silver stains (Bielschowsky or Bodian techniques) [128, 132] or by immunohistochemistry for neurofilament [133]. S100-positive Schwann cells and CD34-positive dendritic stromal cells are also present within the fascicles, as in normal nerves [135].

Differential Diagnosis

Occasionally, solitary circumscribed neuroma or neurofibroma enters the differential. The salient discriminatory features are shown in Table 15.5.

Table 15.5 The differential diagnosis of traumatic neuroma

	Traumatic neuroma	Solitary circumscribed neuroma	Neurofibroma
Clinical differences	• History of trauma	• No clear association with trauma	• In neurofibromatosis, the vulva can be affected as part of a multifocal process
	• Usually painful	• Asymptomatic	• Can be pruritic
	• Develop at sites of nerve transection	• Genital involvement has been reported	
Morphologic differences	• Overall lesion is not encapsulated	• Circumscribed and partially encapsulated by thin perineurium	• Some are circumscribed
	• Haphazard nerve bundles compartmentalized by fibrous tissue	• Compact bundles of Schwann cells without intervening fibrous tissue	• Encapsulation depends on type[a]
		• Frequent interfascicular clefting	• Proliferation of neural elements in a fibrillary or myxoid background
			• Fibrotic stroma is not a feature; if collagen is present, it is usually dispersed in loose clusters
			• Mast cells are common
Immunohistochemical differences	• EMA + perineurium surrounds individual nerve fascicles	• Discontinuous EMA + perineurium restricted to periphery of the lesion	• EMA expression in few cells, usually scattered throughout the lesion

[a]Intraneural neurofibroma is ensheathed by a thick epineurium or perineurium (EMA +)
Data from: Refs. [51, 128, 132, 133]

Summary

Clinical Presentation

- Patients experience pain ranging from mild to excruciating.
- The painful site may be a palpable nodule or thickening, or it may not be palpable at all.

Histologic Features

- Cross-sectioning through a tangle of nerve fibers appears microscopically as a haphazard arrangement of nerve fascicles.
- The contents of the nerve fascicles mirror those of a normal nerve.
- The stroma surrounding the fascicles is usually fibrotic but can show myxoid change.

Differential Diagnosis

- Solitary circumscribed neuroma and neurofibroma may be considered.

Takeaway Essentials

Clinically Relevant Pearls

- Suspect traumatic neuroma for any painful vulvar lesion accompanied by a history of episiotomy, female genital mutilation, or other trauma.

Pathology Interpretation Pearls

- This tumorlike condition can be distinguished from other neural lesions by recognizing the fibrotic stroma separating discrete nerve fascicles.

Immunohistochemical/Molecular Findings

- Within the fascicles, immunostains for neurofilament, S100, and CD34 highlight axons, Schwann cells, and dendritic stromal cells, respectively.
- Surrounding each fascicle is a perineurial layer, which expresses EMA and GLUT-1 (also D2-40 and claudin-1).

Langerhans Cell Histiocytosis

Clinical Features

Ever since the 1987 recommendation by the Writing Group of the Histiocyte Society [138], Langerhans cell histiocytosis (LCH) has been the unifying term for a group of clinically heterogeneous diseases that were once considered as separate entities under the names Hand-Schüller-Christian disease, Letterer-Siwe disease, eosinophilic granuloma, histiocytosis X, Hashimoto-Pritzker syndrome, self-healing histiocytosis, and pure cutaneous histiocytosis. The prototypical LCH patient is a male child or young adult with bony involvement, either localized or multifocal. However, all ages, both genders, and nearly all organ systems have been affected, including the skin, lung, lymph nodes, liver, spleen, mucosal sites, and endocrine glands [139, 140]. Cutaneous manifestations are relatively common [141], but involvement of the female genital tract is unusual [139], with most cases representing part of a multifocal process [142, 143]. Localized disease affects the vulva more often than the vagina, cervix, endometrium, or ovary [142]. Approximately 30 cases of isolated vulvar LCH have been described, affecting a range of ages from infancy to elderly (average 50 years) [142–144]. Nearly half of vulvar lesions present as ulcers (Fig. 15.14), followed by erythematous plaques, irregular nodules, papules, and macules [144], frequently accompanied by pruritus or pain [143, 144]. The clinical picture can simulate venereal disease, eczema, or Paget disease [142, 144–146].

Prognosis or Course

Axiotis et al. [145] proposed four nosological groups for LCH of the genital tract based on clusters of clinical findings, but they found no prognostic differences among these groups. Despite its limited utility [142], this grouping paradigm has appeared in multiple subsequent reports [146–151]. LCH usually follows an indolent course, with few disease-related deaths [139,

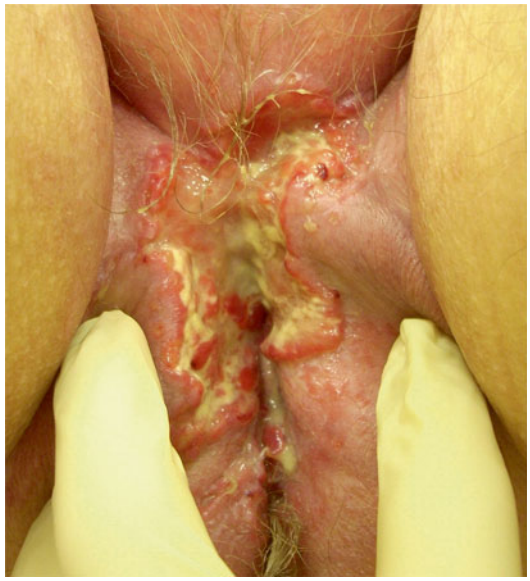


Fig. 15.14 Vulvar Langerhans cell histiocytosis. This painful and pruritic ulceration involves mainly the vestibular portion of the vulva (Used with permission from El-Safadi et al. Management of adult primary vulvar Langerhans cell histiocytosis: review of the literature and a case history. Eur J Obstet Gynecol Reprod Biol. 2012;163(2):123–8)

140, 152]. In a recent review of 27 cases of purely vulvar LCH, 18 cases achieved complete remission following initial treatment, but over half recurred [144]. In general, the combination of osseous and extraosseous involvement often heralds progressive disease [140]. Vulvar treatments are chosen largely on an ad hoc basis and range from topical corticosteroids to radical vulvectomy, with or without chemotherapy and radiation. Thalidomide and its analogs have demonstrated success [142–144].

Etiology/Pathogenesis

The discovery of recurring BRAF mutations in over half of LCH cases, together with evidence of LCH cell clonality, supports a neoplastic basis for LCH [153]. The origin of pathologic cells (LCH cells) remains undetermined. LCH cells demonstrate phenotypic and behavioral characteristics of both resting and activated normal Langerhans cells (LCs), which originally led to the assumption that the normal LC is the precursor for LCH [153]. However, a 2010 study by Allen et al. [154] challenged this assumption by revealing disparate mRNA expression profiles between the two cell types, with LCH cells demonstrating overexpression of several genes encoding myeloid dendritic cell markers. Thus, LCH cells may derive from myeloid dendritic cell precursors. At present, there is insufficient evidence to draw firm conclusions.

The overall inflammatory picture of LCH by light microscopy has led to suggestions of infectious, environmental, or immune causes [153]. The literature does not support an infectious cause [155, 156]. Immune dysregulation has been postulated based on evidence of reduced numbers of circulating suppressor T-cells in LCH patients [157], but given the role of LCs in affecting T-cell function, the LCH cells may instead drive the immune dysfunction [153]. Environmental cytokines may have a role in LCH pathobiology, particularly IL-17A, as indicated by studies showing high levels of this cytokine in the plasma of LCH patients and the ability of this cytokine to induce dendritic cell fusion in vitro, possibly explaining the appearance of multinucleated giant cells in LCH. The source of production of IL-17A is unclear [153].

Histopathology

Aggregates and clusters of histiocytoid[1] (LCH) cells occupy the dermal or subepithelial connective tissues, often with epithelial infiltration [158, 159]. Unlike normal LCs, which exhibit dendritic morphology, LCH cells have a round shape. They demonstrate abundant, pale, eosinophilic cytoplasm with indistinct cell borders and a characteristic reniform or convoluted nucleus (Fig. 15.15a, b) [139, 160]. These cells are the *sine qua non* of LCH. Varying proportions of mature eosinophils are interspersed throughout, often accompanied by lymphocytes, macrophages, and multinucleated giant cells [139]. Mitotic figures, if present, usually pertain to the

[1]Histiocytoid is used in place of histiocytic because although dendritic cells are considered histiocytes (together with macrophages), common usage today of "histiocyte" connotes a monocyte-derived tissue macrophage.

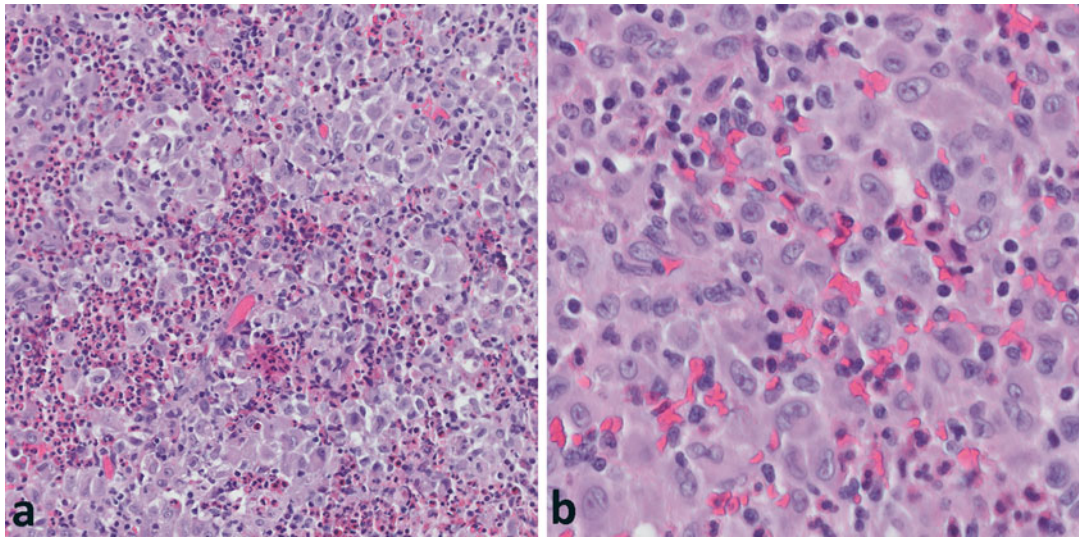


Fig. 15.15 Langerhans cell histiocytosis. (**a**) Clusters and aggregates of histiocytoid cells intermingle with numerous eosinophils, which may appear granulomatous. (**b**) Within this field are reniform and convoluted nuclei. Depending on the plane of tissue sectioning, the reniform nuclei may resemble coffee beans

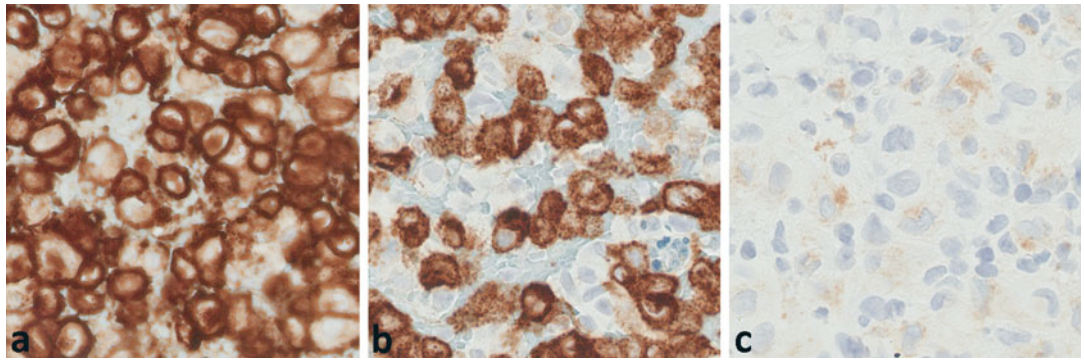


Fig. 15.16 Immunophenotype of Langerhans cell histiocytosis. (**a**) Strong, membranous CD1a expression illustrates the round, rather than dendritic, shape of the LCH cells. (**b**) Langerin expression is observed in a membranous and cytoplasmic distribution. A reniform nucleus stands out in the *bottom right corner*. (**c**) Faint CD68 positivity is seen in some of the cells bearing reniform nuclei

non-LCH cells. Fibrosis may develop in older lesions [139].

Immunohistochemical Features

Notwithstanding the characteristic histologic picture, definitive diagnosis requires ancillary techniques [160, 161]. The LCH cells consistently express S-100 protein and CD1a (Fig. 15.16a) [139, 162–164]. Diagnosis previously relied on demonstration of tennis-racket-shaped Birbeck granules by electron microscopy, but immunohistochemistry for langerin (CD207), which localizes within Birbeck granules, has largely replaced this practice (Fig. 15.16b) [161, 165–167]. CD68 expression is usually less intense than that seen in macrophages (Fig. 15.16c) [158].

Genetics and Molecular Findings

As mentioned above, over half of LCH cases harbor BRAF mutations. In the remaining cases, the BRAF signaling cascade is activated by

unknown mechanisms [153]. Recurring cytogenetic abnormalities have not been consistently found in LCH [168].

Differential Diagnosis

Clinically, vulvar LCH may simulate venereal disease, such as herpes simplex or syphilitic chancres [145], but infectious agents are not found [155, 159]. Microscopically, florid LC hyperplasia can mimic LCH as a secondary phenomenon in lymphomatoid papulosis [169] and scabies infestation [170]. In these cases, the dendritic processes of the LC cells (highlighted by immunohistochemistry) contrast with the round morphology of LCH cells. The LCH cells might be overlooked in the storm of inflammatory elements [145] or may be misinterpreted as a granulomatous process, but careful evaluation reveals their predominance. Once identified, the differential diagnosis narrows to various histiocytic proliferations, such as Erdheim-Chester disease, juvenile xanthogranuloma, and Rosai-Dorfman disease. The salient distinguishing features are provided in Table 15.6. See also Vignette 1 at the end of this chapter.

Summary

Clinical Presentation

- Vulvar LCH most commonly presents as part of a multisystem process, but localized disease can affect a wide range of ages.
- Ulceration is most common, followed by erythematous plaques, irregular nodules, papules, and macules.

Histologic Features

- The *sine qua non* of LCH is the histiocytoid LCH cell, characterized by its round shape, abundant, pale cytoplasm, and a reniform or convoluted nucleus.
- Inflammatory elements are present, with eosinophils providing a helpful clue.

Differential Diagnosis

- The clinical differential for the vulva includes eczema, venereal disease (such as herpes simplex or syphilitic chancres), and Paget disease.

Table 15.6 Differential diagnosis of vulvar LCH

	LCH	Xanthogranuloma	Rosai-Dorfman disease	Erdheim-Chester disease[a]
Morphology	• Cytoplasm abundant and pale • Nuclei reniform or convoluted • Nucleoli small to inconspicuous • Variable numbers of eosinophils; occasional microabscesses • Giant cells usually of foreign-body or Langerhans configuration	• Cytoplasm moderate to abundant and pale to lipid laden • Nuclei round to ovoid, sometimes indented • May have eosinophils • Touton giant cells	• Cytoplasm abundant and pale or clear with emperipolesis • Nuclei round to oval; can be reniform • Prominent nucleoli • Mixed inflammatory component	• Cytoplasm abundant and foamy • Fibrous tissue present • Mixed inflammatory component • Touton giant cells
Immunohistochemistry	• S100 + • CD1a + • Langerin + • CD68 +/–	• S100 – • CD1a – • Langerin – • CD68 + • Factor XIIIa +	• S100 + • CD1a – • Langerin – • CD68 +	• S100 – • CD1a – • Langerin – • CD68 +

Data from: Refs. [51, 139, 162–166, 171, 172]

[a]LCH and Erdheim-Chester disease can coexist, leading some to propose that they exist on a continuum [173, 174]

- The microscopic differential includes histiocytic proliferations such as juvenile xanthogranuloma, Rosai-Dorfman disease, and Erdheim-Chester disease.

Takeaway Essentials

Clinically Relevant Pearls

- The polymorphous clinical spectrum lends itself to a diagnostic challenge, resulting in frequent delays in diagnosis.
- Consider LCH in any persistent, atypical, or multifocal vulvar lesion.

Pathology Interpretation Pearls

- Careful evaluation must be undertaken to discern the LCH cells within the inflammatory background.
- There are no histologic features that can predict LCH behavior, which ranges from spontaneous remission to fatal dissemination.

Immunohistochemical/Molecular Findings

- The majority of LCH cells express S100, CD1a, and langerin (langerin immunohistochemistry has largely replaced the need to seek Birbeck granules by electron microscopy).
- Histiocytic proliferations in the differential diagnosis are ruled out by expression pattern of CD1a and langerin.

Tumors of Unknown Origin

Epithelioid Sarcoma

Clinical Features

Originally defined as a sarcoma of young adult males with a predilection for the aponeuroses of extremities, epithelioid sarcoma is now recognized in two forms: (1) the distal (or "classic") type and (2) the proximal type [175–177]. These two types together constitute less than 1 % of all soft tissue sarcomas [178]. The distal type reflects the original description of the tumor, presenting most commonly between ages 10 and 39 (mean 28) as a mass in the dermis, subcutis, or deep soft tissues of the distal extremities, particularly the hands and forearms [179]. A fair number (12 % [179]) present with nonhealing ulcers with raised margins [177], mimicking various inflammatory dermatoses [180]. Relatively recently, the proximal type was delineated as a unique subtype presenting at a somewhat older age (mean 35–40 years) as a deep-seated mass preferentially involving proximal axial body sites such as the trunk, limb girdles, pelvis, and external genitalia [175, 176]. Over 20 vulvar cases have been reported, presenting at an average age of 31 [181], most commonly presenting as a painless, firm mass in the labia majora simulating a benign cyst [182–184]. The mons pubis [184, 185] and labia minora may be involved [186]. Rarely, ulceration accompanies the vulvar mass [187].

Prognosis or Course

Epithelioid sarcoma usually proceeds along a relentless course of successive recurrences with eventual metastases, mainly to the lungs and lymph nodes [176, 177, 181, 188]. This is one of the few soft tissue sarcomas that regularly metastasizes to lymph nodes, reported in up to 60 % of cases [176], in contrast to only ~3 % for other soft tissue sarcomas [178]. Less commonly, epithelioid sarcoma metastasizes to the scalp [177], including from a vulvar primary [189]. A review of 20 vulvar cases by Argenta et al. [181] indicated that 50 % of patients succumbed to the disease within 8 years. Although adverse prognostic factors have not been specifically established for the vulva, those that have been demonstrated for epithelioid sarcoma in general include proximal type, FNCLCC grade 3, tumor size > 5 cm, and mitotic count ≥ 20 per 10 high-power fields [51]. Commensurate with treatment regimens elsewhere, wide surgical excision remains the cornerstone of therapy for vulvar epithelioid sarcoma, with possible radiation therapy for cases with close margins. Lymph node dissection and chemotherapy remain controversial [181, 190].

Etiology/Pathogenesis

The origin of epithelioid sarcoma has been debated since its first description [179, 191], with consideration given to fibroblastic, (fibro)histiocytic, myofibroblastic, synovial, endothelial, and perineurial lineages [177]. Guillous et al. [175] briefly entertained the notion that epithelioid sarcoma could be a primary carcinoma of soft tissues, perhaps representing the spectrum of epithelial-mesenchymal transdifferentiation. At present, epithelioid sarcoma is considered a tumor of mesenchymal cells exhibiting multidirectional differentiation, primarily epithelial [177], the pathogenesis of which has been linked to the loss of tumor suppressor SMARCB1 (also called INI-1), a protein involved in genomic stability and regulation of cell-cycle progression [192, 193].

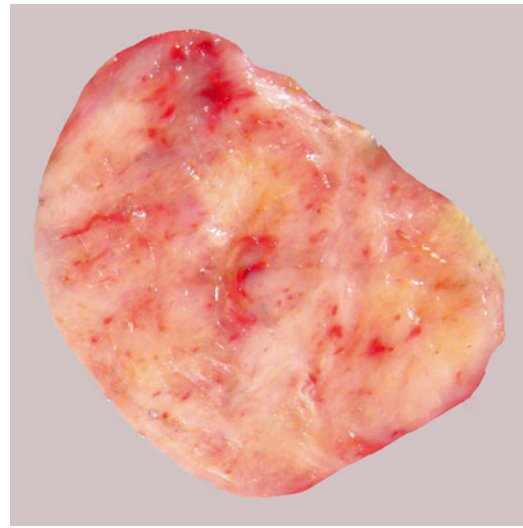


Fig. 15.17 Epithelioid sarcoma. The mass is irregular, is white-tan, and has foci of hemorrhage and necrosis

Histopathology

The usual gross appearance is of a firm, irregular, white-tan, unencapsulated mass, often with hemorrhage and necrosis (Fig. 15.17) [175, 176, 179], arising in the deep soft tissues, subcutis, or dermis [175]. Microscopically, the distal and proximal types exhibit different morphology and, although the vulva is more likely to manifest the proximal type, overlap can occur [177, 194]; occasionally, a single lesion displays hybrid features [195]. The most common and recognizable pattern of the distal type consists of a "pseudogranulomatous" proliferation of polygonal cells growing in waves around central necrosis, reminiscent of necrobiotic granuloma (Fig. 15.18a) [179]. The polygonal cells are relatively uniform, with minimal pleomorphism and voluminous eosinophilic cytoplasm, often with a loss of cohesion. These polygonal cells transition subtly into spindle cells (Fig. 15.18b, c) [177, 179]. In comparison, the proximal type grows in sheets of large, polygonal cells with voluminous cytoplasm, bearing enlarged, often pleomorphic, vesicular nuclei with frequently prominent nucleoli, consistently imparting a carcinoma-like appearance, with a minor spindle cell component (Fig. 15.19a–c) [175, 176]. Rhabdoid cells are frequently observed (Fig. 15.19c), characterized by epithelioid cells

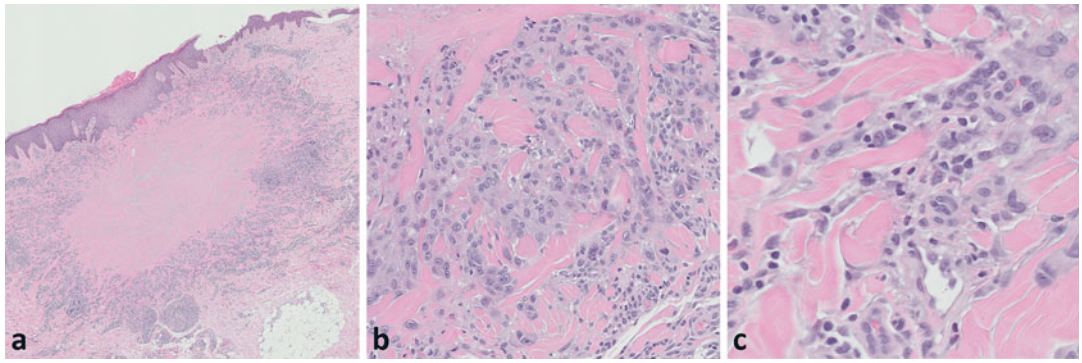


Fig. 15.18 Distal-type epithelioid sarcoma. (**a**) A low-power view discloses a pseudogranulomatous appearance. (**b**, **c**) The epithelioid appearance of cells (*right*) transitions subtly into spindle cells (*left*)

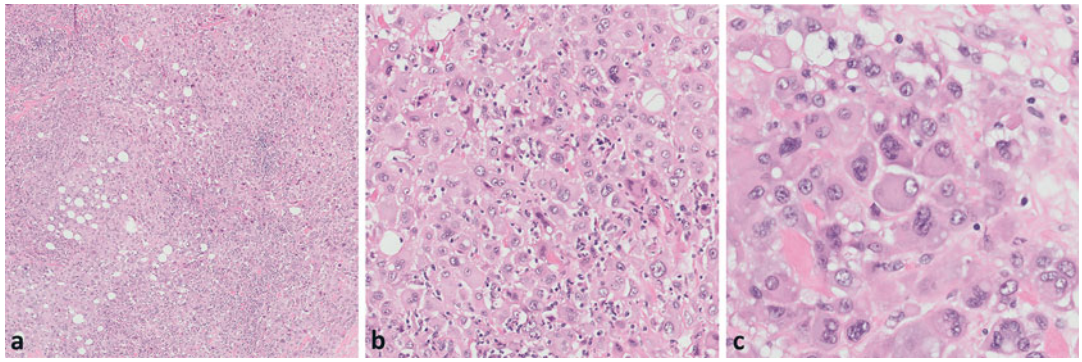


Fig. 15.19 Proximal-type epithelioid sarcoma. (**a**) Low-power view shows sheetlike growth. (**b**, **c**) Higher magnification shows increased pleomorphism compared to the distal type. A rhabdoid cell is present (*center*)

with intracytoplasmic, hyaline-like inclusions compressing and eccentrically displacing the nucleus [175]. Rarely, the distal type demonstrates these rhabdoid cells [176]. The pseudogranulomatous or necrotizing granuloma-like pattern is not as prominent in the proximal type as in the distal type [175, 177]. Both types may demonstrate pseudoangiomatoid spaces, osteoclast-like giant cells, calcification with or without osseous metaplasia, myxoid change, and vascular and perineural invasion [175–177, 179, 194]. Mitotic count varies in both types [188, 196], with > 20 per 10 high-power fields portending a worse prognosis (see above).

Immunohistochemical Features

Congruent with its definition as a mesenchymal tumor with primarily epithelial differentiation, the vast majority of epithelioid sarcomas coexpress vimentin and cytokeratins or EMA, usually diffusely (Figs. 15.20 and 15.21a) [175, 176, 178, 188, 197]. Over half demonstrate CD34 expression. Expression of S100, desmin, SMA, and HMB45, when seen, is usually focal [175, 176, 178, 188, 197]. A key negative marker is INI-1 (also known as hSNF5 and SMARCB1), with nearly 90 % of epithelioid sarcomas showing complete absence of its expression, whereas normal cells express this protein (Fig. 15.21b) [178, 195, 197].

Genetics and Molecular Findings

The discovery of INI-1 protein loss by immunohistochemistry was fueled by cytogenetic studies describing chromosomal aberrations of 22q, which harbors the tumor suppressor gene *SMARCB1/INI-1* [198, 199]. Alterations of 22q, however, are also characteristic of malignant rhabdoid tumor/extrarenal rhabdoid tumor (MRT/ERT), although the mechanisms of 22q inactivation differ. In MRT/ERT, 98 % of cases demonstrate genomic changes of both *SMARCB1/INI-1* alleles [200], whereas biallelic genomic changes in epithelioid sarcoma have been far less consistently demonstrated [196, 201, 202]. In fact, a study by Papp et al. [201] found that 25 of 31 epithelioid sarcoma cases had at least one intact SMARC allele. The results of the study led the authors to favor an epigenetic mechanism of gene silencing by microRNAs to explain the second inactivating event.

Differential Diagnosis

As indicated by the title of the first paper on epithelioid sarcoma [191], the tumor can mimic a granuloma or carcinoma. The former can be excluded by diffuse reactivity for epithelial markers [177]. The latter is almost always CD34 negative [177], and unlike epithelioid sarcoma, carcinomas retain INI-1 expression [195]. Melanoma can usually be distinguished from epithelioid sarcoma by immunohistochemistry, with a notable pitfall in cases of rhabdoid melanoma, which may express cytokeratins and often lacks S100 and HMB45 expression [177, 203]. In such cases, INI-1 expression is key. Of note, INI-1 loss has been noted in up to 50 % of epithelioid malignant peripheral nerve sheath tumors [195]. Table 15.7 reviews the salient discriminating features.

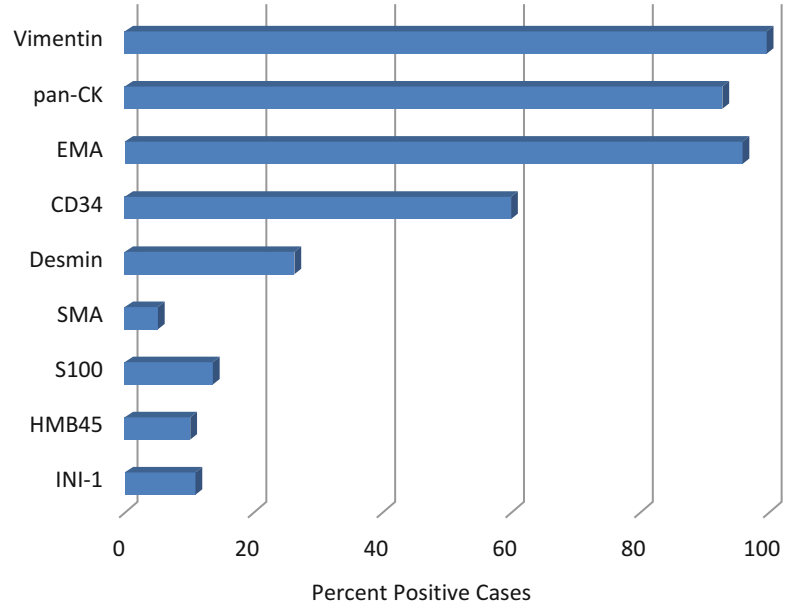


Fig. 15.20 Immunophenotype of epithelioid sarcoma, compiled from several references [175, 176, 178, 188, 195, 197]

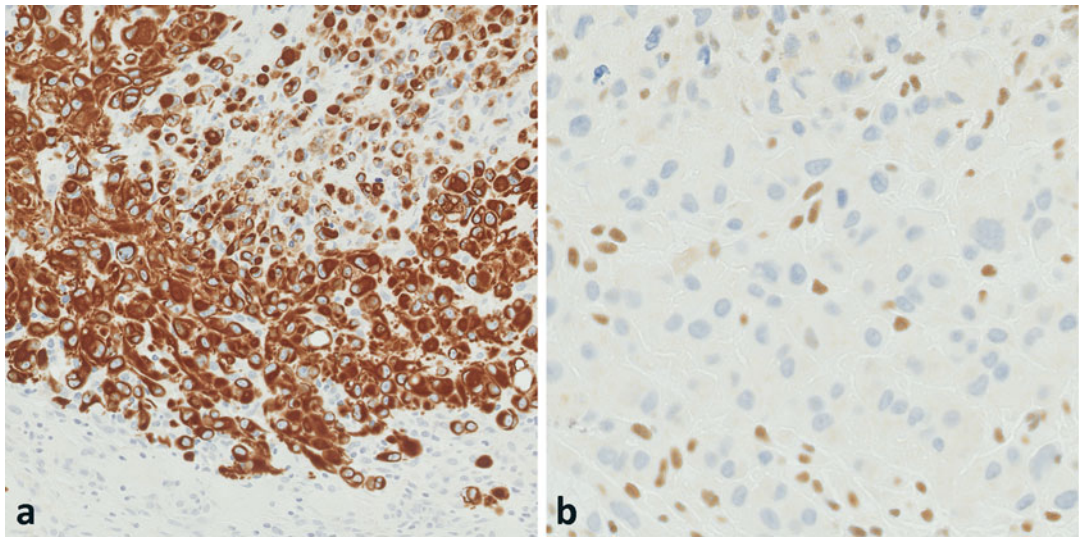


Fig. 15.21 (**a**) Pan-cytokeratin immunostaining highlights epithelioid and spindle cells. (**b**) INI-1 expression is lost in the tumor cells but retained in lymphocytes and stromal cells

Table 15.7 Mimickers of epithelioid sarcoma

	Epithelioid sarcoma	Carcinoma	Melanoma	Necrobiotic granuloma
Morphology	• Epithelioid cells • +/– Pleomorphism • +/– Rhabdoid features • Spindled component	• Epithelioid cells • Pleomorphism • Intercellular bridges • Intraepithelial component	• Can be epithelioid, spindled, or rhabdoid	• Histiocytes can be epithelioid, non-epithelioid, or foamy • Variable giant cells
Immunohistochemistry	• Epithelial markers + (CK, EMA) • CD34 + in 60 % • INI-1 loss	• Epithelial markers + • CD34 – • INI-1 retention	• Occasional cytokeratin + (usually focal) • Melanoma markers + (S100, HMB45, etc.) • INI-1 retention	• Epithelial markers – • CD34 –

Data from: Refs. [36, 177, 195]

Summary

Clinical Presentation

- Patients present in young adulthood with nonspecific complaints related to a painless mass that mimics a benign cyst.
- The distal type preferentially affects distal extremities, and the proximal type preferentially affects the trunk, limb girdles, pelvis, and external genitalia.
- Although the vulva is most likely to manifest the proximal type, overlap can occur.

Histologic Features

- The classic morphology of the distal type is a "pseudogranulomatous" proliferation of polygonal cells growing in waves around central necrosis, with subtle transition of polygonal cells into spindle cells.
- The proximal type demonstrates sheets of large, polygonal (or rhabdoid) cells with voluminous cytoplasm, bearing enlarged, often pleomorphic, vesicular nuclei with frequently prominent nucleoli.

Differential Diagnosis

- Consider epithelioid sarcoma in the differential diagnosis for necrobiotic granuloma, carcinoma, and melanoma, depending on clinical context.

Takeaway Essentials

Clinically Relevant Pearls

- Epithelioid sarcoma is a relentlessly persistent neoplasm with a high rate of metastasis leading to death.
- Consider epithelioid sarcoma in any case previously diagnosed as a granulomatous process that manifested unexpectedly aggressive behavior.

Pathology Interpretation Pearls

- Histologic features predictive of aggressive behavior include proximal-type morphology, FNCLCC grade 3, tumor size > 5 cm, and mitotic count ≥ 20 per 10 high-power fields.

Immunohistochemical/Molecular Findings

- Almost all cases show diffuse expression of epithelial markers (EMA, pan-CK).
- More than half are CD34 +.
- The vast majority show loss of INI-1 by immunohistochemistry, reflecting alterations in the expression of the INI-1/SMARCB1 gene on chromosome 22q11.

Ewing Sarcoma

Clinical Features

Nearly a century ago, Ewing sarcoma and primitive neuroectodermal tumor (PNET) debuted separately, and their respective literatures evolved in parallel for decades [114, 204]. Over time, the two entities began to converge and ultimately collided nosologically with the discovery of common genetic underpinnings [114, 204, 205]. These entities, together with Askin tumor (chest wall lesions), are now referred to collectively as the Ewing family of tumors, or simply as Ewing sarcoma—the preferred term of the World Health Organization for tumors of soft tissue and bone [51]. Ewing sarcoma preferentially affects the bones of children and adolescents, with only about one in five cases occurring outside of bone [206]. Primary cutaneous [207, 208] and gynecological cases [209] are rare. Vulvar cases total nearly 20 to date [209–214], but not all of these cases have molecular confirmation or even immunohistochemical support of the diagnosis [209, 215–217]. The reported patients present mainly in adolescence or young adulthood with a mass of the labium majus or minus [209, 211]. Clinical examination discloses a seemingly benign, mobile, cyst-like mass [210, 211, 218, 219] or a suspicious, infiltrative, exophytic, ulcerated mass [213, 215]. There may be a history of rapid enlargement [210, 213]. Some patients report pain and bleeding [215, 219].

Prognosis or Course

Ewing sarcoma historically behaves aggressively and carries a gloomy prognosis, but the past 3 decades have witnessed marked improvement in 5-year survival rates, which now hover around 70 %. Far fewer patients survive metastatic disease (roughly one-third), which about one-fourth of patients have at presentation [206, 220]. Despite limited data, some authors have tentatively suggested a better outcome for vulvovaginal Ewing sarcoma compared with those arising in usual sites [8, 22]. Treatment for vulvar cases parallels that of Ewing sarcoma elsewhere, consisting of induction chemotherapy followed by either surgery or radiation (or both), depending on size, location, and response to chemotherapy [114, 209, 211].

Etiology/Pathogenesis

The search for the progenitor cell of Ewing sarcoma continues, but evidence suggests a mesenchymal or neural-crest-derived stem cell [221–223]. The two are not mutually exclusive, as mesenchymal stem cells may arise from either mesoderm or neural crest [224–226]. The neoplastic cells bear an underlying chromosomal translocation (described below), producing a fusion protein that alters gene expression. The altered genetic program transforms cells into a relatively undifferentiated phenotype with variable neuroectodermal differentiation [227–229].

Histopathology

Tumors can measure up to 20 cm in the vulva [230]. They may be circumscribed or infiltrative, with a nodular or lobulated cut surface [114, 209]. Microscopically, Ewing sarcoma exhibits a morphologic continuum, with an undifferentiated phenotype at one end and a neuroectodermal phenotype at the other [231]. The undifferentiated tumors capture the essence of a quintessential "small round blue cell tumor," consisting of a homogeneous population of undifferentiated cells without any specific architecture (*classical Ewing sarcoma*) (Fig. 15.22a–c). Rosette formation signals neuroectodermal differentiation (traditionally called *PNET*) (Fig. 15.23). Cases with cellular or architectural heterogeneity outside of this spectrum are referred to as *atypical Ewing sarcoma* [114, 231–233]. Table 15.8 elaborates the different morphologic patterns. Atypical histology carries prognostic relevance, whereas other patterns, mitotic count, and necrosis do not correlate with clinical outcome [232].

Immunohistochemical Features

The vast majority of cases display diffuse and strong immunoreactivity for CD99 and Fli-1 (Figs. 15.24 and 15.25). Caveolin-1, a relatively newer marker, correlates highly with CD99 expression. Pan-cytokeratin expression, when present, is mainly focal [232, 233]. Interestingly,

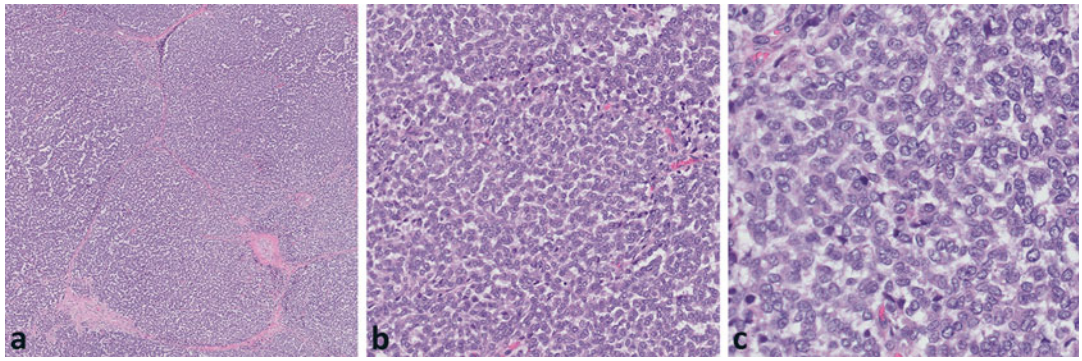


Fig. 15.22 (**a**, **b**) Low-power view discloses sheets of small, round, blue cells. (**c**) Higher power reveals monomorphic cells with scant cytoplasm and indistinct cell borders

the PNET and atypical patterns show more reactivity for CD57 [232], reflecting neuroectodermal differentiation [114]. Although none of these markers alone can nail down the diagnosis, a panel serves as a mandatory initial step [114, 231, 232]. In cases with characteristic histopathology, expression of at least three of four in the panel of CD99, Fli-1, caveolin-1, and CD57 may sufficiently rule in the diagnosis of Ewing sarcoma [232]. For atypical or equivocal cases, molecular confirmation is essential [231, 232, 234, 235].

Genetics and Molecular Findings

Molecular testing provides a gold standard not only for cases with equivocal histologic features, but also for those affecting unusual anatomic sites, such as the vulva [209]. The defining chromosomal translocation of Ewing sarcoma, t(11;22), fuses the EWSR1 and FLI-1 genes [236]. EWSR1 belongs to the TET family, and FLI-1 belongs to the ETS family, leading to the concept that TET::ETS fusions drive Ewing sarcoma. TET::ETS fusions involving relatives of EWSR1 and FLI-1 have been reported and accepted as Ewing sarcoma (usually consisting of EWSR1 fused to an ETS member). Fusions involving TET::non-ETS and non-TET::non-ETS have also been described in cases resembling Ewing sarcoma, but these have not yet been accepted into the Ewing family. For this reason, the term *Ewing-like sarcoma* has emerged. Pending further research, these cases are to be considered and managed as Ewing sarcomas [114, 237–241]. The promiscuity of the EWSR1 gene extends beyond Ewing and Ewing-like sarcomas, with EWSR1 fusions typifying a number of other soft tissue tumors [114]. For this reason, FISH should be accompanied by RT-PCR in order to reveal the fusion partner [236, 242, 243] for cases in which the diagnosis hinges upon the molecular results. Rarely, molecular testing can be negative in cases exhibiting classical Ewing features. After excluding other possible

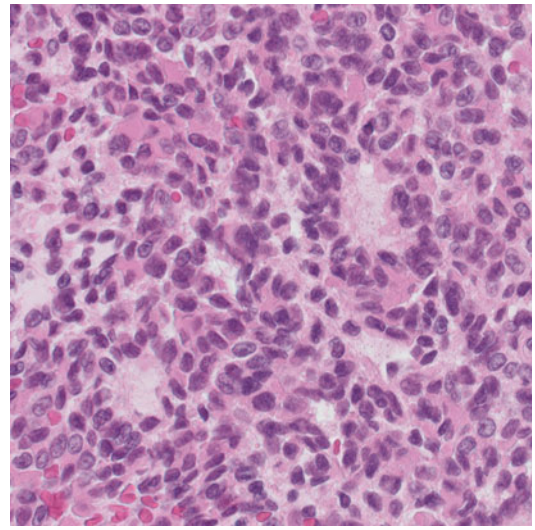


Fig. 15.23 Ewing sarcoma, PNET pattern. Vaguely defined rosettes reflect an attempt at neuroectodermal differentiation

Table 15.8 Morphologic heterogeneity in Ewing sarcoma

	Morphologic patterns		
	Classical	PNET	Atypical
Architectural features	• Sheets and lobules with diffuse cell growth	• Rosette-like formations (ill-defined rings of cells oriented toward a central fibrillary core, akin to Homer Wright neural rosettes in neuroblastomas) • Classical pattern intermixed	Unusual components such as • Desmoplastic or sclerotic stroma • Adamantinoma-like areas (palisades of cells among collagen basal-like membrane) • Pseudovascular structures • Osteoid deposits
Cytologic features	• Homogeneous, small, round cells • Scant or moderately abundant, clear to pale cytoplasm (PAS +) • Indistinct cell borders		• Heterogeneous, larger, variety of shapes ○ Spindle, epithelioid, or rhabdoid cells • Clear to eosinophilic cytoplasm
Nuclear features	• Small with round nuclear contours • Fine chromatin • Inconspicuous nucleoli		• Large with round or irregular contours • One or more prominent nucleoli
Mitotic figures	• Sparse		• Variable
Prognostic relevance	• None		• Unfavorable

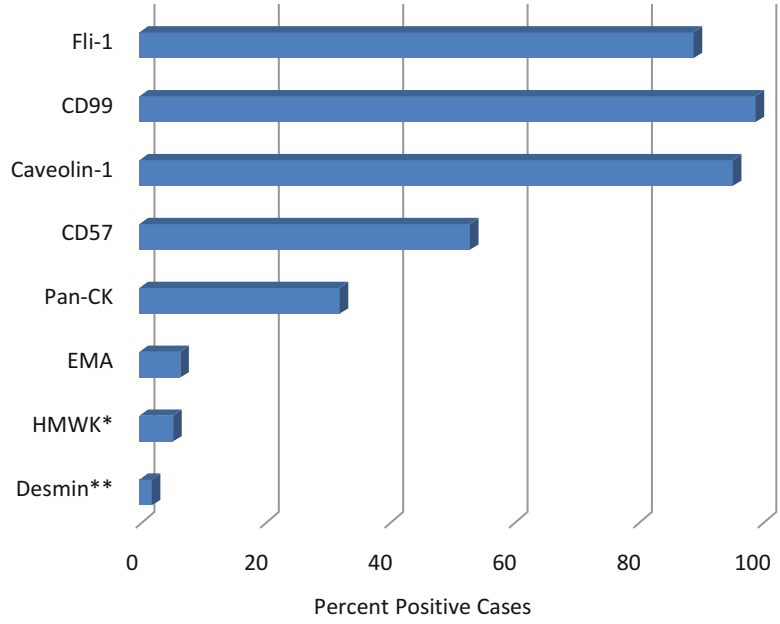


Fig. 15.24 Immunophenotype of Ewing sarcoma. Data compiled from molecularly confirmed cases only [231–233]. *HMWK was only expressed in "adamantinoma-like" Ewing sarcoma. **Weak expression was seen in a single case with classical histology

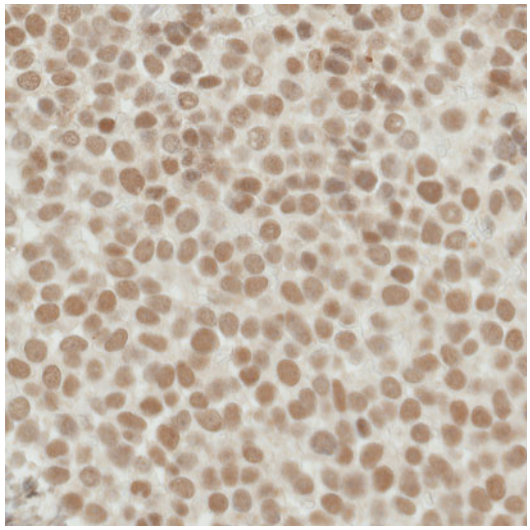


Fig. 15.25 Fli-1 is a nuclear marker. Fli-1 immunostain also highlights normal lymphocytes and endothelial cells [234]

diagnoses, these cases may be signed out as: "Undifferentiated round cell sarcoma with features of Ewing sarcoma" [114].

Differential Diagnosis

Among the entities discussed in this chapter, synovial sarcoma (poorly differentiated) and alveolar rhabdomyosarcoma enter the differential for Ewing sarcoma. Table 15.9 aims to resolve this differential. Other round cell tumors to consider include Merkel cell carcinoma and lymphoblastic lymphoma. Merkel cell carcinoma typically affects an older cohort and can be readily distinguished from Ewing sarcoma by CK20 expression (perinuclear and dot-like) [231, 244, 245]. Lymphoblastic lymphoma is vanishingly rare in the vulvovaginal region [246] and can be excluded with negativity for TdT and CD43 [114, 234].

Table 15.9 The differential diagnosis of Ewing sarcoma

	Ewing sarcoma	Alveolar rhabdomyosarcoma	Synovial sarcoma (poorly differentiated)
Clinical features	• Adolescence or young adulthood	• Childhood or adolescence	• Young adulthood
Cellular architecture	• ± Rosette formation	• Rosettes not observed • Dishesive cells align along fibrovascular septa (overlaps with atypical Ewing sarcoma) • Solid variant lacks alveolar pattern	• Rosettes not observed • Overlapping cells
Cellular morphology	• Primitive, small, round, blue cells • PAS + cytoplasm	• Primitive, small, round, blue cells • Rarely exhibit myogenic differentiation (increasing amounts of eosinophilic cytoplasm) • ± Wreath-like multinucleated cells	• Primitive, small, round to oval cells • Can be spindled or rhabdoid
Immunohistochemical differences	• Fli-1 + • Desmin – [a]	• Desmin + • Myogenin +	• TLE-1 +
Molecular signatures	• t(11;22) or variant	• t(2;13), t(1;13)	• t(X;18)

Data from: Ref. [114]

[a]Desmin positivity in Ewing sarcoma is exceedingly rare but reported [115]

Summary

Clinical Presentation

- Patients present in adolescence or young adulthood with nonspecific complaints related to mass effects.
- Clinical inspection may reveal a suspicious mass or a deceptively benign, cyst-like lesion.

Histologic Features

- Typical histology is that of a quintessential small round blue cell tumor, consisting of a homogenous population of undifferentiated cells without any specific architecture.
- Rosette formation reflects an attempt at neuroectodermal differentiation.
- Atypical cases show relatively heterogeneous architecture and cytologic features, which broadens the differential diagnosis.

Differential Diagnosis

- Consider other small round blue cell tumors such as alveolar rhabdomyosarcoma, poorly differentiated synovial sarcoma, Merkel cell carcinoma, and lymphoblastic lymphoma.

Takeaway Essentials

Clinically Relevant Pearls

- Maintain a high index of suspicion for any vulvar mass, even in the face of seemingly benign clinical exam.
- This notoriously aggressive neoplasm requires prompt and aggressive treatment.

Pathology Interpretation Pearls

- Identification of atypical morphology warrants mention in the final report, as this feature corresponds to a worse outcome.

Immunohistochemical/Molecular Findings

- For classical histology, positivity in three of four in a panel of CD99, Fli-1, caveolin-1, and CD57 can rule in the diagnosis.
- Vulvar cases should incorporate molecular testing since this is an unusual location for this diagnosis.

Synovial Sarcoma

Clinical Features

Classically a tumor of the deep soft tissues of the extremities in young adults, synovial sarcoma can arise at any location and any age [247, 248]. More than half of all cases occur between ages 10 and 40, with an average age of 34 [248]. Following the deep soft tissues of the extremities, the other sites involved include the trunk followed by the head and neck region [248]. Unusual sites include the gynecological tract [247], where vulvar cases [249–255] slightly outnumber those in the vagina [254, 256, 257], uterus [258], ovary [259], and fallopian tubes [260]. Among the nine reported vulvar cases,[2] the average patient presents at age 34 with a seemingly benign, circumscribed labial mass frequently mistaken for a Bartholin cyst or lipoma [249–255]. Labial masses can extend to the mons pubis, vaginal introitus, or urethra. Imaging may reveal involvement of deeper structures such as the inferior pubic ramus [251, 253]. The mass may be painless or painful depending on its size and precise location. One patient found her 8-cm vulvar mass owing to discomfort following intercourse [253]. Further contributing to a deceptively benign impression is that the mass may grow slowly, with an average duration of symptoms of 2–4 years [247, 254].

Prognosis/Course

Historically a lethal tumor with a gloomy prognosis, its 10-year survival rate of 0–15 % in the older literature [247] is currently 52 % [248], likely reflecting advances in multimodal management, which, for the vulva, consists of wide-excision

[2]One case was apparently published twice under different names [252, 253], both of which were counted in the totals by Asher [250] and Sumathi [254].

partial or radical vulvectomy plus chemotherapy, with or without radiotherapy [253, 254]. Notwithstanding such advances, recurrences and metastases are common, typically occurring after 3 and 5 years, respectively [261].

Etiology/Pathogenesis

Initially described in 1910 by Lejars and Rubens-Duval, synovial sarcoma has been likened to synovium owing to its epithelial and mesenchymal components. Early reports describe synovial sarcoma in intra-articular locations, further supporting the concept of a synovial origin [247]. However, owing to numerous ultrastructural and immunohistochemical features, the concept of synovial origin and differentiation has been long abandoned. Instead, it is now widely accepted that the cell of origin is a multipotent stem cell capable of either epithelial or mesenchymal differentiation, driven by its signature chromosomal aberration (discussed below) [247, 262, 263]. Regardless, the term synovial sarcoma lingers as a specific entity [247] entrenched in our literature.

Histopathology

Vulvar masses range in size from 1.2 to 9 cm and demonstrate a solid or cystic consistency (Fig. 15.26) [249, 251]. The microscopic patterns rest on a continuum of identifiable epithelial differentiation: (1) biphasic, (2) monophasic, and (3) poorly differentiated, all of which have been described in the vulva [4, 9–14]. The first pattern (biphasic) is the most recognizable, characteristically exhibiting groups of glandular structures, with open lumina and secretions, dispersed among tightly packed spindle cells (Fig. 15.27a, b) [247, 264]. The epithelial component can also form clefts, cysts, solid cords, nests, or papillary structures, rarely with focal squamous differentiation [247, 265, 266]. The cells have abundant cytoplasm, defined cell borders, and ovoid nuclei with vesicular chromatin [247, 264].

The mesenchymal component appears blue and hypercellular, composed of swirling fascicles of monotonous, ovoid, fusiform cells with scant cytoplasm and indistinct cell borders, rendering an appearance of tightly packed, overlapping cells (Fig. 15.28a, b) [247]. Among the spindle cells may exist hemangiopericytoma-like (staghorn)

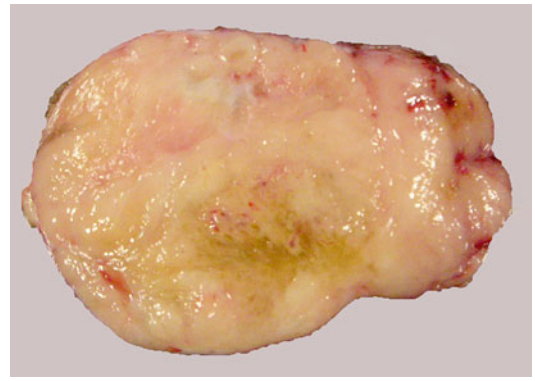


Fig. 15.26 Synovial sarcoma, gross specimen

vessels, myxoid change, herringbone-like growth, hyalinized collagen, calcification, and ossification (Fig. 15.29a–d) [247, 250, 264, 267]. Although extensive ossification with osteoid production is exceedingly rare, Milchgrub et al. [268] warn that its presence may lead to a misdiagnosis of osteosarcoma. The mesenchymal component without an epithelial component constitutes the second pattern (the monophasic pattern).

The third pattern (poorly differentiated) demonstrates increased nuclear pleomorphism, clumped chromatin, and high mitotic rate (usually > 10/10 high-power fields) [269]. There are three possible subtypes of the poorly differentiated pattern, which are detailed in Table 15.10. Necrosis and hemangiopericytoid vessels tend to be more common in the poorly differentiated pattern, while mast cells, calcification, and hyaline collagen bands tend to manifest in the biphasic or monophasic patterns. Poorly differentiated foci portend more aggressive behavior [270].

Immunohistochemical Features

Not surprisingly, the epithelial component consistently demonstrates expression of epithelial markers, such as EMA and cytokeratins (Fig. 15.30) [247, 271, 273]. The mesenchymal component shows at least focal positivity for one epithelial marker 98 % of the time (EMA, E-cadherin, or pan-keratin AE1/AE3) [271]. Also, the complexity of the expressed keratins decreases along the spectrum of biphasic to monophasic to poorly differentiated histology, with the poorly differentiated patterns expressing only limited, focal expression of simple keratins

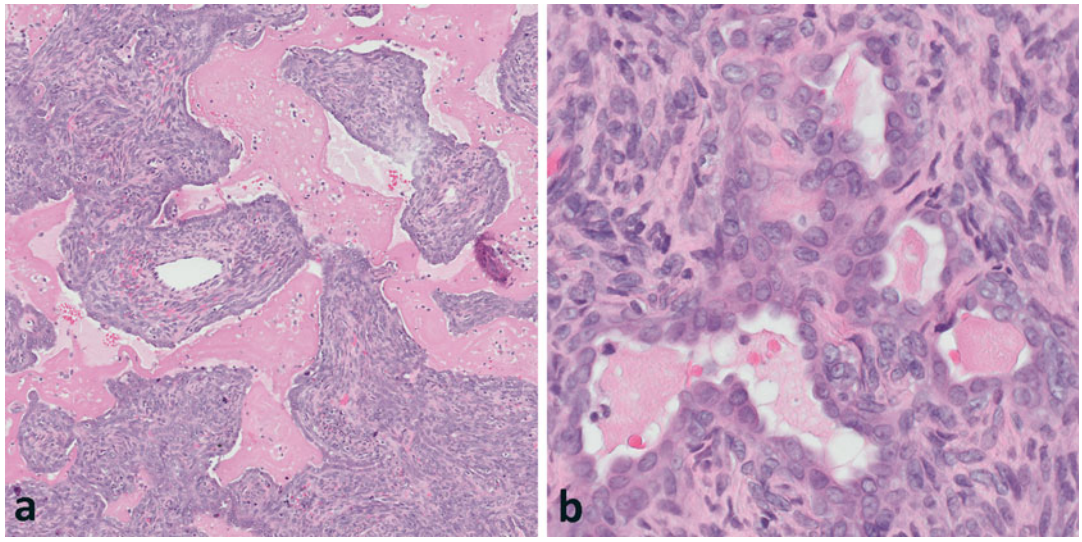


Fig. 15.27 Biphasic synovial sarcoma. (**a**) Glandular formations with luminal secretions are dispersed among tightly packed spindle cells. (**b**) The glands are lined by bland, cuboidal cells

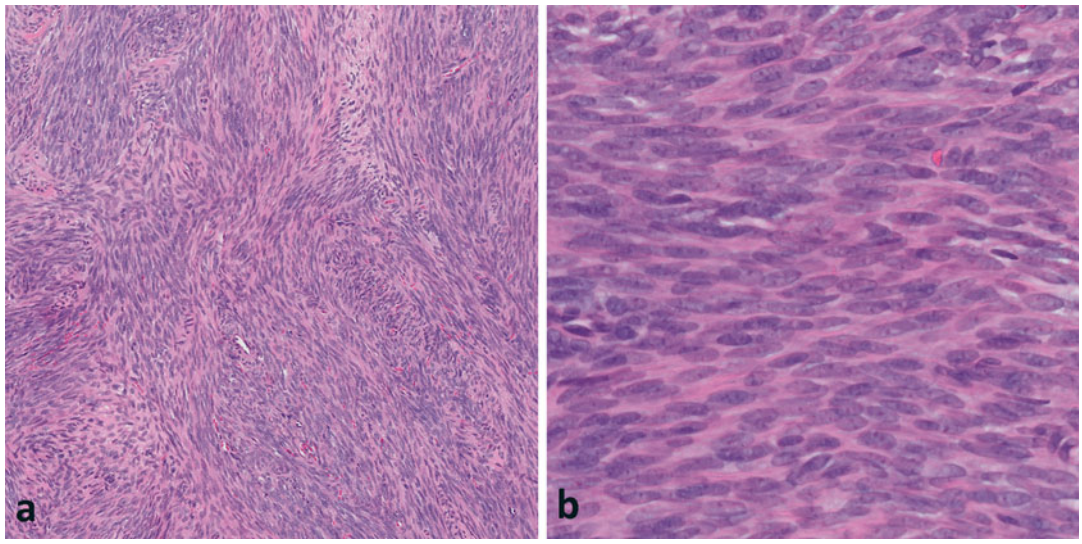


Fig. 15.28 (**a**) The mesenchymal component of synovial sarcoma is hypercellular and blue. (**b**) The cells are tightly packed and appear to overlap

and nearly no expression of complex keratins (Table 15.10) [274]. Ki67 staining identifies increased proliferation in poorly differentiated areas [269, 275]. A relatively newer marker for synovial sarcoma, TLE1, has emerged from gene profiling studies, displaying a sensitivity of 82–100 % [271, 272, 276–278], with most cases staining diffusely [272, 276]. Debate over the specificity of TLE1 centers on its expression in malignant peripheral nerve sheath tumor (MPNST), which frequently enters the differential. While most studies on TLE1 have found that less than 5 % of MPNSTs show more than weak staining [272, 277–279], the Kosemehmetoglu group [276] contends that 30 % of MPNSTs stain diffusely. Despite the small size of the Kosemehmetoglu group's study, the results illustrate a pitfall. Fortunately, for challenging cases,

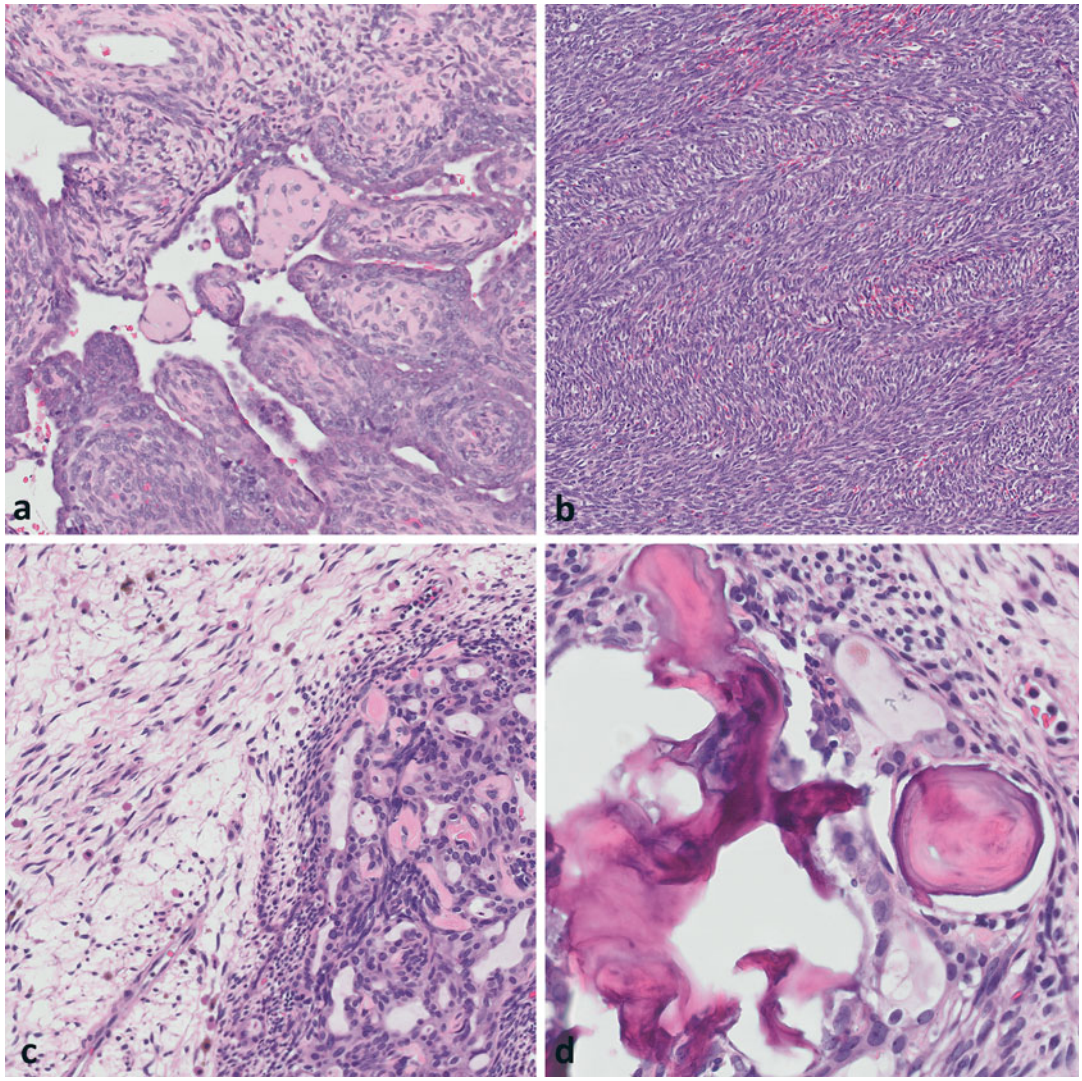


Fig. 15.29 Variability in synovial sarcoma. (**a**) Although papillary in appearance, there are spindle cells where fibrovascular cores should be. (**b**) Herringbone appearance. (**c**) Myxoid change is seen on the *left*, whereas a biphasic component is seen on the *right*. (**d**) Scattered calcifications can be seen, some with a psammomatous appearance

immunohistochemistry serves as a screening tool, while molecular testing remains confirmatory [272, 277, 280].

Genetics and Molecular Findings

Since the unearthing of t(X;18)(p11;q11) and its corresponding SYT-SSX fusion transcript, molecular testing has become a staple of synovial sarcoma diagnosis [280–282]. The perfect specificity claimed in the early reports was later challenged by a report alleging the presence of the fusion transcript in MPNST [283], which has since been rebutted by numerous larger studies confirming the absence of the SYT-SSX fusion transcripts in any other mesenchymal tumor [281, 284–287]. The fusion gene results in a transcript with putative "activator-repressor" effects on transcription [263]. It is debatable whether the fusion subtype impacts tumor behavior. Ladanyi et al. [284] found a survival advantage for patients with fusion partner SSX2 compared to SSX1, a

Table 15.10 The histologic spectrum of synovial sarcoma

Increasing epithelial differentiation and expression of complex keratins

	Biphasic	Monophasic	Poorly differentiated

Architectural features	• Epithelial component
	  ○ Glands with open lumina (or variations)
	• Mesenchymal component
	  ○ Blue and hypercellular, with monotonous, ovoid cells
		• No epithelial component by light microscopy
		• Mesenchymal component
		  ○ Blue and hypercellular, with monotonous, ovoid cells
			• No epithelial component by light microscopy
			• Mesenchymal component blue and hypercellular, with
			  ○ Increased nuclear pleomorphism
			  ○ Clumped chromatin
			  ○ High mitotic rate
			• 3 variants
			  ○ Spindle
			  ○ Round cell
			  ○ Rhabdoid

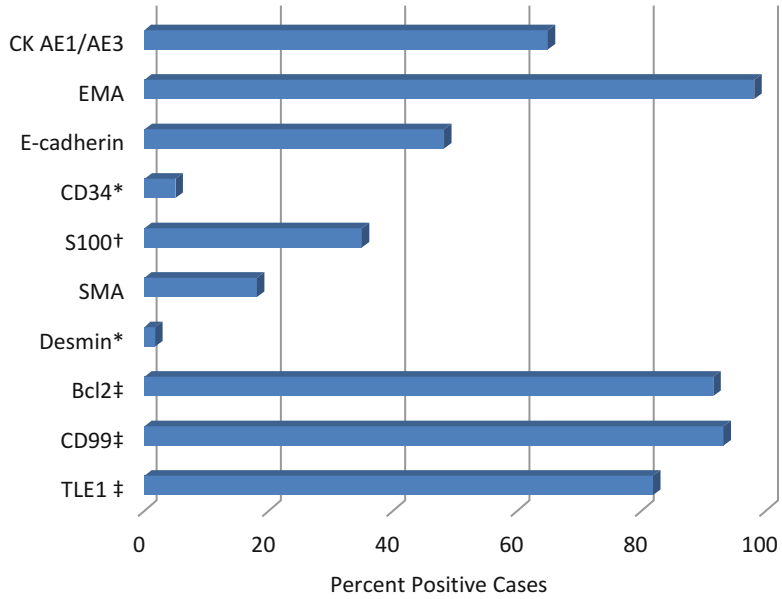


Fig. 15.30 Immunophenotype of synovial sarcoma. Percentages were calculated from data on 60 molecularly proven monophasic and poorly differentiated synovial sarcomas [271]. TLE-1 data are from molecularly confirmed cases [272]. *Focal only. †Usually focal. ‡Diffuse

trend also observed by others [288, 289]. However, Guillou and colleagues [290] assert that grade, not fusion type, matters and point out that the Ladanyi group did not stratify for grade. Guillou's assertion was later substantiated by Huevel et al., who showed that tumor stage, size, and FNCLCC grade are prognostically relevant, not SSX fusion type [291].

Differential Diagnosis

The histologic pattern dictates the differential diagnosis. The biphasic pattern can mimic carcinosarcoma [247, 254], and if myxoid change is prominent, mixed tumor of the vagina [254, 264]. Most carcinosarcomas of the female genital tract arise in the uteri of older adult women, but primary vulvovaginal cases have been

Table 15.11 Monophasic synovial sarcoma versus mimickers

	Synovial sarcoma	MPNST	SFT
Distinguishing morphologic features	• Compact, overlapping cells with oval nuclei	• "Buckled" or "arrowhead" nuclei	• Staghorn vessels more than focal
Immunohistochemistry	• TLE1 + (82–100 %) • Epithelial markers + • CD99 + • S100 ± • CD34 –	• TLE1– (rare +, focal or diffuse) • Epithelial markers rarely + (focal) • CD99 ± • S100 ±	• TLE + up to 40 % • Epithelial markers + rarely • CD99 + • CD34 +
Molecular	• t(X;18)	• Complex	• Diverse/complex

Data from: Refs. [271, 272, 276–278]

reported [292–294]. In carcinosarcoma, there is usually more cytologic atypia [247]. Mixed tumor of the vagina arises in the lower third of the vagina in postmenopausal women, consisting of bland epithelial and mesenchymal elements. In these tumors, desmin and h-caldesmon tend to be positive while CD99 is negative [295].

The monophasic pattern can resemble MPNST, solitary fibrous tumor (SFT), and leiomyosarcoma [247]. Leiomyosarcoma is discussed earlier in the chapter. Table 15.11 compares synovial sarcoma to MPNST and SFT.

Poorly differentiated synovial sarcoma can resemble a number of small round cell tumors, including Ewing sarcoma, as discussed previously. Whenever the diagnosis of synovial sarcoma is challenged by other tumor types, molecular testing is recommended [280]. See also Vignette 3 at the end of the chapter.

Summary

Clinical Presentation

- The typical patient is a young adult woman with a deceptively benign-appearing labial mass.

Histologic Features

- The morphologic spectrum of synovial sarcoma is reviewed in Table 15.10.
- Tumor stage, size, and FNCLCC grade are prognostically relevant.

Differential Diagnosis

- For the biphasic pattern, consider carcinosarcoma, and if myxoid, consider mixed tumor of the vagina.
- For the monophasic pattern, consider MPNST, SFT, and leiomyosarcoma.
- For the poorly differentiated pattern, consider small round cell tumors.

Takeaway Essentials

Clinically Relevant Pearls

- Do not dismiss a slow-growing, cyst-like mass.

Pathology Interpretation Pearls

- Consider synovial sarcoma in any spindle cell tumor of the vulvar region.
- Monophasic and poorly differentiated patterns require immunohistochemical screening followed by molecular confirmation.

Immunohistochemical/Molecular Findings

- Expression of epithelial markers (EMA, pan-cytokeratin, or E-cadherin) decreases along the continuum from biphasic → monophasic → poorly differentiated patterns.
- TLE1 expression can help in proper context but has limited sensitivity and specificity.
- Molecular testing for t(X;18) or its SYT-SSX fusion transcript is recommended for any case in which another tumor type challenges the diagnosis.

Case Vignettes

Vignette 1

Clinical History: An 8-month-old girl is seen in the dermatology clinic for a persistent rash of the external genitalia. The infant had previously been seen in primary care, at which time a diagnosis of diaper dermatitis was rendered. Despite regular application of barrier creams and antifungal ointments, the rash remained unchanged.

Physical examination reveals multiple, erythematous papules on the bilateral labia majora (Fig. 15.31). Additional skin examination discloses yellowish scaling of the scalp and bilateral axillae. Potassium hydroxide (KOH) preparation from the scalp is negative. Simultaneous viral culture and punch biopsy are taken from the labial papules. Viral culture is negative. The biopsy specimen is shown in Fig. 15.32.

What additional studies can resolve the differential? Consider immunohistochemical staining for S100, CD1a, langerin, and CD68. The results of immunostaining are as follows: the mononuclear aggregates mark with S100, langerin, and CD1a. CD68 highlights scattered multinucleated cells.

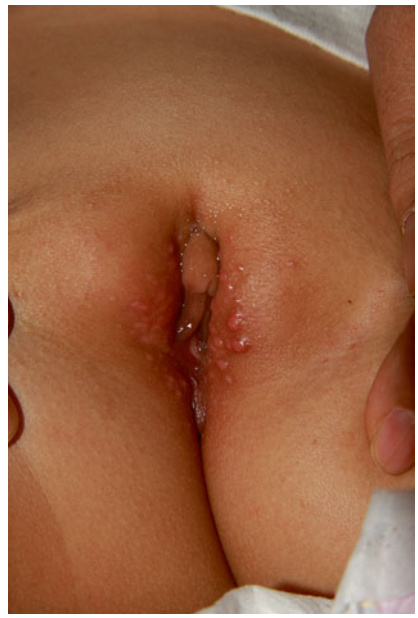


Fig. 15.31 Vignette 1. Multiple, erythematous papules on the bilateral labia majora (Used with permission from Hwang C et al. Isolated Langerhans cell histiocytosis of the vulva in an infant. Ped Derm. 2009;26(6):751–3)

(continued)

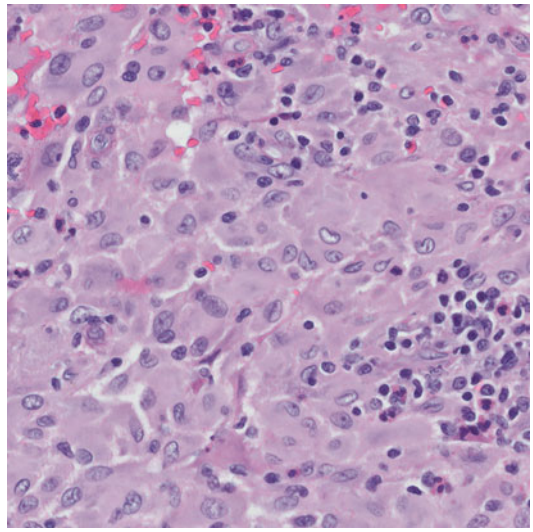


Fig. 15.32 Vignette 1. Beneath an intact epithelium is a band-like infiltrate of mononuclear cells with moderate to abundant pale, eosinophilic cytoplasm with indistinct cell borders. The nuclei exhibit indentations, imparting a kidney-bean shape. Scattered inflammatory cells are noted throughout, with occasional eosinophils

Diagnosis: Langerhans cell histiocytosis (LCH).

Discussion: Involvement of other organs must be assessed with laboratory and imaging studies. Blood tests include CBC, CMP, ESR, and coagulation studies. Imaging entails abdominal ultrasound (to assess the liver, spleen, and intra-abdominal lymph nodes), chest radiograph, and skeletal radiograph survey [161]. Skin manifestations of LCH often resemble dermatitis, most frequently involving the scalp, diaper area, or axilla. This may lead to a clinical diagnosis of seborrheic dermatitis or diaper dermatitis [146, 161, 296]. The presence of concomitant vulvar and scalp lesions is documented [145, 159]. However, owing to the rarity of this diagnosis, especially in infants, a clinician may diagnose the more common seborrheic or diaper dermatitis, resulting in diagnostic delay. In patients with vulvar involvement, an average of 2 years elapses between initial presentation and correct diagnosis [144]. Owing to a polymorphous clinical presentation of LCH, recognizing its potential to occur in the vulva is key. Consideration must be given to this diagnosis in any patient with an atypical or multifocal rash.

Vignette 2

Clinical History: A 13-year-old female is brought to the hospital by ambulance after collapsing in the shower. Her parents found her unresponsive "in a pool of blood." Intravenous fluids and packed red cells are administered. Physical examination is remarkable for a hemorrhagic mass involving the vaginal introitus and vulva. The patient undergoes emergency surgery. The resected specimen is shown in Fig. 15.33. Representative histopathology and immunohistochemistry are shown in Figs. 15.34a–c, 15.35a, b, and 15.36a, b.

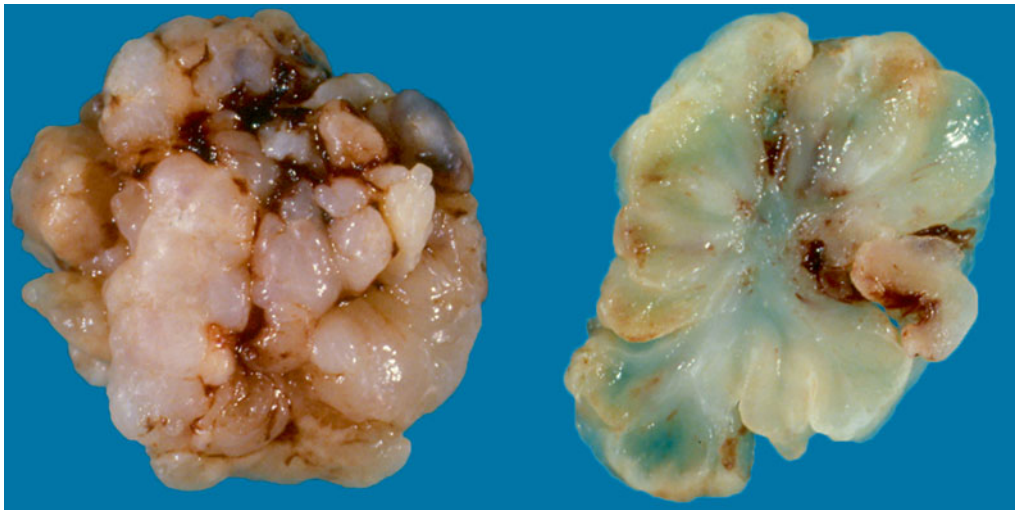


Fig. 15.33 Vignette 2. Gross photographs from the resected mass (Photos courtesy of Dr. Jerome B. Taxy and Dr. Nicole A. Cipriani)

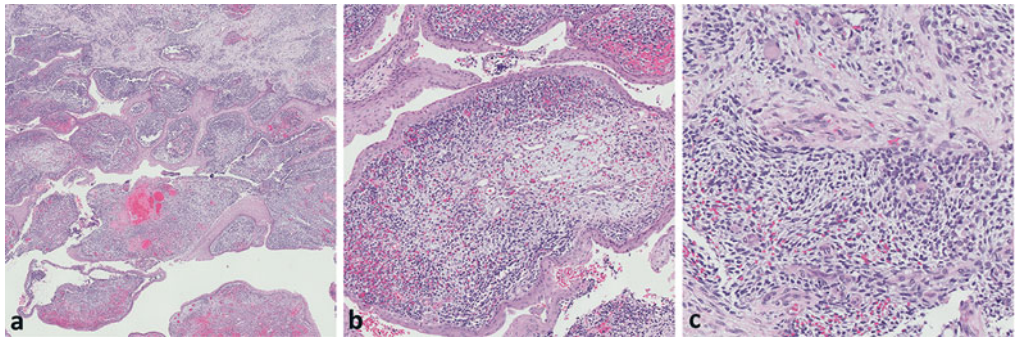


Fig. 15.34 Vignette 2. (**a**) Low-power view reveals a polypoid mass with variable cellularity. (**b**) Higher-power examination reveals a condensation of cells beneath the epithelium. (**c**) The mass otherwise consists of variably dense populations of primitive-appearing, stellate cells. Occasional larger cells with eosinophilic cytoplasm are identified

(continued)

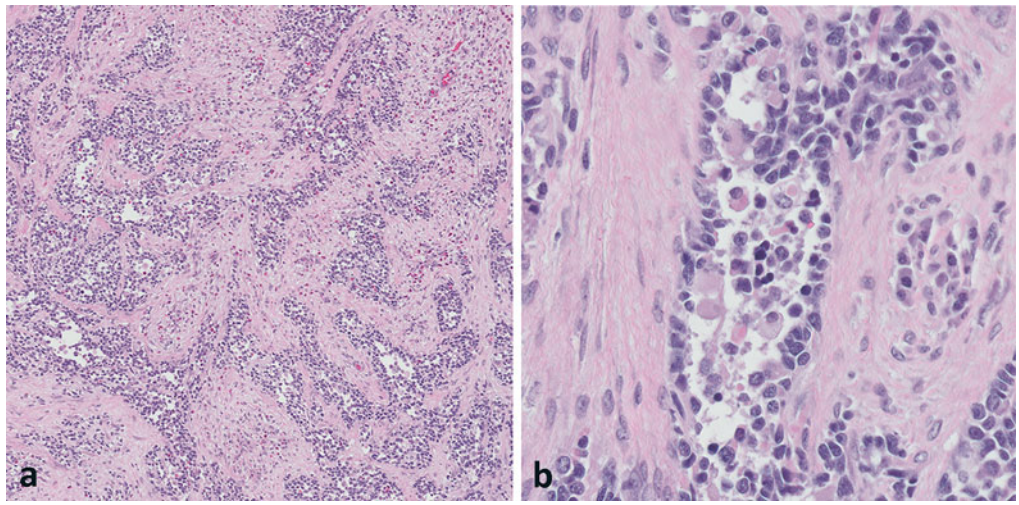


Fig. 15.35 Vignette 2. (**a**) Foci of alveolar architecture are noted. (**b**) Neoplastic cell align along fibrous septa

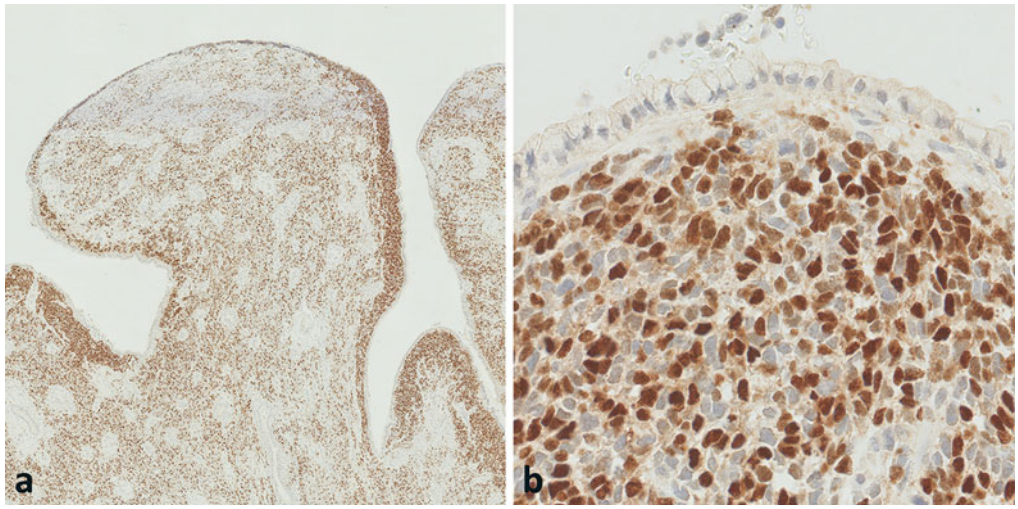


Fig. 15.36 Vignette 2. (**a**) Increased myogenin expression mirrors the cellular condensation noted in Fig. 15.34b. Compare the microscopic image to the cut surface of the specimen in Fig. 15.33. (**b**) Myogenin expression is nuclear

Diagnosis: Rhabdomyosarcoma, embryonal and alveolar types.

Discussion: The morphology is consistent with both alveolar and embryonal (botryoid variant) RMS. Myogenin is one of the best markers for confirming the histologic impression, with some studies suggesting that expression is more diffuse in alveolar cases compared to embryonal [102–104]. Genetic assessment has become more meaningful for treatment purposes rather than for histologic classification. The clinical outcome for embryonal RMS and translocation-negative alveolar RMS is more favorable than that of translocation-bearing alveolar RMS. Therefore, molecular testing is the next best step for this case.

Vignette 3

Clinical History: A 26-year-old woman presents with complaints of a 4-month history of a painless labial mass. Clinical examination discloses a mobile, circumscribed, cyst-like mass. An excisional biopsy is performed, and the light microscopic images are shown in Fig. 15.37a, b.

What is your differential diagnosis? The differential diagnosis for small round blue cell tumors includes Ewing sarcoma, alveolar rhabdomyosarcoma, synovial sarcoma (poorly differentiated), Merkel cell carcinoma, and lymphoblastic lymphoma.

Which additional studies would you perform? Consider an immunohistochemical panel that includes the markers listed in Table 15.12. Based on the results in this case, rhabdomyosarcoma is highly unlikely. Lymphoblastic lymphoma can be excluded. Merkel cell carcinoma is unlikely on the basis of immunohistochemistry and patient age. The remaining two possibilities, Ewing sarcoma and synovial sarcoma, can both express cytokeratins. Although the focal TLE1 staining is not characteristic for synovial sarcoma, that diagnosis cannot yet be excluded. The next best step is to send material for molecular diagnostics. Weeks later, the report documents a t(X;18).

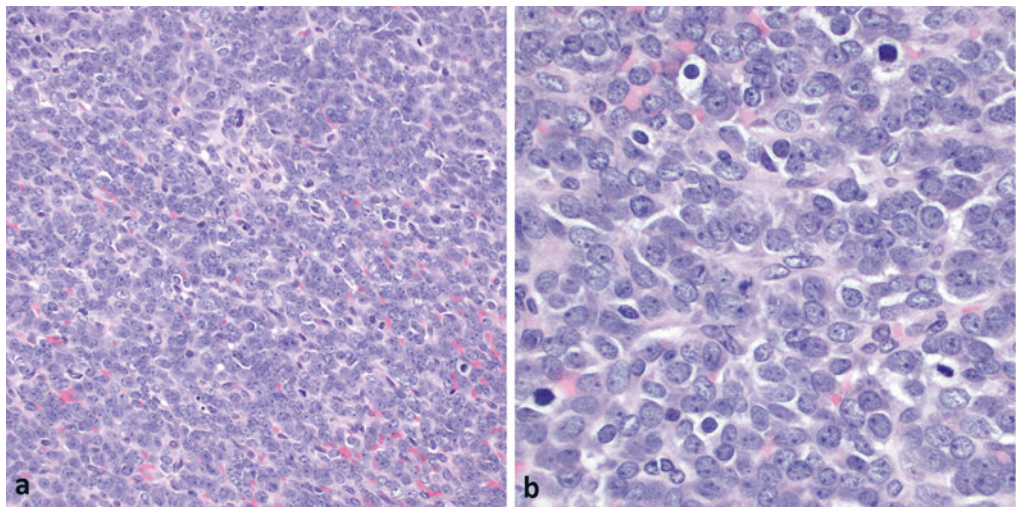


Fig. 15.37 Vignette 3. (**a**) Low-power microscopy reveals sheets of tightly packed, small, round, blue cells without any architecture. (**b**) High-power viewing discloses monomorphic cells with scant cytoplasm and indistinct cell borders. The cells appear to overlap. The nuclei are round, with fine to stippled chromatin

Table 15.12 Immunohistochemical studies

Marker	Purpose	Result
Fli-1	Ewing sarcoma	Equivocal
TLE1	Synovial sarcoma	Focal
Desmin, myogenin	Rhabdomyosarcoma	Negative
TdT, CD43	Lymphoblastic lymphoma	Negative
CK20, CD56	Merkel cell carcinoma	Patchy CD20+(not clearly dot-like) CD56 negative
Pan-cytokeratin	Screen for neuroendocrine carcinoma, synovial sarcoma	Patchy but strong positivity

(continued)

Diagnosis: Synovial sarcoma (poorly differentiated).

Discussion: This is a prime example demonstrating the morphologic and immunophenotypic overlap in sarcomas. Molecular testing is required in any case in which the presumptive diagnosis is challenged by any other diagnosis. Immunohistochemistry narrowed the differential but did not provide a conclusion. Molecular testing is the gold standard in such cases.

References

1. McCluggage WG. Recent developments in vulvovaginal pathology. Histopathology. 2009;54(2):156–73.
2. Fitzhugh VA, Heller DS. Mesenchymal lesions of the vulva. J Low Genit Tract Dis. 2011;15(2):134–45.
3. McCluggage WG. A review and update of morphologically bland vulvovaginal mesenchymal lesions. Int J Gynecol Pathol. 2005;24(1):26–38.
4. Nucci MR, Fletcher CD. Vulvovaginal soft tissue tumours: update and review. Histopathology. 2000;36(2):97–108.
5. Nielsen GP, Young RH. Mesenchymal tumors and tumor-like lesions of the female genital tract: a selective review with emphasis on recently described entities. Int J Gynecol Pathol. 2001;20(2):105–27.
6. Bulun SE. Uterine fibroids. N Engl J Med. 2013;369(14):1344–55.
7. Newman PL, Fletcher CD. Smooth muscle tumours of the external genitalia: clinicopathological analysis of a series. Histopathology. 1991;18(6):523–9.
8. Nielsen GP, Rosenberg AE, Koerner FC, Young RH, Scully RE. Smooth-muscle tumors of the vulva. A clinicopathological study of 25 cases and review of the literature. Am J Surg Pathol. 1996;20(7):779–93.
9. Tavassoli FA, Norris HJ. Smooth muscle tumors of the vulva. Obstet Gynecol. 1979;53(2):213–7.
10. Hornick JL, Fletcher CD. Criteria for malignancy in nonvisceral smooth muscle tumors. Ann Diagn Pathol. 2003;7(1):60–6.
11. Koc O, Sengul N, Gurel S. Perineal leiomyoma mimicking complex Bartholin mass. Int Urogynecol J. 2010;21(4):495–7.
12. McKenzie M, Pintilie H, Wilkinson N, Lane G, Orton J, El-Ghobashy A. A rare case of vulval leiomyosarcoma: management and an updated review of the literature. J Obstet Gynaecol. 2011;31(7):675–6.
13. Tjalma WA, Colpaert CG. Myxoid leiomyosarcoma of the vulva. Gynecol Oncol. 2005;96(2):548–51.
14. Shankar S, Todd PM, Rytina E, Crawford RA. Leiomyosarcoma of the vulva. J Eur Acad Dermatol Venereol. 2006;20(1):116–7.
15. Salehin D, Haugk C, William M, Hemmerlein B, Thill M, Diedrich K, et al. Leiomyosarcoma of the vulva. Eur J Gynaecol Oncol. 2012;33(3):306–8.
16. Dhar KK, Sau AK, Dey P, Vasishta K. Leiomyosarcoma of the labium minus. Int J Gynaecol Obstet. 1994;44(2):166–7.
17. Francis SA, Wilcox FL, Sissons M. Bartholin's gland leiomyoma: a diagnostic and management dilemma. J Obstet Gynaecol Res. 2012;38(6):941–3.
18. Nemoto T, Shinoda M, Komatsuzaki K, Hara T, Kojima M, Ogihara T. Myxoid leiomyoma of the vulva mimicking aggressive angiomyxoma. Pathol Int. 1994;44(6):454–9.
19. Hopkins-Luna AM, Chambers DC, Goodman MD. Epithelioid leiomyoma of the vulva. J Natl Med Assoc. 1999;91(3):171–3.
20. Chandrasekar A, Devakannan T. Leiomyoma of clitoris, an unusual cause of clitoromegaly in a post menopausal patient - a case report and review of the literature. Int J Med Health Sci. 2012;1(3):104–7.
21. Di Gilio AR, Cormio G, Resta L, Carriero C, Loizzi V, Parisi AM, et al. Rapid growth of myxoid leiomyosarcoma of the vulva during pregnancy: a case report. Int J Gynecol Cancer. 2004;14(1):172–5.
22. Horton E, Dobin SM, Debiec-Rychter M, Donner LR. A clonal translocation (7;8)(p13;q11.2) in a leiomyoma of the vulva. Cancer Genet Cytogenet. 2006;170(1):58–60.
23. Zhou J, Ha BK, Schubeck D, Chung-Park M. Myxoid epithelioid leiomyoma of the vulva: a case report. Gynecol Oncol. 2006;103(1):342–5.
24. Faber K, Jones MA, Spratt D, Tarraza HM. Vulvar leiomyomatosis in a patient with esophagogastric leiomyomatosis: review of the syndrome. Gynecol Oncol. 1991;41(1):92–4.
25. Hoelscher AC, Hoelscher AH, Drebber U, Bludau M, Schroeder W. Hereditary esophageal-vulvar syndrome. Ann Thorac Surg. 2012;94(3):e65–7.
26. Barricks RL. Multiple leiomyomata with associated clitoral hypertrophy. J Iowa Med Soc. 1973;63(11):535–8.
27. Cochat P, Guibaud P, Garcia Torres R, Roussel B, Guarner V, Larbre F. Diffuse leiomyomatosis in Alport syndrome. J Pediatr. 1988;113(2):339–43.
28. Miner JH. Alport syndrome with diffuse leiomyomatosis. When and when not? Am J Pathol. 1999;154(6):1633–5.
29. Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasms. A clinicopathologic study of 213 cases. Am J Surg Pathol. 1994;18(6):535–58.

30. Chiang S, Oliva E. Recent developments in uterine mesenchymal neoplasms. Histopathology. 2013;62(1):124–37.
31. Wilkinson E, Hardt N. Vulva. In: Mill S, editor. Histology for pathologists. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
32. Sandberg AA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyosarcoma. Cancer Genet Cytogenet. 2005;161(1):1–19.
33. Sandberg AA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyoma. Cancer Genet Cytogenet. 2005;158(1):1–26.
34. Siegle JC, Cartmell L. Vulvar leiomyoma associated with estrogen/progestin therapy. A case report. J Reprod Med. 1995;40(2):147–8.
35. McGinley KM, Bryant S, Kattine AA, Fitzgibbon JF, Googe PB. Cutaneous leiomyomas lack estrogen and progesterone receptor immunoreactivity. J Cutan Pathol. 1997;24(4):241–5.
36. Folpe AL, Cooper K. Best practices in diagnostic immunohistochemistry: pleomorphic cutaneous spindle cell tumors. Arch Pathol Lab Med. 2007;131(10):1517–24.
37. Perez-Montiel MD, Plaza JA, Dominguez-Malagon H, Suster S. Differential expression of smooth muscle myosin, smooth muscle actin, h-caldesmon, and calponin in the diagnosis of myofibroblastic and smooth muscle lesions of skin and soft tissue. Am J Dermatopathol. 2006;28(2):105–11.
38. Hisaoka M, Wei-Qi S, Jian W, Morio T, Hashimoto H. Specific but variable expression of h-caldesmon in leiomyosarcomas: an immunohistochemical reassessment of a novel myogenic marker. Appl Immunohistochem Mol Morphol. 2001;9(4):302–8.
39. Nicolas MM, Tamboli P, Gomez JA, Czerniak BA. Pleomorphic and dedifferentiated leiomyosarcoma: clinicopathologic and immunohistochemical study of 41 cases. Hum Pathol. 2010;41(5):663–71.
40. Flucke U, van Krieken JH, Mentzel T. Cellular angiofibroma: analysis of 25 cases emphasizing its relationship to spindle cell lipoma and mammary-type myofibroblastoma. Mod Pathol. 2011;24(1):82–9.
41. Iwasa Y, Fletcher CD. Cellular angiofibroma: clinicopathologic and immunohistochemical analysis of 51 cases. Am J Surg Pathol. 2004;28(11):1426–35.
42. McCluggage WG, Connolly L, McBride HA. HMGA2 is a sensitive but not specific immunohistochemical marker of vulvovaginal aggressive angiomyxoma. Am J Surg Pathol. 2010;34(7):1037–42.
43. Park HR, Park YK. Assessment of diagnostic utility of anti-CD34 in soft tissue tumors. J Korean Med Sci. 1995;10(6):436–41.
44. Tardío JC. Leiomyomatous nodules in a cellular angiofibroma: a hitherto unreported finding. Virchows Arch. 2009;454(5):595–8.
45. Sneige N, Yaziji H, Mandavilli SR, Perez ER, Ordonez NG, Gown AM, et al. Low-grade (fibromatosis-like) spindle cell carcinoma of the breast. Am J Surg Pathol. 2001;25(8):1009–16.
46. Iwata J, Fletcher CD. Immunohistochemical detection of cytokeratin and epithelial membrane antigen in leiomyosarcoma: a systematic study of 100 cases. Pathol Int. 2000;50(1):7–14.
47. Lin GY, Sun X, Badve S. Pathologic quiz case. Vaginal wall mass in a 47-year-old woman. Vaginal rhabdomyoma. Arch Pathol Lab Med. 2002;126(10):1241–2.
48. Gad A, Eusebi V. Rhabdomyoma of the vagina. J Pathol. 1975;115(3):179–81.
49. Konrad EA, Meister P, Hübner G. Extracardiac rhabdomyoma: report of different types with light microscopic and ultrastructural studies. Cancer. 1982;49(5):898–907.
50. Willis J, Abdul-Karim FW, di Sant'Agnese PA. Extracardiac rhabdomyomas. Semin Diagn Pathol. 1994;11(1):15–25.
51. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, editors. WHO classification of tumours of soft tissue and bone. Lyon: IARC; 2013.
52. Gold JH, Bossen EH. Benign vaginal rhabdomyoma: a light and electron microscopic study. Cancer. 1976;37(5):2283–94.
53. De M, Tribedi B. Skeletal muscle tissue tumour. Br J Surg. 1940;28:17–28.
54. Urbanke A. True rhabdomyoma of the uterus. Zentralbl Allg Pathol. 1962;103:241–3.
55. Hański W, Hagel-Lewicka E, Daniszewski K. Rhabdomyomas of female genital tract. Report on two cases. Zentralbl Pathol. 1991;137(5):439–42.
56. Lu DY, Chang S, Cook H, Alizadeh Y, Karam AK, Moatamed NA, et al. Genital rhabdomyoma of the urethra in an infant girl. Hum Pathol. 2012;43(4):597–600.
57. Huang TY, Chen JT, Ho WL. Ovarian serous cystadenoma with mural nodules of genital rhabdomyoma. Hum Pathol. 2005;36(4):433–5.
58. López JI, Brouard I, Eizaguirre B. Rhabdomyoma of the vagina. Eur J Obstet Gynecol Reprod Biol. 1992;45(2):147–8.
59. Chabrel CM, Beilby JO. Vaginal rhabdomyoma. Histopathology. 1980;4(6):645–51.
60. Iversen UM. Two cases of benign vaginal rhabdomyoma. Case reports. APMIS. 1996;104(7–8):575–8.
61. Patrelli TS, Franchi L, Gizzo S, Kiener A, Berretta R, Piantelli G, et al. Rhabdomyoma of the vagina. Case report and short literature review. Ann Pathol. 2012;32(1):53–7.
62. López Varela C, López de la Riva M, La Cruz Pelea C. Vaginal rhabdomyomas. Int J Gynaecol Obstet. 1994;47(2):169–70.
63. Losi L, Choreutaki T, Nascetti D, Eusebi V. Recurrence in a case of rhabdomyoma of the vagina. Pathologica. 1995;87(6):704–8.
64. Maitra A, Kumar V. Diseases of infancy and childhood. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease. 8th ed. Philadelphia: Saunders Elsevier; 2010.

65. Sokol A, Shveiky D, Glob. libr. women's med., (ISSN: 1756-2228) 2008; DOI 10.3843/GLOWM. 10000.
66. McCluggage WG, Longacre TA, Fisher C. Myogenin expression in vulvovaginal spindle cell lesions: analysis of a series of cases with an emphasis on diagnostic pitfalls. Histopathology. 2013;63(4):545–50.
67. Suarez Vilela D, Gimenez Pizarro A, Rio SM. Vaginal rhabdomyoma and adenosis. Histopathology. 1990; 16(4):393–4.
68. Hettmer S, Teot LA, van Hummelen P, MacConaill L, Bronson RT, Dall'Osso C, et al. Mutations in hedgehog pathway genes in fetal rhabdomyomas. J Pathol. 2013;231(1):44–52.
69. Ostör AG, Fortune DW, Riley CB. Fibroepithelial polyps with atypical stromal cells (pseudosarcoma botryoides) of vulva and vagina. A report of 13 cases. Int J Gynecol Pathol. 1988;7(4):351–60.
70. Han SH, Song HJ, Hong WK, Lee HS, Choi GS, Shin JH. Rhabdomyomatous mesenchymal hamartoma of the vagina. Pediatr Dermatol. 2009;26(6):753–5.
71. Rosenberg AS, Kirk J, Morgan MB. Rhabdomyomatous mesenchymal hamartoma: an unusual dermal entity with a report of two cases and a review of the literature. J Cutan Pathol. 2002;29(4): 238–43.
72. Stout AP. Rhabdomyosarcoma of the skeletal muscles. Ann Surg. 1946;123(3):447–72.
73. Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975-2005. Cancer. 2009;115(18):4218–26.
74. Harms D. Alveolar rhabdomyosarcoma: a prognostically unfavorable rhabdomyosarcoma type and its necessary distinction from embryonal rhabdomyosarcoma. Curr Top Pathol. 1995;89:273–96.
75. Ferrari A, Dileo P, Casanova M, Bertulli R, Meazza C, Gandola L, et al. Rhabdomyosarcoma in adults. A retrospective analysis of 171 patients treated at a single institution. Cancer. 2003;98(3):571–80.
76. Raney RB, Walterhouse DO, Meza JL, Andrassy RJ, Breneman JC, Crist WM, et al. Results of the Intergroup Rhabdomyosarcoma Study Group D9602 protocol, using vincristine and dactinomycin with or without cyclophosphamide and radiation therapy, for newly diagnosed patients with low-risk embryonal rhabdomyosarcoma: a report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group. J Clin Oncol. 2011;29(10):1312–8.
77. Weiss AR, Lyden ER, Anderson JR, Hawkins DS, Spunt SL, Walterhouse DO, et al. Histologic and clinical characteristics can guide staging evaluations for children and adolescents with rhabdomyosarcoma: a report from the Children's Oncology Group Soft Tissue Sarcoma Committee. J Clin Oncol. 2013;31(26):3226–32.
78. Smith AC, Squire JA, Thorner P, Zielenska M, Shuman C, Grant R, et al. Association of alveolar rhabdomyosarcoma with the Beckwith-Wiedemann syndrome. Pediatr Dev Pathol. 2001;4(6):550–8.
79. Choufani S, Shuman C, Weksberg R. Beckwith-Wiedemann syndrome. Am J Med Genet C Semin Med Genet. 2010;154C(3):343–54.
80. Kuroiwa M, Sakamoto J, Shimada A, Suzuki N, Hirato J, Park MJ, et al. Manifestation of alveolar rhabdomyosarcoma as primary cutaneous lesions in a neonate with Beckwith-Wiedemann syndrome. J Pediatr Surg. 2009;44(3):e31–5.
81. Furlong MA, Mentzel T, Fanburg-Smith JC. Pleomorphic rhabdomyosarcoma in adults: a clinicopathologic study of 38 cases with emphasis on morphologic variants and recent skeletal muscle-specific markers. Mod Pathol. 2001;14(6):595–603.
82. Furlong MA, Fanburg-Smith JC. Pleomorphic rhabdomyosarcoma in children: four cases in the pediatric age group. Ann Diagn Pathol. 2001;5(4):199–206.
83. Ferguson SE, Gerald W, Barakat RR, Chi DS, Soslow RA. Clinicopathologic features of rhabdomyosarcoma of gynecologic origin in adults. Am J Surg Pathol. 2007;31(3):382–9.
84. Ghaemmaghami F, Karimi Zarchi M, Ghasemi M. Lower genital tract rhabdomyosarcoma: case series and literature review. Arch Gynecol Obstet. 2008;278(1):65–9.
85. Kirsch CH, Goodman M, Esiashvili N. Outcome of female pediatric patients diagnosed with genital tract rhabdomyosarcoma based on analysis of cases registered in SEER database between 1973 and 2006. Am J Clin Oncol. 2014;37(1):47–50.
86. Gong Y, Chao J, Bauer B, Sun X, Chou PM. Primary cutaneous alveolar rhabdomyosarcoma of the perineum. Arch Pathol Lab Med. 2002;126(8):982–4.
87. Al-Tonbary Y, Zalata K, Sarhan M, El-Ashery R, Fouda A. Rhabdomyosarcoma of the clitoris. Hematol Oncol Stem Cell Ther. 2008;1(2):133–5.
88. Copeland LJ, Sneige N, Stringer CA, Gershenson DM, Saul PB, Kavanagh JJ. Alveolar rhabdomyosarcoma of the female genitalia. Cancer. 1985;56(4): 849–55.
89. Youngstrom EA, Bartkowski DP. Vulvar embryonal rhabdomyosarcoma: a case report. J Pediatr Urol. 2013;9(4):e144–6.
90. Puranik RB, Naik S, Kulkarni S, Kulkarni MH. Alveolar rhabdomyosarcoma of vulva. Indian J Pathol Microbiol. 2010;53(1):167–8.
91. Imachi M, Tsukamoto N, Kamura T, Shigematsu T, Funakoshi K, Nakano H. Alveolar rhabdomyosarcoma of the vulva. Report of two cases. Acta Cytol. 1991;35(3):345–9.
92. Sultan I, Qaddoumi I, Yaser S, Rodriguez-Galindo C, Ferrari A. Comparing adult and pediatric rhabdomyosarcoma in the surveillance, epidemiology and end results program, 1973 to 2005: an analysis of 2,600 patients. J Clin Oncol. 2009;27(20):3391–7.
93. Newton WA, Gehan EA, Webber BL, Marsden HB, van Unnik AJ, Hamoudi AB, et al. Classification of rhabdomyosarcomas and related sarcomas. Pathologic aspects and proposal for a new classification–an Intergroup Rhabdomyosarcoma Study. Cancer. 1995;76(6):1073–85.

94. Charytonowicz E, Cordon-Cardo C, Matushansky I, Ziman M. Alveolar rhabdomyosarcoma: is the cell of origin a mesenchymal stem cell? Cancer Lett. 2009;279(2):126–36.
95. Parham DM, Barr FG. Classification of rhabdomyosarcoma and its molecular basis. Adv Anat Pathol. 2013;20(6):387–97.
96. Parham DM, Ellison DA. Rhabdomyosarcomas in adults and children: an update. Arch Pathol Lab Med. 2006;130(10):1454–65.
97. Bond SJ, Seibel N, Kapur S, Newman KD. Rhabdomyosarcoma of the clitoris. Cancer. 1994; 73(7):1984–6.
98. Kodet R, Newton WA, Hamoudi AB, Asmar L, Jacobs DL, Maurer HM. Childhood rhabdomyosarcoma with anaplastic (pleomorphic) features. A report of the Intergroup Rhabdomyosarcoma Study. Am J Surg Pathol. 1993;17(5):443–53.
99. Qualman S, Lynch J, Bridge J, Parham D, Teot L, Meyer W, et al. Prevalence and clinical impact of anaplasia in childhood rhabdomyosarcoma : a report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group. Cancer. 2008;113(11):3242–7.
100. Fadare O, Bonvicino A, Martel M, Renshaw IL, Azodi M, Parkash V. Pleomorphic rhabdomyosarcoma of the uterine corpus: a clinicopathologic study of 4 cases and a review of the literature. Int J Gynecol Pathol. 2010;29(2):122–34.
101. Nascimento AF, Fletcher CD. Spindle cell rhabdomyosarcoma in adults. Am J Surg Pathol. 2005;29(8):1106–13.
102. Morotti RA, Nicol KK, Parham DM, Teot LA, Moore J, Hayes J, et al. An immunohistochemical algorithm to facilitate diagnosis and subtyping of rhabdomyosarcoma: the Children's Oncology Group experience. Am J Surg Pathol. 2006;30(8):962–8.
103. Kumar S, Perlman E, Harris CA, Raffeld M, Tsokos M. Myogenin is a specific marker for rhabdomyosarcoma: an immunohistochemical study in paraffin-embedded tissues. Mod Pathol. 2000;13(9):988–93.
104. Cessna MH, Zhou H, Perkins SL, Tripp SR, Layfield L, Daines C, et al. Are myogenin and myoD1 expression specific for rhabdomyosarcoma? A study of 150 cases, with emphasis on spindle cell mimics. Am J Surg Pathol. 2001;25(9):1150–7.
105. Wang NP, Marx J, McNutt MA, Rutledge JC, Gown AM. Expression of myogenic regulatory proteins (myogenin and MyoD1) in small blue round cell tumors of childhood. Am J Pathol. 1995;147(6):1799–810.
106. Cui S, Hano H, Harada T, Takai S, Masui F, Ushigome S. Evaluation of new monoclonal anti-MyoD1 and anti-myogenin antibodies for the diagnosis of rhabdomyosarcoma. Pathol Int. 1999;49(1): 62–8.
107. Seidal T, Mark J, Hagmar B, Angervall L. Alveolar rhabdomyosarcoma: a cytogenetic and correlated cytological and histological study. Acta Pathol Microbiol Immunol Scand A. 1982;90(5):345–54.
108. Sorensen PH, Lynch JC, Qualman SJ, Tirabosco R, Lim JF, Maurer HM, et al. PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the Children's Oncology Group. J Clin Oncol. 2002; 20(11):2672–9.
109. Barr FG. Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. Oncogene. 2001;20(40):5736–46.
110. Williamson D, Missiaglia E, de Reyniès A, Pierron G, Thuille B, Palenzuela G, et al. Fusion gene-negative alveolar rhabdomyosarcoma is clinically and molecularly indistinguishable from embryonal rhabdomyosarcoma. J Clin Oncol. 2010;28(13):2151–8.
111. Skapek SX, Anderson J, Barr FG, Bridge JA, Gastier-Foster JM, Parham DM, et al. PAX-FOXO1 fusion status drives unfavorable outcome for children with rhabdomyosarcoma: a Children's Oncology Group report. Pediatr Blood Cancer. 2013;60(9):1411–7.
112. Hawkins DS, Spunt SL, Skapek SX, Committee CSTS. Children's Oncology Group's 2013 blueprint for research: soft tissue sarcomas. Pediatr Blood Cancer. 2013;60(6):1001–8.
113. Boué DR, Parham DM, Webber B, Crist WM, Qualman SJ. Clinicopathologic study of ectomesenchymomas from Intergroup Rhabdomyosarcoma Study Groups III and IV. Pediatr Dev Pathol. 2000;3(3):290–300.
114. Tsokos M, Alaggio RD, Dehner LP, Dickman PS. Ewing sarcoma/peripheral primitive neuroectodermal tumor and related tumors. Pediatr Dev Pathol. 2012;15 Suppl 1:108–26.
115. Machado I, Llombart-Bosch A. Myogenic differentiation in Ewing sarcoma family of tumors. Am J Surg Pathol. 2011;35(3):464; author reply -5
116. Bahrami A, Gown AM, Baird GS, Hicks MJ, Folpe AL. Aberrant expression of epithelial and neuroendocrine markers in alveolar rhabdomyosarcoma: a potentially serious diagnostic pitfall. Mod Pathol. 2008;21(7):795–806.
117. Weiss SW, Goldblum JR. Benign tumors of peripheral nerves. Enzinger and Weiss's soft tissue tumors. 5th ed. St. Louis: Mosby; 2007.
118. Soroush M, Modirian E, Masoumi M. Neuroma in bilateral upper limb amputation. Orthopedics. 2008;31(12):1193.
119. Dharmarathna HM, Tripathi N, Atkinson P. Painful, traumatic neuroma of an episiotomy scar: a case report. J Reprod Med. 2007;52(5):456–7.
120. Lefhalm B, Fleckenstein G, Kuhn W. Neurinoma in the area of an episiotomy scar. A case report. Geburtshilfe Frauenheilkd. 1996;56(10):566–8.
121. Sonnendecker EW, Cohen RJ, Dreyer L, Sher RC, Findlay GH. Neuroma of the vulva. A case report. J Reprod Med. 1993;38(1):33–6.
122. Fernández-Aguilar S, Noël JC. Neuroma of the clitoris after female genital cutting. Obstet Gynecol. 2003;101(5 Pt 2):1053–4.
123. Abdulcadir J, Pusztaszeri M, Vilarino R, Dubuisson JB, Vlastos AT. Clitoral neuroma after female genital

mutilation/cutting: a rare but possible event. J Sex Med. 2012;9(4):1220–5.
124. World Health Organization. Female genital mutilation. 2014. [updated 2014 Feb; cited 2014 Feb]. Available from: http://www.who.int/mediacentre/factsheets/fs241/en/
125. Rotter K, Sanhueza R, Robles K, Godoy M. A descriptive study of traumatic lower limb amputees from the Hospital Hel Trabajador: clinical evolution from the accident until rehabilitation discharge. Prosthet Orthot Int. 2006;30(1):81–6.
126. Fenrich K, Gordon T. Canadian Association of Neuroscience review: axonal regeneration in the peripheral and central nervous systems–current issues and advances. Can J Neurol Sci. 2004;31(2):142–56.
127. Ortiz-Hidalgo C, Weller RO. Peripheral nervous system. In: Mills SE, editor. Histology for pathologists. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
128. Argenyi ZB, Santa Cruz D, Bromley C. Comparative light-microscopic and immunohistochemical study of traumatic and palisaded encapsulated neuromas of the skin. Am J Dermatopathol. 1992;14(6):504–10.
129. Mathews GJ, Osterholm JL. Painful traumatic neuromas. Surg Clin North Am. 1972;52(5):1313–24.
130. Baron R. Mechanisms of disease: neuropathic pain–a clinical perspective. Nat Clin Pract Neurol. 2006;2(2):95–106.
131. Baron R. Peripheral neuropathic pain: from mechanisms to symptoms. Clin J Pain. 2000;16(2 Suppl):S12–20.
132. Reed ML, Jacoby RA. Cutaneous neuroanatomy and neuropathology. Normal nerves, neural-crest derivatives, and benign neural neoplasms in the skin. Am J Dermatopathol. 1983;5(4):335–62.
133. Rodríguez-Peralto JL, Riveiro-Falkenbach E, Carrillo R. Benign cutaneous neural tumors. Semin Diagn Pathol. 2013;30(1):45–57.
134. Fogt F, Capodieci P, Loda M. Assessment of perineurial invasion by GLUT-1 immunohistochemistry. Appl Immunohistochem. 1995;3:194–7.
135. Hirose T, Tani T, Shimada T, Ishizawa K, Shimada S, Sano T. Immunohistochemical demonstration of EMA/Glut1-positive perineurial cells and CD34-positive fibroblastic cells in peripheral nerve sheath tumors. Mod Pathol. 2003;16(4):293–8.
136. Jokinen CH, Dadras SS, Goldblum JR, van de Rijn M, West RB, Rubin BP. Diagnostic implications of podoplanin expression in peripheral nerve sheath neoplasms. Am J Clin Pathol. 2008;129(6):886–93.
137. Macarenco RS, Ellinger F, Oliveira AM. Perineurioma: a distinctive and underrecognized peripheral nerve sheath neoplasm. Arch Pathol Lab Med. 2007;131(4):625–36.
138. Writing Group of the Histiocyte Society. Histiocytosis syndromes in children. Lancet. 1987;1(8526):208–9.
139. Lieberman PH, Jones CR, Steinman RM, Erlandson RA, Smith J, Gee T, et al. Langerhans cell (eosinophilic) granulomatosis. A clinicopathologic study encompassing 50 years. Am J Surg Pathol. 1996;20(5):519–52.
140. Howarth DM, Gilchrist GS, Mullan BP, Wiseman GA, Edmonson JH, Schomberg PJ. Langerhans cell histiocytosis: diagnosis, natural history, management, and outcome. Cancer. 1999;85(10):2278–90.
141. Querings K, Starz H, Balda BR. Clinical spectrum of cutaneous Langerhans' cell histiocytosis mimicking various diseases. Acta Derm Venereol. 2006;86(1):39–43.
142. Montero AJ, Díaz-Montero CM, Malpica A, Ramirez PT, Kavanagh JJ. Langerhans cell histiocytosis of the female genital tract: a literature review. Int J Gynecol Cancer. 2003;13(3):381–8.
143. Chang JC, Blake DG, Leung BV, Plaza JA. Langerhans cell histiocytosis associated with lichen sclerosus of the vulva: case report and review of the literature. J Cutan Pathol. 2013;40(2):279–83.
144. El-Safadi S, Dreyer T, Oehmke F, Muenstedt K. Management of adult primary vulvar Langerhans cell histiocytosis: review of the literature and a case history. Eur J Obstet Gynecol Reprod Biol. 2012;163(2):123–8.
145. Axiotis CA, Merino MJ, Duray PH. Langerhans cell histiocytosis of the female genital tract. Cancer. 1991;67(6):1650–60.
146. Venizelos ID, Mandala E, Tatsiou ZA, Acholos V, Goutzioulis M. Primary langerhans cell histiocytosis of the vulva. Int J Gynecol Pathol. 2006;25(1):48–51.
147. Dietrich JE, Edwards C, Laucirica R, Kaufman RH. Langerhans cell histiocytosis of the vulva: two case reports. J Low Genit Tract Dis. 2004;8(2):147–9.
148. Padula A, Medeiros LJ, Silva EG, Deavers MT. Isolated vulvar Langerhans cell histiocytosis: report of two cases. Int J Gynecol Pathol. 2004;23(3):278–83.
149. Pan Z, Sharma S, Sharma P. Primary langerhans cell histiocytosis of the vulva: report of a case and brief review of the literature. Indian J Pathol Microbiol. 2009;52(1):65–8.
150. Santillan A, Montero AJ, Kavanagh JJ, Liu J, Ramirez PT. Vulvar Langerhans cell histiocytosis: a case report and review of the literature. Gynecol Oncol. 2003;91(1):241–6.
151. Foley S, Panting K, Bell H, Leonard N, Franks A. Rapid resolution of primary vulval adult Langerhans cell histiocytosis with very potent topical corticosteroids. Australas J Dermatol. 2011;52(1):e8–14.
152. Komp DM. Langerhans cell histiocytosis. N Engl J Med. 1987;316(12):747–8.
153. Badalian-Very G, Vergilio JA, Fleming M, Rollins BJ. Pathogenesis of Langerhans cell histiocytosis. Annu Rev Pathol. 2013;8:1–20.
154. Allen CE, Li L, Peters TL, Leung HC, Yu A, Man TK, et al. Cell-specific gene expression in Langerhans cell histiocytosis lesions reveals a distinct profile compared with epidermal Langerhans cells. J Immunol. 2010;184(8):4557–67.

155. Jeziorski E, Senechal B, Molina TJ, Devez F, Leruez-Ville M, Morand P, et al. Herpes-virus infection in patients with Langerhans cell histiocytosis: a case-controlled sero-epidemiological study, and in situ analysis. PLoS One. 2008;3(9):e3262.
156. Jenson HB, McClain KL, Leach CT, Deng JH, Gao SJ. Evaluation of human herpesvirus type 8 infection in childhood Langerhans cell histiocytosis. Am J Hematol. 2000;64(4):237–41.
157. Osband ME, Lipton JM, Lavin P, Levey R, Vawter G, Greenberger JS, et al. Histiocytosis-X. N Engl J Med. 1981;304(3):146–53.
158. Geissmann F, Lepelletier Y, Fraitag S, Valladeau J, Bodemer C, Debré M, et al. Differentiation of Langerhans cells in Langerhans cell histiocytosis. Blood. 2001;97(5):1241–8.
159. Hoang MP, Owen SA, Haisley-Royster C, Allen MH, Shea CR, Selim MA. Papular eruption of the scalp accompanied by axillary and vulvar ulcerations. Arch Dermatol. 2001;137(9):1241–6.
160. Chu T. Langerhans cell histiocytosis. Australas J Dermatol. 2001;42(4):237–42.
161. Haupt R, Minkov M, Astigarraga I, Schäfer E, Nanduri V, Jubran R, et al. Langerhans cell histiocytosis (LCH): guidelines for diagnosis, clinical work-up, and treatment for patients till the age of 18 years. Pediatr Blood Cancer. 2013;60(2):175–84.
162. Xu X, Liu WP, Yang QP, Wang WY, Liao DY, Zhao S, et al. Langerhans cell histiocytosis: a clinicopathologic and immunohistochemical analysis of 258 cases. Zhonghua Bing Li Xue Za Zhi. 2012; 41(2):91–6.
163. Pileri SA, Grogan TM, Harris NL, Banks P, Campo E, Chan JK, et al. Tumours of histiocytes and accessory dendritic cells: an immunohistochemical approach to classification from the International Lymphoma Study Group based on 61 cases. Histopathology. 2002;41(1):1–29.
164. Orii T, Takeda H, Kawata S, Maeda K, Yamakawa M. Differential immunophenotypic analysis of dendritic cell tumours. J Clin Pathol. 2010;63(6): 497–503.
165. Chikwava K, Jaffe R. Langerin (CD207) staining in normal pediatric tissues, reactive lymph nodes, and childhood histiocytic disorders. Pediatr Dev Pathol. 2004;7(6):607–14.
166. Lau SK, Chu PG, Weiss LM. Immunohistochemical expression of Langerin in Langerhans cell histiocytosis and non-Langerhans cell histiocytic disorders. Am J Surg Pathol. 2008;32(4):615–9.
167. Valladeau J, Ravel O, Dezutter-Dambuyant C, Moore K, Kleijmeer M, Liu Y, et al. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. Immunity. 2000;12(1):71–81.
168. da Costa CE, Szuhai K, van Eijk R, Hoogeboom M, Sciot R, Mertens F, et al. No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies. Genes Chromosomes Cancer. 2009;48(3):239–49.
169. Jokinen CH, Wolgamot GM, Wood BL, Olerud J, Argenyi ZB. Lymphomatoid papulosis with CD1a+ dendritic cell hyperplasia, mimicking Langerhans cell histiocytosis. J Cutan Pathol. 2007;34(7):584–7.
170. Bhattacharjee P, Glusac EJ. Langerhans cell hyperplasia in scabies: a mimic of Langerhans cell histiocytosis. J Cutan Pathol. 2007;34(9):716–20.
171. Janssen D, Harms D. Juvenile xanthogranuloma in childhood and adolescence: a clinicopathologic study of 129 patients from the Kiel pediatric tumor registry. Am J Surg Pathol. 2005;29(1):21–8.
172. Castilla EA, Ormsby A. Adult xanthogranuloma of the vulva: case report and review. Pathology. 2002;34(1):86–7.
173. Yin J, Zhang F, Zhang H, Shen L, Li Q, Hu S, et al. Hand-Schüller-Christian disease and Erdheim-Chester disease: coexistence and discrepancy. Oncologist. 2013;18(1):19–24.
174. Janku F, Munoz J, Subbiah V, Kurzrock R. A tale of two histiocytic disorders. Oncologist. 2013;18(1):2–4.
175. Guillou L, Wadden C, Coindre JM, Krausz T, Fletcher CD. "Proximal-type" epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features. Clinicopathologic, immunohistochemical, and ultrastructural study of a series. Am J Surg Pathol. 1997;21(2):130–46.
176. Hasegawa T, Matsuno Y, Shimoda T, Umeda T, Yokoyama R, Hirohashi S. Proximal-type epithelioid sarcoma: a clinicopathologic study of 20 cases. Mod Pathol. 2001;14(7):655–63.
177. Fisher C. Epithelioid sarcoma of Enzinger. Adv Anat Pathol. 2006;13(3):114–21.
178. Sakharpe A, Lahat G, Gulamhusein T, Liu P, Bolshakov S, Nguyen T, et al. Epithelioid sarcoma and unclassified sarcoma with epithelioid features: clinicopathological variables, molecular markers, and a new experimental model. Oncologist. 2011;16(4):512–22.
179. Chase DR, Enzinger FM. Epithelioid sarcoma. Diagnosis, prognostic indicators, and treatment. Am J Surg Pathol. 1985;9(4):241–63.
180. Shmookler BM, Gunther SF. Superficial epithelioid sarcoma: a clinical and histologic stimulant of benign cutaneous disease. J Am Acad Dermatol. 1986;14(5 Pt 2):893–8.
181. Argenta PA, Thomas S, Chura JC. Proximal-type epithelioid sarcoma vs. malignant rhabdoid tumor of the vulva: a case report, review of the literature, and an argument for consolidation. Gynecol Oncol. 2007;107(1):130–5.
182. Ulbright TM, Brokaw SA, Stehman FB, Roth LM. Epithelioid sarcoma of the vulva. Evidence suggesting a more aggressive behavior than extra-genital epithelioid sarcoma. Cancer. 1983;52(8):1462–9.
183. Tholpady A, Lonergan CL, Wick MR. Proximal-type epithelioid sarcoma of the vulva: relationship to malignant extrarenal rhabdoid tumor. Int J Gynecol Pathol. 2010;29(6):600–4.
184. Kim HJ, Kim MH, Kwon J, Kim JY, Park K, Ro JY. Proximal-type epithelioid sarcoma of the vulva

with INI1 diagnostic utility. Ann Diagn Pathol. 2012;16(5):411–5.
185. Andrisani A, Serena A, Ambrosini G, Capobianco G, Chiarelli S. Proximal-type epithelioid sarcoma of the mons pubis: report of a case. Eur J Gynaecol Oncol. 2011;32(3):339–42.
186. Piver MS, Tsukada Y, Barlow J. Epithelioid sarcoma of the vulva. Obstet Gynecol. 1972;40(6):839–42.
187. Hall DJ, Grimes MM, Goplerud DR. Epithelioid sarcoma of the vulva. Gynecol Oncol. 1980;9(2): 237–46.
188. Rekhi B, Gorad BD, Chinoy RF. Clinicopathological features with outcomes of a series of conventional and proximal-type epithelioid sarcomas, diagnosed over a period of 10 years at a tertiary cancer hospital in India. Virchows Arch. 2008;453(2):141–53.
189. Weissmann D, Amenta PS, Kantor GR. Vulvar epithelioid sarcoma metastatic to the scalp. A case report and review of the literature. Am J Dermatopathol. 1990;12(5):462–8.
190. Ong AC, Lim TY, Tan TC, Wang S, Raju GC. Proximal epithelioid sarcoma of the vulva: a case report and review of current medical literature. J Obstet Gynaecol Res. 2012;38(7):1032–5.
191. Enzinger FM. Epitheloid sarcoma. A sarcoma simulating a granuloma or a carcinoma. Cancer. 1970;26(5):1029–41.
192. Brenca M, Rossi S, Lorenzetto E, Piccinin E, Piccinin S, Rossi FM, et al. SMARCB1/INI1 genetic inactivation is responsible for tumorigenic properties of epithelioid sarcoma cell line VAESBJ. Mol Cancer Ther. 2013;12(6):1060–72.
193. Vries RG, Bezrookove V, Zuijderduijn LM, Kia SK, Houweling A, Oruetxebarria I, et al. Cancer-associated mutations in chromatin remodeler hSNF5 promote chromosomal instability by compromising the mitotic checkpoint. Genes Dev. 2005;19(6):665–70.
194. Miettinen M, Fanburg-Smith JC, Virolainen M, Shmookler BM, Fetsch JF. Epithelioid sarcoma: an immunohistochemical analysis of 112 classical and variant cases and a discussion of the differential diagnosis. Hum Pathol. 1999;30(8):934–42.
195. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol. 2009;33(4):542–50.
196. Kohashi K, Izumi T, Oda Y, Yamamoto H, Tamiya S, Taguchi T, et al. Infrequent SMARCB1/INI1 gene alteration in epithelioid sarcoma: a useful tool in distinguishing epithelioid sarcoma from malignant rhabdoid tumor. Hum Pathol. 2009;40(3):349–55.
197. Chbani L, Guillou L, Terrier P, Decouvelaere AV, Grégoire F, Terrier-Lacombe MJ, et al. Epithelioid sarcoma: a clinicopathologic and immunohistochemical analysis of 106 cases from the French sarcoma group. Am J Clin Pathol. 2009;131(2):222–7.
198. Modena P, Lualdi E, Facchinetti F, Galli L, Teixeira MR, Pilotti S, et al. SMARCB1/INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. Cancer Res. 2005;65(10):4012–9.
199. Sigauke E, Rakheja D, Maddox DL, Hladik CL, White CL, Timmons CF, et al. Absence of expression of SMARCB1/INI1 in malignant rhabdoid tumors of the central nervous system, kidneys and soft tissue: an immunohistochemical study with implications for diagnosis. Mod Pathol. 2006;19(5): 717–25.
200. Jackson EM, Sievert AJ, Gai X, Hakonarson H, Judkins AR, Tooke L, et al. Genomic analysis using high-density single nucleotide polymorphism-based oligonucleotide arrays and multiplex ligation-dependent probe amplification provides a comprehensive analysis of INI1/SMARCB1 in malignant rhabdoid tumors. Clin Cancer Res. 2009;15(6): 1923–30.
201. Papp G, Changchien YC, Péterfia B, Pecsenka L, Krausz T, Stricker TP, et al. SMARCB1 protein and mRNA loss is not caused by promoter and histone hypermethylation in epithelioid sarcoma. Mod Pathol. 2013;26(3):393–403.
202. Sullivan LM, Folpe AL, Pawel BR, Judkins AR, Biegel JA. Epithelioid sarcoma is associated with a high percentage of SMARCB1 deletions. Mod Pathol. 2013;26(3):385–92.
203. Banerjee SS, Harris M. Morphological and immunophenotypic variations in malignant melanoma. Histopathology. 2000;36(5):387–402.
204. Dehner LP. Primitive neuroectodermal tumor and Ewing's sarcoma. Am J Surg Pathol. 1993;17(1): 1–13.
205. Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, et al. The Ewing family of tumors–a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med. 1994;331(5):294–9.
206. Jawad MU, Cheung MC, Min ES, Schneiderbauer MM, Koniaris LG, Scully SP. Ewing sarcoma demonstrates racial disparities in incidence-related and sex-related differences in outcome: an analysis of 1631 cases from the SEER database, 1973–2005. Cancer. 2009;115(15):3526–36.
207. Shingde MV, Buckland M, Busam KJ, McCarthy SW, Wilmott J, Thompson JF, et al. Primary cutaneous Ewing sarcoma/primitive neuroectodermal tumour: a clinicopathological analysis of seven cases highlighting diagnostic pitfalls and the role of FISH testing in diagnosis. J Clin Pathol. 2009;62(10): 915–9.
208. Terrier-Lacombe MJ, Guillou L, Chibon F, Gallagher G, Benhattar J, Terrier P, et al. Superficial primitive Ewing's sarcoma: a clinicopathologic and molecular cytogenetic analysis of 14 cases. Mod Pathol. 2009;22(1):87–94.
209. McCluggage WG, Sumathi VP, Nucci MR, Hirsch M, Dal Cin P, Wells M, et al. Ewing family of tumours involving the vulva and vagina: report of a series of four cases. J Clin Pathol. 2007;60(6):674–80.
210. Fong YE, López-Terrada D, Zhai QJ. Primary Ewing sarcoma/peripheral primitive neuroectodermal tumor of the vulva. Hum Pathol. 2008;39(10):1535–9.

211. Kelling K, Noack F, Altgassen C, Kujath P, Bohlmann MK, Hoellen F. Primary metastasized extraskeletal Ewing sarcoma of the vulva: report of a case and review of the literature. Arch Gynecol Obstet. 2012;285(3):785–9.
212. Cetiner H, Kir G, Gelmann EP, Ozdemirli M. Primary vulvar Ewing sarcoma/primitive neuroectodermal tumor: a report of 2 cases and review of the literature. Int J Gynecol Cancer. 2009;19(6):1131–6.
213. Dadhwal V, Bahadur A, Gupta R, Bansal S, Mittal S. Peripheral neuroectodermal tumor of the vulva: a case report. J Low Genit Tract Dis. 2010;14(1):59–62.
214. Halil S, Kucuk M, Arvas M, Aydin O, Calay ZZ. Peripheral primitive neuroectodermal tumor (PNET) of the vulva: a case report. Eur J Gynaecol Oncol. 2011;32(1):117–8.
215. Moodley M, Jordaan A. Ewing's sarcoma of the vulva–a case report. Int J Gynecol Cancer. 2005; 15(6):1177–8.
216. Paredes E, Duarte A, Couceiro A, Fernandes D, Alves A, Bastos S. A peripheral neuroectodermal tumor of the vulva. Acta Med Port. 1995;8(3): 161–3.
217. Habib K, Finet JF, Plantier F, Spatz A, Sfoggia D, Fitoussi F. Rare lesion of the vulva. Arch Anat Cytol Pathol. 1992;40(2–3):158–9.
218. Che SM, Cao PL, Chen HW, Liu Z, Meng D. Primary Ewing's sarcoma of vulva: a case report and a review of the literature. J Obstet Gynaecol Res. 2013;39(3):746–9.
219. Scherr GR, d'Ablaing G, Ouzounian JG. Peripheral primitive neuroectodermal tumor of the vulva. Gynecol Oncol. 1994;54(2):254–8.
220. Esiashvili N, Goodman M, Marcus RB. Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance Epidemiology and End Results data. J Pediatr Hematol Oncol. 2008;30(6):425–30.
221. von Levetzow C, Jiang X, Gwye Y, von Levetzow G, Hung L, Cooper A, et al. Modeling initiation of Ewing sarcoma in human neural crest cells. PLoS One. 2011;6(4):e19305.
222. Meltzer PS. Is Ewing's sarcoma a stem cell tumor? Cell Stem Cell. 2007;1(1):13–5.
223. Tirode F, Laud-Duval K, Prieur A, Delorme B, Charbord P, Delattre O. Mesenchymal stem cell features of Ewing tumors. Cancer Cell. 2007;11(5): 421–9.
224. Morikawa S, Mabuchi Y, Niibe K, Suzuki S, Nagoshi N, Sunabori T, et al. Development of mesenchymal stem cells partially originate from the neural crest. Biochem Biophys Res Commun. 2009;379(4): 1114–9.
225. Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell. 2007;129(7):1377–88.
226. Nagoshi N, Shibata S, Kubota Y, Nakamura M, Nagai Y, Satoh E, et al. Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. Cell Stem Cell. 2008;2(4):392–403.
227. Jedlicka P. Ewing Sarcoma, an enigmatic malignancy of likely progenitor cell origin, driven by transcription factor oncogenic fusions. Int J Clin Exp Pathol. 2010;3(4):338–47.
228. Lessnick SL, Ladanyi M. Molecular pathogenesis of Ewing sarcoma: new therapeutic and transcriptional targets. Annu Rev Pathol. 2012;7:145–59.
229. Sankar S, Bell R, Stephens B, Zhuo R, Sharma S, Bearss DJ, et al. Mechanism and relevance of EWS/FLI-mediated transcriptional repression in Ewing sarcoma. Oncogene. 2013;32(42):5089–100.
230. Lazure T, Alsamad IA, Meuric S, Orbach D, Fabre M. Primary uterine and vulvar Ewing's sarcoma/peripheral neuroectodermal tumors in children: two unusual locations. Ann Pathol. 2001;21(3):263–6.
231. Machado I, Noguera R, Mateos EA, Calabuig-Fariñas S, López FI, Martínez A, et al. The many faces of atypical Ewing's sarcoma. A true entity mimicking sarcomas, carcinomas and lymphomas. Virchows Arch. 2011;458(3):281–90.
232. Llombart-Bosch A, Machado I, Navarro S, Bertoni F, Bacchini P, Alberghini M, et al. Histological heterogeneity of Ewing's sarcoma/PNET: an immunohistochemical analysis of 415 genetically confirmed cases with clinical support. Virchows Arch. 2009;455(5):397–411.
233. Folpe AL, Goldblum JR, Rubin BP, Shehata BM, Liu W, Dei Tos AP, et al. Morphologic and immunophenotypic diversity in Ewing family tumors: a study of 66 genetically confirmed cases. Am J Surg Pathol. 2005;29(8):1025–33.
234. Folpe AL, Hill CE, Parham DM, O'Shea PA, Weiss SW. Immunohistochemical detection of FLI-1 protein expression: a study of 132 round cell tumors with emphasis on CD99-positive mimics of Ewing's sarcoma/primitive neuroectodermal tumor. Am J Surg Pathol. 2000;24(12):1657–62.
235. Mhawech-Fauceglia P, Herrmann F, Penetrante R, Beck A, Sait S, Block AM, et al. Diagnostic utility of FLI-1 monoclonal antibody and dual-colour, break-apart probe fluorescence in situ (FISH) analysis in Ewing's sarcoma/primitive neuroectodermal tumour (EWS/PNET). A comparative study with CD99 and FLI-1 polyclonal antibodies. Histopathology. 2006;49(6):569–75.
236. Gamberi G, Cocchi S, Benini S, Magagnoli G, Morandi L, Kreshak J, et al. Molecular diagnosis in Ewing family tumors: the Rizzoli experience–222 consecutive cases in four years. J Mol Diagn. 2011;13(3):313–24.
237. Sankar S, Lessnick SL. Promiscuous partnerships in Ewing's sarcoma. Cancer Genet. 2011;204(7):351–65.
238. Pierron G, Tirode F, Lucchesi C, Reynaud S, Ballet S, Cohen-Gogo S, et al. A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. Nat Genet. 2012;44(4):461–6.
239. Wang L, Bhargava R, Zheng T, Wexler L, Collins MH, Roulston D, et al. Undifferentiated small round

cell sarcomas with rare EWS gene fusions: identification of a novel EWS-SP3 fusion and of additional cases with the EWS-ETV1 and EWS-FEV fusions. J Mol Diagn. 2007;9(4):498–509.
240. Sumegi J, Nishio J, Nelson M, Frayer RW, Perry D, Bridge JA. A novel t(4;22)(q31;q12) produces an EWSR1-SMARCA5 fusion in extraskeletal Ewing sarcoma/primitive neuroectodermal tumor. Mod Pathol. 2011;24(3):333–42.
241. Antonescu C. Round cell sarcomas beyond Ewing: emerging entities. Histopathology. 2014;64(1): 26–37.
242. Bridge RS, Rajaram V, Dehner LP, Pfeifer JD, Perry A. Molecular diagnosis of Ewing sarcoma/primitive neuroectodermal tumor in routinely processed tissue: a comparison of two FISH strategies and RT-PCR in malignant round cell tumors. Mod Pathol. 2006;19(1):1–8.
243. Machado I, Noguera R, Pellin A, Lopez-Guerrero JA, Piqueras M, Navarro S, et al. Molecular diagnosis of Ewing sarcoma family of tumors: a comparative analysis of 560 cases with FISH and RT-PCR. Diagn Mol Pathol. 2009;18(4):189–99.
244. Hierro I, Blanes A, Matilla A, Muñoz S, Vicioso L, Nogales FF. Merkel cell (neuroendocrine) carcinoma of the vulva. A case report with immunohistochemical and ultrastructural findings and review of the literature. Pathol Res Pract. 2000;196(7): 503–9.
245. Khoury-Collado F, Elliott KS, Lee YC, Chen PC, Abulafia O. Merkel cell carcinoma of the Bartholin's gland. Gynecol Oncol. 2005;97(3):928–31.
246. Chalubinski K, Breitenecker G, Tatra G. Malignant lymphomas of the vulva and vagina. Geburtshilfe Frauenheilkd. 1992;52(10):630–1.
247. Fisher C. Synovial sarcoma. Ann Diagn Pathol. 1998;2(6):401–21.
248. Sultan I, Rodriguez-Galindo C, Saab R, Yasir S, Casanova M, Ferrari A. Comparing children and adults with synovial sarcoma in the Surveillance, Epidemiology, and End Results program, 1983 to 2005: an analysis of 1268 patients. Cancer. 2009;115(15):3537–47.
249. Kawauchi S, Ihara K, Nishikawa K, Sugino N, Takahashi M, Sasakil K. Synovial sarcoma arising in the vulva cytogenetically confirmed by SYT breakapart rearrangement fluorescence in situ hybridization: a case report and discussion of diagnostic methods. Oncol Lett. 2012;4(5):955–9.
250. Asher V, van Schalkwyk G, Bali A. Synovial sarcoma of the vulva: a case report. J Med Case Rep. 2011;5:95.
251. Ambani DS, White B, Kaplan AL, Alberto A. A case of monophasic synovial sarcoma presenting as a vulvar mass. Gynecol Oncol. 2006;100(2):433–6.
252. White BE, Kaplan A, Lopez-Terrada DH, Ro JY, Benjamin RS, Ayala AG. Monophasic synovial sarcoma arising in the vulva: a case report and review of the literature. Arch Pathol Lab Med. 2008;132(4): 698–702.
253. Holloway CL, Russell AH, Muto M, Albert M, Viswanathan AN. Synovial cell sarcoma of the vulva: multimodality treatment incorporating preoperative external-beam radiation, hemivulvectomy, flap reconstruction, interstitial brachytherapy, and chemotherapy. Gynecol Oncol. 2007;104(1):253–6.
254. Sumathi VP, Fisher C, Williams A, Meis JM, Ganesan R, Kindblom LG, et al. Synovial sarcoma of the vulva and vagina: a clinicopathologic and molecular genetic study of 4 cases. Int J Gynecol Pathol. 2011;30(1):84–91.
255. Nielsen GP, Shaw PA, Rosenberg AE, Dickersin GR, Young RH, Scully RE. Synovial sarcoma of the vulva: a report of two cases. Mod Pathol. 1996;9(10): 970–4.
256. Minig L, Farnetano G, Peiretti M, Roviglione G, Zanagnolo V, Pelosi G, et al. Poorly differentiated synovial sarcoma of the vagina: a case report and a clinical literature review. Ecancermedicalscience. 2008;2:99.
257. Pelosi G, Luzzatto F, Landoni F, Staffa N, Maggioni A, Braidotti P, et al. Poorly differentiated synovial sarcoma of the vagina: first reported case with immunohistochemical, molecular and ultrastructural data. Histopathology. 2007;50(6):808–10.
258. Dundr P, Fischerová D, Povýšil C, Tvrdík D, Cibula D. Primary synovial sarcoma of the uterus. Pathol Oncol Res. 2012;18(2):529–33.
259. Smith CJ, Ferrier AJ, Russell P, Danieletto S. Primary synovial sarcoma of the ovary: first reported case. Pathology. 2005;37(5):385–7.
260. Mitsuhashi A, Nagai Y, Suzuka K, Yamazawa K, Nojima T, Nikaido T, et al. Primary synovial sarcoma in fallopian tube: case report and literature review. Int J Gynecol Pathol. 2007;26(1):34–7.
261. Krieg AH, Hefti F, Speth BM, Jundt G, Guillou L, Exner UG, et al. Synovial sarcomas usually metastasize after >5 years: a multicenter retrospective analysis with minimum follow-up of 10 years for survivors. Ann Oncol. 2011;22(2):458–67.
262. dos Santos NR, de Bruijn DR, van Kessel AG. Molecular mechanisms underlying human synovial sarcoma development. Genes Chromosomes Cancer. 2001;30(1):1–14.
263. de Bruijn DR, Nap JP, van Kessel AG. The (epi) genetics of human synovial sarcoma. Genes Chromosomes Cancer. 2007;46(2):107–17.
264. Chan JA, McMenamin ME, Fletcher CD. Synovial sarcoma in older patients: clinicopathological analysis of 32 cases with emphasis on unusual histological features. Histopathology. 2003;43(1):72–83.
265. Povýsil C. Synovial sarcoma with squamous metaplasia. Ultrastruct Pathol. 1984;7(2–3):207–13.
266. Mirra JM, Wang S, Bhuta S. Synovial sarcoma with squamous differentiation of its mesenchymal glandular elements. A case report with light-microscopic, ultramicroscopic, and immunologic correlation. Am J Surg Pathol. 1984;8(10):791–6.
267. Varela-Duran J, Enzinger FM. Calcifying synovial sarcoma. Cancer. 1982;50(2):345–52.

268. Milchgrub S, Ghandur-Mnaymneh L, Dorfman HD, Albores-Saavedra J. Synovial sarcoma with extensive osteoid and bone formation. Am J Surg Pathol. 1993;17(4):357–63.
269. de Silva MV, McMahon AD, Paterson L, Reid R. Identification of poorly differentiated synovial sarcoma: a comparison of clinicopathological and cytogenetic features with those of typical synovial sarcoma. Histopathology. 2003;43(3):220–30.
270. de Silva MV, McMahon AD, Reid R. Prognostic factors associated with local recurrence, metastases, and tumor-related death in patients with synovial sarcoma. Am J Clin Oncol. 2004;27(2):113–21.
271. Pelmus M, Guillou L, Hostein I, Sierankowski G, Lussan C, Coindre JM. Monophasic fibrous and poorly differentiated synovial sarcoma: immunohistochemical reassessment of 60 t(X;18)(SYT-SSX)-positive cases. Am J Surg Pathol. 2002;26(11):1434–40.
272. Foo WC, Cruise MW, Wick MR, Hornick JL. Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics. Am J Clin Pathol. 2011;135(6):839–44.
273. Ordóñez NG, Mahfouz SM, Mackay B. Synovial sarcoma: an immunohistochemical and ultrastructural study. Hum Pathol. 1990;21(7):733–49.
274. Miettinen M, Limon J, Niezabitowski A, Lasota J. Patterns of keratin polypeptides in 110 biphasic, monophasic, and poorly differentiated synovial sarcomas. Virchows Arch. 2000;437(3):275–83.
275. Skytting BT, Bauer HC, Perfekt R, Nilsson G, Larsson O. Ki-67 is strongly prognostic in synovial sarcoma: analysis based on 86 patients from the Scandinavian Sarcoma group register. Br J Cancer. 1999;80(11):1809–14.
276. Kosemehmetoglu K, Vrana JA, Folpe AL. TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms. Mod Pathol. 2009;22(7):872–8.
277. Jagdis A, Rubin BP, Tubbs RR, Pacheco M, Nielsen TO. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. Am J Surg Pathol. 2009;33(12):1743–51.
278. Knösel T, Heretsch S, Altendorf-Hofmann A, Richter P, Katenkamp K, Katenkamp D, et al. TLE1 is a robust diagnostic biomarker for synovial sarcomas and correlates with t(X;18): analysis of 319 cases. Eur J Cancer. 2010;46(6):1170–6.
279. Terry J, Saito T, Subramanian S, Ruttan C, Antonescu CR, Goldblum JR, et al. TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma emerging from gene expression profiling studies. Am J Surg Pathol. 2007;31(2):240–6.
280. Coindre JM, Pelmus M, Hostein I, Lussan C, Bui BN, Guillou L. Should molecular testing be required for diagnosing synovial sarcoma? A prospective study of 204 cases. Cancer. 2003;98(12):2700–7.
281. Sun B, Sun Y, Wang J, Zhao X, Zhang S, Liu Y, et al. The diagnostic value of SYT-SSX detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) for synovial sarcoma: a review and prospective study of 255 cases. Cancer Sci. 2008;99(7):1355–61.
282. Amary MF, Berisha F. Bernardi FeC, Herbert A, James M, Reis-Filho JS, et al. Detection of SS18-SSX fusion transcripts in formalin-fixed paraffin-embedded neoplasms: analysis of conventional RT-PCR, qRT-PCR and dual color FISH as diagnostic tools for synovial sarcoma. Mod Pathol. 2007;20(4):482–96.
283. O'Sullivan MJ, Kyriakos M, Zhu X, Wick MR, Swanson PE, Dehner LP, et al. Malignant peripheral nerve sheath tumors with t(X;18). A pathologic and molecular genetic study. Mod Pathol. 2000;13(11):1253–63.
284. Ladanyi M, Antonescu CR, Leung DH, Woodruff JM, Kawai A, Healey JH, et al. Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. Cancer Res. 2002;62(1):135–40.
285. Tamborini E, Agus V, Perrone F, Papini D, Romanò R, Pasini B, et al. Lack of SYT-SSX fusion transcripts in malignant peripheral nerve sheath tumors on RT-PCR analysis of 34 archival cases. Lab Invest. 2002;82(5):609–18.
286. Guillou L, Coindre J, Gallagher G, Terrier P, Gebhard S, de Saint Aubain Somerhausen N, et al. Detection of the synovial sarcoma translocation t(X;18) (SYT;SSX) in paraffin-embedded tissues using reverse transcriptase-polymerase chain reaction: a reliable and powerful diagnostic tool for pathologists. A molecular analysis of 221 mesenchymal tumors fixed in different fixatives. Hum Pathol. 2001;32(1):105–12.
287. Coindre JM, Hostein I, Benhattar J, Lussan C, Rivel J, Guillou L. Malignant peripheral nerve sheath tumors are t(X;18)-negative sarcomas. Molecular analysis of 25 cases occurring in neurofibromatosis type 1 patients, using two different RT-PCR-based methods of detection. Mod Pathol. 2002;15(6):589–92.
288. Nilsson G, Skytting B, Xie Y, Brodin B, Perfekt R, Mandahl N, et al. The SYT-SSX1 variant of synovial sarcoma is associated with a high rate of tumor cell proliferation and poor clinical outcome. Cancer Res. 1999;59(13):3180–4.
289. Inagaki H, Nagasaka T, Otsuka T, Sugiura E, Nakashima N, Eimoto T. Association of SYT-SSX fusion types with proliferative activity and prognosis in synovial sarcoma. Mod Pathol. 2000;13(5):482–8.
290. Guillou L, Benhattar J, Bonichon F, Gallagher G, Terrier P, Stauffer E, et al. Histologic grade, but not SYT-SSX fusion type, is an important prognostic factor in patients with synovial sarcoma: a multicenter, retrospective analysis. J Clin Oncol. 2004;22(20):4040–50.
291. ten Heuvel SE, Hoekstra HJ, Bastiaannet E, Suurmeijer AJ. The classic prognostic factors tumor

stage, tumor size, and tumor grade are the strongest predictors of outcome in synovial sarcoma: no role for SSX fusion type or Ezrin expression. Appl Immunohistochem Mol Morphol. 2009;17(3): 189–95.
292. Neesham D, Kerdemelidis P, Scurry J. Primary malignant mixed Müllerian tumor of the vagina. Gynecol Oncol. 1998;70(2):303–7.
293. Sebenik M, Yan Z, Khalbuss WE, Mittal K. Malignant mixed mullerian tumor of the vagina: case report with review of the literature, immunohistochemical study, and evaluation for human papilloma virus. Hum Pathol. 2007;38(8):1282–8.
294. Sotiropoulou M, Haidopoulos D, Vlachos G, Pilalis A, Rodolakis A, Diakomanolis E. Primary malignant mixed Mullerian tumor of the vagina immunohistochemically confirmed. Arch Gynecol Obstet. 2005;271(3):264–6.
295. Oliva E, Gonzalez L, Dionigi A, Young RH. Mixed tumors of the vagina: an immunohistochemical study of 13 cases with emphasis on the cell of origin and potential aid in differential diagnosis. Mod Pathol. 2004;17(10):1243–50.
296. French JA, Softich S. Nonhealing diaper rash with associated hepatosplenomegaly. Am Fam Physician. 2009;80(12):1481.

Index

M.P. Hoang and M.A. Selim (eds.), *Vulvar Pathology*,
DOI 10.1007/978-1-4939-1807-2, © Springer Science+Business Media New York 2015