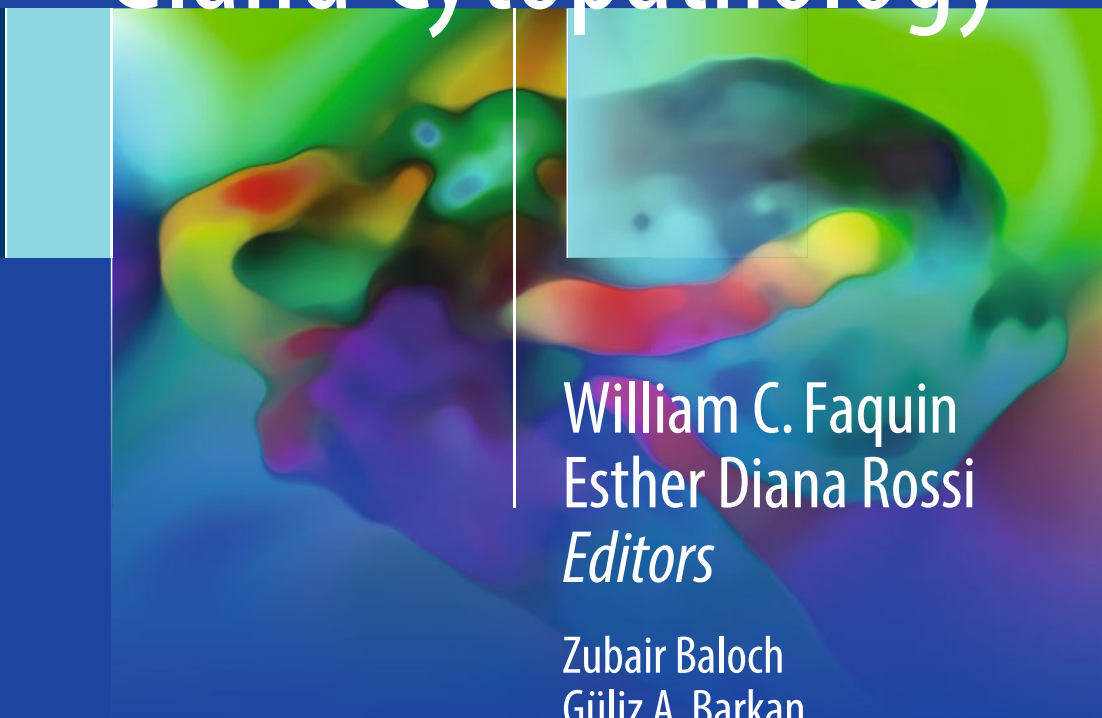
The Milan System for Reporting Salivary Gland Cytopathology



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THE INTERNATIONAL ACADEMY OF CYTOLOGY
1957

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ISBN 978-3-319-71284-0 ISBN 978-3-319-71285-7 (eBook)
https://doi.org/10.1007/978-3-319-71285-7

Library of Congress Control Number: 2017964365

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Dedication for Milan Atlas

– William C. Faquin

To my wonderful husband and favorite poet, Judson, and in loving memory of my remarkable mother, Mary Clay.

To quote WB Yeats, "How can we know the dancer from the dance?"

Esther D. Rossi

To my father, Antonio, the first Rossi in my family who became a professor and taught me the sense of duties and the real meaning of the words "perseverance, resiliency and believe in yourself."

To my lovely mom, Francesca, and my sister, Marta, the gems and passion of my life. They are always the safe harbor and the bright lighthouse of my life and the deepest and warmest joy of my days.

Foreword

> It had long since come to my attention that people of accomplishment rarely sat back and let things happen to them. They went out and happened to things. –Leonardo da Vinci

This atlas is the culmination of collaborative efforts by an international consortium to standardize classification and reporting of salivary gland cytopathology. It represents a remarkable achievement despite challenges imposed by the diversity, complexity, and significant cytomorphologic overlap of salivary gland lesions. The discriminating crux of salivary gland cytopathology is the identification and triage of those lesions requiring significant clinical management. Fine-needle aspiration biopsy, even with its diagnostic limitations, is still the most effective, minimally invasive procedure to accomplish this task. The continuous discovery of genetic alterations and the resultant molecular tests improve the surgical and cytopathologist's ability to render diagnoses of some salivary gland neoplasms with high specificity. Yet the challenge remains the ever-increasing demand to do more and more with limited material. This means fine-needle aspiration cytopathology will remain at the forefront of diagnostic procedures. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) presents logical, pragmatic, and flexible reporting terminology that allows the pathologist and the clinician to communicate effectively with improved patient care as the desired outcome.

The evolution of the Milan System transpired as if guided by da Vinci's premise. What began as a chat among friends in Bologna, Italy, in early 2015, germinated into a distinct plan formulated by colleagues during the 2015 USCAP Annual Meeting in Boston. The time was ripe to address the challenges of reporting salivary gland cytopathology in a patient-centric environment. The maturation of this fledgling concept was rapid. A handful of experts met in Milan in September, during the 2015 European Congress of Cytology, and the *Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)* was actualized. The American Society of Cytopathology and the International Academy of Cytology were supportive of the development of the Milan System, and society leaders were members of the initial nucleus of cytopathologists who developed the categories and recruited an international cadre of experts to form the working group. The Milan System was developed using the highly successful framework initiated in Bethesda in the mid-1990s that

resulted in *The Bethesda System for Reporting Cervical Cytology* and the refined process of the 2010 *The Bethesda System for Reporting Thyroid Cytopathology.*

Less than one year after the initial idea was articulated, the Milan System working group had its first meeting during USCAP 2016 in Seattle and set an ambitious timeline for completion. The Milan System was also unveiled to the cytopathology community by Dr. Faquin at the American Society for Cytopathology (ASC) Companion Meeting: "Time to Standardize the Cytology Reporting for Salivary Glands: Introduction of the Milan System." In San Antonio during USCAP 2017, the working group had completed the majority of their work on time and on target! This systematic atlas emerged as a major advancement in salivary gland cytopathology. One of the hallmarks of MSRSGC is that reporting categories are evidence-based with emphasis on risk stratification to promote appropriate management targeted to optimal patient care.

This text and the accompanying web atlas, readily available on the ASC website, have been designed to take the reader step by step through the new system's terminology. Readers will become familiar with the six main diagnostic categories, adjusted from other guidelines and reporting systems to address some of the unique issues in salivary gland cytopathology. The risk of malignancy (ROM) statistics have been gleaned from the current literature and will no doubt be adjusted as further studies are published after the MSRSGC has been implemented. Experienced and novice cytopathologists alike will discover that pearls abound among these iridescent pages. The high-quality images were selected carefully to illustrate the criteria, problems, and pitfalls associated with this often problematic subdiscipline.

The acceptance of an internationally developed reporting system provides an innovative vocabulary that will have a major impact on patient care through lucid, standardized communication that allows future diagnostic and therapeutic refinements to be implemented rapidly and efficiently.

With this atlas, the coeditors, Drs. Faquin and Rossi, "happened to things" and their immediate legacy will surely impact diagnosis and management of salivary gland tumors.

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Preface

This atlas is the result of a collaborative effort by a group of cytopathologists, surgical pathologists, molecular pathologists, and head and neck surgeons with the shared goal of developing a practical and uniform reporting system for salivary gland cytopathology. The atlas is cosponsored by the American Society of Cytopathology and the International Academy of Cytology. The initial idea for this effort was conceived during the annual USCAP Meeting held in Boston, MA, in March 2015. Subsequently, a taskforce of eight experts in salivary gland cytology was selected, and the first meeting of the taskforce, coordinated by Drs. Faquin and Rossi, took place on September 20, 2015, in Milan, Italy, during the European Congress of Cytology. The Milan System Taskforce appreciated the importance of including international members, and as such, 47 experts in the field of salivary gland cytology from 15 countries were invited to participate as coauthors of the atlas. Two online surveys related to the practice of salivary gland cytology were conducted (Rossi et al. 2017), and the results of these surveys formed the initial framework for the Milan System for Reporting Salivary Gland Cytopathology.

The atlas is organized by six general diagnostic categories: "Non-Diagnostic," "Non-Neoplastic," "Atypia of Undetermined Significance (AUS)," "Neoplasm: Benign," "Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)," "Suspicious for Malignancy," and "Malignant." It includes definitions, morphologic criteria, and explanations for each of these categories. Specific chapters are dedicated to the application of ancillary studies, clinical management, and histological considerations.

The challenges posed by the inherent complexity of salivary gland FNA are complicated by the lack of a standardized, tiered diagnostic framework by which salivary gland FNA can be reported. The establishment of the Milan System for Reporting Salivary Gland Cytopathology represents an essential step towards addressing these challenges, with the objective of increasing the overall effectiveness of salivary gland FNA and fostering better communication with clinicians and between institutions in order to improve overall patient care. It is the hope of all the

contributors to this atlas that it will be a practical and useful reporting system, meeting the needs of the international cytology community and bettering the lives of the patients we serve.

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Abbreviations

ACC	Acinic cell carcinoma
AdCC	Adenoid cystic carcinoma
AFB	Acid fast bacteria
AR	Androgen receptor
AUS	Atypia of undetermined significance
BCA	Basal cell adenoma
BCAdC	Basal cell adenocarcinoma
Ca-ex-PA	Carcinoma ex pleomorphic adenoma
CAMSG	Cribriform adenocarcinoma of minor salivary glands
CCC	Clear cell carcinoma
CISH	Chromogenic in situ hybridization
CMV	Cytomegalovirus
CNB	Core needle biopsy
CT	Computed tomography
DLBCL	Diffuse large B-cell lymphoma
DOG1	Discovered on GIST1
EBER	Epstein-Barr-encoded RNA
EBV	Epstein-Barr virus
EMZBCL	Extranodal marginal zone B-cell lymphoma
EMC	Epithelial-myoepithelial carcinoma
ER	Estrogen receptor
FC	Flow cytometry
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescent in situ hybridization
FNA	Fine-needle aspiration
GATA3	GATA binding protein 3
GFAP	Glial fibrillary acidic protein
HCCC	Hyalinizing clear cell carcinoma
HMGA2	High-mobility group AT-hook 2
HMWK	High molecular weight cytokeratin
IARC	International Agency for Research on Cancer

IC	Immunochemistry
ISH	In situ hybridization
LEC	Lymphoepithelial carcinoma
LEF-1	Lymphoid enhancer-binding factor 1
LESA	Lymphoepithelial sialadenitis
LGMEC	Low-grade mucoepidermoid carcinoma
LMWK	Low molecular weight cytokeratin
MALT	Mucosa-associated lymphoid tissue
MASC	Mammary analogue secretory carcinoma (*see* SC)
MGB	Mammaglobin
MC	Myoepithelial carcinoma
MEC	Mucoepidermoid carcinoma
MGB	Mammaglobin
MZL	Marginal zone lymphoma
MRI	Magnetic resonance imaging
MSRSGC	The Milan System for Reporting Salivary Gland Cytopathology
MYO	Myoepithelioma
MZL	Marginal zone lymphomas
N:C	Nuclear-cytoplasmic ratio
NGS	Next-generation sequencing
NOS	Not-otherwise specified
PA	Pleomorphic adenoma
PanK	Pancytokeratin
PAS	Periodic acid-Schiff
PACA	Polymorphous (low-grade) adenocarcinoma
PAS-D	Periodic acid-Schiff with diastase
PCR	Polymerase chain reaction
PET	Positron emission tomography
PLAG1-	Pleomorphic adenoma gene 1
PLGA	Polymorphous (low-grade) adenocarcinoma (*se*e PACA)
PR	Progesterone receptor
PSA	Prostate-specific antigen
PTAH	Phosphotungstic acid hematoxylin (PTAH)
RCC	Renal cell carcinoma
ROM	Risk of malignancy
ROSE	Rapid on-site evaluation
RT-PCR	Reverse transcription polymerase chain reaction
SC	Secretory carcinoma
SCC	Squamous cell carcinoma
SDC	Salivary duct carcinoma
SGN	Salivary gland neoplasms
SGT	Salivary gland tumor
SM	Suspicious for malignancy
SMA	Smooth muscle actin
SMG	Submandibular glands

STAT-5a	Signal transducer and activator of transcription 5a
SUMP	Salivary gland neoplasm of uncertain malignant potential
TTF-1	Thyroid transcription factor-1
USG	Ultrasound guidance
WHO	World Health Organization
WT	Warthin tumor

Chapter 1
The Milan System for Reporting Salivary Gland Cytopathology

Zubair Baloch, Andrew S. Field, Nora Katabi, and Bruce M. Wenig

Introduction

Fine-needle aspiration (FNA) has become widely accepted as an efficient first line diagnostic test in the management of salivary gland lesions. It can differentiate between neoplastic and non-neoplastic salivary gland lesions, and in cases of a neoplasm, FNA can diagnose many common benign tumors [1–12]. In most cases, FNA can also differentiate between low- and high-grade carcinomas. Neoplastic salivary gland lesions are usually managed surgically, while non-neoplastic ones are managed conservatively, usually without surgical intervention. Knowing whether a

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_1

carcinoma is low- or high-grade can determine the extent of surgery, including decisions on preservation of the facial nerve in the case of parotid tumors, and indications for neck dissection. In a subset of benign neoplasms such as pleomorphic adenoma (PA) and Warthin tumor (WT), the specific FNA diagnosis allows for the option of managing the tumor nonsurgically by clinical follow-up and imaging, depending upon patient wishes and health status [1–6]. The risk of malignancy (ROM) prior to FNA for a salivary gland mass varies depending upon its size and location: 20–25% in the parotid gland, 40–50% in the submandibular gland, and 50–81% in the sublingual and minor salivary glands [1, 3, 8–12].

Salivary gland FNA test performance shows a range of sensitivities and specificities depending upon a variety of factors including: technical experience of the operator performing the FNA, quality of the cytologic preparations, experience of the evaluating cytopathologist, morphologic heterogeneity of the lesion, and presence of a cystic component [1–16]. The reported overall sensitivity of salivary gland FNA in most series ranges from 86% to 100%, and the specificity ranges from 90% to 100% [1–19]. False negative and false positive diagnoses are uncommon. The sensitivity and specificity to differentiate neoplastic from non-neoplastic salivary gland lesions are 79–100% and 71–100%, respectively, while the accuracy of FNA in distinguishing benign from malignant salivary gland lesions ranges from 81% to 100% [1–8, 12]. In contrast, the accuracy of salivary gland FNA when used to specifically subtype a neoplasm shows a wider range, varying from 48% to 94% [1–5, 12]. The challenges posed by the inherent complexity of salivary gland FNA are further complicated by the lack of a standardized, tiered diagnostic framework by which salivary gland FNA can be reported. The establishment of a classification system for reporting salivary gland FNA represents an essential step towards improving the overall effectiveness of salivary gland FNA, leading to improved patient care. The reporting system should emphasize risk stratification rather than specific diagnoses, providing a ROM for each ascending risk category rather than a binary benign or malignant assessment for each individual case [1–19].

A new reporting system for salivary gland cytology specimens is the subject of this atlas. It has been developed by an international consortium of experienced health care professionals, and is designated *The Milan System for Reporting Salivary Gland Cytopathology* (MSRSGC) [19]. The objective of the MSRSGC is to foster better communication between clinicians and between institutions in order to improve overall patient care. The MSRSGC consists of six diagnostic categories, including a "Non-Neoplastic" category and a "Neoplasm" category that is split into "Benign" and "Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)" (Table 1.1). It is an evidence-based system derived from the literature which correlates diagnostic categories with ROM and clinical management strategies (Table 1.2) [2, 3, 5, 6, 12, 20].

Table 1.1 Milan System for Reporting Salivary Gland Cytopathology: diagnostic categories, definitions, and explanatory notes

Diagnostic category and definition	Explanatory notes
I. *Non-Diagnostic* Insufficient cellular material for a cytologic diagnosis	• This diagnostic category should only be used after all the material has been processed and examined • Exceptions include matrix material and mucinous cyst contents
II. *Non-Neoplastic* Benign entities such as chronic sialadenitis, reactive lymph node, granulomas, and infection	• The ROM for this category would be expected to be low if strict inclusion criteria are applied • Specimens will include those lacking cytomorphologic evidence of a neoplastic process • Inflammatory, metaplastic, and reactive changes • Specimens showing evidence of reactive lymphoid tissue (flow cytometry is recommended based on clinical and morphologic suspicion)
III. *Atypia of Undetermined Significance (AUS)* (≤10% of all salivary gland FNA samples); containing limited atypia; indefinite for a neoplasm	• Samples are indefinite for a neoplasm; a neoplastic process cannot be excluded after examination of all the cellular material • A majority of these FNAs will represent reactive atypia or poorly sampled neoplasms
IV. *Neoplasm*	
A. *Benign* Reserved for benign neoplasms diagnosed based on established cytologic criteria	• This category will include classic cases of pleomorphic adenoma, Warthin tumor, lipoma, etc
B. *Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)* Reserved for FNA samples that are diagnostic of a neoplasm; however, diagnosis of a specific entity cannot be made	• This diagnosis should be used for cases where a malignant neoplasm cannot be excluded. • A majority of these cases will include cellular benign neoplasms, neoplasms with atypical features, and low-grade carcinomas
V. *Suspicious for Malignancy (SM)* This category is for FNA samples showing features that are highly suggestive of, but not unequivocal for malignancy.	• The FNA report should state which type of malignant tumor is suspected or provide a differential diagnosis • A majority of specimens in this category will be high-grade carcinoma on histopathologic follow-up (An attempt should be made on histopathologic examination to subclassify the neoplasm following complete surgical excision into specific types and grades of carcinoma for cytologic-histologic correlation).
VI. *Malignant* This category is for FNA specimens that are diagnostic of malignancy	• An attempt should be made to subclassify the neoplasm into specific types and grades of carcinoma: e.g., low-grade (low-grade mucoepidermoid carcinoma) vs. high-grade (salivary duct carcinoma) "Other" malignancies such as lymphomas, metastases, and sarcomas are also included in this category and should be specifically designated

ROM risk of malignancy, *FNA* fine-needle aspiration

Table 1.2 The Milan System for Reporting Salivary Gland Cytopathology: implied risk of malignancy and recommended clinical management

Diagnostic category	Risk of malignancy (%)[a]	Management[b]
I. Non-Diagnostic[c]	25	Clinical and radiologic correlation/repeat FNA
II. Non-Neoplastic	10	Clinical follow-up and radiologic correlation
III. Atypia of undetermined significance (AUS)	20[d]	Repeat FNA or surgery
IV. Neoplasm		
A. Neoplasm: Benign	<5	Surgery or clinical follow-up[e]
B. Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	35	Surgery[f]
V. Suspicious for malignancy (SM)	60	Surgery[f]
VI. Malignant	90	Surgery[f, g]

Diagnostic Categories: Diagnostic category numbers should not be used without the category designation in cytology reports. FNA fine-needle aspiration

[a]The following ranges for risk of malignancy for diagnostic categories have been cited in the literature: Non-Diagnostic 0–67%; Non-Neoplastic 0–20%; AUS 10–35%; Neoplasm: Benign 0–13%; SUMP 0–100%; Suspicious for Malignancy 0–100%; and Malignant 57–100% (Colella et al. [2]; Griffith et al. [3]; Liu et al. [5]; Rossi et al. [6]; Wei et al. [12], Schmidt et al. [20])

[b]For detailed discussion see Chap. 9, "Clinical Management"

[c]Specimen adequacy criteria have not been validated

[d]A limited number of studies in the literature have classified cases either as atypical or inconclusive

[e]A subset of patients may be followed clinically

[f]Intraoperative consultation may be helpful to determine the extent of surgery

[g]Extent of surgery depends upon type and grade of malignant tumor

Format of Report

To communicate clearly, each salivary gland FNA report should include one of the general diagnostic categories of MSRSGC, which is associated with an implied ROM, in addition to a specific diagnosis.

Example of a report for pleomorphic adenoma FNA:

- Satisfactory for Evaluation
- Interpretation: NEOPLASM, BENIGN
- Diagnosis: Pleomorphic adenoma

The ROM (see Table 1.2) may represent an overestimation because it is based on cases that have undergone surgical excision, and may have been impacted by publication bias, patient demographics, and institutional referral patterns. Therefore, the actual ROM is expected in practice be in the mid-range of what is reported in the literature.

The cytology report should also include:

- A statement of the adequacy of the specimen
- A brief description of the cytological features present
- A specific diagnosis as to the nature of the non-neoplastic process or neoplasm present
- Or if the above mentioned is not possible—a concise comment on the reason for the categorization of the lesion.

While the diagnostic categories are numbered (I–VI), we do not recommend that a salivary gland FNA report should consist solely of the category number without the accompanying category name, which would greatly diminish the communication between cytopathologist and treating clinician.

The general diagnostic categories of MSRSGC provide useful inherent information for appropriate clinical management. The reporting of ROM with the general diagnostic categories is optional and left to the discretion of the individual pathologist or laboratory. The dedicated chapters on the MSRSGC diagnostic categories provide a framework for subcategorization and for sample reports, which may serve as a useful guide for reporting salivary gland FNA specimens.

Indications for Salivary Gland FNA

FNA is used in conjunction with both clinical and radiologic findings in the initial evaluation of any mass in the major and minor salivary glands [1–5]. Overall, a majority of salivary gland nodules occur in the superficial compartment and less often in the deep compartment of the parotid gland. Cytopathologists performing FNAs of these masses should be familiar with the basic anatomy of the parotid gland and its surrounding structures (Fig. 1.1) [21]. Patients presenting for FNA may complain of a palpable mass with or without pain in the head and neck region, or in some cases, partial paralysis or paresthesia most commonly involving the facial nerve [3–6]. Alternatively, the mass may have been palpated by a clinician or found on imaging studies. Clinicians will occasionally send patients for FNA who do not have a palpable or radiologically detectable mass. FNA should be discouraged in these instances because it has the potential to lead to a false negative diagnosis.

Sampling Techniques for Salivary Gland FNA

A critical aspect of salivary gland FNA is adequate sampling and appropriate sample preparation. The FNA should be performed ideally by a cytopathologist, radiologist, or clinician well trained in the FNA technique. Ultrasound is a useful adjunct for the procedure, especially for cystic or difficult to palpate masses, but it is not absolutely essential to use ultrasound guidance for the FNA of palpable

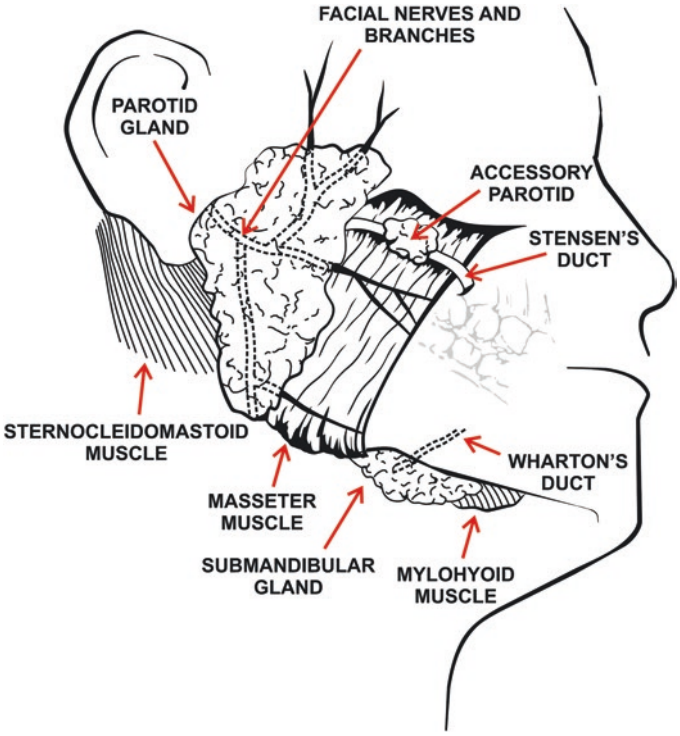

Fig. 1.1 Anatomic relationship of the parotid gland and surrounding structures, including branches of the facial nerve, masseter muscle, Stensen's duct, and submandibular gland. (From Faquin and Powers [21], with permission)

salivary gland lesions. Ideally, the FNA should utilize a 23 or 25 gauge needle, usually attached to a 10 cm^3 syringe, and often using a syringe holder to assist in applying a vacuum during the procedure (Fig. 1.2). In some cases, a needle by itself can be used (French or Zajdela technique). The key to the procedure is the puncture and rapid movement of the needle forwards and backwards passaging the full depth of the lesion, with aspiration applied where necessary to drain a cystic component, or to facilitate obtaining cellular material. Rapid on-site evaluation (ROSE) is recommended when possible because an immediate assessment of adequacy can be made, reducing the need for repeat FNA procedures and facilitating triage of material for cell blocks, flow cytometry, and ancillary studies.

Core needle biopsy (CNB) is a relatively new technique for diagnosing salivary gland lesions. CNB usually obtains larger tissue samples than FNA, and potentially may provide more tissue than a cell block or direct scraping of smears for immunohistochemical (IC) and molecular studies [9]. However, given the increased potential complications of CNB, which include the possibility of facial nerve injury and tumor seeding along the biopsy track, FNA is still the recommended standard procedure.

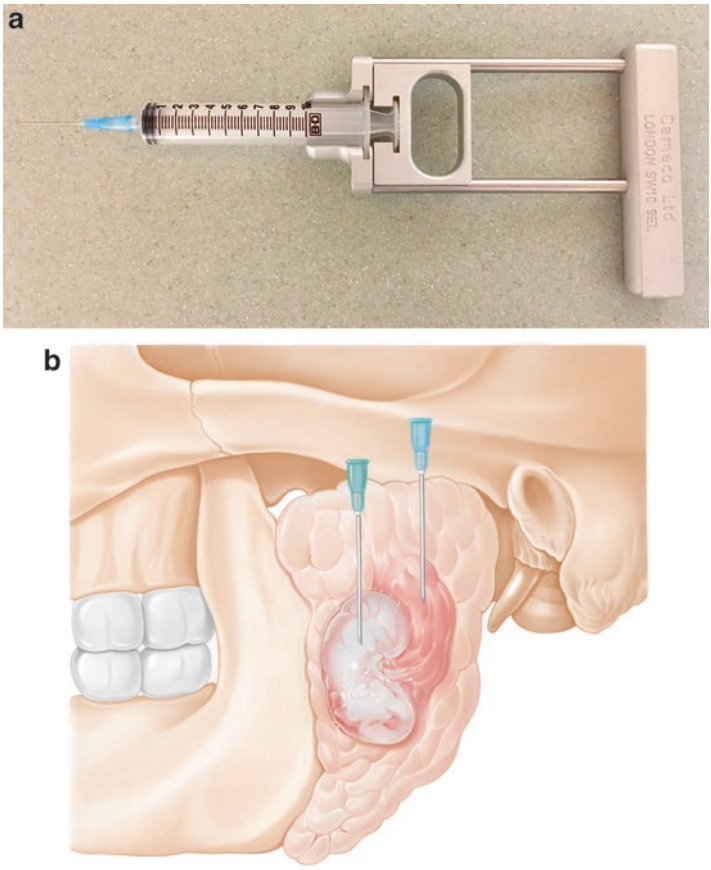

Fig. 1.2 (**a**) Standard FNA equipment showing a Cameco syringe holder with a 10 cm^3 syringe and attached 25 G needle. One hand should be used to palpate and fix the nodule, while the other hand grasps the Cameco holder to place the needle and perform the biopsy using suction. (**b**) Schematic showing the use of the Zajdela technique to aspirate a parotid gland lesion using a needle without suction. (Courtesy of Ms. Antonia Conti, CMI)

FNA Sample Preparation

A combination of air-dried and alcohol-fixed direct smears is the mainstay of salivary gland FNA, but they can also be supplemented by liquid-based preparations. Use of direct smears helps to maximize the accuracy of the FNA. Several features, including the inherent qualities of any matrix material, cytoplasmic features, and the nature of a proteinaceous or mucinous background, can be better appreciated using air-dried preparations. Alcohol-fixed preparations are useful for the assessment of nuclear qualities and the degree of cytologic atypia. In addition, preparation of a cell block can be helpful for selected cases where ancillary tests including molecular studies are needed.

FNA Specimen Preparation

- Air-dried smears with May-Grunwald-Giemsa or Diff Quik staining (rapid turn-around time, highlights matrix, cytoplasmic vacuoles, and background mucin)
- Alcohol-fixed smears with Papanicolaou staining (highlights nuclear details)
- Liquid-based preparations (removal of obscuring blood and assessment of nuclear features)
- Cell block (histochemical and IC stains; molecular studies)
- Needle rinses (flow cytometry and microbiologic studies)

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Chapter 2
Non-Diagnostic

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_2

General Background

Adequate cellularity from a target lesion is essential for an accurate interpretation; however, specific criteria for the adequacy of a salivary gland fine-needle aspiration (FNA) have yet to be defined. Both qualitative and quantitative aspects of the specimen are important for defining its adequacy [1, 2]. Many factors—including the aspiration technique (manual versus ultrasound guided), caliber of the FNA needle, nature of the lesion (solid versus cystic), sample collection/preservation method, preparation technique artifacts, and presence of obscuring blood or other material—can influence the adequacy of a salivary gland aspirate. The cellularity alone may not be enough to qualify a salivary gland FNA specimen as adequate if it does not correlate with clinical and radiologic findings [3–6, 18]. Rare high-grade cells from a salivary gland cancer may be sufficient to diagnose an FNA as "Suspicious for Malignancy" or "Malignant," while an aspirate containing abundant non-neoplastic salivary gland elements might be classified as "Non-Diagnostic" for not being representative of the lesion.

An absolute number of cells needed for salivary gland FNA adequacy has not been validated and established in the literature. A recent survey of cytopathologists showed that many practitioners tend to use criteria similar to those recommended in *The Bethesda System for Reporting Thyroid Cytology*—a minimum of six groups of cells with ten cells each [7, 8]. It is recommended that until more data is available a minimum of 60 lesional cells, could be used as a reasonable and objective measure of adequacy. It is hoped that adhering to a practical set of criteria for sample adequacy, even if empirical, will help to ensure a low false-negative rate, and lead to better overall patient care. Based upon Non-Diagnostic rates for other cytology reporting systems as well as the authors own experiences, it is estimated that the rate of Non-Diagnostic salivary gland FNAs should be approximately 10% or lower.

Definition

A Non-Diagnostic salivary gland aspirate is one that for qualitative and/or quantitative reasons provides insufficient diagnostic material to provide an informative interpretation.

Cytologic Criteria

- Rare or absent cells (Fig. 2.1); less than 60 lesional cells
- Poorly prepared slides with artifacts (e.g., air-drying, obscuring blood, and poor staining) that preclude the evaluation of the cellular component (Figs. 2.2 and 2.3)
- Non-neoplastic (normal) salivary gland elements in the setting of a clinically or radiologically defined mass (Fig. 2.4)

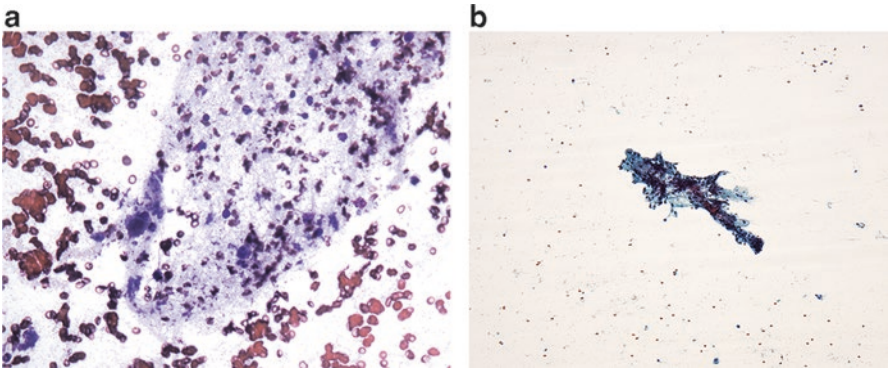

Fig. 2.1 Non-Diagnostic. (**a**) Blood, debris, and rare inflammatory cells are present, but insufficient for classification (smear, Romanowsky stain). (**b**) Hypocellular aspirate showing background blood and scant non-lesional cells (smear, Papanicolaou stain)

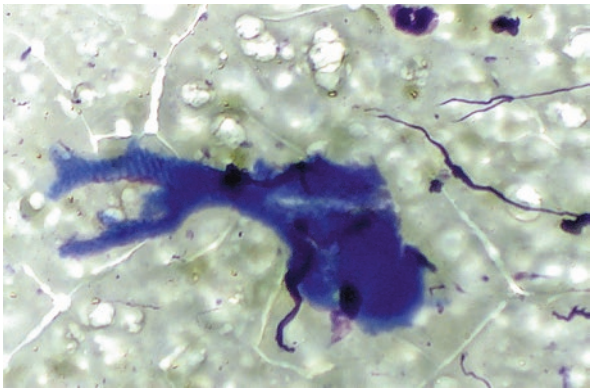

Fig. 2.2 Non-Diagnostic. The aspirate contains dense nonspecific material, background debris, and extensive air-drying artifact (smear, Romanowsky stain)

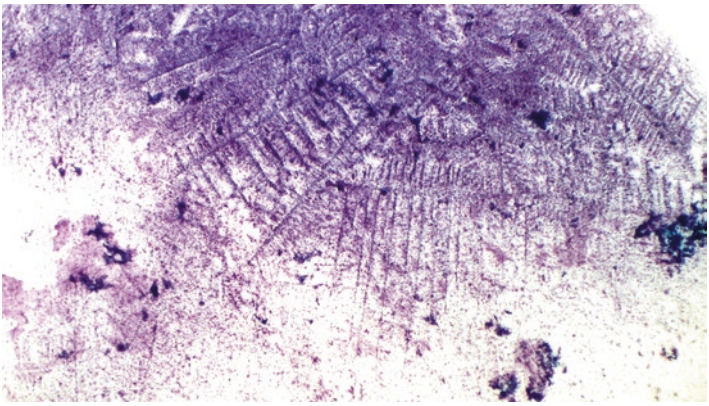

Fig. 2.3 Non-Diagnostic. Hypocellular aspirate with background proteinaceous material and debris with ferning artifact. There are insufficient lesional cells present for classification (smear, Romanowsky stain)

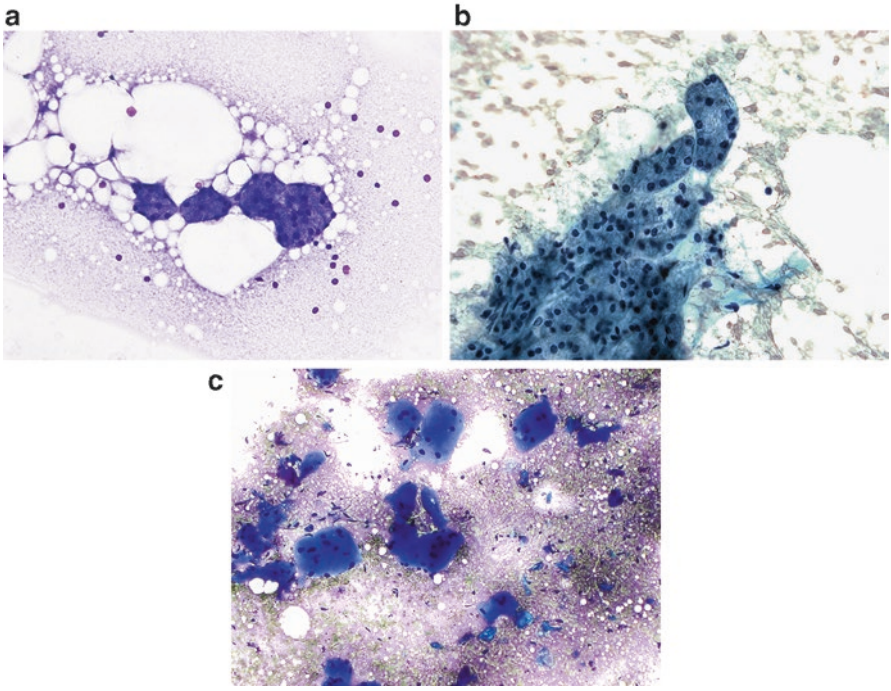

Fig. 2.4 Non-Diagnostic. (**a**) This aspirate in a patient with a discrete mass consists only of blood and non-neoplastic (normal) salivary gland elements. (smear, Romanowsky stain). (**b**) This aspirate shows non-neoplastic (normal) salivary gland acini in a lobular arrangement with focal ductal cells. This aspirate would not be considered representative of a clinically defined mass lesion (smear, Papanicolaou stain). (**c**) This aspirate shows scattered fragments of skeletal muscle, blood, and debris (smear, Romanowsky stain)

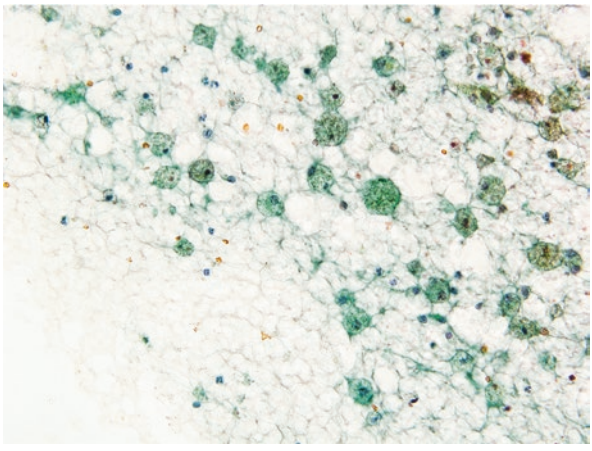

Fig. 2.5 Non-Diagnostic. Non-mucinous cyst contents showing histiocytes, debris, and few inflammatory cells (smear, Papanicolaou stain)

- Non-mucinous cyst fluid without an epithelial component should be subcategorized as "Non-Diagnostic, cystic fluid only" (Fig. 2.5)

Exceptions to these cytologic criteria include the following:

a. Any salivary gland aspirate with significant cytologic atypia cannot be classified as "Non-Diagnostic" (see Chap. 4, Atypia of Undetermined Significance)
b. Mucinous cyst fluid contents without an epithelial component should be interpreted as "Atypia of Undetermined Significance (AUS)" instead of "Non-Diagnostic" (see Chap. 4, Atypia of Undetermined Significance).
c. The presence of abundant inflammatory cells without an epithelial component can be interpreted as adequate.
d. In the absence of neoplastic cells, the presence of a matrix component suggestive of a neoplasm should not be classified as "Non-Diagnostic."

Explanatory Notes

Salivary gland aspirates containing adequate cellular material are required to ensure a low false-negative rate for FNA as well as for triage of patients for appropriate management. Specific criteria (e.g., minimal number of epithelial/lesional cells or minimal amount of matrix) have not been established in the literature [1–16]. The authors recommend using the cellularity criteria of a minimum of 60 cells representative of the lesion [8, 17]. FNA specimens containing only benign, non-neoplastic salivary gland tissue (benign acinar cells in a lobular arrangement with ductal cells and other normal salivary gland elements) should generally be interpreted as "Non-Diagnostic", since most likely it is a sampling error and not representative of the lesion of interest. This is especially true when there is clinical or radiologic evidence of a defined mass. An aspirate, however, consisting of only non-neoplastic salivary gland elements can also be encountered in various conditions, including sialadenosis, accessory parotid gland tissue, sialolithiasis, lipomatosis, and hamartoma [3–6, 11–16]. It is important to have good clinical correlation to assist in recognizing these non-neoplastic conditions as a cause of salivary gland swelling in order to avoid unnecessary surgical intervention as well as repeat FNAs.

An aspirate of bilateral salivary gland enlargement without a defined mass and yielding only benign salivary gland elements can be classified as "Non-Neoplastic" rather than "Non-Diagnostic" with proper clinical correlation. It is prudent that a cautionary note about a possible sampling error be added to the case.

Regardless of the perceived inadequacy of a salivary gland aspirate, the presence of cytological atypia should always be considered adequate and reported as AUS, or as one of the other diagnostic categories (Fig. 2.6). In these cases, a comment should be added describing the limiting factors (e.g., scant cellularity) and the nature of the atypical features.

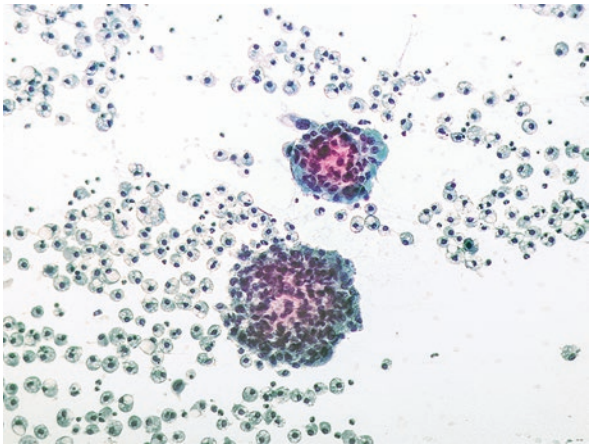

Fig. 2.6 Atypia of Undetermined Significance (AUS). This aspirate of a cyst shows histiocytes and two rare clusters of atypical epithelial cells. The presence of atypia precludes the classification of this aspirate as Non-Diagnostic. Depending upon the number of epithelial cells and degree of atypia, this aspirate would be best classified as either "Atypia of Undetermined Significance," "Salivary Gland Neoplasm of Uncertain Malignant Potential," or "Suspicious for Malignancy" (smear, Papanicolaou stain)

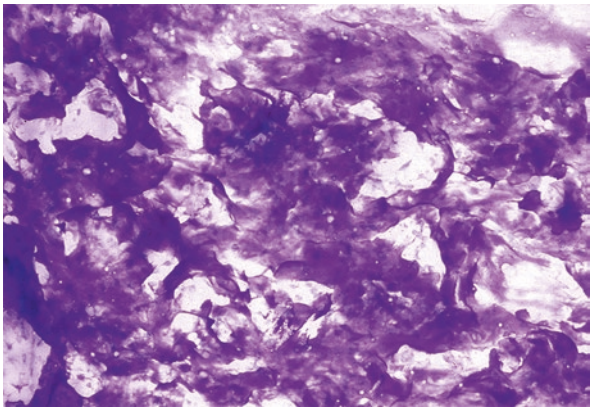

Fig. 2.7 Neoplasm: Benign. This aspirate consists of abundant acellular metachromatic matrix only. This finding is indicative of a neoplasm, and is characteristic of pleomorphic adenoma (smear, Romanowsky stain)

When a salivary gland aspirate consists of abundant matrix material without a cellular component (Fig. 2.7), the findings should be classified within one of *The Milan System for Reporting Salivary Gland Cytopathology* categories other than "Non-Diagnostic". Aspirates with this cytologic feature, depending upon the matrix qualities, are indicative of neoplasia. It is important that an aspirate yielding only cyst fluid with or without histiocytes and inflammatory cells be characterized as mucinous or non-mucinous in consideration of a possible mucoepidermoid

Table 2.1 Three examples of samples that warrant a note or comment

Reason	Note
Presence of only benign salivary tissue	The finding of "non-neoplastic" salivary gland elements only, does not explain the presence of a clinically or radiologically defined mass
Necrotic debris only	The finding of necrotic debris only is considered "Non-Diagnostic", but it raises the possibility of a neoplastic process. Clinical and radiologic correlations are needed
Non-mucinous cyst contents	Repeat fine-needle aspiration under radiologic guidance is recommended if clinically indicated

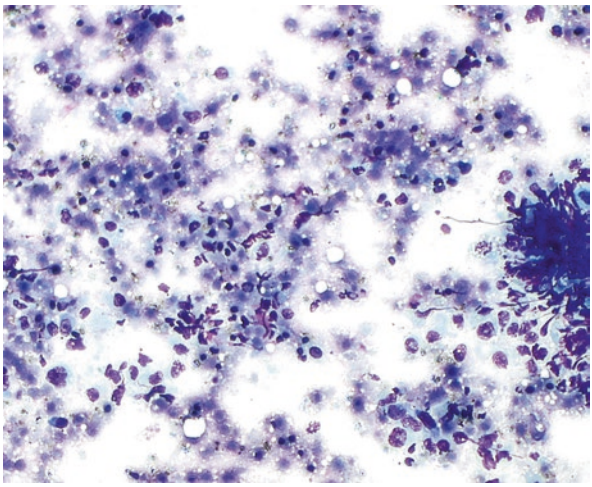

Fig. 2.8 Non-Diagnostic. Aspirates showing only necrosis and few inflammatory cells should be classified as "Non-Diagnostic". A note can be added to the case that the presence of necrotic debris raises the possibility of an infarcted neoplasm (smear, Romanowsky stain)

carcinoma (MEC) or other salivary gland neoplasms with cystic change. If the nature of the cyst fluid is not clear, an explanatory comment should be provided.

All aspirates of salivary gland cysts are best performed and interpreted in the context of clinical and ultrasound features. Cyst fluid analysis with biochemical testing can be incorporated into the diagnostic report. Salivary gland FNA specimens from a solid mass, with inadequate cells and with very scant mucoid material, should be classified as "Non-Diagnostic." In addition, aspirates of salivary gland masses containing only necrotic debris with no viable cells should also be diagnosed as "Non-Diagnostic." A comment can be added to the diagnosis since the cytologic findings may represent an infarcted neoplasm such as an oncocytoma, Warthin tumor, or carcinoma (Table 2.1) (Fig. 2.8).

Clinical Management

A repeat FNA is indicated for salivary gland aspirates classified as "Non-Diagnostic." The use of ultrasound guidance and/or rapid on-site evaluation (ROSE) is recommended for the repeat FNA to prevent a second Non-Diagnostic sample. For this group of patients, additional imaging such as computed tomography or magnetic resonance imaging may be useful. An open biopsy or surgical resection may be recommended for repeated Non-Diagnostic cases where the clinical and radiological information are sufficiently suspicious for a neoplastic or possible malignant lesion. (see Chap. 9, Clinical Management).

Sample Reports

Example 1 (solid lesion):
Evaluation limited by scant or absent cellularity
NON-DIAGNOSTIC
Insufficient cellularity for diagnosis. See note.
Note: Repeat fine-needle aspiration under radiologic guidance is recommended if clinically indicated.

Example 2:
Evaluation limited by non-neoplastic salivary gland elements only.
NON-DIAGNOSTIC
Non-neoplastic benign salivary gland elements only. See note.
Note: The finding of "non-neoplastic" salivary gland elements only, does not explain the presence of a clinically or radiologically defined mass. Therefore, the FNA sample is not considered representative of the lesion detected on clinical and/or radiologic examination. Repeat fine-needle aspiration under radiologic guidance is recommended if clinically indicated.

Example 3:
Evaluation limited by preservation artifact
NON-DIAGNOSTIC
Minimal poorly preserved cells, insufficient for diagnosis. See note.
Note: This specimen is Non-Diagnostic due to both scant cellularity and poor sample preservation.

Example 4 (cystic lesion)
Evaluation limited by lack of epithelial or lesional cells—cyst fluid only
NON-DIAGNOSTIC, CYST FLUID
Acellular, non-mucinous cyst fluid. See note.
Note: Repeat fine-needle aspiration under radiologic guidance is recommended if clinically indicated.

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Chapter 3
Non-Neoplastic

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_3

General Background

The non-neoplastic lesions of the salivary glands are relatively common, and can clinically mimic a neoplasm due to the presence of a distinct mass [1–5]. Acute and chronic sialadenitis that also include granulomatous disease are the most common non-neoplastic lesions [6] (Table 3.1). Acute sialadenitis typically results from

Table 3.1 Causes of sialadenitis

Acute
Suppurative
Staphylococcus aureus
Strepvtococcus sp.
Nonsuppurative
Paramyxovirus
Cytomegalovirus
Epstein–Barr virus
Chronic
Obstructive sialadenopathy
Stones
Trauma/injury
Infection
Other causes of duct obstruction including irradiation, tumors, and IgG4-related disease
Granulomatous
Obstructive sialadenopathy
Stones, extravasated mucin, secretory products
Other causes of duct obstruction including tumors
Specific infections
Mycobacterial
Cat-scratch disease
Toxoplasmosis
Tularemia
Fungal
Sarcoidosis
Systemic disease
Wegener's granulomatosis (granulomatosis with polyangiitis)
Crohn's disease

bacterial infection and is rarely sampled by fine-needle aspiration (FNA) due to its typical clinical presentation. Chronic sialadenitis can result from causes that lead to salivary duct obstruction, most often sialolithiasis, but in some cases can be related to systemic causes such as IgG4-related autoimmune disease. Granulomatous inflammation of the salivary gland is uncommon; the causes include mucoceles, infections, and sarcoidosis [7–12].

The average risk of malignancy (ROM) for aspirates of salivary gland lesions classified as "Non-Neoplastic" is approximately 10% with study ranges from 0 to 20% [8–13]. It is crucial that the ROM should be interpreted within the context of the patient population selected to undergo a salivary gland FNA since there is often a suspicion of malignancy. Many of the non-neoplastic salivary gland conditions can also be secondary to synchronous neoplastic processes. One of the goals of the Milan System is to improve test performance. A careful clinical and radiologic correlation is necessary to avoid the pitfall of a false negative FNA result when reporting a salivary gland FNA as "Non-Neoplastic."

General Definition

The designation "Non-Neoplastic" is used for specimens that show benign non-neoplastic changes, including those associated with acute or chronic reactive responses to inflammation, structural alterations, and infection. The designation "Non-Neoplastic" is intended to be used in conjunction with available clinical and radiologic information.

Sialolithiasis

Sialolithiasis, the formation of ductal calculi, is often associated with salivary gland enlargement and pain, and clinical symptoms can mimic a neoplasm [6]. The stones are usually composed of calcium phosphate and calcium carbonate admixed with other minor components. Sialolithiasis occurs primarily in the submandibular gland (up to 80% in Wharton's duct), less often in the parotid gland (approximately 20% in Stensen's duct), and very rarely in sublingual glands. Imaging studies such as computed tomography (CT) are very accurate in detecting ductal calculi and corresponding duct dilatation.

Cytologic Criteria

- Hypocellular aspirate
- Scant or absent acinar cells

- Groups of benign ductal cells and/or metaplastic squamous, ciliated, or mucinous cells
- Inflammatory background ± mucin
- Calcifications (stone fragments)

Explanatory Note

In the very early stages of disease, aspirates of a salivary gland mass due to sialolithiasis may yield only normal-appearing salivary gland tissue. In such situations, the major diagnostic consideration is a sampling error. In long-standing cases, the gland is involved by chronic inflammation (i.e., chronic sialadenitis), squamous metaplastic changes in the ductal epithelium, and parenchymal atrophy (Fig. 3.1). The diagnosis of sialolithiasis is usually straightforward when clinical and radiological findings are available. Fragments of stone or crystalline debris are present in the FNA specimen in approximately 50% of cases (Fig. 3.2). However, when stone fragments are absent, epithelial changes (especially metaplastic squamous and mucinous cells) and a mucoid background can be difficult to distinguish from a low-grade mucoepidermoid carcinoma (LGMEC) (see Chap. 7, Malignant). Squamous metaplastic cells with atypia can also raise the possibility of metastatic squamous cell carcinoma, although the degree of cytologic atypia usually is mild in cases of sialolithiasis (Fig. 3.3). In some instances, a diagnosis of "Atypia of Undetermined Significance (AUS)" with an explanatory note may be necessary (see Chap. 4, Atypia of Undetermined Significance).

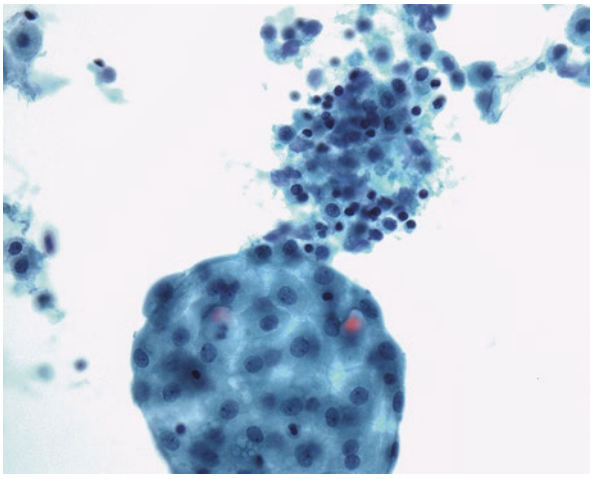

Fig. 3.1 Non-Neoplastic. This aspirate of sialolithiasis contains a cluster of metaplastic ductal cells with background acute and chronic inflammation (smear, Papanicolaou stain)

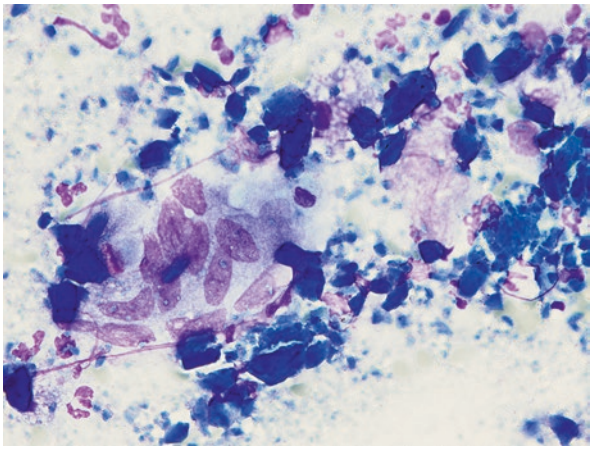

Fig. 3.2 Non-Neoplastic. This aspirate of sialolithiasis shows stone fragments and a multinucleated giant cell (smear, Papanicolaou stain)

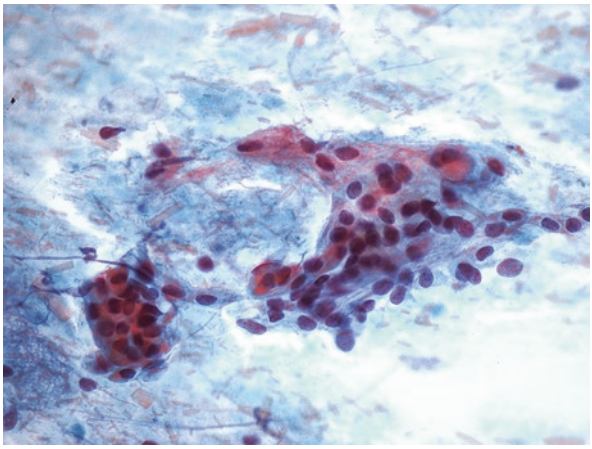

Fig. 3.3 Non-Neoplastic. This smear shows metaplastic ductal cells from an aspirate of sialolithiasis (smear, Papanicolaou stain)

Acute Sialadenitis

Acute sialadenitis most frequently involves the parotid gland followed by the submandibular gland [6, 14]. It is rarely evaluated by FNA since it is usually diagnosed based upon typical clinical symptoms and is treated with antibiotics. It can occur as suppurative or nonsuppurative forms. Acute suppurative sialadenitis is most often caused by oral cavity bacteria such as *Staphylococcus aureus* or *Streptococcus* sp. It is more common in older patients with dehydration, poor oral hygiene, malnutrition, oral neoplasms, liver cirrhosis, and diabetes mellitus. Acute nonsuppurative sialadenitis is more common in children, and is often associated with viral infections including those caused by paramyxovirus (mumps), cytomegalovirus (CMV), and Epstein–Barr virus (EBV) (mononucleosis). In addition, acute sialadenitis secondary to obstruction of the submandibular (Wharton's) duct by stones or strictures has been reported.

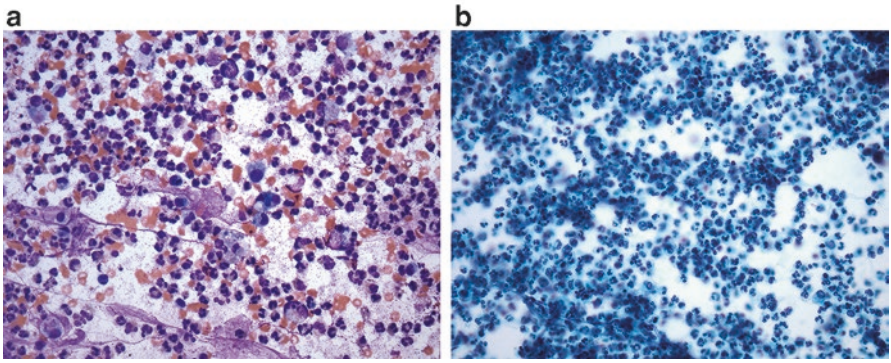

Fig. 3.4 Non-Neoplastic. These aspirates of acute sialadenitis (**a**) (smear, Romanowsky stain) and (**b**) (Papanicolaou stain) show abundant acute inflammation with occasional histiocytes and background debris, but no evidence of a neoplastic process. Clinical follow-up and radiologic correlation are needed to ensure that the aspirate is representative

Cytologic Criteria

- Abundant neutrophils ± bacteria (Fig. 3.4)
- Histiocytes
- Necroinflammatory debris (suppurative)
- Granulation tissue (later stages)

Explanatory Notes

Although infrequently aspirated, FNA of acute sialadenitis, which is usually painful, is used to exclude an underlying neoplastic condition. Aspiration of any residual mass should be performed after resolution of the inflammatory process since tumor diathesis in high-grade cancers can mimic acute sialadenitis. In contrast, caution should be exercised not to overinterptet the reactive atypia and degenerative changes in ductal cells (Fig. 3.5) due to acute inflammation as a neoplastic process. Special stains for bacteria as well as material for microbiologic culture and sensitivity testing can be useful.

Chronic Sialadenitis Including IgG4-Related Disease

Chronic sialadenitis is primarily a condition involving the submandibular glands [15]. It is most common in middle-aged adults with a slightly increased incidence in males. The clinical history and physical examination will often suggest the diagnosis;

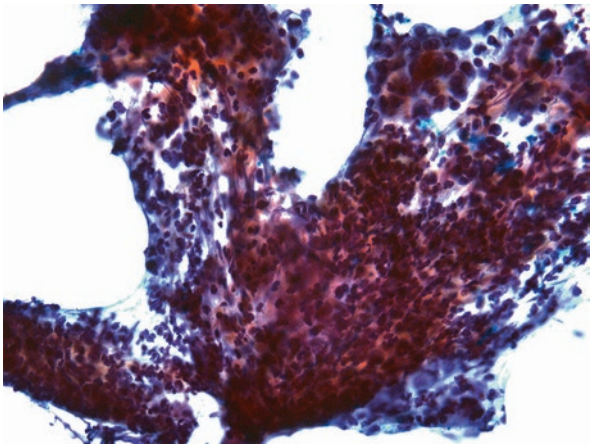

Fig. 3.5 Non-Neoplastic. This smear shows focal ductal cells (*upper right*) with reactive atypia in a background of marked acute sialadenitis (smear, Papanicolaou stain)

however, some cases will present as a firm mass simulating a neoplasm. It has a strong association with obstruction of a major duct secondary to sialolithiasis. Other potential causes of duct obstruction and chronic sialadenitis include radiation, surgery, trauma, autoimmune disorders, and bulimia. Chronic obstructive sialadenitis, chronic recurrent sialadenitis, and chronic sclerosing sialadenitis are the three main forms of the disease. Chronic sclerosing sialadenitis is also known as Küttner tumor, and some cases represent a form of IGg4-related disease, which can be either localized or occasionally systemic. Chronic sclerosing sialadentitis is often bilateral and causes a generalized firmness of the gland. The presence of IgG4-positive plasma cells and elevated serum levels of IgG4 would suggest IgG-4-related disease whose diagnosis is made based upon specific clinical features and histopathologic criteria.

Cytologic Criteria

- Hypocellular
- Small groups of ductal cells, may be basaloid or metaplastic
- Absent or scant acinar cells
- Chronic inflammation (including lymphocytes and plasma cells)
- Fibrotic stromal fragments

Explanatory Notes

The combination of a hypocellular aspirate with absent acinar cells, small cohesive ductal groups, and mild chronic inflammation is characteristic of chronic sialadenitis (Fig. 3.6), but clinical and radiologic correlations are needed to exclude a

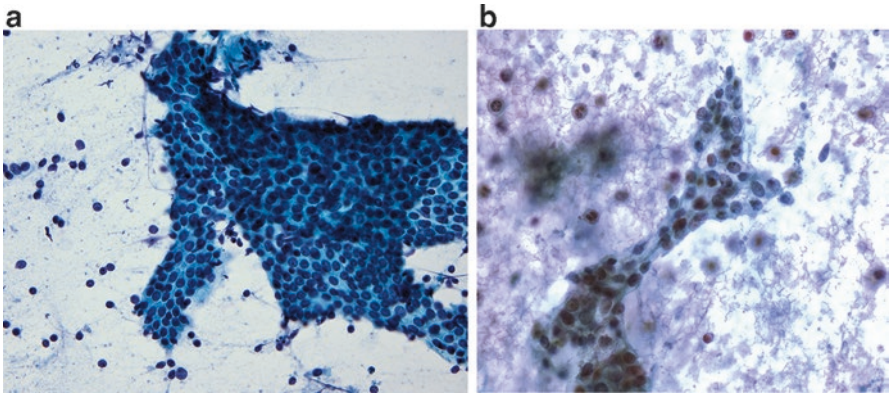

Fig. 3.6 Non-Neoplastic. (**a**) This aspirate of chronic sialadenitis shows a sheet of cytologically bland ductal cells. (**b**) This aspirate of chronic sialadenitis demonstrates reactive ductal atypia (smear, Papanicolaou stain)

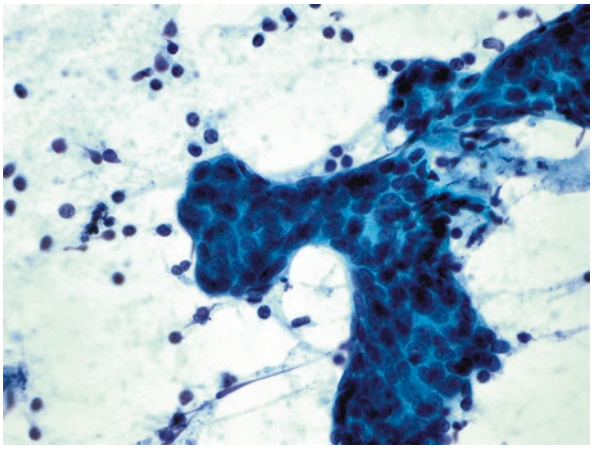

Fig. 3.7 Non-Neoplastic. This smear of chronic sialadenitis demonstrates a smaller atrophic ductal group with basaloid qualities and background chronic inflammation; avoid misinterpreting this as a basaloid neoplasm (smear, Papanicolaou stain)

non-representative FNA sample. The most common pitfall for chronic sialadenitis is misinterpretation of the metaplastic or atrophic ductal cells (Fig. 3.7) as a basaloid neoplasm (see Chap. 4). In contrast, chronic sialadentitis generally lacks the degree of cellularity and larger three-dimensional epithelial groups found in aspirates of a basaloid neoplasm.

In addition to stone fragments and inflammatory cells, a subset of chronic sialadenitis cases and benign inflamed cysts can have amylase crystalloids (Fig. 3.8), which are non-birefringent crystalline structures with rectangular, needlelike, and rhomboid geometric shapes [16, 17]. Amylase crystalloids are primarily associated with benign, non-neoplastic conditions, although they have occasionally been reported in Warthin tumor and pleomorphic adenoma (PA). It is important that inflamed cases with amylase crystalloids include a comment in the diagnosis that clinical and radiologic correlations are needed to help exclude a neoplastic condition. Other crystalloids that

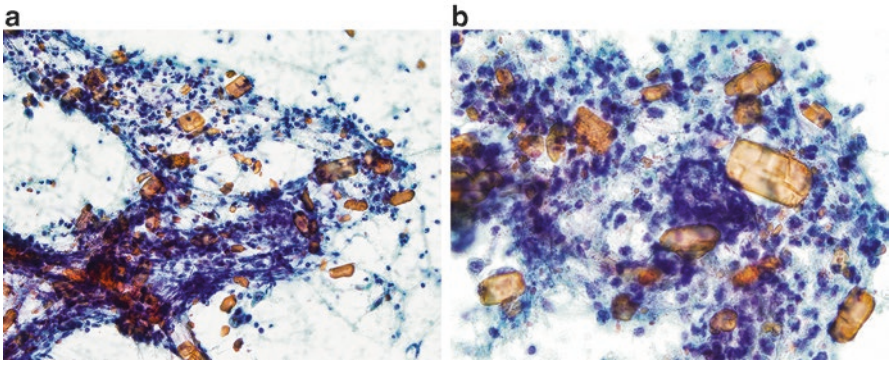

Fig. 3.8 Non-Neoplastic. Amylase crystalloids (**a**, **b**) are non-birefringent crystalline structures with rectangular, needle-shaped, rhomboid, and platelike shapes. They are most commonly associated with non-neoplastic inflammatory conditions as in this case (smear, Papanicolaou stain)

can be seen in salivary gland aspirates include floret-shaped tyrosine crystalloids as well as collagenous crystalloids, and calcium oxalate crystals. Unlike amylase crystalloids, tyrosine crystalloids are more commonly associated with neoplastic conditions, most often with PA, but with some malignant neoplasms as well [17].

Granulomatous Sialadenitis

Granulomatous inflammation can involve the salivary gland parenchyma or associated lymph nodes. Patients usually present with a slow-growing mass [14]. It is commonly a response to extravasated ductal contents, particularly mucin, secondary to obstructive sialadenopathy, which can result from a variety of causes including specific infections (e.g., mycobacterial, actinomycosis, cat-scratch disease, toxoplasmosis, tularemia) or less commonly a systemic granulomatous disease such as sarcoidosis. In very rare cases, granulomatous inflammation can be due to certain neoplastic conditions such as Hodgkin lymphoma, T-cell lymphoma, and a subset of metastatic carcinomas (e.g., nasopharyngeal carcinoma).

Cytologic Criteria

- Hypocellular (scant acinar and ductal cells)
- Groups of epithelioid histiocytes
- Variable amounts of acute and chronic inflammatory cells
- ± Multinucleated giant cells
- ± Necrotic background debris

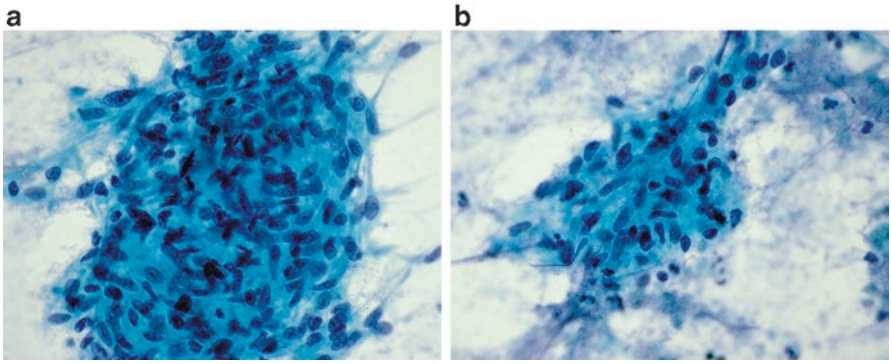

Fig. 3.9 Non-Neoplastic. (**a**) This aspirate of granulomatous sialadenitis shows a large group of epithelioid histiocytes; an infectious agent should be excluded. (**b**) Aspirates of sarcoidosis yield loose collections of epithelioid histiocytes, and usually lack background necrosis (noncaseating) (smear, Papanicolaou stain)

Explanatory Notes

The diagnosis of granulomatous sialadenitis relies on identifying groups of epithelioid histiocytes (Fig. 3.9a). Obstructive sialadenopathy with extravasation of ductal contents is the most common cause of a granulomatous reaction, which can result from calculi or less often tumors. In cases with more marked granulomas, care should be taken to avoid misinterpreting the epithelioid histiocytes with their moderate amounts of eosinophilic cytoplasm and curved nuclei as an epithelial neoplasm. Mycobacterial infection (tuberculous or nontuberculous) is the most common etiology for infectious granulomatous sialadenitis, although special stains (AFB) infrequently reveal diagnostic acid fast bacteria. Other granulomatous infections of the salivary glands are rare. Cat-scratch fever and tularemia can be associated with suppurative granulomatous inflammation, including peripherally palisading epithelioid histiocytes, centrally located neutrophils, and an associated mixed chronic inflammation. When infectious etiologies are suspected, special stains can be performed using cell block material or using liquid based slides. In addition, the cytopathologist should consider submitting material for microbiology cultures and/or polymerase chain reaction (PCR) testing; otherwise, the patient might be subjected to a repeat FNA to obtain additional material.

Sarcoidosis (Fig. 3.9b) is among the most common systemic causes of granulomatous sialadenitis. Aspirates yield loose collections of epithelioid histiocytes, and usually lack background necrosis (noncaseating). Sarcoidosis is a diagnosis of exclusion, and requires clinical and microbiologic correlation as well as special stains to exclude an infectious cause.

Reactive Lymph Node Hyperplasia

Enlarged intra- and peri-parotid lymph nodes are a common non-neoplastic cause of a salivary gland mass (Table 3.2). They are frequently sampled by FNA to confirm benign disease, to diagnose infection, or to rule-out either metastatic disease or lymphoma [18]. The etiology of parotid gland lymph node hyperplasia can be non-specific, or it can be a response to clinical or subclinical bacterial or viral infection often involving the skin of the face or scalp. Mononucleosis, tuberculosis, and cat-scratch disease, among others, can also result in such a reaction.

Cytologic Criteria

Aspirates of reactive lymph node hyperplasia are usually cellular and contain (Figs. 3.10 and 3.11):

- Mixed population of lymphocytes with predominance of small mature forms
- Tingible body macrophages
- Lymphohistiocytic aggregates representing the cytologic correlate of germinal centers
- Background lymphoglandular bodies

Explanatory Notes

The presence of a heterogeneous population of lymphocytes, tingible body macrophages, and dendritic cells suggests the diagnosis of reactive lymphoid hyperplasia. In most cases, the predominant cell population will consist of a mixture of small mature B- and T-lymphocytes. Clinical correlation is needed along with demonstration of polyclonality by flow cytometry or immunohistochemical studies. Caution is recommended, particularly when evaluating aspirates of lymph nodes in the elderly, lymph

Table 3.2 Causes of reactive lymph node hyperplasia

Nonspecific
Specific
Bacterial and fungal lymphadenitis
Infectious mononucleosis
Mycobacterial lymphadenitis
Cat scratch disease
Sarcoidosis
Rosai–Dorfman disease (sinus histiocytosis with massive lymphadenopathy)
Kikuchi lymphadenitis

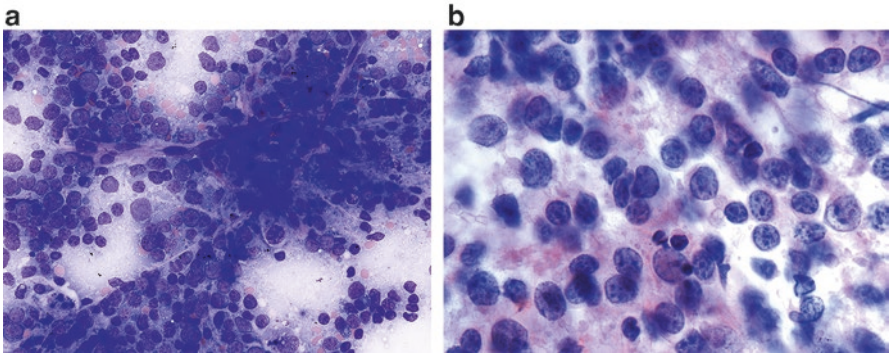

Fig. 3.10 Non-Neoplastic. These aspirates of reactive lymph node hyperplasia (**a**) (smear, Romanowsky stain) (Courtesy of William Geddie, MD, Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada) and (**b**) (smear, Papanicolaou stain) show a mixed population of mostly small and intermediate-size lymphocytes admixed with follicular dendritic cells. Flow cytometry can be used to confirm a polyclonal population

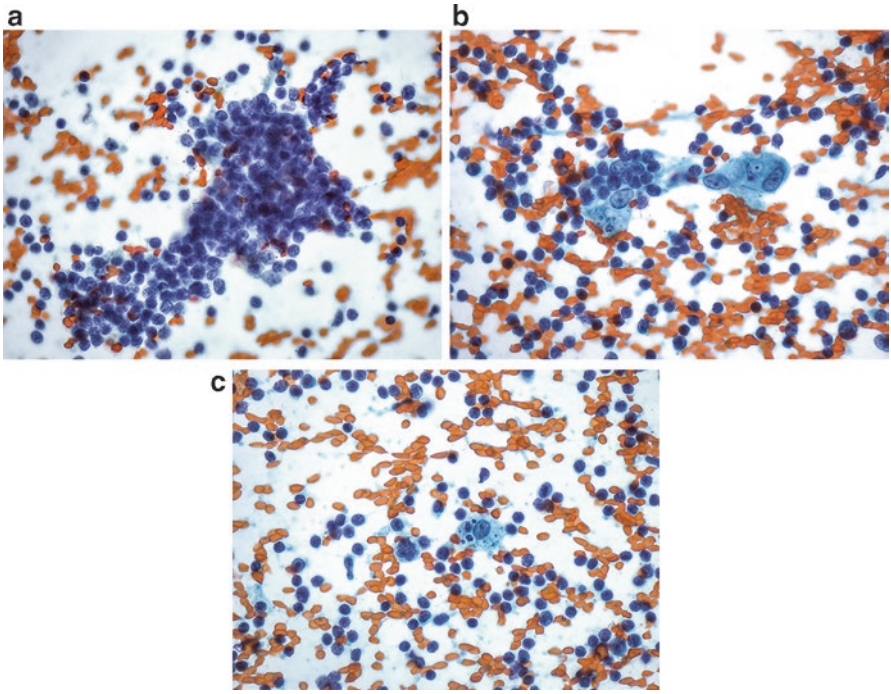

Fig. 3.11 Non-Neoplastic. These aspirates of reactive lymph node hyperplasia show (**a**) a cohesive group of lymphocytes and follicular dendritic cells representing a germinal center fragment. (**b**, **c**) Tingible body macrophages are present in a background of predominantly small mature lymphocytes and occasional follicular dendritic cells (smear, Papanicolaou stain)

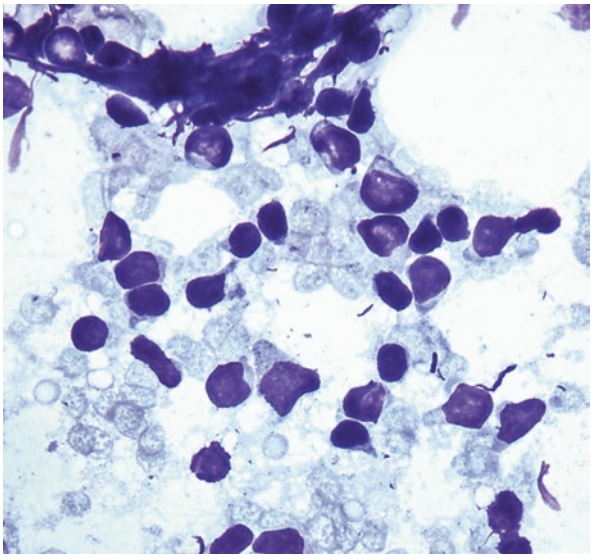

Fig. 3.12 Atypia of Undetermined Significance (AUS). This lymph node aspirate shows an increased proportion of larger lymphocytes. In the absence of flow cytometry to exclude lymphoma, such aspirates should be classified as AUS (smear, Romanowsky stain)

nodes larger than 3 cm, and multiple enlarged or matted lymph nodes. In addition, patients with autoimmune disease such as Sjögren's syndrome are at increased risk of developing primary parotid gland lymphomas. Occasionally, reactive lymphoid hyperplasia can contain an increased proportion of larger cells, either lymphoid or histiocytic (Fig. 3.12), which can lead to a diagnosis of AUS. Infectious mononucleosis due to EBV can produce markedly atypical cells. It is also important to note that a subset of lymphomas can yield an aspirate with a heterogeneous appearance mimicking reactive lymphoid hyperplasia, namely extranodal marginal zone lymphoma as well as others such as Hodgkin lymphoma, some T-cell lymphomas, and T-cell rich B-cell lymphoma. For any case of a salivary gland lymph node aspirate where lymphoma is in the differential diagnosis, flow cytometry using an aliquot of unfixed material is highly recommended.

Clinical correlation and follow-up are important in patients with lymphadenopathy, and a note suggesting additional evaluation for patients with persistent lymphadenopathy can be useful. This is particularly true in cases where immunophenotyping is not performed, as well as for certain unsuspected lymphomas such as Hodgkin lymphoma where flow cytometry can be negative.

Benign Lymphoepithelial Lesion/Lymphoepithelial Sialadenitis (LESA)

Lymphoepithelial sialadenitis (LESA) is a benign condition characterized by a lymphocytic infiltrate associated with parenchymal atrophy and foci of ductal hyperplasia with intraepithelial lymphocytes (Fig. 3.13). It is an autoimmune lesion and is

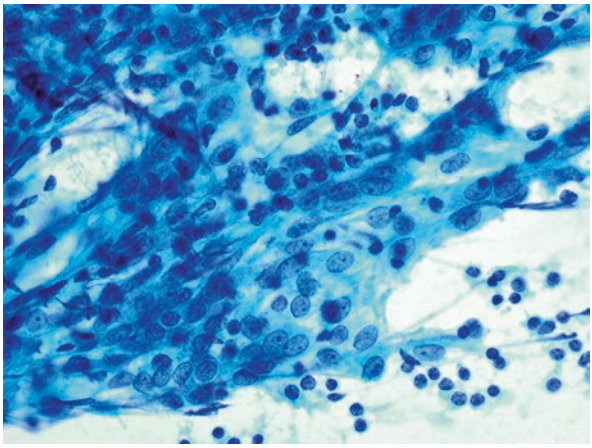

Fig. 3.13 Non-Neoplastic. This aspirate demonstrates the lymphoepithelial lesion of lymphoepithelial sialadenitis, which consists of a bland sheet of ductal epithelial cells with admixed small lymphocytes (smear, Papanicolaou stain)

often related to Sjögren's syndrome; it is more common in women, and affects the parotid glands in about 90% of cases [19]. Bilateral disease is typical, although one gland may be more severely affected than the other. Patients experience recurrent, often progressive, parotid gland enlargement with varying degrees of discomfort or pain. Patients with Sjögren's syndrome have an increased risk of developing lymphoma, particularly extranodal marginal zone lymphoma.

Cytologic Criteria

The hallmark cytologic features of LESA are:

- Cellular aspirate
- Lymphoepithelial lesions consisting of cohesive sheets of ductal cells, often with squamous metaplastic changes, and with small mature lymphocytes percolating through the epithelial sheets (Fig. 3.14).
- Mixed population of lymphocytes, dendritic cells, and tingible body macrophages with predominance of small mature lymphocytes
- Lymphohistiocytic aggregates
- Acinar cells are usually absent

Explanatory Notes

The lymphoepithelial lesions in aspirates of LESA will often have squamous metaplastic features. The ductal epithelial cells will exhibit a uniform atypia, including enlarged nuclei with variably distinct nucleoli that overall resembles reparative changes. In some cases, the lymphoepithelial lesions can raise a differential

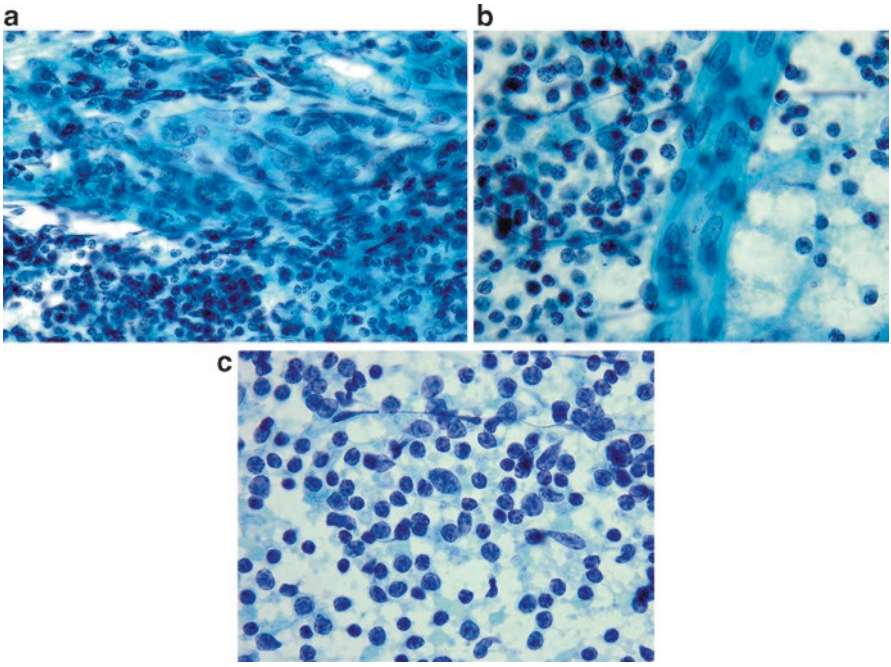

Fig. 3.14 Non-Neoplastic. (**a**, **b**) The lymphoepithelial lesions of lymphoepithelial sialadenitis (LESA) are sheets of ductal cells that can exhibit squamous metaplastic features. (**c**) The associated lymphoid population in LESA is a mixed pattern with a predominance of small mature lymphocytes (smear, Papanicolaou stain)

diagnosis of metastatic carcinoma to a lymph node, particularly when reviewed outside of the clinical context of LESA. In contrast to metastatic carcinoma, the epithelial cells in aspirates of LESA lack significant nuclear pleomorphism, mitotic activity, hyperchromasia, and background necrosis. Given the increased risk of primary lymphoma in patients with LESA, care should be taken to assess the aspirate for polyclonality using flow cytometry, and for a population of lymphocytes with atypical features.

In contrast to LESA, which is usually solid, aspirates of lymphoepithelial cysts (including those associated with HIV) will lack the large sheetlike lymphoepithelial lesions of LESA, and consist of proteinaceous cyst contents with admixed degenerating squamous cells, keratin debris, as well lymphocytes and lymphohistiocytic aggregates (Fig. 3.15). In some cases, a glandular cyst lining component, which can be ciliated, may also be encountered. In middle-aged and older patients, care should be taken to exclude the possibility of metastatic squamous cell carcinoma, which will usually exhibit more marked squamous atypia than in a lymphoepithelial cyst.

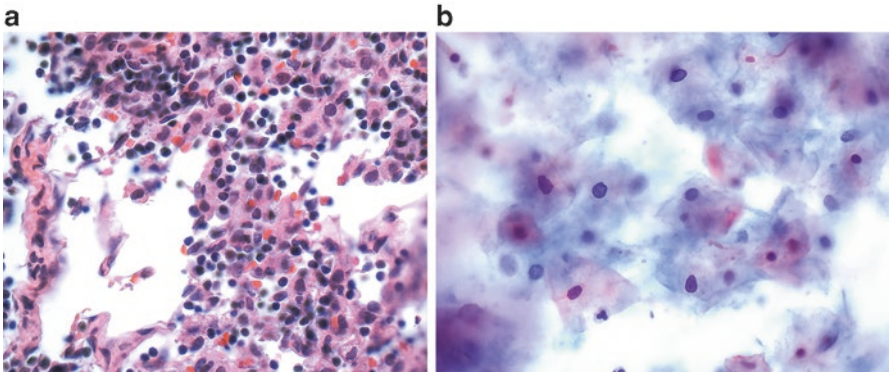

Fig. 3.15 Non-Neoplastic. (**a**) Aspirates of lymphoepithelial cysts consist of a mixed population of lymphocytes and variable numbers of dendritic cells. (**b**) Some cases may show only cyst contents with abundant bland nucleate and anucleate squamous cells. Clinical context is important to exclude a squamous cell carcinoma (smear, Papanicolaou stain)

Entities Sometimes Classified as "Non-Neoplastic"

Sialadenosis

Sialadenosis or sialosis is an uncommon persistent, non-inflammatory, non-neoplastic enlargement of salivary glands [7]. Sialadenosis primarily affects the parotid glands, often bilaterally, although it can occasionally occur in the submandibular glands. Sialadenosis is almost always associated with an underlying systemic disorder such as diabetes, hypothyroidism, malnutrition, obesity, pregnancy, alcohol abuse, cirrhosis, HIV infection, or with several medications (especially antihypertensives). Clinically, the salivary gland swelling develops gradually, without a defined mass, and is usually painless.

Cytologic Criteria (Fig. 3.16)

- Cellular aspirate
- Clusters of enlarged (hypertrophic) acinar cells
- Normal cytoarchitectural arrangement of acini is maintained
- Background of stripped acinar cell nuclei
- Fibroadipose tissue
- Features suggestive of neoplasm, cyst, or inflammatory lesion are absent

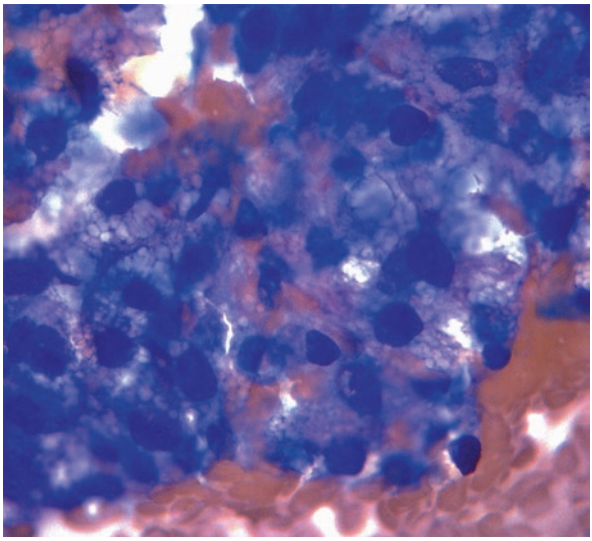

Fig. 3.16 Non-Neoplastic. This aspirate of sialadenosis shows a cluster of large vacuolated acinar cells. Clinical correlation is needed to interpret this aspirate (smear, Romanowsky stain)

Explanatory Notes

The enlarged acinar size in sialadenosis may be difficult to appreciate by FNA, but the condition is suspected clinically. Clinical and radiologic correlations are essential in diagnosing sialadenosis, since the major differential diagnosis is a sampling error (i.e., "Non-Diagnostic," see Chap. 2, Non-Diagnostic). Therefore, for aspirates containing only non-neoplastic salivary gland elements, the cytopathologist should usually classify the aspirate as "Non-Diagnostic" when a discrete mass is present (i.e., suggesting a possible sampling error), or as "Non-Neoplastic" in the absence of a discrete mass and with appropriate clinicoradiological information. In either case, a comment describing the possibility of a sampling error is strongly recommended (see sample report).

Because numerous acinar cells are present in sialadenosis, care must be taken not to confuse this entity with a well-differentiated acinic cell carcinoma (see Chap. 7). Most importantly, the cells of sialadenosis maintain a normal cytologic and histologic cytoarchitectural arrangement, including a normal ductal component, while the neoplastic cells of acinic cell carcinoma do not. Other entities in the differential diagnosis of sialadenosis include accessory parotid gland, hamartoma, lipoma/lipomatosis, and sialolithiasis. Accessory parotid gland tissue may present clinically as a mass, and can occur anywhere along the parotid (Stensen's) duct overlying the masseter muscle.

Oncocytosis

Oncocytosis is primarily encountered in older adults. It is considered a hyperplastic change in which there are variable degrees of oncocytic metaplasia of acinar and ductal cells (Fig. 3.17). Depending upon the extent of oncocytosis, distinction from oncocytoma (a true neoplasm) is often not possible since the two entities overlap clinically and histologically [20]. Because of this, most FNA cases of oncocytosis will be placed into the "Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)" category (See Chap. 5, Neoplasm).

Cytologic Criteria

- Acinar and ductal cells with abundant, granular, eosinophilic cytoplasm
- Normal cytoarchitectural arrangement of acini and ductal cells is maintained
- Variable amounts of benign ductal cells and fibroadipose tissue
- Features suggestive of neoplasm, cyst, or inflammatory lesion are absent

Explanatory Notes

Oncocytosis in the salivary gland is more common with increasing age. The differential diagnosis includes oncocytoma as well as oncocytic changes that can occur in several primary salivary gland neoplasms, including pleomorphic adenoma and mucoepidermoid carcinoma. Recognizing the admixture of oncocytic acinar and ductal cells in a "normal" architectural pattern is the key to avoid misdiagnosing oncocytosis as an oncocytic neoplasm.

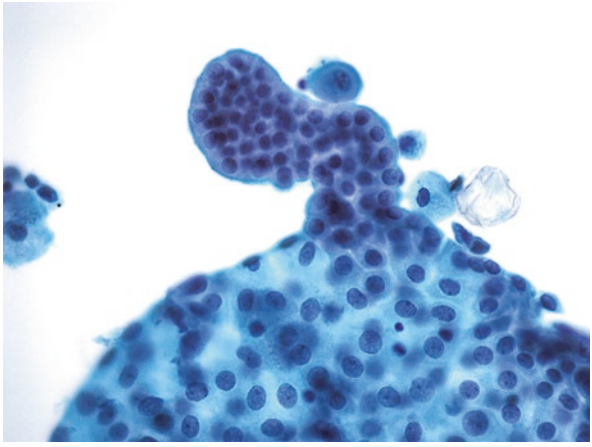

Fig. 3.17 Non-Neoplastic. This aspirate of oncocytosis from a multinodular gland shows a sheetlike collection of oncocytes merging with a small fragment of ductal epithelium (smear, Papanicolaou stain)

Clinical Management

Salivary gland lesions diagnosed as "Non-Neoplastic" by FNA should be followed clinically by repeat physical examination, cross-sectional imaging, or a combination of both depending upon the nature of the lesion. Any change in either the clinical or radiologic features should prompt repeat sampling, especially given the risk of sampling error in this subset of salivary gland lesions.

Sample Reports

Example 1:
Satisfactory for evaluation
NON-NEOPLASTIC
Abundant acute inflammation and reactive changes consistent with acute sialadenitis. See note.
Note: Correlation with microbiologic studies is suggested.

Example 2:
Evaluation limited by scant cellularity
NON-NEOPLASTIC
Consistent with chronic sialadenitis. See note.
Note: Clinical and radiological correlations are recommended to ensure that the aspirate is representative of the lesion.

Example 3:
Satisfactory for evaluation
NON-NEOPLASTIC
Granulomatous inflammation. See note.
Note: Non-necrotizing granulomas are present admixed with acute and chronic inflammation. Diagnostic considerations include a non-specific reaction secondary to obstructive sialadenopathy, infection, and sarcoidosis. Correlation with microbiologic studies is suggested.

Example 4:
Satisfactory for evaluation
NON-NEOPLASTIC
Consistent with reactive lymphoid hyperplasia. See note.
Note: Corresponding flow cytometry is benign, supporting the diagnosis. Clinical follow-up is recommended, and if lymphadenopathy persists, additional evaluation may be indicated.

Example 5:
Satisfactory for evaluation
NON-NEOPLASTIC
Consistent with lymphoepithelial sialadenitis. See note.
Note: Corresponding flow cytometry is benign, supporting the diagnosis.

Example 6:
Satisfactory for Evaluation.
NON-NEOPLASTIC
Benign salivary gland tissue suggestive of sialadenosis. See Note.
Note: Based on the clinical presentation of bilateral salivary gland enlargement without a discrete mass and with enlarged acinar cells microscopically, the findings are suggestive of sialadenosis. Clinical and radiologic correlations are needed to ensure that the FNA sample is representative of the lesion.

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Chapter 4
Atypia of Undetermined Significance

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General Background

A goal of salivary gland fine-needle aspiration (FNA) is to determine if an aspirated lesion is neoplastic or not since this can impact clinical management [1–5]. In the Milan System, salivary gland FNA samples that are indefinite for a neoplastic condition are classified as "Atypia of Undetermined Significance" (AUS). The AUS category will potentially reduce the number of false-negative diagnoses in the "Non-Neoplastic" category as well as reducing the number of false positive diagnoses in the "Neoplasm" category.

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_4

The AUS category is heterogeneous in nature. FNAs in this category will often be associated with pre-analytical factors (e.g., FNA technique, smearing technique, air drying artifact, obscuring background) or the inherent characteristics of the lesion (e.g., cystic, fibrotic, necrotic), resulting in scant numbers of well-preserved cells. Samples classified as "AUS" will usually show morphological overlap between non-neoplastic and neoplastic processes [1–10].

Definition

The diagnostic category "Atypia of Undetermined Significance (AUS)" applies to a salivary gland FNA that lacks either qualitative or quantitative cytomorphologic features to be diagnosed with confidence as non-neoplastic or neoplastic. In addition, the FNA exhibits an atypical cytomorphologic feature that excludes the possibility of classifying it as "Non-Diagnostic." Most samples will represent reactive atypia or poorly sampled neoplasms.

Cytologic Criteria

The diagnosis of AUS can be used in the following scenarios:

- Reactive and reparative atypia indefinite for a neoplasm (Fig. 4.1)
- Squamous, oncocytic, or other metaplastic changes indefinite for a neoplasm (Figs. 4.2, 4.3, and 4.4)
- Low cellularity specimens suggestive of, but not diagnostic of a neoplasm (Fig. 4.5)
- Specimens with preparation artifacts hampering distinction between a non-neoplastic and neoplastic process (Fig. 4.6)
- Mucinous cystic lesions with an absent or very scant epithelial component (Fig. 4.7)
- Salivary gland lymph nodes or lymphoid lesions that are indefinite for a lymphoproliferative disorder (Fig. 4.8)

Explanatory Notes

Salivary gland FNAs with suboptimal cellularity or with artifacts can raise uncertainty as to whether the aspirated lesion is neoplastic or not. Sparsely cellular aspirates composed of basaloid cells can raise a differential diagnosis that includes chronic sialadenitis and a basaloid neoplasm (Fig. 4.9). In many instances, the distinction is clear. Most cases of chronic sialadenitis will be hypocellular with

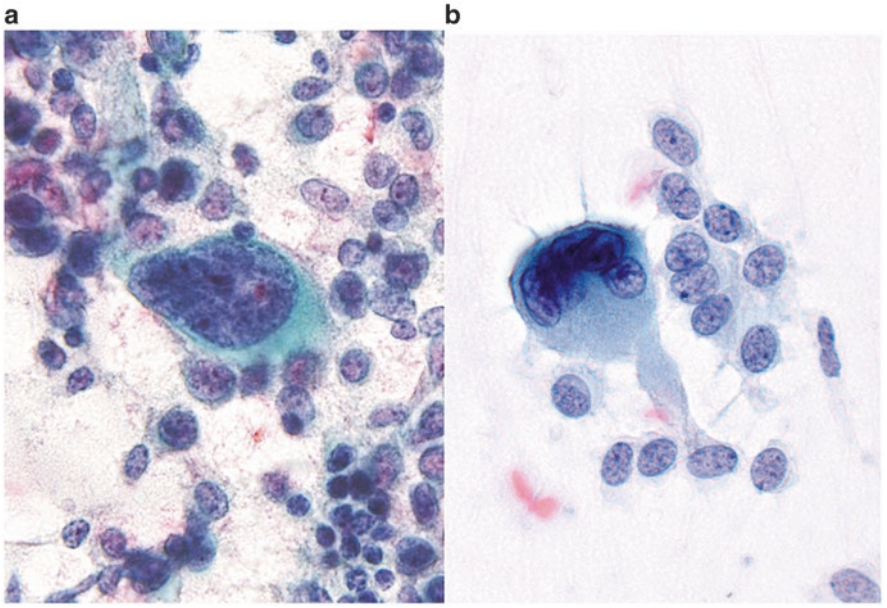

Fig. 4.1 Atypia of Undetermined Significance. These two images (**a**, **b**) show rare atypical cells in an inflammatory background, indefinite for a neoplasm (smears, Papanicolaou stain)

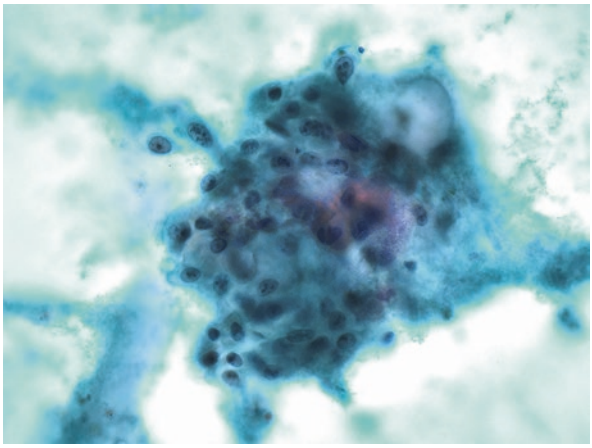

Fig. 4.2 Atypia of Undetermined Significance. Group of epithelioid cells, indefinite for a neoplasm (smear, Papanicolaou stain)

background chronic inflammation and few small cohesive groups of ductal cells, often with a basaloid quality. In contrast, most aspirates of a basaloid neoplasm are cellular and composed of complex basaloid groups, often with associated matrix. For cases where the distinction between a reactive process and a basaloid neoplasm is uncertain, an AUS diagnosis is appropriate. Similarly, salivary gland aspirates with various metaplastic changes including squamous, oncocytic, and sebaceous features, can be challenging and raise the differential diagnosis of a poorly sampled

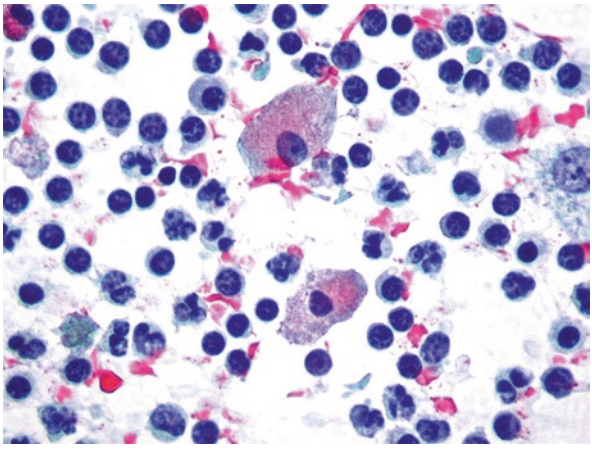

Fig. 4.3 Atypia of Undetermined Significance. The aspirate shows occasional epithelial cells with oncocytic features in a background with numerous lymphocytes, indefinite for a neoplasm (smear, Papanicolaou stain). The surgical follow-up was a Warthin tumor

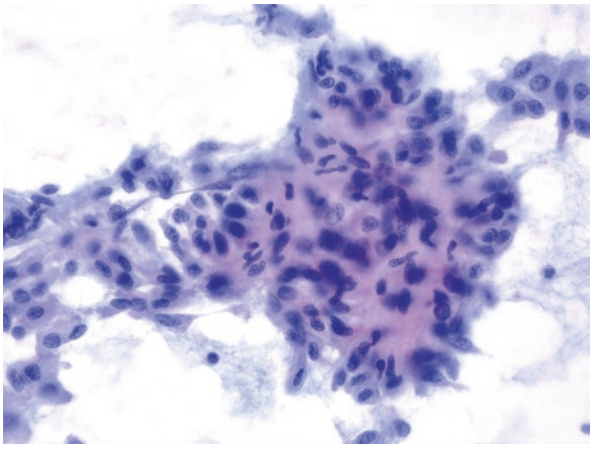

Fig. 4.4 Atypia of Undetermined Significance. This aspirate contains occasional groups of bland epithelial cells with oncocytic features. The findings are indefinite for an oncocytoma versus oncocytic metaplasia (smear, Papanicolaou stain)

neoplasm including mucoepidermoid carcinoma, pleomorphic adenoma, and Warthin tumor [5–8]. The presence of a scant population of spindle cells can also suggest both reactive processes such as nodular fasciitis or granulomatous inflammation and neoplastic conditions including myoepithelioma, schwannoma, and solitary fibrous tumor (Fig. 4.10).

For salivary gland aspirates containing a prominent lymphoid component, several lesions, both non-neoplastic and neoplastic, should be considered in the differential diagnosis [10] (Table 4.1). Non-neoplastic lesions include chronic sialadenitis, lymphoepithelial sialadenitis (LESA), lymphoepithelial cysts, as well as reactive intraparotid or periparotid lymph nodes, and these are usually classified as "Non-Neoplastic." However, for cases where there is limited atypia of the lymphoid component (Fig. 4.11) or where the polyclonal nature of the aspirate is in doubt and a lymphoproliferative disorder cannot be excluded, such FNA samples can be classified as AUS. When evaluating these lymphocyte-predominant aspirates, attention

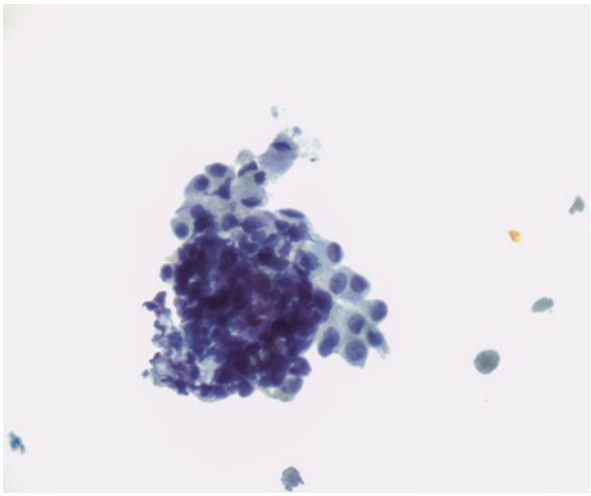

Fig. 4.5 Atypia of Undetermined Significance. This hypocellular aspirate shows a very rare group of mildly atypical epithelial cells with associated "lymphocytic tangles," suggestive but not diagnostic of a neoplasm (smear, Papanicolaou stain)

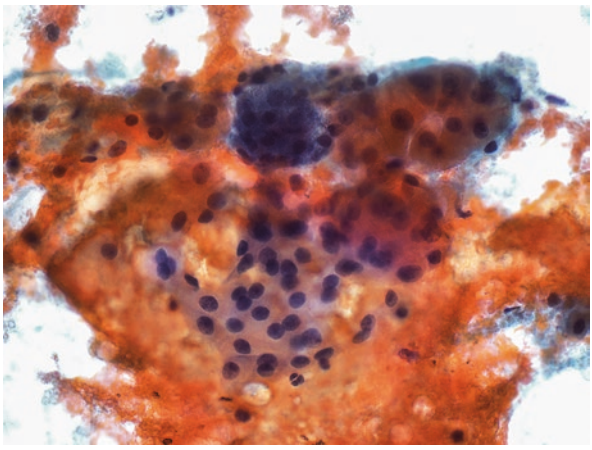

Fig. 4.6 Atypia of Undetermined Significance. The epithelial cells in this aspirate are suggestive of a neoplastic process but abundant blood limits the evaluation (smear, Papanicolaou stain)

should be given to the degree of cellular heterogeneity, the pattern of lymphocytes as dispersed or in aggregates, and the degree of atypia of the lymphoid population. In addition, clinical correlation is needed. Aspirates of enlarged reactive intraparotid and periparotid lymph nodes are common. Most reactive lymph node aspirates show a polymorphous population of lymphocytes, lymphohistiocytic aggregates, tingible body macrophages, plasma cells, and lymphoglandular bodies in the background (see Chap. 3). However, the latter cytomorphologic pattern overlaps with features of nodal and extranodal marginal zone lymphomas (MZL) that can be very difficult to identify in cytology samples. Immunophenotyping, usually by flow cytometry, is typically needed to distinguish between reactive lymph node hyperplasia and MZL. If this is not possible, an AUS or, alternatively, a "Suspicious for Malignancy" interpretation with a note (see sample report) is recommended for these aspirates (see Chap. 6).

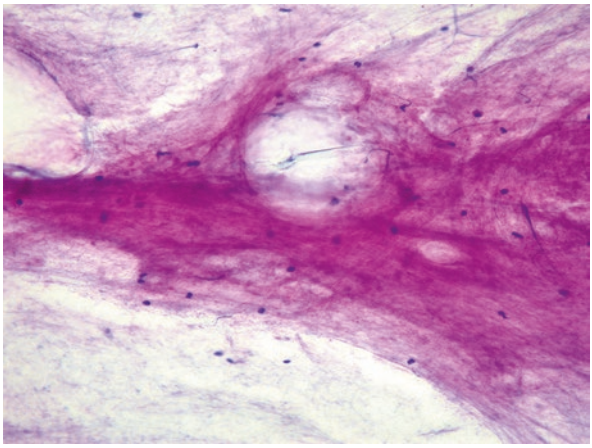

Fig. 4.7 Atypia of Undetermined Significance. This aspirate contains abundant mucin without any epithelial cells. The differential diagnosis includes a benign mucinous cyst; however, a low-grade mucoepidermoid carcinoma cannot be excluded (smear, Romanowsky stain)

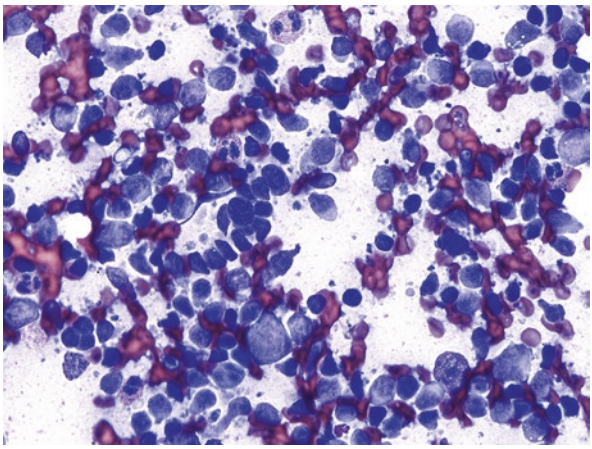

Fig. 4.8 Atypia of Undetermined Significance. Mixed population of lymphocytes with background lymphoglandular bodies and increased numbers of larger lymphocytes. A lymphoma cannot be excluded, particularly in the absence of flow cytometry (smear, Romanowsky stain)

A variety of neoplastic and non-neoplastic lesions of the salivary glands can present with a predominant cystic component, with at least one-third of cystic salivary gland lesions being neoplastic [11] (Table 4.2). FNAs of these lesions often yield serous or mucoid material, frequently of low cellularity. Such aspirates may be obtained from non-neoplastic lesions including mucus retention cysts, mucoceles, ductal cysts, and lymphoepithelial cysts as well as cystic neoplasms such as Warthin tumor, cystic pleomorphic adenoma, low-grade mucoepidermoid carcinoma, and cystadenoma/cystadenocarcinoma. Aspirates with sufficient cellularity usually lead to a specific diagnosis. However, cases containing mucinous cyst contents only and/or a sparse epithelial component can pose diagnostic difficulties. In these cases, the aspirate can be classified as AUS. Aspirates of cystic salivary gland lesions can be generally divided into mucinous and non-mucinous types. Aspirates of non-mucinous cyst contents characterized by watery proteinaceous fluid containing scattered lymphocytes, histiocytes, and debris will be classified as "Non-Diagnostic-cyst

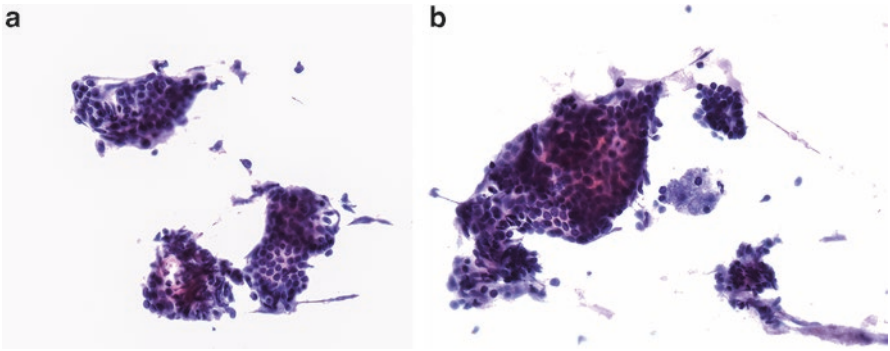

Fig. 4.9 Atypia of Undetermined Significance. These aspirates (**a**, **b**) show groups of basaloid-appearing epithelium that are indefinite for a neoplastic process versus reactive or metaplastic changes (smear, Papanicolaou stain). (*Note: These images are purposefully overexposed to capture nuclear detail*)

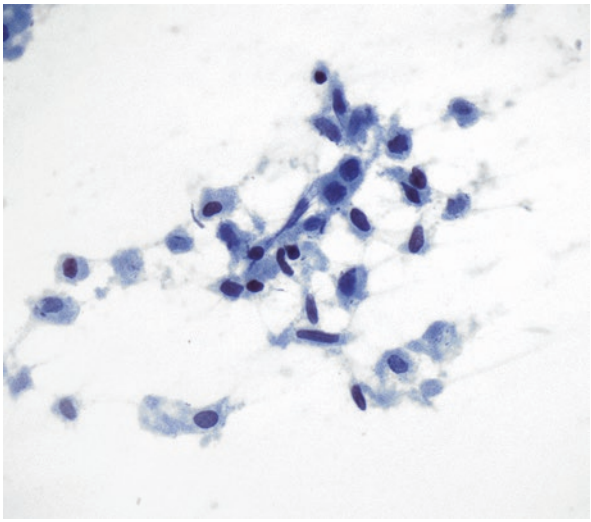

Fig. 4.10 Atypia of Undetermined Significance. This hypocellular aspirate contains occasional epithelioid and spindled cells that are suggestive of a neoplasm (smear, Papanicolaou stain)

contents." When an epithelial component is present but scant (Fig. 4.12), raising the possibility of a neoplasm, the aspirate can be classified as AUS. For aspirates of mucinous cyst contents or where significant amounts of background mucin are present, the possibility of a low-grade mucoepidermoid carcinoma should be considered. Depending upon the clinical context and cytomorphologic features of the aspirated lesion, many of these cases with background mucin that are indefinite for a neoplasm will be classified as AUS.

Lymphoepithelial cysts occur in the area of the parotid gland. Aspirates yield squamous cells, sometimes with reactive atypia, in a lymphoid background [7–11]. The differential diagnosis, particularly when significant squamous atypia is present, includes a cystic metastasis of squamous cell carcinoma (Fig. 4.13). Most cases of

Table 4.1 Differential diagnosis of "lymphocyte-rich aspirates"

Intrinsic
Non-Neoplastic
Chronic sialadenitis
Granulomatous sialadenitis
Lymphoepithelial sialadenitis (LESA)
Lymphoepithelial (HIV-associated) cyst
Neoplastic
Warthin tumor
Mucoepidermoid carcinoma
Acinic cell carcinoma
Malignant lymphoma
Extrinsic
Non-Neoplastic
Reactive lymph node hyperplasia
Neoplastic
Malignant lymphoma of nodal origin

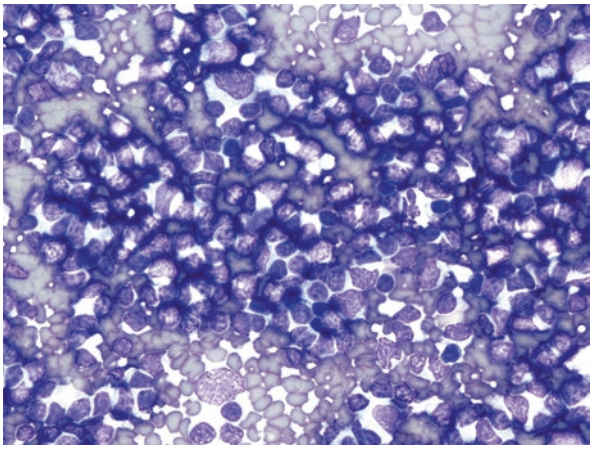

Fig. 4.11 Atypia of Undetermined Significance (AUS). This aspirate shows a mixed lymphoid pattern with an atypical population of intermediate-size lymphocytes. In the absence of flow cytometry, this aspirate can be classified as either "AUS" or "Suspicious for Malignancy" (smear, Romanowsky stain)

metastatic squamous cell carcinoma are recognized as "Malignant;" however, in cases with scant cellularity, limited atypia, or extensive degenerative features, distinction between an inflamed branchial cleft cyst with reactive atypia versus metastatic squamous cell carcinoma may not be possible. A subset of these cases can be classified as AUS. A wide variety of other less common scenarios where a neoplasm cannot be ruled out after examination of all the cellular material can be encountered and may be appropriate for classification within the AUS category.

As in other cytology reporting systems, the proportion of FNAs diagnosed as AUS is anticipated to be low, and a benchmark of <10% of all salivary gland FNAs is appropriate for this category. The use of AUS should be limited, and

Table 4.2 Differential diagnosis of lesions that can yield "cystic" fluid

Intrinsic
Non-Neoplastic
Obstructive sialadenopathy (retention cyst, mucocele)
Salivary duct cyst
Lymphoepithelial (HIV-associated) cyst
Polycystic disease
Neoplastic
Warthin tumor
Pleomorphic adenoma
Mucoepidermoid carcinoma
Acinic cell carcinoma
Cystadenoma/cystadenocarcinoma
Secretory carcinoma
Extrinsic
Non-Neoplastic
Branchial cleft cyst
Neoplastic
Necrotic metastatic carcinoma in intraparotid or periparotid lymph node

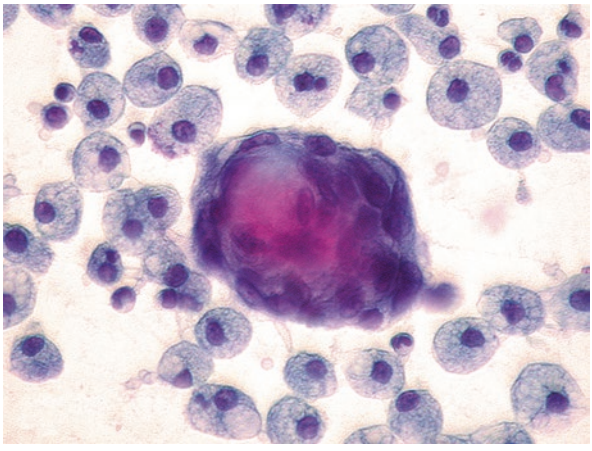

Fig. 4.12 Atypia of Undetermined Significance. This hypocellular cyst aspirate contains rare atypical epithelial groups that are suggestive of, but not diagnostic of, a cystic neoplasm (smear, Papanicolaou stain)

cytopathologists should make every attempt to classify specimens using other more specific categories whenever possible. There may be a role for intralaboratory monitoring of the AUS rate to avoid overuse of this category. It is recommended that the entire FNA specimen be processed for cytomorphologic interpretation before rendering a diagnosis of AUS. The ROM for the AUS category is expected to be in a range that falls between the ROMs of the non-neoplastic and

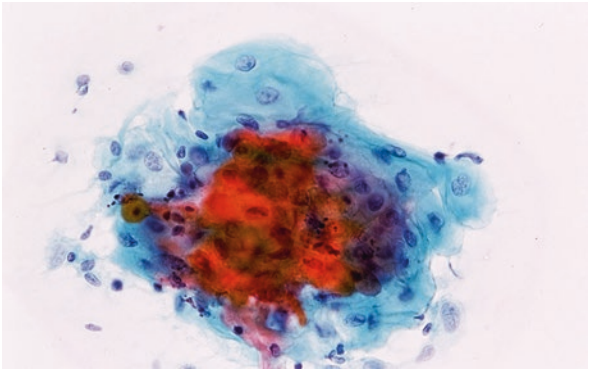

Fig. 4.13 Atypia of Undetermined Significance. This image showing a collection of cytologically bland keratinizing squamous cells raises a differential diagnosis of metastatic squamous cell carcinoma versus reactive squamous atypia in a benign squamous cyst. Clinical context and quality of the fine-needle aspiration sample will influence the cytologic classification (smear, Papanicolaou stain)

the neoplastic categories, which we estimate to be approximately 20%; however, given the lack of literature pertaining to salivary gland aspirates classified as AUS, the ROM is not yet well defined.

Management

A diagnosis of AUS should lead to careful correlation with clinical and radiologic findings. Depending upon the overall risk assessment, it may result in a repeat FNA, core-needle biopsy, open biopsy, or surgical excision. For cystic lesions, aspiration of any residual mass using ultrasound guidance can help to achieve a more specific cytologic diagnosis. In aspirates with an atypical lymphoid population, flow cytometry, immunochemistry, or tissue biopsy should be considered to rule out a lymphoproliferative disorder.

Sample Reports

Example 1:
Evaluation limited by scant cellularity
ATYPIA OF UNDETERMINED SIGNIFICANCE
Histiocytes ± scant epithelial cells in a background of abundant mucin. See Note.
Note: The differential diagnosis of mucin-containing cysts includes mucocele, mucus retention cysts, and low-grade mucoepidermoid carcinoma. Clinical and radiological correlations are needed. Aspiration of a residual mass, if present, may help to achieve a more specific diagnosis.

Example 2:
Evaluation limited by scant cellularity
ATYPIA OF UNDETERMINED SIGNIFICANCE
Few clusters of basaloid cells with mild atypia. See note.
Note: While the aspirate may represent chronic sialadenitis with metaplasia and reactive changes, a salivary gland neoplasm with basaloid features cannot be completely excluded. Recommend clinical and radiologic correlations and additional sampling if clinically indicated.

Example 3:
Evaluation limited by scant cellularity
ATYPIA OF UNDETERMINED SIGNIFICANCE
Scant oncocytic cells with cytological and/or architectural atypia. See note.
Note: While the aspirate may represent oncocytic metaplasia or oncocytic hyperplasia, a neoplastic process cannot be entirely excluded. Recommend clinical and radiologic correlations and additional sampling if clinically indicated.

Example 4 Sample report of a lymphoid-rich aspirate:
Satisfactory for evaluation
ATYPIA OF UNDETERMINED SIGNIFICANCE
Abundant mixed population of lymphocytes with occasional atypical forms. See note.
Note: The aspirate is suggestive of a reactive lymph node, but in the absence of flow cytometry, a low-grade lymphoproliferative disorder cannot be entirely excluded. Clinical and radiological correlations are recommended.

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Chapter 5
Neoplasm

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General Background

Salivary gland neoplasms are rare, more commonly occur in the parotid gland, and comprise approximately 6% of all head and neck tumors, and 0.3% of all malignancies [1–8]. Up to 80% of salivary gland neoplasms arising in the parotid gland are benign, as compared to a significantly increased incidence of malignant tumors in the other major and all minor salivary glands. In adults, pleomorphic adenomas (PA) account for about 50% of all salivary gland neoplasms; Warthin tumor (WT) is the second most common benign tumor. A majority of studies cite mucoepidermoid carcinoma as the most common malignant tumor in both children and adults; however, this can vary depending upon anatomic site and patient cohort [1–3].

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_5

Fine-needle aspiration (FNA) is widely utilized in the management of salivary gland neoplasms. It can effectively distinguish with high specificity (97–98%) a non-neoplastic lesion from a neoplasm and a benign neoplasm from a malignant neoplasm [1–3]. In general, FNA can diagnose the most common benign tumors of the salivary gland, PA, and WT with high specificity (>98%). However, FNA is generally less effective in providing a specific diagnosis for certain other epithelial neoplasms of the salivary gland. The main cited reason for this limitation is the morphologic overlap and diversity among the many different types of salivary gland tumors, sometimes even within the same tumor. Therefore, differentiation between a benign and low-grade malignant neoplasm based on purely cellular and cytoarchitectural features can be challenging in an FNA specimen, particularly when material for ancillary studies is not available. Consequently, such specimens are commonly designated as either a "salivary gland neoplasm" or "suspicious for a neoplasm" with a broad differential diagnosis including both a cellular benign neoplasm and a low-grade epithelial malignancy [1–8].

Based upon the cited literature and published meta-analyses, the FNA diagnosis of a salivary gland neoplasm that is not clearly malignant can be consolidated into the following two general diagnostic categories (Table 5.1) [5–25].

1. Neoplasm: Benign
2. Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)

General Definitions

1. *Neoplasm: Benign*: This diagnosis is made only when an FNA specimen shows characteristic cytomorphologic features of a specific benign epithelial or mesenchymal neoplasm of the salivary gland. The most common being PA and WT.

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Table 5.1 Definitions and entities included in the diagnostic category "Neoplasm" [5–25]

Neoplasm
Benign FNA specimens showing cytomorphologic features of a benign epithelial or mesenchymal neoplasm
1. Epithelial origin[a] a. Pleomorphic Adenoma b. Warthin Tumor c. Oncocytoma 2. Mesenchymal origin a. Lipoma b. Schwannoma c. Lymphangioma d. Hemangioma
Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP) FNA specimens showing cytomorphologic features diagnostic of a neoplastic process, but a malignant neoplasm cannot be excluded
3. Cellular basaloid neoplasm 4. Cellular oncocytic/oncocytoid neoplasm 5. Cellular neoplasm with clear cell features

[a]Due to overlapping cytomorphologic features with malignant tumors, most cases of benign neoplasm classified as basal cell adenoma, myoepithelioma, and cystadenoma on histopathologic examination will be diagnosed as SUMP on FNA (under the subheading of cellular basaloid neoplasm or cellular neoplasm with clear cell features) (see Tables 5.2 and 5.3)

Table 5.2 Morphologic scenarios and differential diagnosis of cases classified as "basaloid neoplasm" [1–3, 5–8]

Cytomorphologic features[a]	Differential diagnosis[b]
1. Cellular basaloid neoplasm *with* fibrillary stroma	• Cellular pleomorphic adenoma • Epithelial-myoepithelial carcinoma • Basal cell adenoma/adenocarcinoma
2. Cellular basaloid neoplasm *with* hyaline stroma	• Basal cell adenoma/adenocarcinoma • Adenoid cystic carcinoma • Epithelial-myoepithelial carcinoma • Polymorphous adenocarcinoma[c]
3. Cellular basaloid neoplasm *with* mixed/other stroma	• Adenoid cystic carcinoma • Polymorphous adenocarcinoma[c]
4. Cellular basaloid neoplasm *with* scant to no stroma	• Cellular pleomorphic adenoma • Canalicular adenoma • Myoepithelioma • Myoepithelial carcinoma • Adenoid cystic carcinoma

[a]Highly dependent on cytologic preparations
[b]Provided as a guide—may or may not be included in the diagnostic report
[c]Commonly encountered in minor salivary glands

Table 5.3 Morphologic scenarios and differential diagnosis of cases classified as "SUMP: cellular oncocytic/oncocytoid" [14–23]

Cytomorphologic features	Differential diagnosis
Cellular oncocytic/oncocytoid neoplasm *with*	
1. Cystic background (histiocytes, proteinaceous debris, ± inflammatory cells)	• Warthin tumor[a] • Cystadenoma, oncocytic
2. Mucinous background	• Mucoepidermoid carcinoma, oncocytic variant • Rare case of Warthin tumor with focal mucinous metaplastic change[b]
3. Blood or non-specific background	• Oncocytoma • Myepithelioma[c]
4. Granular (usually coarse)/vacuolated cytoplasm	• Acinic cell carcinoma • Secretory carcinoma / Mammary analogue secretory carcinoma (MASC) • Metastatic renal cell carcinoma
5. Appreciable focal nuclear atypia[d]	• Salivary duct carcinoma • High grade mucoepidermoid carcinoma • Metastatic carcinoma

[a]Tumor usually shows lymphocytes in the background and intimately associated with tumor cell groups
[b]Diagnosis requires exclusion of oncocytic mucoepidermoid carcinoma
[c]Rare cases may show prominent oncocytic change
[d]Cases with multifocal or diffuse presence of nuclear atypia should be classified as "suspicious for carcinoma" or "malignant"

2. *Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)*: This diagnosis is reserved for FNA specimens where the cytomorphologic features are diagnostic of a neoplastic process, but the cytologic findings cannot effectively distinguish between a benign and malignant neoplasm. Most malignant tumors included in this diagnostic category will be low-grade carcinomas.

Benign Neoplasms

The following benign neoplasms of epithelial and mesenchymal origin can be diagnosed by FNA based upon established cytomorphologic features.

Pleomorphic Adenoma [3]

Pleomorphic adenoma (PA), also known as benign mixed tumor, is a benign biphasic neoplasm characterized by a variable admixture of ductal epithelial cells, myoepithelial cells, and mesenchymal matrix (see Table 5.1). The designation "metastasizing pleomorphic adenoma," represents a rare salivary gland neoplasm that cytologically as well as histologically resembles a pleomorphic adenoma, but with the propensity for metastasis.

Cytologic Criteria

- Distinctive chondromyxoid matrix: Best appreciated using Romanowsky stains (Diff-Quik®, Giemsa), as a bright magenta matrix with a distinct fibrillary/feathery quality; grey to translucent green in Papanicolaou-stained preparations (Fig. 5.1)
- Myoepithelial cells: Variety of shapes (polygonal, plasmacytoid, round, spindle, and clear), bland nuclear features, often the predominant cell type (Fig. 5.2)
- Ductal epithelial cells: Bland nuclear features, small groups recapitulating ductal structures
- Iconic PA:Modestly cellular with readily identifiable, abundant fibrillar matrix, bland ductal epithelial and myoepithelial cells (Fig. 5.3)

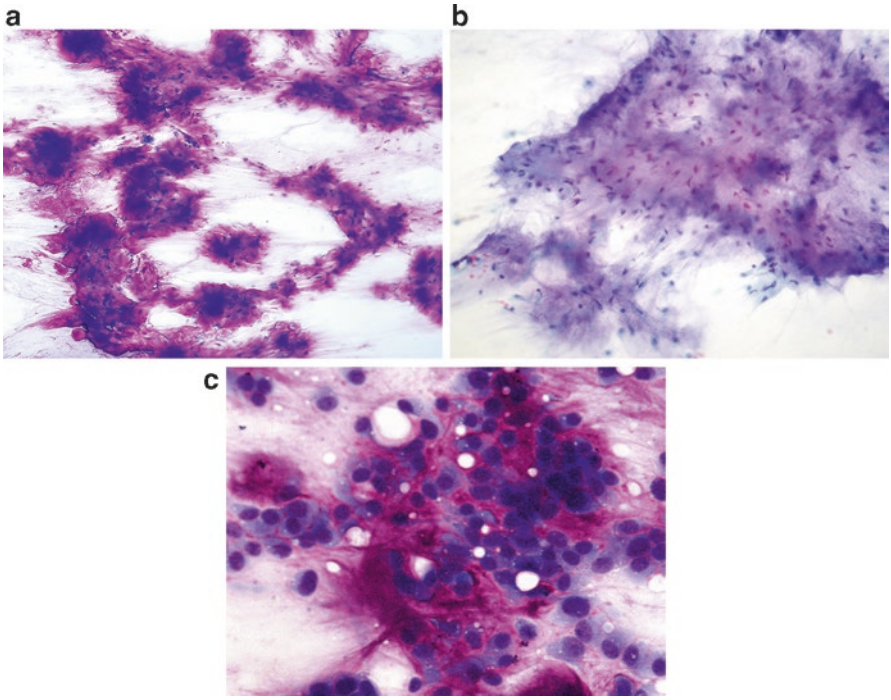

Fig. 5.1 Neoplasm: Benign. Pleomorphic adenoma. Intense metachromatic fibrillary matrix with myoepithelial cells embedded within—(**a**) (smear, Romanowsky stain), (**b**) (smear, Papanicolaou stain). FNA of pleomorphic adenoma showing metachromatic fibrillary matrix with embedded myoepithelial cells. Notice the stroma individually surrounds each cell and the so called "troll hair" appearance of the stroma—(**c**) (smear, Romanowsky stain)

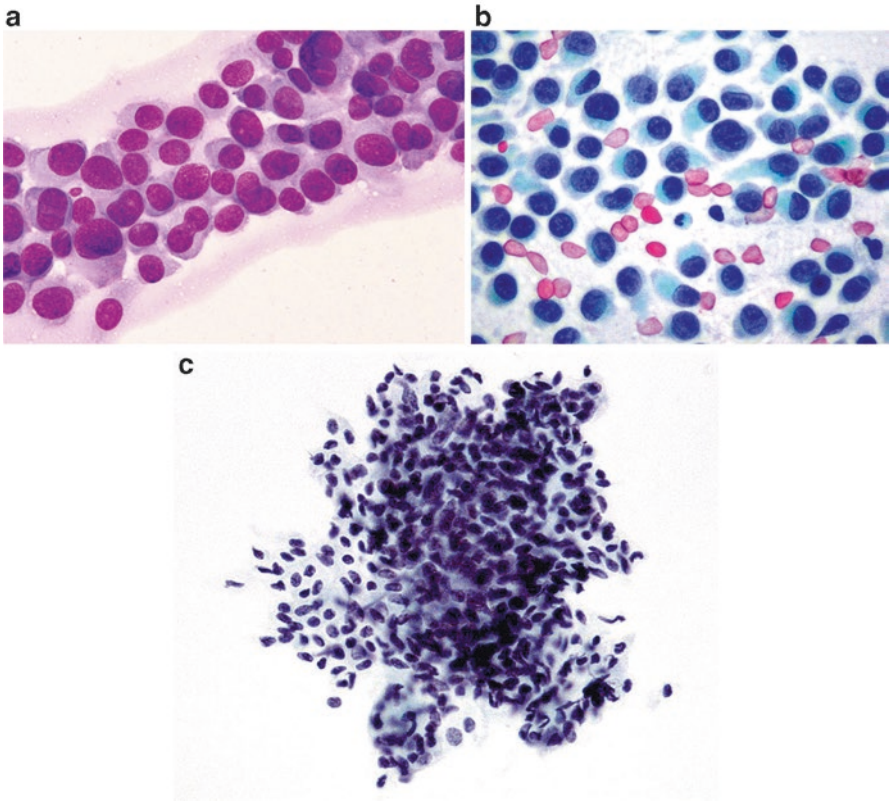

Fig. 5.2 Neoplasm: SUMP. FNA of pleomorphic adenoma showing a highly cellular, matrix-poor tumor with a predominance of plasmacytoid myoepithelial cells—(**a**) (smear, Romanowsky stain), (**b**) (high power, smear, Papanicolaou stain). This pleomorphic adenoma is a cellular, matrix poor specimen with spindled and epithelioid myoepithelial cells—(**c**) (liquid-based preparation, Papanicolaou stain)

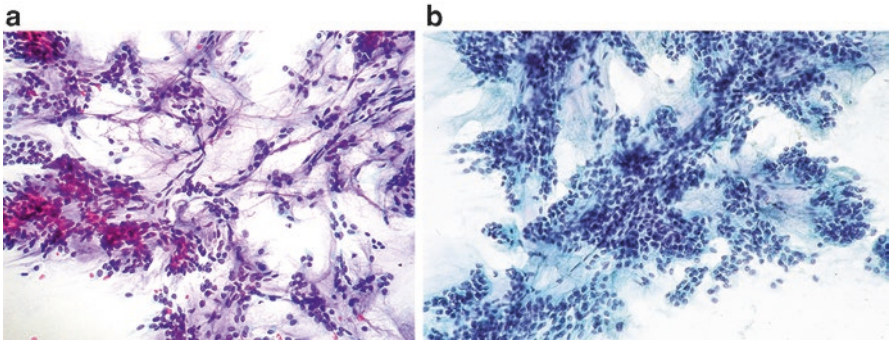

Fig. 5.3 Neoplasm: Benign. (**a**, **b**) Pleomorphic adenoma showing myoepithelial cells and very delicate, pale-staining matrix (smear a, Romanowsky stain, smear b, Papanicolaou stain)

Explanatory Notes

PA is one of several "matrix-producing tumors" that also includes adenoid cystic carcinoma (AdCC), basal cell adenoma/adenocarcinoma, and epithelial-myoepithelial carcinoma. The hallmark and most distinguishing feature of PA is the presence of chondromyxoid matrix, best appreciated using Romanowsky stains (Diff Quik®, Giemsa). The advantage of using both Romanowsky and Papanicolaou stains is that the matrix is easily identified on Romanowsky stains; while Papanicolaou stains highlight the bland nuclear features of the ductal and myoepithelial cells, the latter are usually embedded within the matrix. FNA specimens with classic features of PA can be readily diagnosed as "Neoplasm: Benign." The biphasic nature of PA, combined with the variable ratio of epithelial and myoepithelial cells with mesenchymal matrix, yields a spectrum of cytomorphologic patterns on aspiration, and as a result, there can be overlap with other salivary gland neoplasms. When classic features of PA are not present or when additional "atypical" features are identified, then the FNA should be diagnosed as "Neoplasm: SUMP."

It is most important to exclude AdCC from the differential diagnosis when faced with a matrix-producing tumor since management and prognosis will differ substantially from PA. This becomes challenging when highly cellular aspirates are obtained that have scant to absent matrix (see Fig. 5.2). The differential diagnosis may include the solid variant of AdCC, myoepithelioma, or other basal cell neoplasms. Such cases would be classified as "Neoplasm: SUMP." Occasionally adenoid cystic-like areas within the PA can reveal matrix in tubules or globules mimicking the hyaline globules of AdCC (Fig. 5.4).

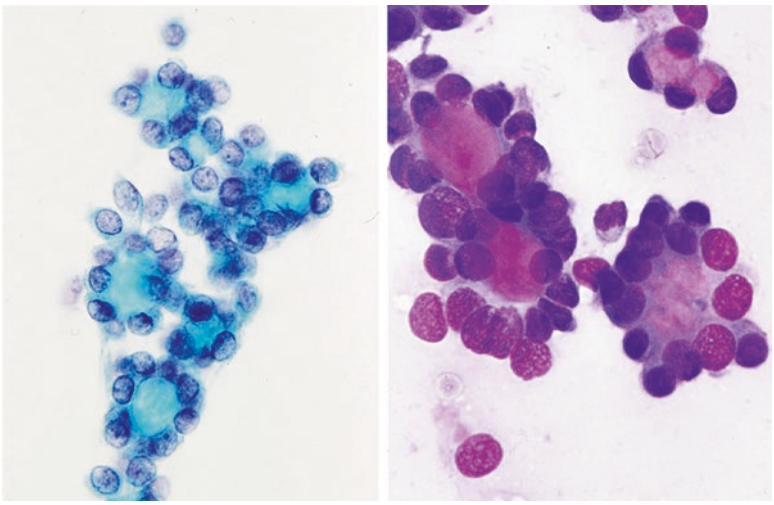

Fig. 5.4 Neoplasm: SUMP. FNA of pleomorphic adenoma having adenoid cystic carcinoma-like areas (smear, Papanicolaou and Romanowsky stains)

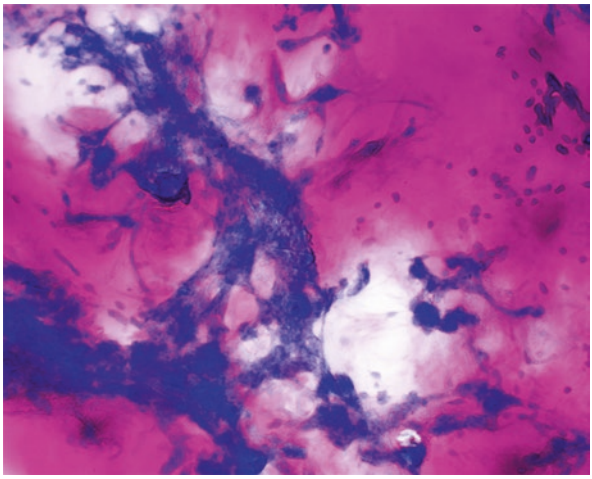

Fig. 5.5 Pleomorphic adenoma. The stroma lacks the usual fibrillary character and mimics thick mucin (smear, Romanowsky stain)

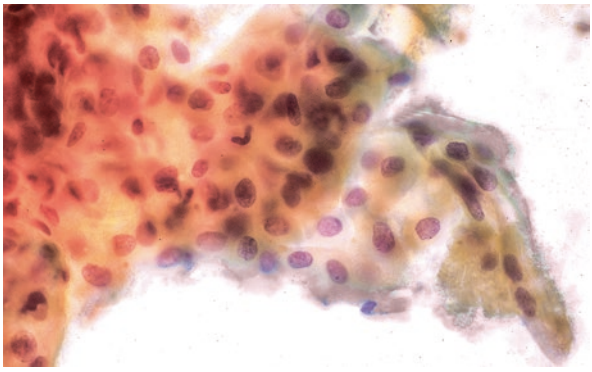

Fig. 5.6 Neoplasm: SUMP. FNA of pleomorphic adenoma showing squamous metaplasia (smear, Papanicolaou stain)

Another potential pitfall occurs when the matrix of PA is thin and mucoid in character (Fig. 5.5). Bland epithelial cells with abundant matrix that have a mucoid rather than fibrillar quality can mimic low-grade mucoepidermoid carcinoma. This is particularly challenging in the presence of squamous or mucinous metaplastic changes; the former is more common and is seen as foci of metaplastic squamous cells (Fig. 5.6) or even clusters of anucleate squames; the latter as goblet cells, often with variable amounts of delicate mucoid matrix in the background. Ancillary studies using IC to demonstrate myoepithelial differentiation combined with PLAG1 or HMGA2 positivity can be helpful (see Chap. 8).

When myoepithelial cells predominate, their morphology and cellularity will dictate the differential diagnosis. While myoepithelioma and cellular PA are consistently in the differential diagnosis, when myoepithelial cells have clear cytoplasm, the diagnostic considerations can include epithelial-myoepithelial carcinoma, sebaceous adenoma/carcinoma, myoepithelial carcinoma, and even metastases such as

renal cell carcinoma. Myoepithelial cells with spindled morphology (Fig. 5.7) and even palisading will have bland nuclear features with a differential diagnosis that includes schwannoma, possibly hemangioma, or even nodular fasciitis, but typically sarcomas and spindle cell carcinomas are excluded based on absence of significant nuclear pleomorphism and mitoses. While atypical myoepithelial cells can occasionally be encountered in PA (Fig. 5.8), the presence of numerous atypical cells (nuclear pleomorphism, distinct nucleoli, mitoses) and/or necrosis is a worrisome sign for malignancy. If the clinical history includes rapid enlargement of a previously stable PA or development of a tumor in a patient with a history of PA, then carcinoma ex PA enters the differential diagnosis.

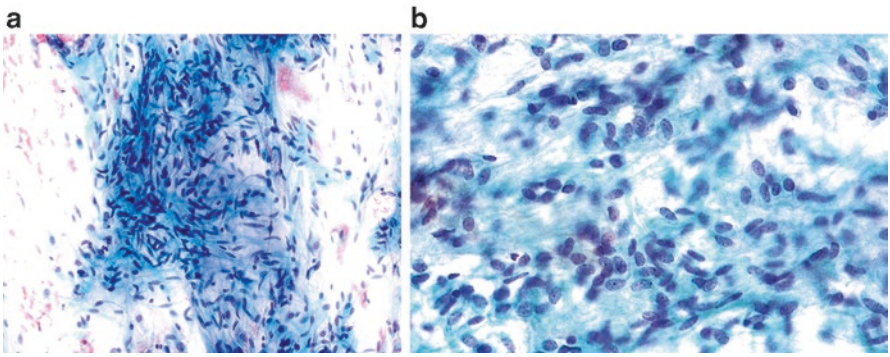

Fig. 5.7 Neoplasm: SUMP. (**a**, **b**) This aspirate of a pleomorphic adenoma has a predominance of spindled myoepithelial cells mimicking a salivary gland neoplasm of mesenchymal origin (smear, Papanicolaou stain)

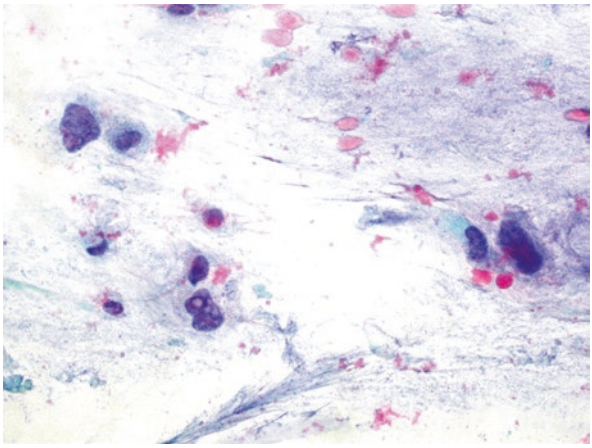

Fig. 5.8 Neoplasm: SUMP. This case of pleomorphic adenoma shows marked nuclear atypia of the myoepithelial cells; in such cases, malignant transformation needs to be excluded (smear, Papanicolaou stain)

Warthin Tumor [1, 3]

Warthin tumor (WT) is the second most common neoplasm of the parotid gland. The majority occur in the 7th to 9th decades of life and patients usually have a significant history of smoking. Patients present with a doughy painless mass that may fluctuate in size.

Cytologic Criteria

- Tripartite appearance with dirty proteinaceous background, lymphocytes, and sheets of oncocytes (Fig. 5.9)
- Oncocytes: Abundant homogeneous granular cytoplasm (orange on Papanicolaou stain) with well-defined borders (Fig. 5.10)

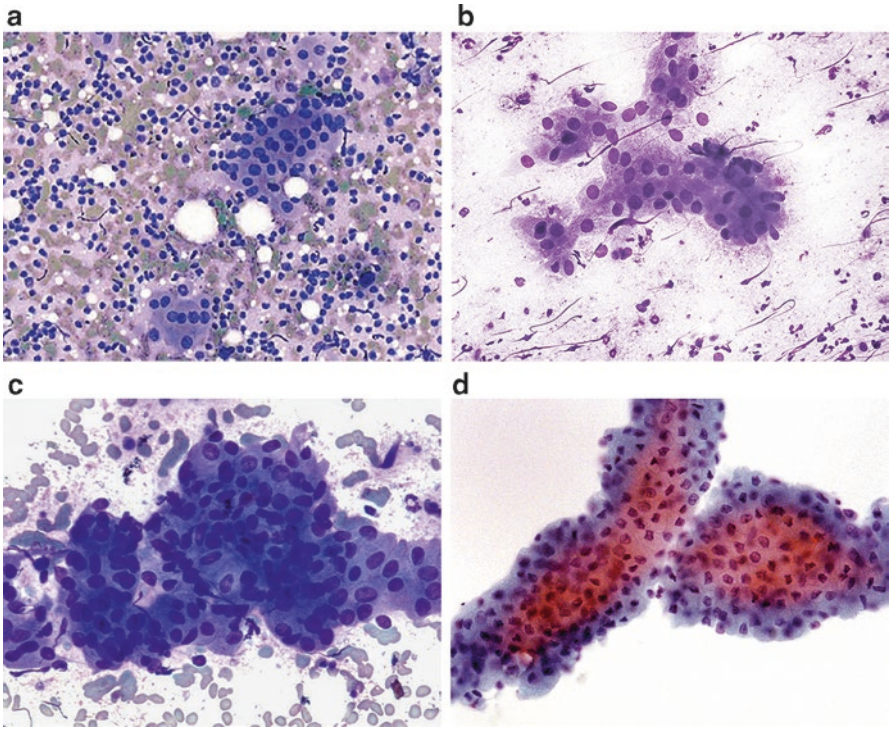

Fig. 5.9 Neoplasm: Benign. (**a**–**c**) FNA of Warthin tumor (WT) showing classic cytomorphologic features consisting of background lymphocytes and groups of oncocytic epithelial cells (smear, Romanowsky stain); (**d**) This case of WT only shows oncocytic cells arranged in papillary groups. Notice the lack of lymphocytes; such cases may be classified as "oncocytic/oncocytoid neoplasm" (liquid-based preparation, Papanicolaou stain)

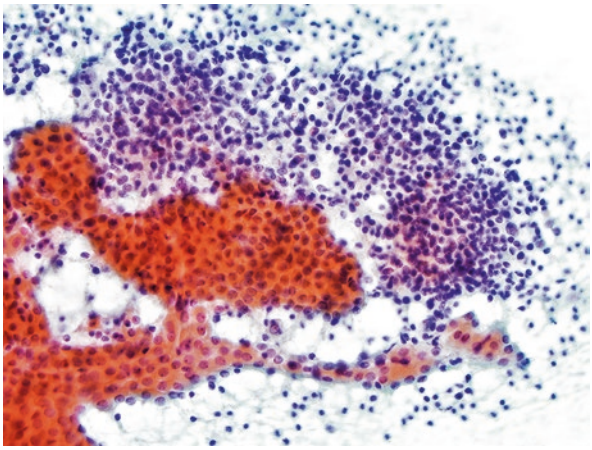

Fig. 5.10 Neoplasm: Benign. This classic aspirate of Warthin tumor consists of oncocytic cells with abundant granular cytoplasm and well-defined borders in a background of lymphocytes (smear, Papanicolaou stain)

- Epithelial cell nuclei: Centrally placed and round, with prominent nucleolus
- Lymphocytes: Mixed population dominated by small mature-appearing cells

Explanatory Notes

WT occurs almost exclusively in the parotid gland and the tripartite appearance is essentially diagnostic. Aspirates with classic features should be diagnosed as "Neoplasm: Benign." Distinction should be made from intraparotid lymph nodes, lymphoepithelial sialadenitis (LESA), oncocytoma, and lymphoepithelial cyst. Intraparotid lymph nodes and LESA lack the oncocytic epithelium and dirty cyst debris characteristic of a WT. Oncocytomas consist of epithelial cells only and lack the dirty cystic background and lymphocytes of WT.

Rarely, WTs undergo spontaneous infarction, and the nodule will often rapidly increase in size after infarction, raising the possibility of a salivary gland malignancy. Material aspirated from an infarcted WT may contain necrotic debris and atypical squamous cells. These cells may raise the differential diagnosis of squamous cell carcinoma (SCC). Distinction is aided by recognition of scattered necrotic columnar cell ghosts and the small number of atypical squamous elements characteristic of infarcted WT. SCC will have larger numbers of atypical squamous cells than are characteristically seen in WT as well as more severe atypia and scattered mitoses. The very rapid enlargement of a preexistent nodule also favors infarction of a WT. Lymphoepithelial cysts or HIV-associated benign lymphoepithelial lesions are characterized by unilocular or multilocular cysts with glandular or squamous lining and hyperplastic lymphoid tissue. Aspirates contain a mixed lymphoid population among which are distributed rare glandular or more commonly squamous cells. The background may be proteinaceous, but the sheets of oncocytic epithelium

characteristic of WT are not seen in aspirates of lymphoepithelial cysts or HIV-associated lymphoepithelial lesions.

Oncocytoma [1, 9, 14, 15]

Nearly 90% of oncocytomas occur within the major salivary glands, but they comprise only 1% of parotid gland neoplasms. Most cases occur in the 6th to 8th decades of life.

Cytologic Criteria

- Irregular sheets and clusters of large polygonal cells with abundant homogeneous granular cytoplasm (Fig. 5.11)

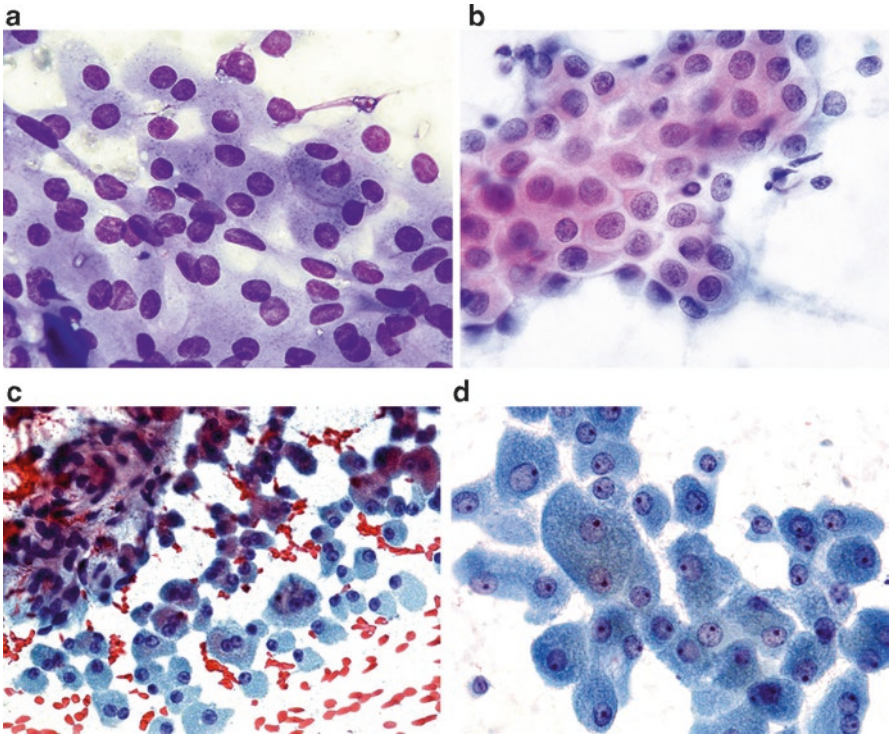

Fig. 5.11 Neoplasm: Benign. FNAs of oncocytoma showing various patterns of a monotonous population of oncocytic cells with abundant granular cytoplasm and well-defined borders arranged in cohesive groups—(**a**) (smear, Romanowsky stain), (**b**–**d**) smear, Papanicolaou stain

- Oncocytes: Well-defined cytoplasmic borders
- Nuclei: Enlarged, round, and distinct nucleolus
- Background: Clean or contains red blood cells
- Nuclear pleomorphism and mitotic figures absent

Explanatory Notes

The differential diagnosis of oncocytoma includes WT, diffuse oncocytosis, and acinic cell carcinoma (ACC). Aspirates of oncocytoma and oncocytosis are virtually identical; however, oncocytoma presents clinically as a distinct circumscribed mass, while oncocytosis is a more multifocal and/or less well-defined lesion. WT contains occasional groups of oncocytes, but differs from oncocytoma by also having a dirty proteinaceous background and a mixed lymphoid population. ACC contains a mixture of polygonal cells with delicate vacuolated cytoplasm. In contrast, oncocytomas lack the cytoplasmic vacuoles of ACC; a Romanowsky stain can be used to highlight this subtle distinction. IC for DOG-1 and SOX10 is positive in ACC, but negative in oncocytomas (see Chap. 8). Oncocytic carcinoma is very rare and is clinically invasive; clinical correlation is needed.

Lipoma [10]

Lipomas are uncommon neoplasms of the salivary glands. They represent 0.5% of salivary gland tumors, and three-quarters of these occur in the parotid gland. They often present as a palpable soft nodule.

Cytologic Criteria

- Lacelike sheets and clusters of very low nuclear-cytoplasmic (N:C) ratio cells with optically clear cytoplasm (Fig. 5.12)
- Individual cells: Single large clear vacuole occupying the entire cytoplasmic volume (Fig. 5.13)
- Nuclei: Small, hyperchromatic, and displaced to the margin of the cell
- Background: May contain droplets of lipid (best seen on Romanowsky stains)

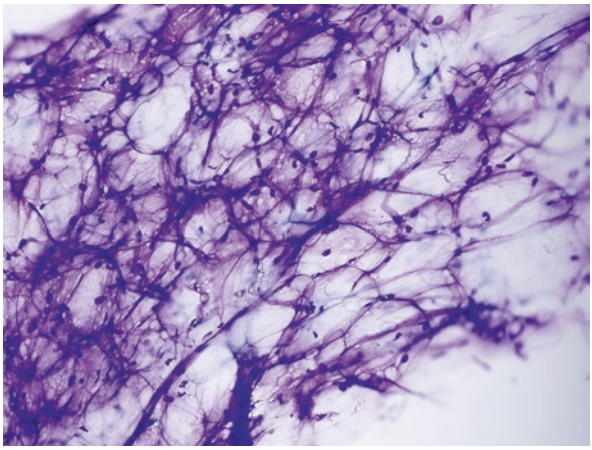

Fig. 5.12 Neoplasm: Benign. FNA of lipoma showing lacelike group of mature adipocytes with abundant clear cytoplasm and small dark nuclei (smear, Romanowsky stain)

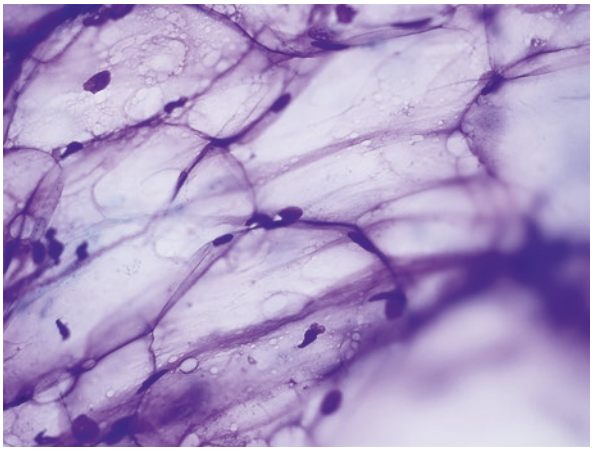

Fig. 5.13 Neoplasm: Benign. This FNA contains a group of adipocytes from a lipoma characterized by large cells with abundant clear cytoplasm. The small dark nuclei are often displaced to the edge of the cell (smear, Romanowsky stain)

Explanatory Note

It may be difficult to distinguish fatty change of the salivary gland from lipoma by FNA. While fatty change will contain normal acinar and ductal elements, lipomas are composed purely of adipose tissue, although rare examples of lipomas with entrapped normal serous acini and ducts have been reported. Clinical correlation is useful.

Schwannoma [12]

Schwannoma represents the most common benign neural tumor of the salivary glands. FNA of a schwannoma is often associated with pain of a radicular type.

Cytologic Criteria

- Scant to moderately cellular aspirate
- Spindle-shaped cells with wispy bipolar cytoplasmic processes (Fig. 5.14)
- Cells: Form cohesive groups and clusters, sometimes with palisading
- Cytoplasm: Pale and ill-defined
- Nuclei: Small, dark, bland, and elongate/spindled; may be bent, curved, or even S-shaped
- Occasional large but bland nuclei can be seen ("ancient change").
- Nucleoli: Small or absent
- Background: Myxoid material

Explanatory Notes

The most common differential diagnosis is with PA and myoepithelioma. Some cases of PA and schwannoma can be very difficult to distinguish. Ancillary studies are helpful in establishing a definitive diagnosis of either PA or schwannoma. Schwannomas are strongly and diffusely positive for S100 and SOX10, and negative for keratin and myoepithelial markers. Other differential diagnostic considerations include sarcomas, which are very rare in the salivary gland. The possibility of a sarcoma should be considered when aspirates are cellular and display significant nuclear atypia often with scattered mitoses and apoptosis.

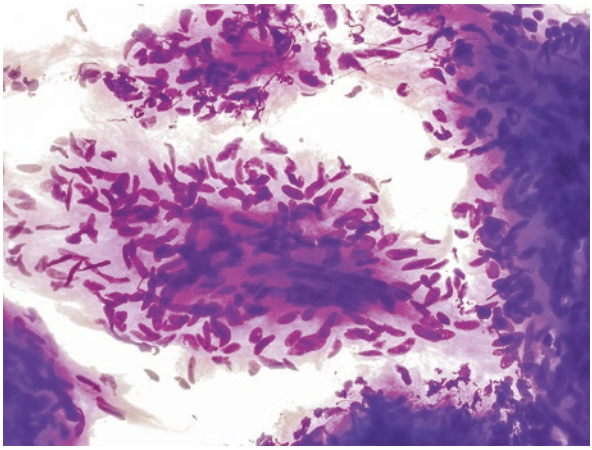

Fig. 5.14 Neoplasm: Benign. This aspirate of schwannoma shows a group of bland spindle cells with wispy cytoplasm. The cytoplasmic borders are indistinct. Nuclei are spindle-shaped and display bends or curves (smear, Romanowsky stain)

Lymphangioma [10, 11]

Lymphangiomas arising within the salivary glands are rare with most occurring in children. They present as slowly growing fluctuant masses. Most arise in the parotid gland.

Cytologic Criteria

- Hypocellular smears with watery background
- Occasional red blood cells
- Scattered mature-appearing lymphocytes
- Rare background clusters of nonneoplastic salivary gland acinar tissue may be present

Explanatory Notes

Aspirates obtained from salivary gland lymphangiomas are frequently Non-Diagnostic, and are composed of watery fluid containing scattered mature-appearing lymphocytes and occasional groups of non-neoplastic acinar cells. Endothelial cells are generally absent from aspirated material. The diagnosis usually requires careful clinical and radiologic correlation.

Hemangioma [13]

Hemangiomas are the most common benign mesenchymal tumor of the salivary gland with most examples arising in the parotid gland [13]. In addition, the majority of hemangiomas occurs in the first decade of life, especially the first year of life. The latter are of the so-called juvenile type and may regress spontaneously. Juvenile hemangiomas may be highly cellular.

Cytologic Criteria

- Aspirate dominated by red blood cells
- Few groups of bland spindle-shaped to polygonal endothelial cells, which may form elongated cord-like structures (Fig. 5.15)
- Individual cells: Oval to spindle-shaped nuclei
- Nuclei: Small, bland, and lack nucleoli
- Scattered histiocytes may be present.

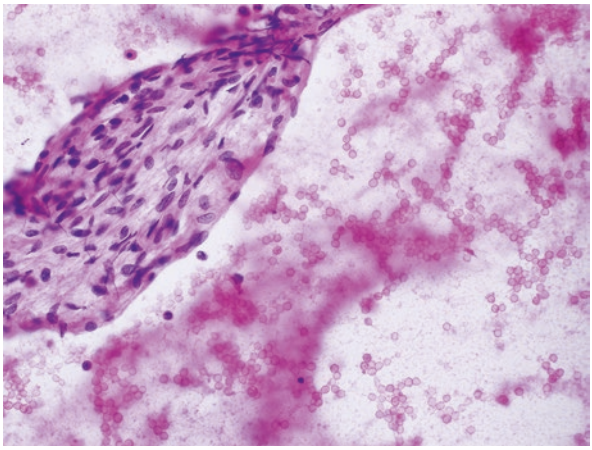

Fig. 5.15 Neoplasm: Benign. Smears obtained from hemangiomas are characteristically bloody, but may contain small aggregates of bland spindle-shaped endothelial cells. Rarely, sheet-like structures composed of oval or spindle-shaped endothelial cells will be present. Clinical and radiologic correlation is needed in the evaluation. (smear, Romanowsky stain)

Explanatory Notes

Aspirates of hemangiomas may be so dominated by blood that the endothelial cell component may be overlooked. When hemangioma is in the clinical or radiologic differential diagnosis, a careful search for groups of bland spindle to oval-shaped endothelial cells should be made.

Salivary Gland Neoplasm of Uncertain Malignant Potential

"Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)" is a diagnostic category reserved for FNA specimens that are diagnostic of a neoplasm; however, a definitive diagnosis of a specific entity cannot be made. This diagnosis should be used for cases in which a malignant neoplasm cannot be excluded. A majority of these cases will include cellular benign neoplasms, neoplasms with monomorphic lesional cells, basaloid neoplasms, oncocytic/oncocytoid neoplasms, neoplasms with clear cell features, neoplasms with atypical features, and low-grade carcinomas.

Cellular Basaloid Neoplasm [3–5, 8, 24]

The SUMP category with a subcategorization of "cellular basaloid neoplasm" should be reserved only for tumors in which a specific diagnosis is not possible, and the differential diagnosis includes both benign and malignant tumors. Cellular basaloid neoplasms are characterized by a predominant population of cells with scant

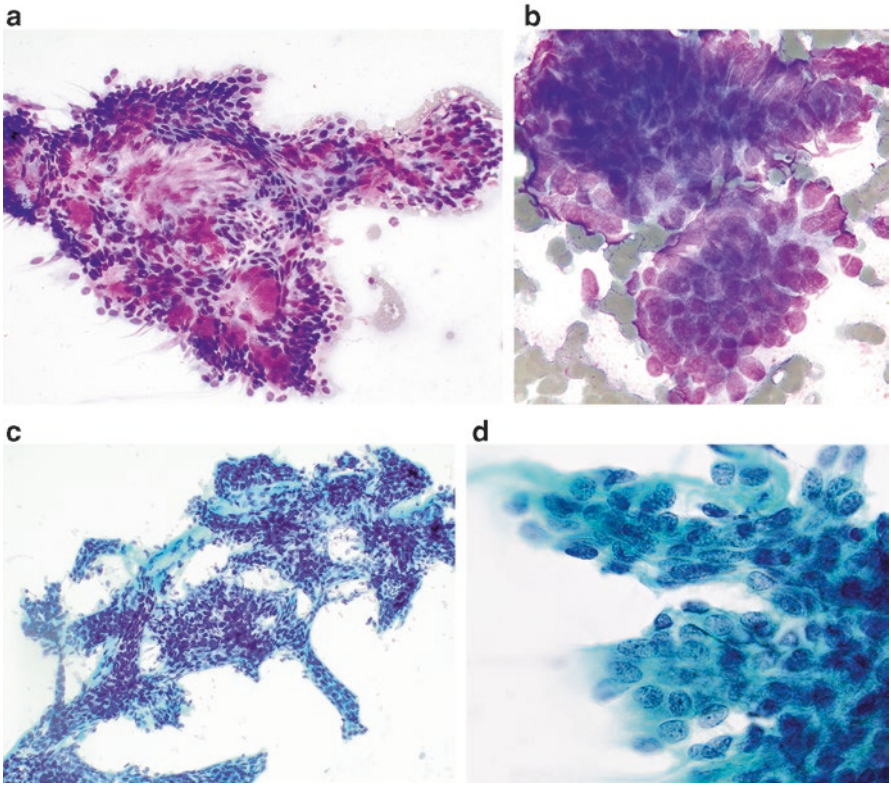

Fig. 5.16 Neoplasm: SUMP. (**a**, **b**) FNA of cellular basaloid neoplasms showing a predominant population of cells with scanty cytoplasm with hyaline stroma (smear, Romanowsky stain); (**c**, **d**) This FNA contains a monotonous population of basaloid cells arranged in cohesive groups with scanty hyaline stroma (smear, Papanicolaou stain)

cytoplasm that confers an immature ("basaloid") cytomorphology. Such tumors can be associated with variable stromal elements that alter the differential diagnosis (Fig. 5.16).

Cytologic Criteria

- The differential diagnosis of basaloid salivary gland neoplasms is among the most challenging areas in salivary gland FNA. There is significant morphologic overlap among cellular basaloid neoplasms that makes rendering a specific diagnosis challenging.

- The cytologic criteria for diagnosing these entities is discussed in detail in those sections devoted to the specific tumor types. Table 5.2 [1–3, 5–8] shows the possible cytomorphologic features and their key associated differential diagnosis for specimens diagnosed as "basaloid neoplasms"; however, all subgroups of basaloid neoplasms have an overlapping differential diagnosis.

Explanatory Notes

It is essential that a specific diagnosis only be rendered in conjunction with consideration of clinical and radiological findings. When uncertainty remains based on the cytomorphologic findings, or when the clinical and radiological findings conflict with the pathologic impression, a diagnosis of "Neoplasm: SUMP" is appropriate. For the purposes of discussing tumors appropriate for classification within the SUMP diagnostic category, it should be noted that a specific diagnosis of a cellular basaloid neoplasm can sometimes be refined using ancillary studies, such as IC and/or molecular testing. Some tumors in the differential diagnosis of cellular basaloid neoplasms are benign neoplasms with a malignant counterpart that cannot be excluded definitively on FNA since the cytomorphologic features are nearly identical. These include basal cell adenoma and basal cell adenocarcinoma as well as some cases of myoepithelioma and myoepithelial carcinoma. Histologic evaluation to exclude invasive growth and lymphovascular or perineural invasion is needed to definitively distinguish between these benign and malignant tumors. In the absence of concerning cytologic findings such as nuclear atypia or background necrosis, or suspicious imaging and clinical findings, the risk of malignancy is considered to be low. In such instances, a diagnosis of SUMP is made at the discretion of the cytologist, but a benign tumor can be favored using a comment on the case (e.g., "favor basal cell adenoma or myoepithelioma") (see Fig. 5.16).

Distinction between various tumors with "basaloid" morphology is more challenging when there is an absence or paucity of characteristic matrix material such as the fibrillar chondromyxoid matrix material of PA or the acellular hyaline spheres of AdCC. When a specific diagnosis cannot be made, the differential diagnosis typically includes benign tumors (cellular PA, basal cell adenoma, canalicular adenoma, myoepithelioma); low-grade malignancies (basal cell adenocarcinoma, epithelial-myoepithelial carcinoma, polymorphous (low-grade) adenocarcinoma); and intermediate to high-grade malignancies (AdCC, particularly the solid variant) (Figs. 5.17 and 5.18). Surgical excision is generally indicated for a SUMP diagnosis. If the extent of surgery depends on a more definitive evaluation, a repeat aspiration may be helpful, particularly if additional ancillary studies such as IC or molecular studies would potentially yield a more specific diagnosis. Alternatively, frozen section examination at the time of surgery may also provide further useful information. A deep cutaneous neoplasm can occasionally mimic a superficial

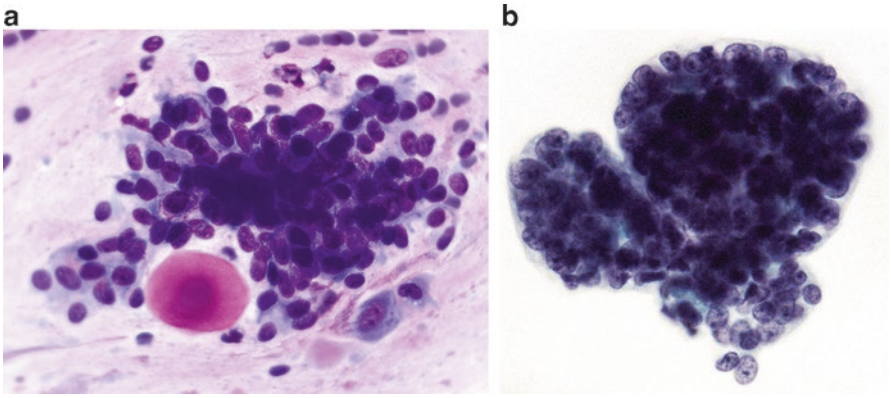

Fig. 5.17 Neoplasm: SUMP. (**a**) This FNA shows basaloid tumor cells associated with well-demarcated hyaline stroma. Depending upon the cellularity and cytomorphologic features combined with clinical findings, the diagnosis of cases such as this can range from "SUMP-basaloid neoplasm" to "suspicious for adenoid cystic carcinoma" (smear, Romanowsky stain). (**b**) This aspirate shows basaloid tumor cells arranged in a 3-dimensional cohesive group with nuclear crowding and minimal to no stroma (liquid-based preparation, Papanicolaou stain)

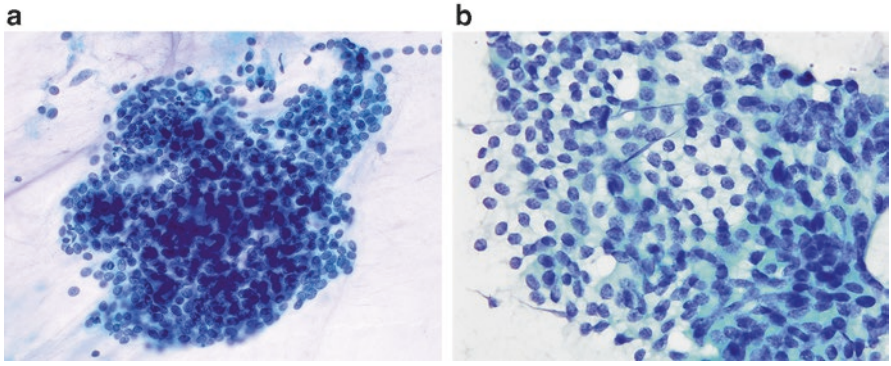

Fig. 5.18 Neoplasm: SUMP. (**a**, **b**) In this FNA of a cellular basaloid neoplasm there is a cohesive group of basaloid cells with no stroma. On histologic follow-up this case was diagnosed as solid variant of adenoid cystic carcinoma (smear, Papanicolaou stain)

parotid gland tumor or even mimic a periparotid or intraparotid lymph node metastasis. Accordingly, cutaneous basal cell carcinoma, pilomatrixoma, basaloid SCC, and high-grade neuroendocrine carcinoma (either primary in the salivary gland, cutaneous Merkel cell carcinoma, or as a distant metastasis) should be considered in the differential diagnosis.

Cellular Oncocytic/Oncocytoid Neoplasm [9, 14–22]

Neoplasms showing oncocytic or oncocytic-like (i.e., oncocytoid) features are common in the salivary glands. While oncocytic features are the main characteristic of some salivary gland tumors (SGT) such as WT and oncocytoma, they can also be an accompanying finding in several other salivary gland neoplasms, including PA, myoepithelioma, and MEC. In addition, some non-oncocytic neoplasms like ACC and metastatic renal cell carcinoma can cytomorphologically mimic true oncocytic tumors. In most of these cases, it is possible to give an accurate diagnosis if characteristic cytomorphologic features are present (see separate related chapters), and/or if the diagnostic pitfalls are carefully assessed, and if ancillary tests are performed. However, there remains a subset of SGT with oncocytic features that cannot be confidently subtyped, and it is appropriate to classify these as "SUMP-oncocytic/oncocytoid neoplasms."

Cytologic Criteria

Aspirates of SGT classified as "Neoplasm: SUMP" with oncocytic/oncocytoid features have the following characteristics (Table 5.3) [14–23]:

- Cellular aspirate
- Neoplastic cells: Oncocytic or oncocytoid features that cannot be classified further (Figs. 5.19 and 5.20)
- Moderate amounts of oncocytic granular cytoplasm
- Round to oval nucleus ± distinct nucleolus
- Oncocytic/oncocytoid neoplastic cells lack high-grade cellular features such as marked nuclear atypia, high mitotic activity, and necrosis.

Explanatory Notes

The SUMP-oncocytic/oncocytoid subcategory should be reserved for cases that include both primary salivary gland oncocytic neoplasms and their mimics, mainly low-grade carcinomas, in the differential diagnosis. In addition, these are tumors where a definitive interpretation such as oncocytoma is not possible. The most common oncocytic neoplasm of salivary glands is WT, which is accurately diagnosed by cytology in most cases. However, in a small subset of cases, all of the usual diagnostic features of WT may not be readily discernible or the tumor may show focal mucinous or squamous metaplasia leading to diagnostic difficulty. In some cases, oncocytes are present in a cystic background without accompanying lymphocytes, even though a lymphocyte-poor WT is favored.

An aspirate of a SGT showing oncocytic neoplastic cells with focal intra-cytoplasmic mucin in a mucinous background should raise concern for an oncocytic mucoepidermoid carcinoma (MEC), and, depending upon the overall cytomorphologic features

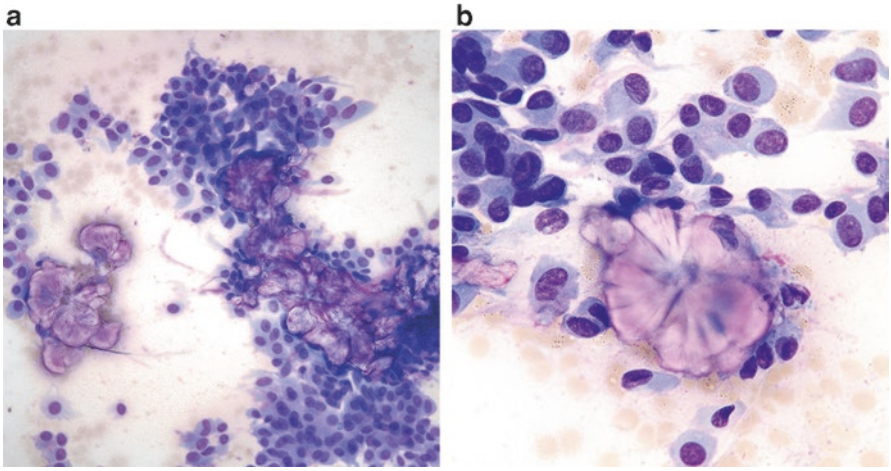

Fig. 5.19 Neoplasm: SUMP. (**a**, **b**) This aspirate shows neoplastic cells with oncocytic/oncocytoid cytoplasm arranged in a cohesive clusters with associated crystalline material. The cells demonstrate eccentrically placed nuclei (plasmacytoid appearance). On histologic follow-up this case was diagnosed as myoepithelioma (smear, Romanowsky stain)

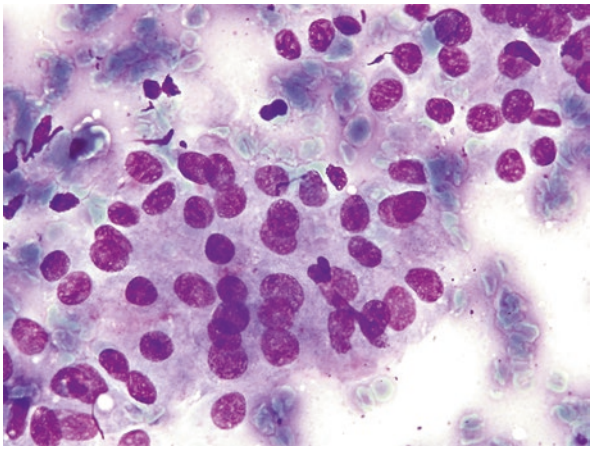

Fig. 5.20 Neoplasm: SUMP. FNA of cellular oncocytic/oncocytoid neoplasm showing loose groups and dispersed neoplastic cells with bland oncocytic features. On histologic follow-up this case was diagnosed as acinic cell carcinoma (smear, Romanowsky stain)

present, a diagnosis of "SUMP-oncocytic/oncocytoid neoplasm" may be warranted. Oncocytic carcinoma of the salivary gland displays a spectrum of cytologic features ranging from bland to overtly malignant. Rarely, cases of oncocytic carcinoma are cytomorphologically identical to oncocytoma and cannot be distinguished without histopathologic examination demonstrating invasion or evidence of metastasis. Clinical findings may be useful to provide evidence of an infiltrative cancer. Nuclear atypia, mitotic activity, or necrosis are not features of oncocytoma, and when present are suggestive of malignancy. However, if features indicative of malignancy are not encountered, the "SUMP-oncocytic/oncocytoid" designation can be used to classify such cases with a recommendation to correlate with clinical and radiologic features.

A SGT with oncocytic features can be difficult to differentiate from ACC due to the shared features of low nuclear grade and abundant oncocytic cytoplasm (see Fig. 5.20). Aspirates of ACC usually display cells with delicate vacuolated or pale cytoplasm, indistinct cytoplasmic borders, and nuclei that are sometimes larger than those in oncocytes. The background usually contains many stripped nuclei (especially in smear preparations), and some cases may contain background lymphocytes. Ancillary studies can be very helpful to arrive at a definitive diagnosis of ACC (i.e., "Malignant"); however, in cases with limited cellularity and/or lacking material for ancillary studies, a diagnosis of "SUMP-oncocytic/oncocytoid neoplasm" can be made with a comment that ACC is in the differential diagnosis.

Secretory carcinoma (SC) (aka mammary analogue secretory carcinoma, or MASC) can exhibit oncocytic features in FNA specimens. SC usually consists of an admixture of cells with granular and eosinophilic cytoplasm, cells with multivacuolated cytoplasm, and some cells with intracellular mucin. These features are frequently misinterpreted as ACC or oncocytic MEC. PAs and myoepitheliomas may show oncocytic metaplasia, but generally other specific characteristic features of these neoplasms such as the presence of fibrillary metachromatic matrix lead to the correct interpretation. Rarely, metastatic carcinomas with eosinophilic cytoplasm can also mimic primary oncocytic neoplasms. These can be readily distinguished using ancillary studies, especially IC), correlated with clinical findings.

Cellular Neoplasm with Clear Cell Features [23–25]

SGT with clear cell features are uncommon. These tumors include a broad range of benign and malignant neoplasms with overlapping cytomorphologic features. Lesional cells with clear or vacuolated cytoplasm are the key diagnostic feature (Figs. 5.21, 5.22, 5.23, 5.24, and 5.25). Because they are uncommon, clear cell neoplasms should represent a minor subgroup of the SUMP category. Since most of the neoplasms predicted to be placed into this subcategory are low-grade malignancies, the risk of malignancy (ROM) may be at the higher end of the ROM for SUMP (20–40%); however, the risk of high-grade malignancy for this diagnostic subcategory is expected to be low.

Cytologic Criteria

- Cellular aspirate diagnostic of a neoplasm but characteristic cytomorphologic features of a specific tumor entity (see specific tumors in other chapters of this book) are absent.
- Neoplastic cells with clear cell features: Clear, foamy, granular, or vacuolated cytoplasm, or any combination thereof; features are not characteristic of true oncocytes (see Figs. 5.21, 5.22, 5.23, 5.24, and 5.25)

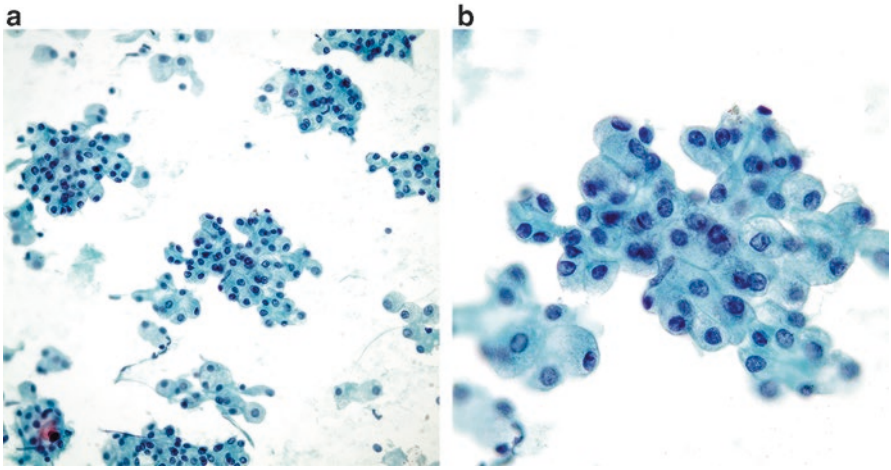

Fig. 5.21 Neoplasm: SUMP. (**a**, **b**) FNA of a cellular neoplasm with clear cell to oncocytoid features showing sheets of epithelial cells with finely vacuolated cytoplasm. Nuclei are enlarged, but retain smooth nuclear membranes. The histologic follow-up of this case was acinic cell carcinoma (smear, Papanicolaou stain)

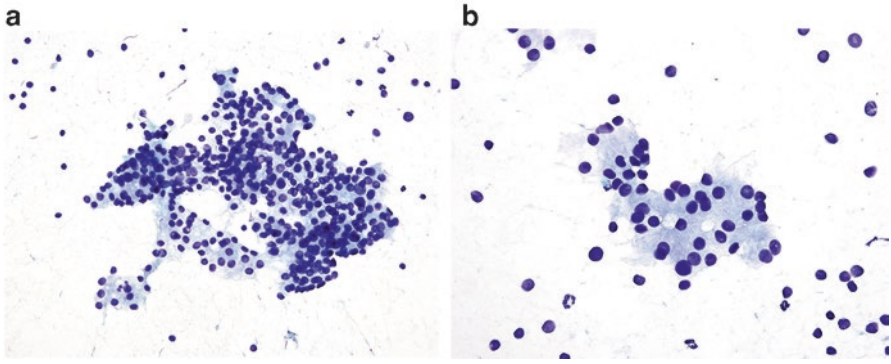

Fig. 5.22 Neoplasm: SUMP. This aspirate contains loosely cohesive groups of cells with indistinct finely vacuolated, pale-staining cytoplasm imparting a clear quality. Nuclei are small to medium-sized with even chromatin. No nuclear pleomorphism is seen. The histologic follow-up was acinic cell carcinoma. (**a**) (smear, Papanicolaou stain) and (**b**) (smear, Papanicolaou stain). This case could also be classified as "Suspicious for Malignancy" based on one's level of suspicion of acinic cell carcinoma

- Nuclear cytologic grade: Low to moderate.
- Absence of high-grade features (e.g., necrosis, marked nuclear atypia, mitotic activity)
- Ancillary studies, if performed, do not allow classification into another diagnostic category (e.g., "benign" or "malignant").

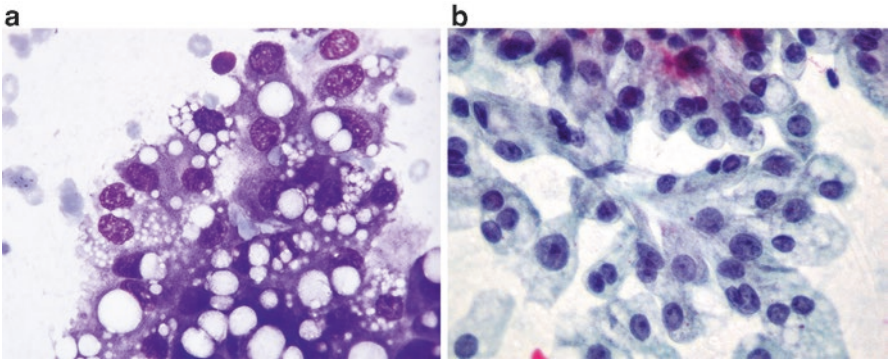

Fig. 5.23 Neoplasm: SUMP. This FNA shows a neoplastic proliferation of cells with delicate pale cytoplasm containing variably-sized clear vacuoles and round nuclei with inconspicuous nucleoli and minimal nuclear pleomorphism. (**a**) (smear, Romanowsky stain) and (**b**) (smear, Papanicolaou stain). The histologic follow-up was acinic cell carcinoma

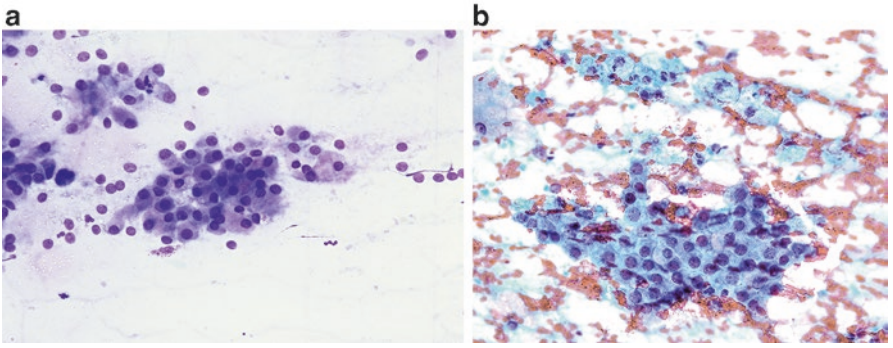

Fig. 5.24 Neoplasm: SUMP. FNA of a neoplasm with variably oncocytoid to clear cell features. A monotonous population of neoplastic cells arranged in cohesive groups with finely granular cytoplasm. The background shows thin mucin and clear histiocytic-type cells that raise a differential diagnosis of mucoepidermoid carcinoma (**a**) (smear Romanowsky stain and (**b**) (smear, Papanicolaou stain)

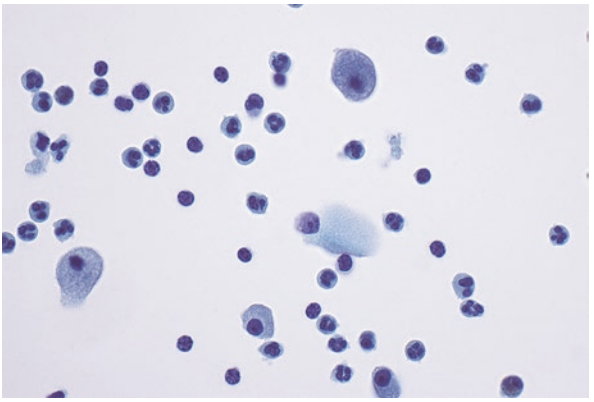

Fig. 5.25 Neoplasm: SUMP. FNA of a neoplasm with scattered large cells with finely vacuolated clear to pale cytoplasm (smear, Papanicolaou stain)

Explanatory Notes

Salivary gland aspirates composed of a prominent population of cells with clear cytoplasm should be evaluated with caution since the differential diagnosis is broad and the distinction between the various clear cell tumors can be challenging. Cytoplasmic "clearing" represents a non-specific change resulting from one or a combination of cellular alterations including: (1) intracytoplasmic lipid, mucin, or glycogen; (2) intracellular edema; (3) paucity of intracellular organelles. Depending upon the type of cytologic preparation and the type of neoplasm, the cytoplasm of the neoplastic cells can range from coarsely granular to foamy (see Figs. 5.21 and 5.23), vacuolated, optically clear, or a combination thereof. The clear cytoplasmic change is best appreciated on Papanicolaou and H&E (cell block) staining, while May-Grünwald-Giemsa (MGG) staining will usually impart a non-specific pale blue hue to the cytoplasm, with the exception of mucin or lipid. There exists a significant overlap between tumors with clear cell features. In the evaluation of an aspirate containing clear cell features and for which definitive tumor classification cannot be made by standard cytologic evaluation, additional material should be obtained, if possible, for ancillary marker studies (see Chap. 8). Defining the nature of the clear cell change (e.g., lipid, mucin, or glycogen) using histochemical stains (PAS and PAS-diastase, mucicarmine) can be helpful to limit the differential diagnosis of clear cell neoplasms followed by a focused IC panel. If a specific tumor classification is not possible based upon quantitative or qualitative cytomorphological features and any ancillary studies performed, the aspirate can be classified as "SUMP," "Suspicious for Malignancy," or "Malignant," depending upon the degree of nuclear atypia, differential diagnostic considerations, and the estimated ROM.

Clinical Management

FNA cases classified as "Neoplasm: Benign" should have MRI or CT studies performed to assess the extent of the lesion prior to complete excision of the benign lesion with nerve preservation (see Chap. 9, Clinical Management). A subset of patients who are not surgical candidates or who are unable to accept the risk of potential nerve injury might be clinically followed without surgical management.

The management of FNA cases classified as "Neoplasm: SUMP" is similar, but includes a greater degree of clinical decision-making. Preoperative imaging by MRI or CT should be performed on this group of patients to evaluate the extent of the tumor as well as assessing the neck. Nerve-sparing surgical resection is indicated unless the patient is not a surgical candidate. Intraoperative frozen section can be used in cases diagnosed as SUMP to potentially better define the histologic classification and margin status, and to determine if a neck dissection is indicated.

Sample Reports

Sample Report Example: Benign Neoplasm

Example 1:
Satisfactory for evaluation
NEOPLASM: BENIGN
Pleomorphic adenoma.

Sample Report Examples: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)

Example 2:
Satisfactory for evaluation
NEOPLASM: SALIVARY GLAND NEOPLASM OF UNCERTAIN MALIGNANT POTENTIAL (SUMP)
Cellular basaloid neoplasm. See note.
Note: The specimen shows a monomorphic population of basaloid cells with minimal nuclear atypia associated with fibrillary matrix. No mitoses or tumor necrosis is seen. The findings are suggestive of a cellular pleomorphic adenoma; however, other matrix-producing basaloid tumors such as basal cell adenoma, basal cell adenocarcinoma, and epithelial-myoepithelial carcinoma cannot be completely excluded.

Example 3:
Satisfactory for evaluation
NEOPLASM: SALIVARY GLAND NEOPLASM OF UNCERTAIN MALIGNANT POTENTIAL (SUMP)
Cellular neoplasm with clear cell features. See note.
Note: The specimen shows a low-grade biphasic neoplasm with clear cell features. The differential diagnosis includes pleomorphic adenoma and myoepthelioma; however, epithelial-myoepithelial carcinoma cannot be completely excluded.

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Chapter 6
Suspicious for Malignancy

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General Background

The categories "Atypia of Undetermined Significance (AUS)," "Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)," and "Suspicious for Malignancy (SM)" represent indeterminate diagnostic categories in the Milan System [1]. They are used to stratify the risk of malignancy (ROM) and to inform the treating clinician that a particular specimen cannot be placed into a more specific benign or malignant diagnostic category due to diagnostic limitations such as sparse cellularity or various specimen artifacts (see Chaps. 4 and 5). The SM category is a traditional diagnostic category used in nearly all cytology reporting systems and, as such, its characteristics are well known to practicing cytologists [2–7]. The purpose of separating SM from the Malignant category is to preserve the high

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_6

positive predictive value (PPV) of a fine-needle aspiration (FNA) classified as Malignant while at the same time offering a diagnostic option with a relatively high ROM for those FNAs where the cytomorphologic criteria fall short in quantity and/or quality for a Malignant diagnosis [8–16]. In the Milan System, the ROM for the SM category approaches 60% [1]. With the growing availability of immunohistochemical and molecular markers for salivary gland tumors (see Chap. 8), a subset of FNAs classified as SM may benefit from the application of ancillary testing to yield a more specific interpretation.

Definition

A salivary gland FNA is classified as SM when some, but not all the criteria for a specific diagnosis of malignancy are present, and yet the overall cytologic features are suggestive of malignancy.

Cytologic Criteria

When making a diagnosis of SM, the FNA should be described as suspicious for a primary salivary gland malignancy, or suspicious for a metastasis, or lymphoma [8–12]. A significant proportion of SM cases will be suboptimal samples of a high-grade malignancy. Aspects of a salivary gland FNA leading to an interpretation of SM include:

- Markedly atypical cells with poor smear preparation, poor cell preservation, fixation artifact, or obscuring inflammation and blood (Figs. 6.1 and 6.2)
- Presence of limited cytologic features of a specific malignant lesion (e.g., adenoid cystic carcinoma, mucoepidermoid carcinoma, and acinic cell carcinoma) in an otherwise sparsely cellular aspirate (Figs. 6.3, 6.4, and 6.5)
- Presence of markedly atypical and/or suspicious cytologic features in a subset of cells but admixed with features of a benign salivary gland lesion (Fig. 6.6).

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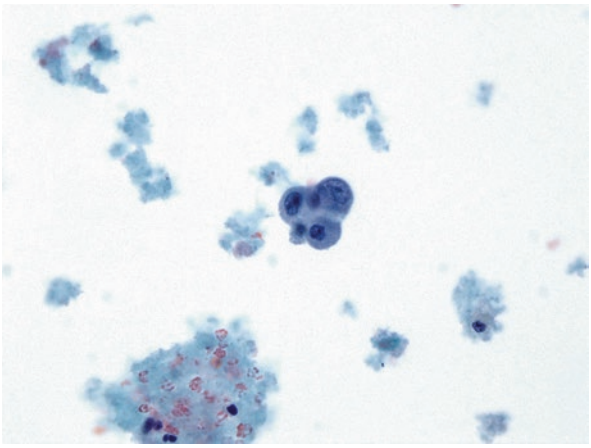

Fig. 6.1 Suspicious for Malignancy. The smear shows rare markedly atypical cells suggestive of carcinoma, but the classification is limited by scant cellularity (smear, Papanicolaou stain)

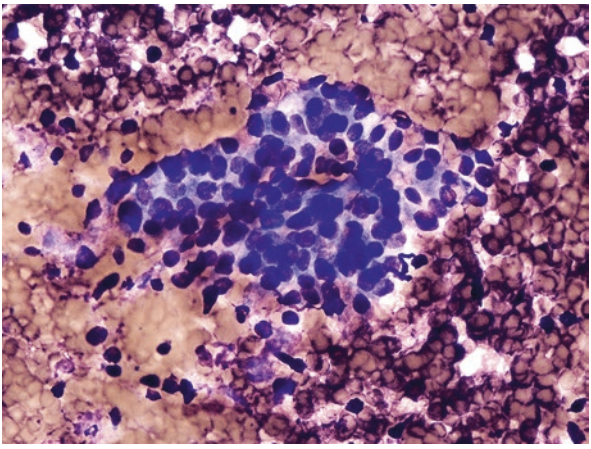

Fig. 6.2 Suspicious for Malignancy. The smear contains markedly atypical cells suspicious for high-grade carcinoma, but with obscuring blood limiting the assessment (smear, Romanowsky stain)

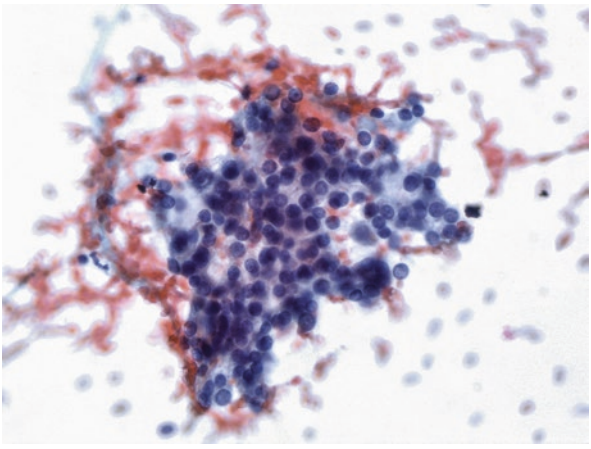

Fig. 6.3 Suspicious for Malignancy. The smear shows a group of epithelial cells suggestive of acinic cell carcinoma, but hypocellularity and background blood in the absence of ancillary studies limits the evaluation (smear, Papanicolaou stain)

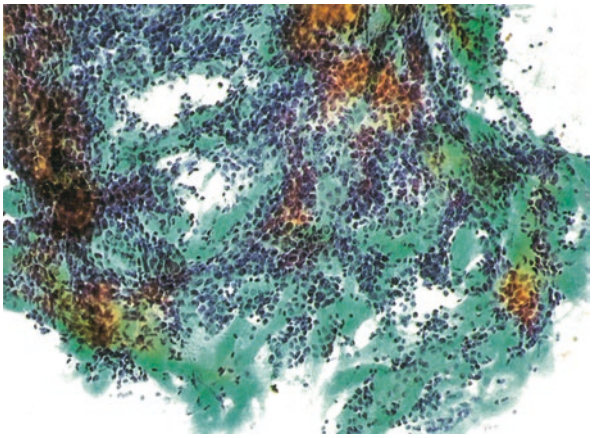

Fig. 6.4 Suspicious for Malignancy. This smear is composed of basaloid cells and abundant matrix spheres with a pattern suspicious for adenoid cystic carcinoma (smear, Papanicolaou stain)

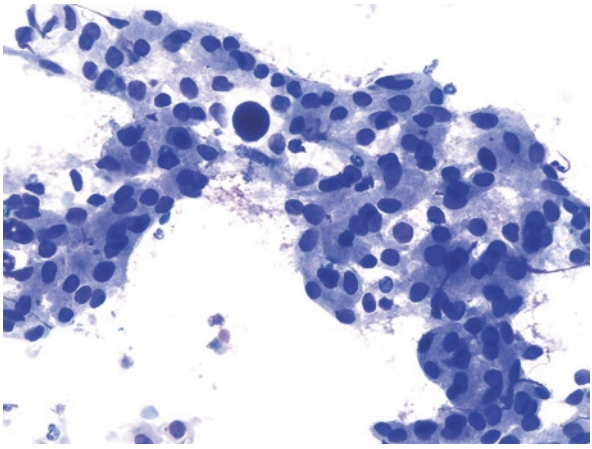

Fig. 6.5 Suspicious for Malignancy. The smear consists of epithelial cells with epidermoid features, suggestive of mucoepidermoid carcinoma (smear, Romanowsky stain)

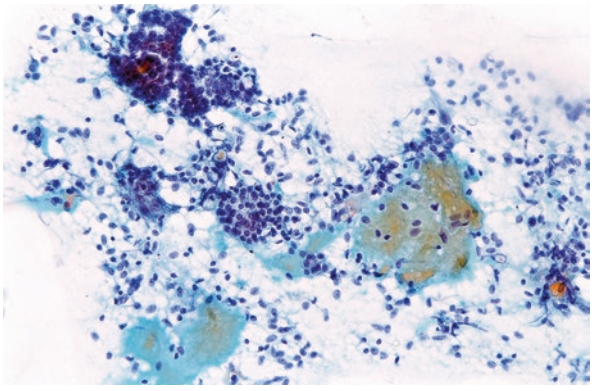

Fig. 6.6 Suspicious for Malignancy. The smear shows presence of markedly atypical (*upper left*) cytologic features in a subset of cells admixed with features of pleomorphic adenoma (smear, Papanicolaou stain)

Atypical features can include prominent nucleoli or macronucleoli, anisonucleosis, increased nuclear to cytoplasmic ratio, nuclear molding, prominent nuclear pleomorphism, atypical mitosis, and clumped, coarse chromatin (Fig. 6.7).

- Scant sample with atypical features suggestive of a neuroendocrine neoplasm (Fig. 6.8)

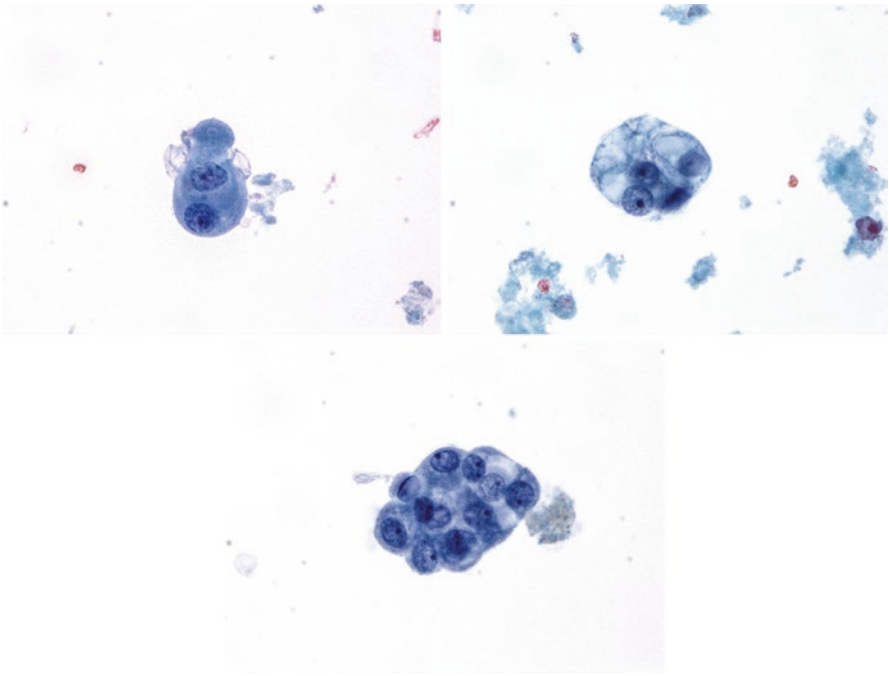

Fig. 6.7 Suspicious for Malignancy. This aspirate is hypocellular but contains occasional small groups of markedly atypical cells suspicious for carcinoma. The corresponding resection showed a high-grade mucoepidermoid carcinoma (smear, Papanicolaou stain)

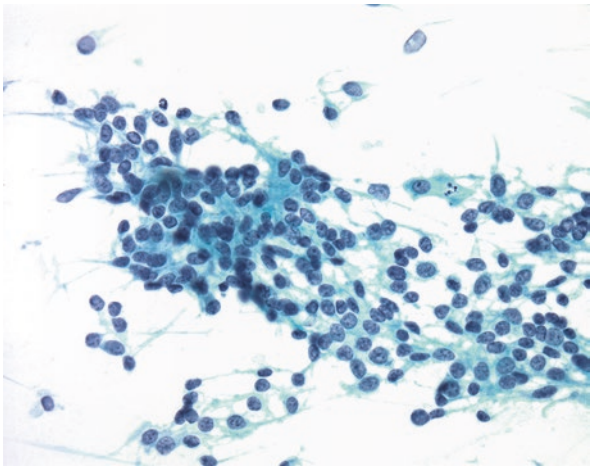

Fig. 6.8 Suspicious for Malignancy. This smear shows neoplastic cells containing nuclei with "salt and pepper" chromatin suggestive of neuroendocrine differentiation (smear, Papanicolaou stain)

A differential diagnosis of lymphoma is usually considered in salivary gland aspirates with a prominent population of lymphocytes or atypical lymphoid cells with microscopic fragments of lymphocyte cytoplasm ("lymphoglandular bodies") in the background [17, 18]. Immunophenotyping, usually by flow cytometry, is key to making a diagnosis of most lymphomas in cytologic specimens. Thorough clinical correlation is also essential. Successful subclassification of lymphoma may require performance of ancillary immunohistochemical and molecular studies. Many of the aspirates of lymphoma classified as SM lack sufficient material for the performance of these ancillary studies [17, 18]. A detailed cytology review of lymphoma diagnosis is beyond the scope of this atlas, but some of the cytomorphologic features suggestive of a lymphoma include:

- A population of enlarged atypical lymphoid cells as seen in large cell lymphomas (Fig. 6.9)
- A monomorphic lymphoid population. This may be made up of small/intermediate lymphocytes as in intermediate grade follicular lymphoma (Fig. 6.10), or showing angulated, indented nuclei resembling centrocytes suggesting mantle cell lymphoma, or small lymphocytes with round nuclei and coarse chromatin suggesting small lymphocytic lymphoma
- A heterogeneous lymphoid population with atypical forms (Fig. 6.11). Extranodal marginal zone lymphomas (ENMZL) are especially characterized by a heterogeneous cell population including small to intermediate size centrocyte-like cells, and a smaller number of larger lymphoid cells, plasmacytoid cells, tingible body macrophages, dendritic cells, and plasma cells.

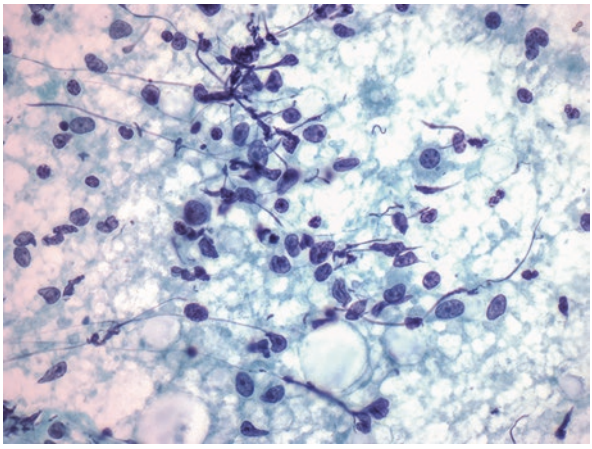

Fig. 6.9 Suspicious for Malignancy. This smear shows a population of enlarged atypical lymphoid cells suspicious for a large cell lymphoma (smear, Papanicolaou stain)

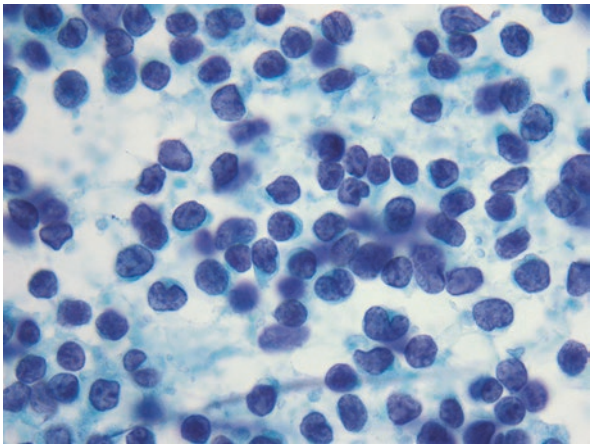

Fig. 6.10 Suspicious for Malignancy. This aspirate shows a monotonous population of intermediate-size lymphocytes that, based upon cytomorphology alone, are highly suspicious for lymphoma. Additional ancillary studies including immunophenotyping are needed for classification (smear, Papanicolaou stain)

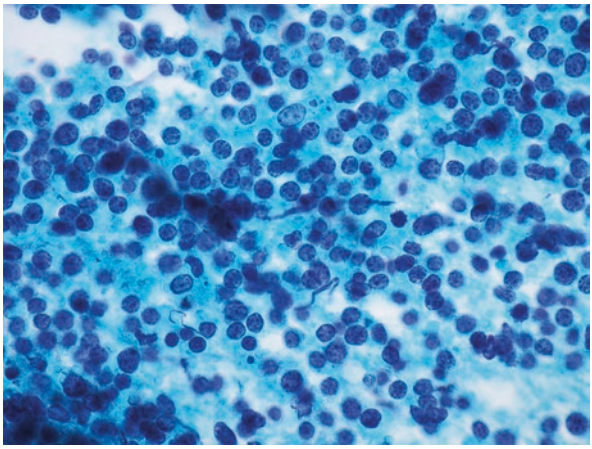

Fig. 6.11 Suspicious for Malignancy. This smear shows a polymorphous pattern with a predominance of intermediate-size lymphoid cells as can be seen in marginal zone lymphomas. Ancillary studies are needed for further classification (smear, Papanicolaou stain)

Explanatory Notes

A diagnosis of SM can be made in aspirates showing focal marked cellular atypia in a less than optimal specimen. Once significant atypia, suggesting malignancy, is identified in an FNA that is hypocellular or poorly prepared, the case is no longer insufficient or "Non-Diagnostic." SM also usually indicates that the cytology is characterized by a higher degree of atypia than in those aspirates in the AUS and SUMP categories, thus highly suggestive of a malignant lesion. SM should not be used for cases where the overall cytomorphological features are better classified as AUS or SUMP. The latter are associated with a significantly lower ROM than cases classified as SM. The cytomorphological stratification of AUS, SUMP, and SM can be subtle and in some cases subjective, but careful scrutiny of the cytomorphological features and proper application of ancillary techniques will aid in accurate

classification. In some cases, the diagnosis of "Suspicious for Malignancy" may be upgraded to "Malignant" once the results of any ancillary studies become available. Whenever rapid on-site evaluation (ROSE) is offered, it can be used to improve the quality and quantity of the FNA specimen, and assist in triaging material for additional diagnostic studies.

A majority of salivary gland FNAs classified as SM will be samples of high-grade cancers that have some limiting factors precluding a definitive diagnosis of malignancy. A subset of cases will be lower-grade salivary gland cancers that exhibit many of the characteristic cytologic features of a particular salivary gland cancer, but for qualitative or quantitative reasons are not sufficient to be diagnostic (Fig. 6.12). Most commonly, aspirates of low-grade mucoepidermoid carcinoma, acinic cell carcinoma, and adenoid cystic carcinoma will fall into the latter subset. Other tumors such as aspirates of neuroendocrine carcinoma, which are rare in the salivary gland, are usually diagnostic of malignancy provided that adequate material is available for ancillary studies. The most common form of neuroendocrine carcinoma in the salivary gland is poorly differentiated neuroendocrine carcinoma with Merkel cell-like features, and by cytomorphology alone it would typically be interpreted as malignant unless it were a compromised specimen.

Salivary gland aspirates containing a prominent lymphoid population will require ancillary studies for a definitive diagnosis of lymphoma. Otherwise, classification of the aspirate as SM can be used for cases where there is a cytologic pattern suggesting lymphoma such as the presence of large atypical lymphoid cells or a monomorphic lymphoid population. Most often, there will be a heterogeneous lymphoid population, and the differential diagnosis will include a benign process such as reactive lymphoid hyperplasia, chronic sialadenitis, or Sjogren's syndrome. Occasionally, such cases can exhibit sufficient atypical cytomorphologic and clinical features as to be suspicious for lymphoma, but flow cytometry or other methods of immunophenotypic analysis are essential to ultimately rule in or rule out lymphoma. If the

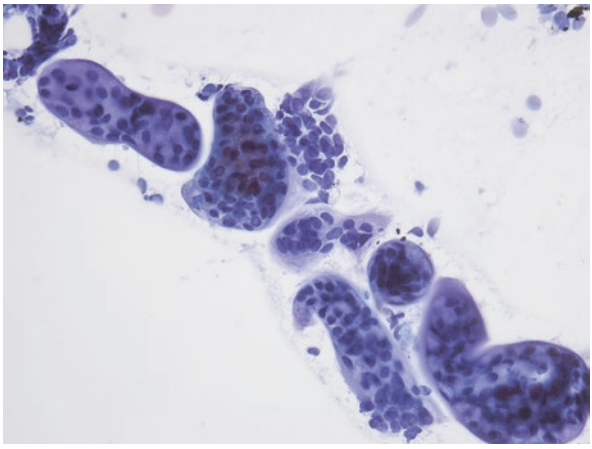

Fig. 6.12 Suspicious for Malignancy. This smear shows cytologic features that are highly suspicious for adenoid cystic carcinoma, but the specimen is limited to a single Papanicolaou-stained smear (smear, Papanicolaou stain)

FNA has not been submitted for flow cytometry, repeat FNA with flow is the best approach, and if there is local expertise, core biopsy can be added. Correlation with hematopathology is recommended, and in some cases, surgical excision of the lesion will be indicated for definitive diagnosis and subclassification for those lesions that are lymphoma. While rarely involving the salivary glands or intraparotid lymph nodes, classic Hodgkin lymphoma has distinctive cytomorphologic features that would lead to a diagnosis of at least "Suspicious for Hodgkin lymphoma" in most cases. Flow cytometry would generally not be useful for confirming the diagnosis of Hodgkin lymphoma, but material for other ancillary studies would be indicated; excisional biopsy may be needed for a definitive diagnosis.

Clinical Management

The cytologic diagnosis of SM is not equivalent to "Malignant," even though it is suggestive of a malignant lesion and the risk of malignancy is high. It cannot be used alone as a basis for radical surgery, chemotherapy, or radiotherapy (see Chap. 9). In response to a diagnosis of SM, consideration should be given as to whether or not obtaining additional material by repeat FNA, core biopsy, open biopsy, or surgical excision would be most useful. For cases with repeat FNA, every effort should be made to obtain adequate material for any ancillary studies that would be indicated. Clinical and radiologic correlations are of course important, and when surgery is performed, intraoperative frozen section can be considered in appropriate cases.

Sample Reports

Example 1:
Satisfactory for evaluation
SUSPICIOUS FOR MALIGNANCY
Rare markedly atypical cells, suspicious for high-grade carcinoma.

Example 2:
Satisfactory for evaluation
SUSPICIOUS FOR MALIGNANCY
Suspicious for high-grade mucoepidermoid carcinoma/adenoid cystic carcinoma/salivary duct carcinoma.

Example 3:
Evaluation limited by scant cellularity
SUSPICIOUS FOR MALIGNANCY
Atypical cells in a mucinous background, suspicious for low-grade mucoepidermoid carcinoma.

Example 4:
Satisfactory for evaluation
SUSPICIOUS FOR MALIGNANCY
Rare large atypical lymphocytes, suspicious for non-Hodgkin lymphoma. See note.
Note: Further evaluation using immunophenotyping studies by flow cytometry or immunochemistry in a repeat FNA or tissue sample is recommended.

Example 5:
Satisfactory for evaluation
SUSPICIOUS FOR MALIGNANCY
Monomorphic population of atypical small lymphoid cells, suspicious for non-Hodgkin lymphoma. See note.
Note: Additional tissue sampling either by repeat FNA or tissue biopsy is recommended for further evaluation with ancillary studies including flow cytometry.

Example 6:
Evaluation limited by scant well-preserved cells
SUSPICIOUS FOR MALIGNANCY
Cyst contents with occasional atypical squamous cells and dyskeratotic cells, suspicious for metastatic keratinizing squamous cell carcinoma.

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Chapter 7
Malignant

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General Background

Malignant salivary gland tumors include a diverse group of primary neoplasms involving both the major and minor salivary glands [1–4]. In addition, various secondary neoplasms (e.g., metastatic cutaneous squamous cell carcinoma) can also involve the salivary glands or lymph nodes within or closely associated with salivary glands. A majority of malignant tumors occur in the parotid and submandibular glands and account for most of the salivary gland fine-needle aspirations (FNA) [1–9]. This chapter discusses tumors that commonly involve the major salivary glands and can be diagnosed by FNA. As described in the previous chapters, while

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_7

some low-grade salivary gland cancers overlap cytomorphologically with their benign counterparts, many of the more common primary cancers in well-sampled and prepared FNAs with adequate material for ancillary studies will exhibit features that are sufficiently distinctive to be classified as "Malignant." Once the diagnostic threshold has been reached for diagnosing a salivary gland FNA as "Malignant," an attempt to grade it should be made, as it can influence the clinical management (see Chap. 9).

Definition

Salivary gland aspirates classified as "Malignant" contain a combination of cytomorphologic features that, either alone or in combination with ancillary studies, is diagnostic of malignancy. When possible, an attempt should be made to provide the grade of the neoplasm as well as the specific tumor type (e.g., low-grade mucoepidermoid carcinoma).

Low-Grade Carcinomas

Acinic Cell Carcinoma

Acinic cell carcinoma (ACC) comprises approximately 10–15% of all salivary gland epithelial malignancies, and is the second most common malignant salivary gland tumor after mucoepidermoid carcinoma (MEC). In the pediatric age group, it constitutes about a third of salivary gland carcinomas [7, 8]. It shows a slight female predilection (1.5:1) and a wide age distribution; the mean age at diagnosis is 50 years. ACC occurs most commonly in the parotid gland, while many minor salivary gland tumors previously diagnosed as ACC have been reclassified as secretory carcinoma (aka mammary analogue secretory carcinoma). Most ACC present as mobile, soft to firm, well-circumscribed 1–4 cm masses. The tumors are usually asymptomatic and slow-growing; pain, fixation to the surrounding tissues, and facial nerve involvement are considered poor prognostic features and may indicate high grade transformation. ACC can metastasize to cervical lymph nodes, and local tumor recurrences can be seen in up to 35% of cases. Distant metastases are rare; however, they have been reported in the liver and lung.

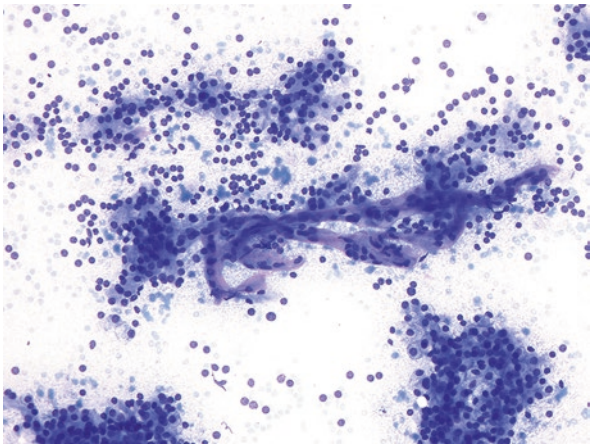

Fig. 7.1 Malignant. Acinic cell carcinoma. Cellular smear with loosely cohesive groups of fragile acinar cells adherent to a delicate capillary meshwork. Note the presence of stripped nuclei in the flocculent background and the conspicuous absence of ductal cells (smear, Romanowsky stain)

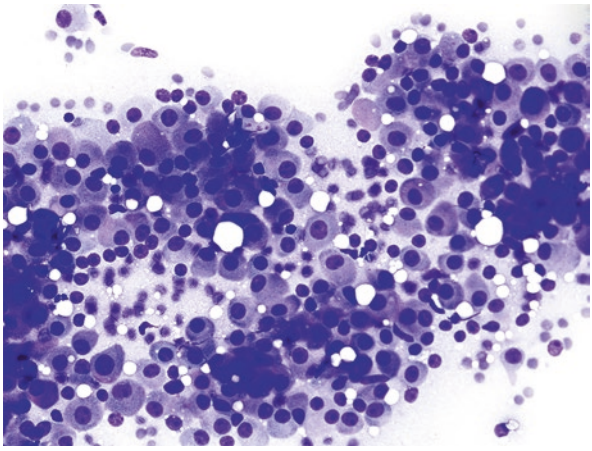

Fig. 7.2 Malignant. Acinic cell carcinoma. Dyshesive well-preserved tumor cells with delicate granular cytoplasm and stripped nuclei. The cells are polygonal with low N:C ratio (smear, Romanowsky)

Cytologic Criteria

ACC is a malignant epithelial neoplasm in which at least some of the neoplastic cells exhibit serous acinar differentiation, characterized by the presence of periodic acid-Schiff (PAS)-positive diastase-resistant cytoplasmic zymogen secretory granules. Most FNA specimens of ACC show the following characteristics:

- Cellular smears with "monotonous" population of epithelial cells (Fig. 7.1)
- Polygonal tumor cells with low nuclear–cytoplasmic (N:C) ratio and abundant delicate vacuolated cytoplasm with basophilic quality (Fig. 7.2)
- Variable numbers of cytoplasmic zymogen granules. These granules are PAS-positive, diastase resistant. (Fig. 7.3)
- Predominantly dispersed or loosely cohesive cell population; no lobular (grape-like) pattern

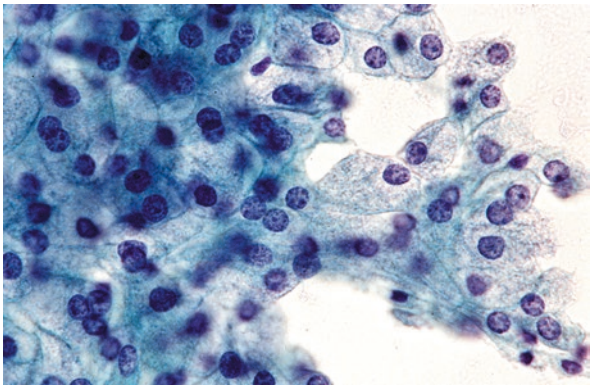

Fig. 7.3 Malignant. Acinic cell carcinoma. Aspirate showing a sheet of cells with abundant delicate cytoplasm with scattered small coarse granules (smear, Papanicolaou stain)

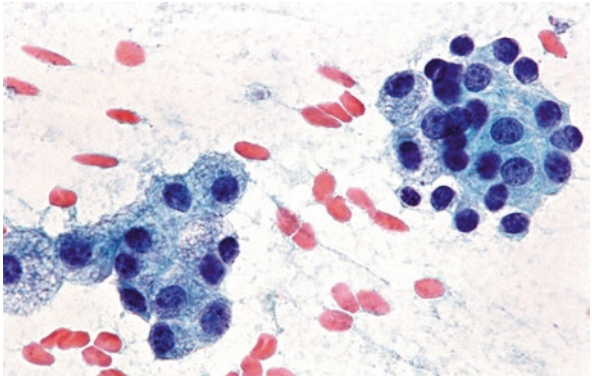

Fig. 7.4 Malignant. This acinic cell carcinoma has three-dimensional clusters of acinar cells with abundant delicate cytoplasm; low N:C ratio; uniform, round-to-oval nuclei, with distinct nucleoli (smear, Papanicolaou stain)

- Capillary meshwork with loosely adherent cells or well-developed papillary formations
- Uniform, round eccentric nuclei with distinct nucleoli (Fig. 7.4)
- Minimal nuclear pleomorphism
- No mitotic activity or necrosis
- Clean or frothy background; stripped nuclei
- Background lymphocytes present in a subset of cases
- Rare psammoma bodies

Explanatory Notes

While ACC usually have a dispersed cell pattern, small crowded groups of cells or papillary clusters around a rich capillary meshwork can sometimes be seen. The tumor cells are large and polygonal to oval with indistinct cell borders, and abundant delicate vacuolated cytoplasm, which has a subtle basophilic quality. Cytoplasmic zymogen granules, which are indicative of serous acinar

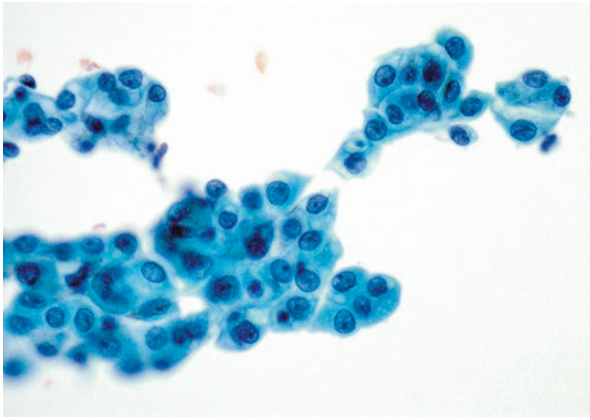

Fig. 7.5 Malignant. This acinic cell carcinoma has loosely cohesive groups of cells with a somewhat higher N:C ratio imparting more of a non-specific glandular appearance (smear, Papanicolaou stain)

differentiation, are usually coarse, stain basophilic in Papanicolaou-stained preparations, but are best seen in Romanowsky-type stains where they appear red or magenta. Unfortunately, zymogen granules are often sparse and/or difficult to detect on routinely stained cytologic preparations. In addition to serous acinar cells, aspirates can also show clear cells, intercalated duct-like cells, and non-specific glandular cells. Intercalated duct-like cells are smaller, cuboidal, have a higher N:C ratio with centrally placed nuclei, and lack the classic cytoplasmic zymogen granules. Non-specific glandular cells are frequently seen; they resemble the intercalated duct-like cells but are larger and rounder (Fig. 7.5). Most ACC have minimal to no nuclear pleomorphism, and usually lack mitoses or necrosis. Numerous naked nuclei may be present in the aspirate and may be difficult to distinguish from lymphocytes. Material should be collected whenever possible for ancillary studies. Demonstration of PAS-positive diastase resistant cytoplasmic zymogen granules is helpful. In contrast to normal salivary gland acini, amylase is not regularly expressed in ACC, and myoepithelial markers (e.g., smooth muscle actin, p63, keratin 5/6, calponin, and S100) are generally negative. The most useful ancillary markers of ACC are DOG1 (anoctamin-1, described in gastrointestinal stromal tumors) and SOX10 (Table 7.1) (see Chap. 8).

ACC are usually deceptively bland tumors, and hence can occasionally be confused with non-neoplastic salivary gland elements or sialadenosis. The latter two entities maintain the characteristic grape-like arrangement of normal acinar cells with associated ductal cells, whereas aspirates of ACC have a more monotonous population of acinar cells with extensive cellular dyshesion and lacking the grape-like cytomorphology of non-neoplastic acinar cells. When the cells are more vacuolated or clear, it can lead to confusion with low-grade MEC, which is positive with mucin stains. Low-grade MEC also shows an admixture of three cell types including intermediate, epidermoid, and mucinous cells. Similarly, vacuolated acinar cells may lead to confusion with sebaceous tumors, which have abundant lipid-rich cytoplasm that is negative for PAS with diastase (PAS-D), and with epithelial-myoepithelial carcinomas, which express myoepithelial markers and are PAS-D negative.

Table 7.1 Immunohistochemical stains in the differential diagnosis of low-grade salivary gland malignancies

	p63/p40	SMA, SMMHC, calponin	S100	CK8/18	CK5/6	CD117	MUCIN	PAS-D	DOG1
ACC	–	–	–	+	–	–	–	+ (Granules)	+
AdCC	+ (ME)	+ (ME)	+	+ (EP)	+ (ME)	+	–	–	–
MEC, LG	+ (SQ)	–	–	+ (MUC)	+ (SQ)	–	+	+	–
SC	–	–	+++	+	–	–	+	±	–
EMC	+ (ME)	+ (ME)	+ (ME)	+ (EP)	+ (ME)	–	–	–	–
MC	+	+	+	–	+	–	–	–	–

PAS-D Periodic acid-Schiff with diastase, *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain, *ACC* Acinic cell carcinoma, *AdCC* Adenoid cystic carcinoma, *MEC LG* Mucoepidermoid carcinoma low-grade, *SC* Secretory carcinoma (mammary analogue secretory carcinoma [MASC]), *EMC* Epithelial-myoepithelial carcinoma, *MC* Myoepithelial carcinoma, *ME* Myoepithelial cells, *EP* Epithelial (luminal) cells, *SQ* squamoid (epidermoid) cells, *MUC* mucin-secreting cells

ACCs share many cytomorphologic features with secretory carcinoma, which, before their recognition as a separate entity, were likely diagnosed as ACC. However, secretory carcinoma lacks PAS-D-positive cytoplasmic zymogen granules, is negative for DOG1, and expresses S100, GATA3, and mammaglobin diffusely. Molecular studies can be performed on cytology material for the ETV6/NTRK translocation, which is specific for secretory carcinoma. Other entities that can enter into the differential diagnosis of ACC are oncocytoma and Warthin tumor. As opposed to the "oncocytoid" cells that can be seen in ACC, true oncocytes as seen in Warthin tumor and oncocytoma have dense non-vacuolated granular cytoplasm and are histochemically positive for PTAH.

ACC with a predominance of intercalated duct-like cells and non-specific glandular cells are among the most difficult ACC to recognize cytologically and will usually be classified as "Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)" or as "Suspicious for Malignancy (SM)."

Metastatic renal cell carcinomas can resemble ACC, and are best differentiated from ACC with the help of immunohistochemistry, clinical history, and supporting imaging studies. In rare cases, ACC can undergo high-grade transformation ("dedifferentiation") (Fig. 7.6), in which case it would be diagnosed by FNA as a high-grade carcinoma.

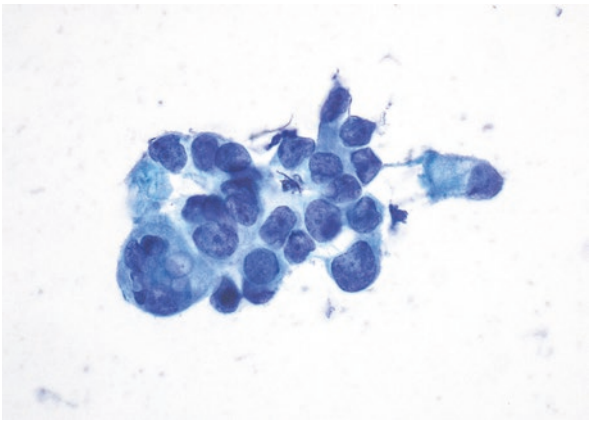

Fig. 7.6 Malignant. This aspirate of acinic cell carcinoma with high-grade transformation shows a loose cluster of epithelial cells with nuclear pleomorphism (smear, Papanicoloau stain)

Secretory Carcinoma

Secretory carcinoma (SC), previously referred to as "mammary analogue secretory carcinoma (MASC)," is a recently described low-grade salivary gland tumor included as a distinct entity [7, 10–12] in the 2017 WHO classification of head and neck tumors [7]. Akin to secretory breast cancer, SC expresses S100 protein, mammaglobin, vimentin, and harbors a t(12; 15) (p13; q25) translocation, which leads to the *ETV6-NTRK3* fusion product. The tumor is found most commonly in the parotid gland, followed by the intraoral minor salivary glands and submandibular gland. Most tumors occur in adults and show an equal gender distribution; the mean age is 47 years (range 14–78 years) [7]. The tumors range in size from 1 to 4 cm. The clinical course of SC is characterized by an indolent behavior, with a moderate risk of local recurrence (15%), lymph node metastasis (20%), and low risk of distant metastasis (5%) [7, 10–12]. High-grade transformation, akin to that seen in other low-grade salivary gland malignancies, has also been described in SC [13, 14].

Cytologic Criteria

SC is composed of microcystic, tubular, and solid structures with eosinophilic colloid-like background secretory material (Fig. 7.7). Cells have low-grade vesicular nuclei with finely granular chromatin and distinctive centrally located nucleoli (Fig. 7.8). Moderate to abundant pale to pink vacuolated or granular cytoplasm is present (Fig. 7.9). Marked nuclear atypia, mitoses, or necrosis is absent or rare.

- Cellular aspirate
- Cells present singly and in tubular, follicular, and papillary groups
- Bland cuboidal, polygonal low N:C cells
- Abundant vacuolated eosinophilic cytoplasm

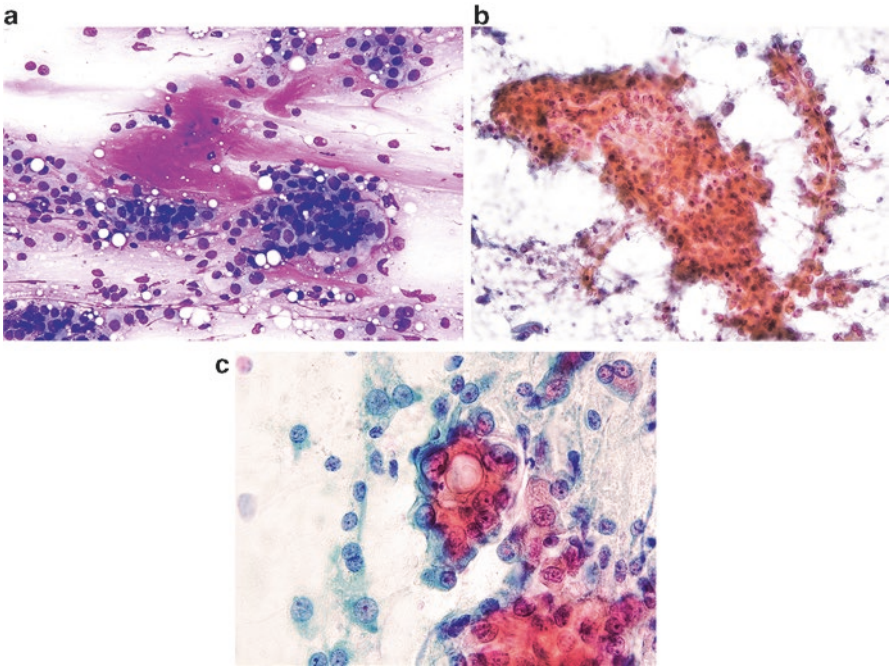

Fig. 7.7 Malignant. Secretory carcinoma (mammary analogue secretory carcinoma [MASC]). These aspirates (**a**–**c**) show different architectural patterns of microcystic, tubular, microfollicular, and solid sheets of glandular cells with eosinophilic colloid-like secretory material (smear, Papanicolaou and Romanowsky stains)

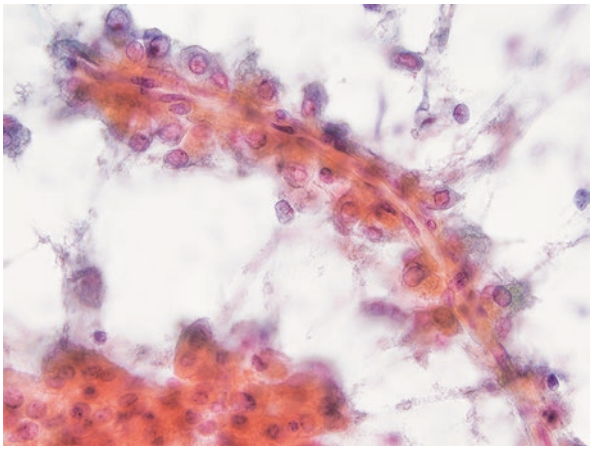

Fig. 7.8 Malignant. This aspirate of secretory carcinoma consists of cells with low-grade vesicular nuclei with finely granular chromatin and distinct nucleoli (smear, Papanicolaou stain)

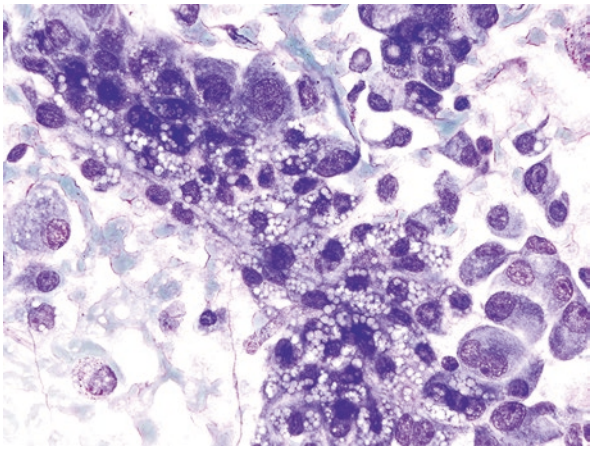

Fig. 7.9 Malignant. This FNA of secretory carcinoma shows cells with moderate to abundant pale, markedly vacuolated cytoplasm (smear, Romanowsky stain)

- Absence of cytoplasmic zymogen granules
- Uniform round eccentrically located nuclei with smooth nuclear contours, fine chromatin, and distinct nucleoli
- Background mucoproteinaceous material
- Presence of *ETV6/NTRK3* translocation

Explanatory Notes

The most common differential diagnostic consideration for SC is ACC, and many cases previously diagnosed as papillary cystic ACC [11] have been reinterpreted as SC after performing the appropriate molecular studies. SC should be suspected in aspirates with cytomorphologic features resembling ACC but lacking the characteristic cytoplasmic basophilic PAS-D-positive zymogen granules. In addition, SC tends to have more pronounced cytoplasmic vacuoles than ACC. Other clues to SC include the presence of papillary structures, particularly for tumors from non-parotid sites. The cytologic differential diagnosis of SC also includes other salivary gland tumors with mucin such as low-grade mucoepidermoid carcinoma, or that are characterized by large eosinophilic cells such as Warthin tumor, oncocytoma, and oncocytic cystadenoma. The multivacuolated cells seen in SC are not characteristic for any of these tumors, and are among the most useful differentiating features, as is the lack of squamous, intermediate, and goblet-type mucinous cells seen in low-grade MEC.

Appropriate immunohistochemical and molecular studies can be used to confirm the diagnosis of SC (see Chap. 8). SC is positive for S100, mammaglobin, and GATA3. SC is usually negative or at most focally positive for DOG1, and lacks reactivity for the myoepithelial markers calponin, CK5/6, and p63. Mucicarmine can be used to demonstrate intracellular and extracellular mucin. In general, the limited

experience available with the FNA diagnosis of SC suggests that its cytomorphologic features are not unique. It is therefore important to ensure the availability of adequate material to allow for the performance of appropriate immunohistochemical stains and molecular testing if the diagnosis of SC is suspected.

Epithelial-Myoepithelial Carcinoma

Epithelial-myoepithelial carcinoma (EMC) is an uncommon low-grade malignancy. It accounts for <5% of all salivary gland malignancies [7, 15]. Approximately 75% of EMCs occur in the parotid gland, while the rest are equally distributed between submandibular gland and minor salivary glands. EMC is a disease of older individuals in the 6th to 7th decade, and with no gender predilection. Patients usually present with a localized slow-growing mass. EMC is a biphasic tumor with an inner layer of cuboidal ductal cells and outer layer of larger clear myoepithelial cells. The ratio of myoepithelial to ductal cells is usually 2:1–3:1. Several histologic variants of EMC have been described.

Cytologic Criteria

EMC shows variable proportions of ductal and myoepithelial cells, but the latter component typically predominates (Fig. 7.10). Aspirates of EMC show the following:

- Cellular aspirate
- Arrangement of bland cells in pseudopapillary groups, sheets, and 3-dimensional groups (Figs. 7.11 and 7.12)

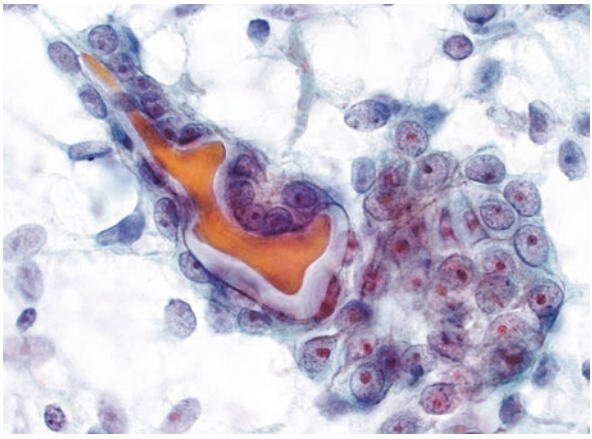

Fig. 7.10 Malignant. Epithelial-myoepithelial carcinoma. The aspirate shows a biphasic tumor with inner cuboidal ductal cells and prominent outer myoepithelial cells (smear, Papanicolaou stain)

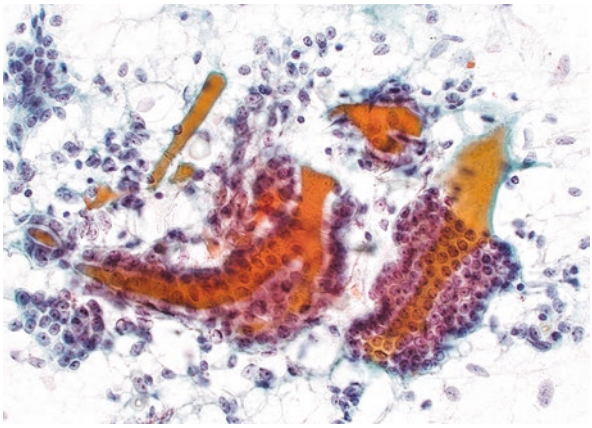

Fig. 7.11 Malignant. Aspirate of epithelial-myoepithelial carcinoma showing biphasic cells organized in pseudopapillary tubules and sheets (smear, Papanicoloau stain)

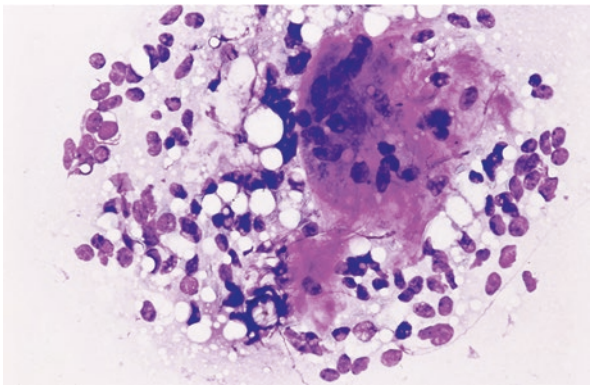

Fig. 7.12 Malignant. This aspirate of epithelial-myoepithelial carcinoma has a prominent biphasic pattern of ductal cells and abundant pale myoepithelial cells as well as focal proteinaceous material (smear, Papanicolaou stain)

- Laminated, acellular stromal cores (Fig. 7.13)
- Predominant population of clear myoepithelial cells
- Minor population of ductal cells with scant cytoplasm
- Background stripped nuclei
- Biphasic nature of the tumor is highlighted by immunostaining with HMW keratins and myoepithelial markers (P63, smooth muscle actin, calponin)

Explanatory Notes

The predominant cell in aspirates of EMC is the myoepithelial cell, which has bland nuclei with open chromatin and an unusually abundant clear or pale cytoplasm. Because of the bland nuclear features, EMC will often be classified as "Neoplasm: SUMP" or as "SM." The delicate nature of the glycogen-rich cytoplasm results in fragile myoepithelial cells and frequent background stripped nuclei. The cuboidal

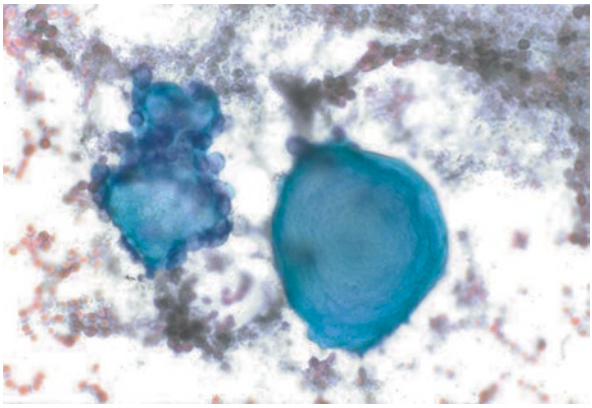

Fig. 7.13 Malignant. This epithelial-myoepithelial carcinoma has prominent concentrically laminated proteinaceous secretions that should be distinguished from the matrix material of adenoid cystic carcinoma (smear, Papanicolaou stain)

ductal component is sometimes more difficult to identify. Concentrically laminated acellular stromal spheres stain pink with Diff-Quik and blue-green with Papanicolaou stains. Material should be collected for ancillary studies to highlight the biphasic nature of the tumor.

Several entities are included in the differential diagnosis of EMC such as adenoid cystic carcinoma (AdCC), myoepithelioma, myoepithelial carcinoma, and cellular pleomorphic adenoma (PA). However, none of the tumors in the differential diagnosis have the prominent population of large clear myoepithelial cells seen in EMC. Unlike EMC, AdCC is a basaloid neoplasm with stromal material lacking the laminated features seen in EMC. Myoepithelial carcinoma and myoepithelioma lack the biphasic pattern of EMC, and the cells are smaller with less abundant pale cytoplasm. EMC can be difficult to distinguish from a cellular PA; however, PA lacks the large clear cells, and unique laminated stromal cores (see Chap. 5).

Given the abundant clear cells in EMC, other tumors with clear cell features such as metastatic renal cell carcinoma (RCC) could also be considered; however, RCC lacks the biphasic pattern and has a distinct immunoprofile. Similarly, clear cell carcinoma which occurs primarily in the minor salivary glands also lacks the biphasic pattern of EMC and harbors a EWSR1-ATF1 translocation. Careful correlation with clinical history coupled with judicious use of immunochemical markers could aid in the diagnosis of EMC.

High-Grade Carcinomas

Salivary Duct Carcinoma

Salivary duct carcinoma (SDC) is a high-grade malignant salivary gland tumor, initially described in 1968 by Kleinsasser, Klein, and Hübner [16] as a tumor analogous to ductal carcinoma of the breast [7, 17–19]. SDC can arise de novo, but up to 50% of cases represent malignant transformation of an existing PA (carcinoma ex

pleomorphic adenoma [Ca-ex-PA]). SDC constitute approximately 10% of all malignant salivary gland tumors, occur in older individuals with a peak incidence in the 7th decade, and are much more common in men. The parotid gland is the most common primary site (80%). SDC presents as a rapidly growing mass frequently with symptoms of nerve involvement. Tumors are usually large and have an infiltrative growth pattern with foci of necrosis. Several histologic variants have been reported, including papillary, micropapillary, mucin-rich, sarcomatoid, and oncocytic; however, these morphologies are typically associated with areas of classic SDC showing an apocrine carcinoma-like growth pattern [7]. Regional or distant metastases may already be present at the time of diagnosis, contributing to the poor prognosis of this tumor. The standard management for resectable tumors is radical surgery with ipsilateral neck dissection, followed by postoperative adjuvant radiotherapy.

Cytologic Criteria

SDC is a high-grade malignant neoplasm resembling mammary ductal carcinoma and has the following cytologic characteristics:

- Cellular aspirate.
- Sheets, three-dimensional crowded and cribriform groups of cells with overtly malignant cytologic features (Figs. 7.14 and 7.15)
- Medium to large polygonal cells with well-defined cell borders and abundant eosinophilic cytoplasm (Fig. 7.16)
- Enlarged round to oval, pleomorphic nuclei with anisonucleosis, hyperchromasia, and prominent nucleolus
- Frequent mitoses
- Necrotic background; stripped enlarged nuclei (Fig. 7.17)

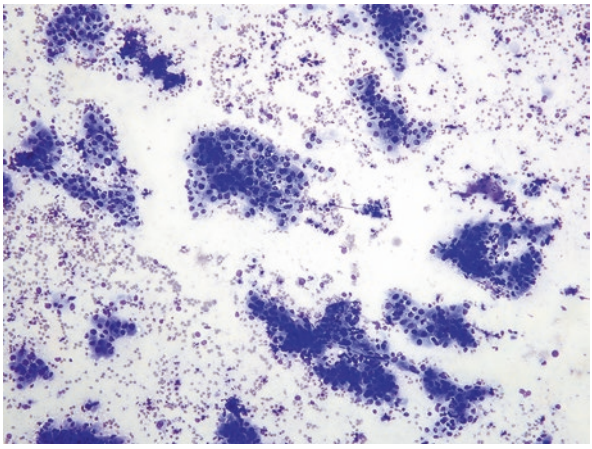

Fig. 7.14 Malignant. Salivary duct carcinoma. The aspirate is cellular with three-dimensional groups of epithelial cells with moderate amounts of cytoplasm and hyperchromatic nuclei in a background of blood and necrosis (smear, Romanowsky stain)

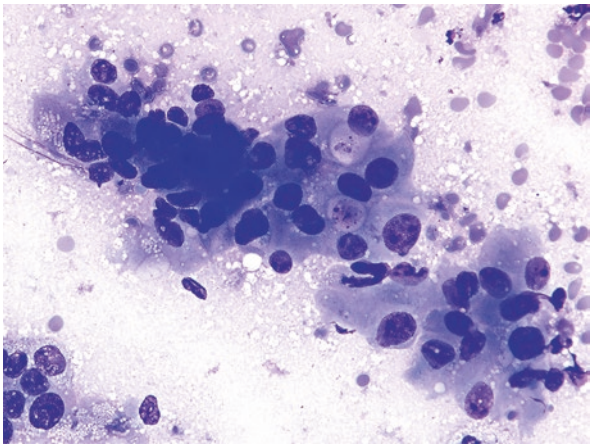

Fig. 7.15 Malignant. This aspirate of salivary duct carcinoma contains groups of high-grade malignant cells with abundant cytoplasm, nuclear pleomorphism, prominent nucleoli, and glandular features (smear, Romanowsky stain)

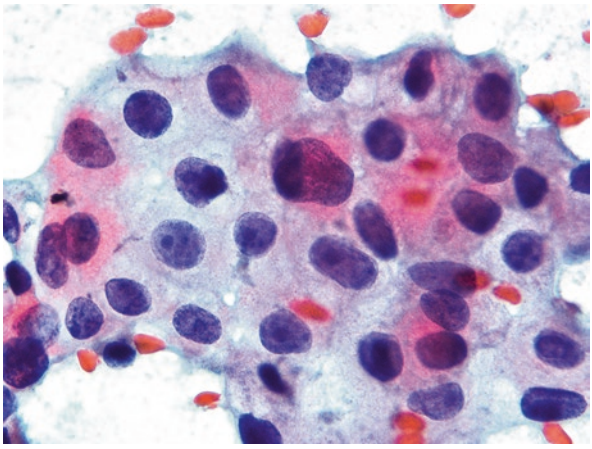

Fig. 7.16 Malignant. The polygonal cells in this FNA of salivary duct carcinoma have large pleomorphic nuclei with prominent nucleoli (smear, Papanicolaou stain)

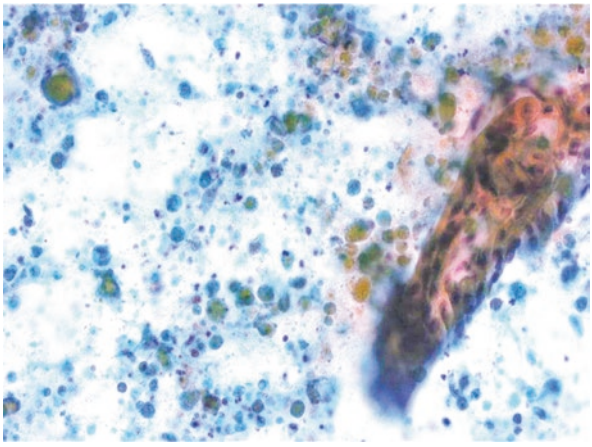

Fig. 7.17 Malignant. This aspirate of salivary duct carcinoma shows abundant background necrosis (smear, Papanicolaou stain)

Explanatory Notes

SDC are easily recognized cytologically as high grade carcinomas, but more specific classification will often require ancillary studies. SDC are positive for androgen receptor (AR), whereas expression of ER or PR is rare. GATA-3 is positive in SDC and >80% are also positive for GCDFP-15 (Table 7.2). Her2/neu expression is frequently seen (Fig. 7.18), but diffuse strong membranous staining or HER2 amplification demonstrated by fluorescent *in situ* hybridization (FISH) is seen in only about 25% of cases. SDC typically have a high proliferation index of over 25% with Ki67/MIB1 staining.

Table 7.2 Immunohistochemical stains in the differential diagnosis of high-grade salivary gland malignancies

	p63/ p40	SMA, SMMHC, calponin	CK8/18	CK5/6	CK20	MUCIN	AR	SYN, CHROMO, CD56, CD57	Site-specific
MEC, HG	+	–	Focal	+	–	Focal	–	–	–
SQCC[a]	+	–	–	+	–	–	–	–	–
SDC	–	–	+	–	–	–	+	–	–
PDC, NE	∓	–	+ (dot)	–	+	–	–	+	–
Metastatic	–	–	+	∓	∓	∓	–	–	+[b]

MEC, HG Mucoepidermoid carcinoma, high grade, *SQCC* Squamous cell carcinoma, *SDC* Salivary duct carcinoma, *PDC, NE* Poorly differentiated carcinoma,neuroendocrine type, *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain, *AR* androgen receptor, *SYN* synaptophysin, *CHROMO* chromogranin

[a]SQCC includes primary and metastatic squamous cell carcinoma, as well as lymphoepithelial carcinoma

[b]TTF1 for lung/thyroid primaries; CDX2 for colorectal primaries, PAX8 for renal primaries; HMB45/MART1 for melanomas

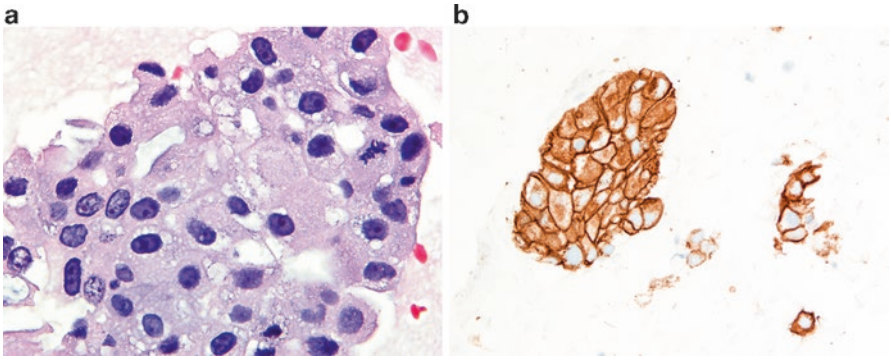

Fig. 7.18 Malignant. Salivary duct carcinoma. (**a**) The cell block section shows a cluster of tumor cells with nuclear pleomorphism, well-defined cellular borders, relatively abundant granular cytoplasm, and nuclei with prominent nucleoli. Note the mitotic figure in the upper right corner. (**b**) Her2neu immunostain showing strong membranous staining in tumor cells (cell block, H&E)

The main differential diagnostic considerations for SDC include high-grade MEC, oncocytic carcinoma, and metastatic carcinoma from the breast, prostate, or lungs. High-grade adenocarcinoma not-otherwise-specified (NOS) also enters the differential diagnosis, but this is a diagnosis of exclusion, which should only be made after the other diagnostic considerations are ruled out. Immunohistochemistry can be very helpful for addressing the differential diagnostic considerations. Both MEC and SDC are composed of large pleomorphic epithelial cells, but SDC lacks the squamoid features of MEC, and intracellular mucin is uncommon in SDC. Keratinization is absent in SDC, and would favor metastatic squamous carcinoma. Oncocytic carcinomas lack the prominent necrosis and ductal features seen in SDC, and they differ in their immunoprofiles.

Metastatic carcinoma from breast or prostate can sometimes enter the differential diagnosis, particularly in a patient with a known history of these cancers. Clinical correlation and interpretation of the cytologic findings in the appropriate clinical context is essential for the diagnosis of high-grade primary and secondary salivary gland cancers [20]. A focused panel of immunochemical markers can usually resolve any difficult cases where the cytomorphology is not definitive (Table 7.2).

Lymphoepithelial Carcinoma

Lymphoepithelial carcinoma (LEC) is a rare salivary gland tumor that comprises <1% of all salivary gland cancers [7, 21, 22]. There is a known predilection for Inuits in the Arctic region and Southern China and Japan. Tumors in endemic populations show a higher frequency of parotid gland involvement, slight female predilection, and nearly 100% association with Epstein-Barr virus (EBV). In the USA, the disease affects predominately Caucasians (82%) in the 6th decade, with no gender predilection. The parotid is also the most frequently affected salivary gland. EBV is typically absent outside of endemic areas. Patients usually present with an enlarging mass of the parotid or submandibular gland with associated cervical lymphadenopathy. Tumors usually range in size from 1–10 cm and often infiltrate the surrounding parenchyma. LEC has a tendency to spread to cervical lymph nodes, but this does not impact the patients' survival. Surgery and radiation therapy remain the treatment of choice.

Cytologic Criteria

LEC is a high-grade primary salivary gland cancer composed of an undifferentiated carcinoma accompanied by a prominent non-neoplastic lymphoplasmacytic stroma. It is cytologically and histologically similar in appearance to nasopharyngeal carcinoma. FNA of LEC shows the following features:

- Cellular aspirate
- Syncytial clumps of polyhedral to spindled cells with scant cytoplasm (Fig. 7.19)
- Pleomorphic, vesicular nuclei with distinct nucleoli (Fig. 7.20)
- Abundant small background lymphocytes and plasma cells

Explanatory Notes

Aspirates of LEC are easily recognized as a high-grade cancer. The cytomorphology is fairly unique, and essentially the same as nonkeratinizing nasopharyngeal carcinoma. The presence of a polymorphous lymphoid background and pleomorphic cells with vesicular nuclei and prominent nucleoli can raise a differential diagnosis of a high-grade lymphoproliferative lesion, especially Hodgkin lymphoma.

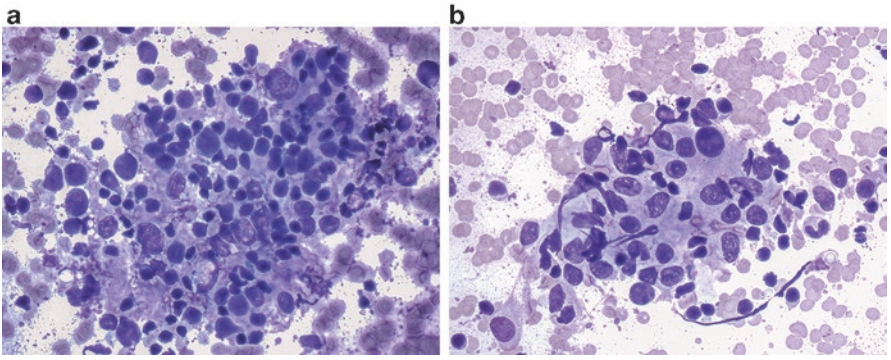

Fig. 7.19 Malignant. (**a**, **b**) FNA of lymphoepithelial carcinoma showing dyshesive and markedly atypical epithelial cells with background lymphocytes (smear, Romanowsky stain)

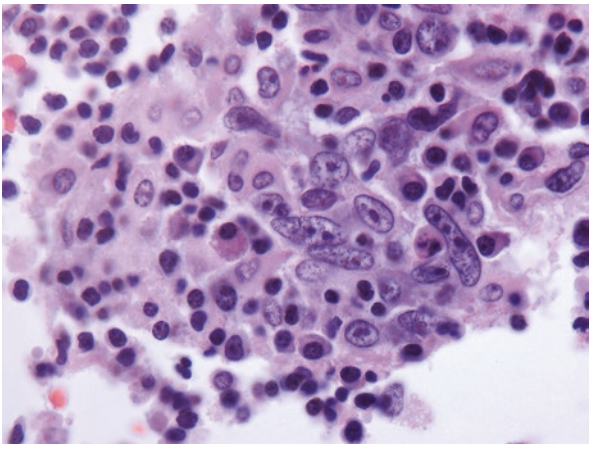

Fig. 7.20 Malignant. Cell block of lymphoepithelial carcinoma showing undifferentiated-appearing epithelial cells in a lymphoid background (H&E stain)

The syncytial clusters of tumor cells in LEC help to distinguish it from Hodgkin lymphoma. Ancillary studies using keratins, p63, and in situ hybridization (ISH) for Epstein–Barr encoded RNA (EBER) can be useful to confirm the cytologic diagnosis of LEC, and to distinguish it from other undifferentiated primary and metastatic tumors.

Carcinoma with High-Grade Transformation

"Dedifferentiation," or the more widely accepted term "high-grade transformation," is defined as the transformation of a well-differentiated tumor into a high-grade malignancy that lacks the distinct histologic characteristics of the original neoplasm [9, 13, 14]. The phenomenon has been described in ACC, AdCC, EMC, polymorphous adenocarcinoma, myoepithelial carcinoma, and SC. Primary salivary gland carcinomas with high-grade transformation follow an especially aggressive clinical course. In some cases, only the high-grade component will be sampled by FNA, and the classification may be limited to "high-grade carcinoma." However, adequately sampled tumors may show features of both the primary tumor and the higher grade component (Fig. 7.21).

Small Cell Neuroendocrine Carcinoma

According to the WHO 2017 classification of head and neck tumors, small cell neuroendocrine carcinoma (SCNC) is a subtype of poorly differentiated carcinoma [7]. It is rare and morphologically similar to its much more common counterparts in the lung and skin (Merkel cell carcinoma). Patients are usually older with a mean

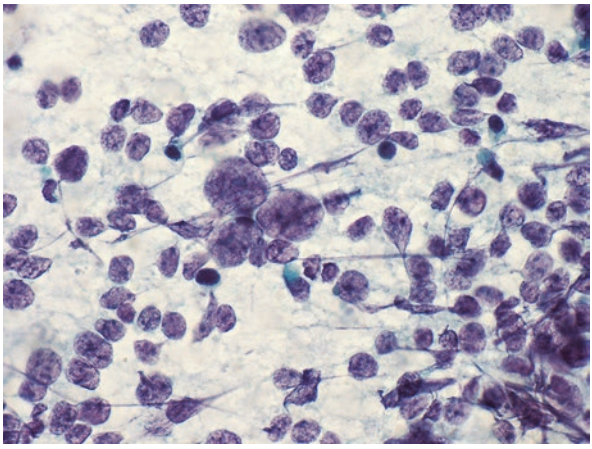

Fig. 7.21 Malignant. This aspirate of an adenoid cystic carcinoma with high-grade transformation shows a population of high-grade pleomorphic tumor cells with an undifferentiated appearance (smear, Papanicolaou stain)

age at presentation in the 5th to 6th decades. SCNC can involve both major and minor salivary glands, but the parotid gland is the predominant site of involvement. Patients typically present with a rapidly growing mass with associated cervical lymphadenopathy and symptoms of facial nerve involvement. SCNC are poorly circumscribed tumors that are typically large (2–5 cm range), and have a poor long-term prognosis.

Cytologic Criteria

SCNC are cytologically identical to small cell carcinomas from other anatomic sites. Aspirates of primary salivary gland SCNC show the following:

- Cellular smears
- Single cells and small clusters
- High N:C ratio cells with scant cytoplasm (Figs. 7.22 and 7.23)
- Oval hyperchromatic nuclei with inconspicuous nucleoli
- Nuclear molding (Fig. 7.24)
- Frequent mitoses, necrosis, and apoptotic blue bodies
- Nuclear streaking and crush artifact

Explanatory Notes

Aspirates of primary salivary gland SCNC are usually readily recognized by the characteristic pattern of high-grade cells with nuclear molding and neuroendocrine nuclear features [23]. Ancillary studies can be used to confirm the diagnosis. The

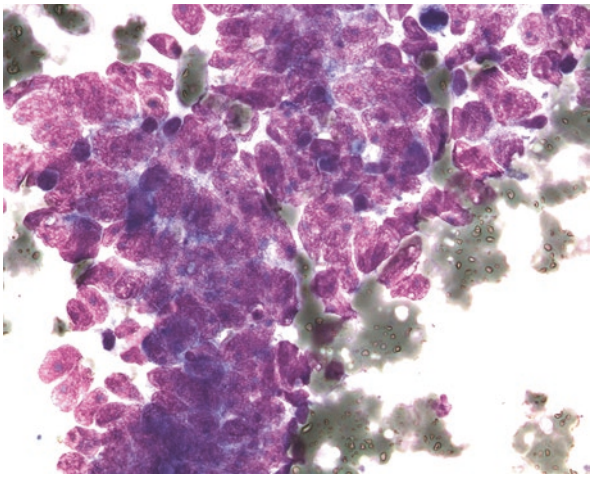

Fig. 7.22 Malignant. This aspirate of small cell carcinoma shows characteristic tumor cells with high N:C ratio, nuclear molding, and scant cytoplasm (smear, Romanowsky stain)

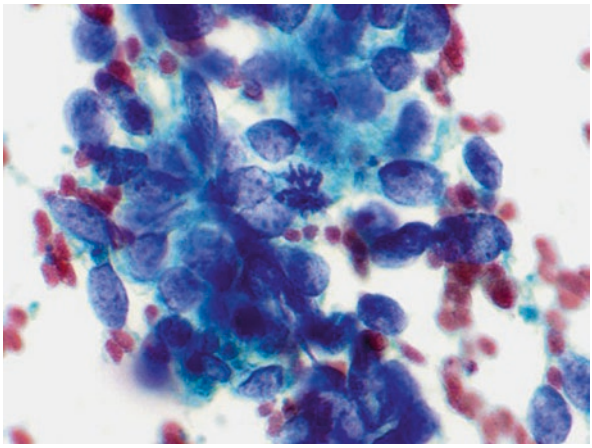

Fig. 7.23 Malignant. This FNA of a small cell carcinoma shows a three-dimensional cluster of tumor cells with high N:C ratio, scant to minimal cytoplasm, mitosis, fine chromatin, and no nucleoli (smear, Papanicolaou stain)

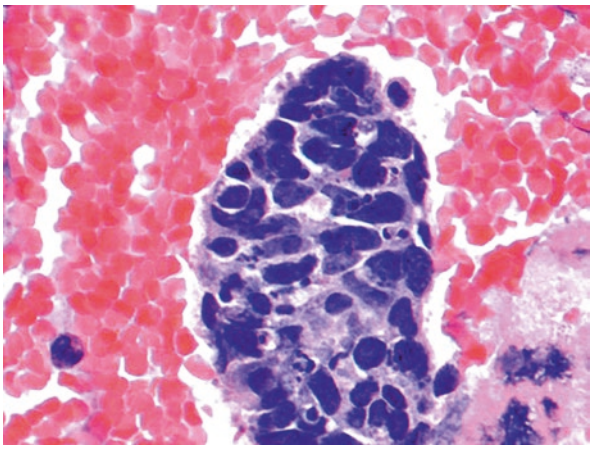

Fig. 7.24 Malignant. This cell block of small cell carcinoma exhibits conspicuous nuclear molding and apoptotic bodies (cell block, H&E stain)

tumor shows dot-like immunoreactivity for keratin, and positive staining with one or more neuroendocrine markers (e.g., synaptophysin, chromogranin, and NSE). Like their cutaneous Merkel cell carcinoma counterpart, SCNC of salivary gland often show dot-like positivity for CK20 [24], but are negative for the Merkel cell polyomavirus. Ki67 shows a very high proliferation index (>50%). The most common differential diagnosis is with metastatic small cell carcinoma, either cutaneous Merkel cell carcinoma or small cell carcinoma from the lung or other anatomic sites. Less often, the differential diagnosis will include other high-grade carcinomas with basaloid features or small round blue cell cancers. A combination of ancillary marker studies and clinical correlation is usually sufficient to resolve the differential diagnosis.

Cancers with Indeterminate or Multiple Grades

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common primary salivary gland malignancy in both adults and children, with a peak incidence in the 2nd decade of life [7, 25, 26]. MEC occur most often in the parotid gland, followed by intraoral minor salivary glands, especially those of the palate. MEC are variably solid and cystic depending upon histologic grade.

Mucoepidermoid carcinomas are graded according to a three-tiered system as low-, intermediate-, and high-grade. The histopathologic grading systems in current use rely on some features that are difficult to appreciate in cytologic samples, such as perineural invasion, lymphovascular invasion, and pattern of invasion, but also include features that can be assessed in cytologic preparations, such as proportion of solid vs. cystic (mucinous) components, presence of necrosis, anaplasia, and mitoses. Using the relative amounts of tumor cells and mucin and the presence of high-grade cytologic features including necrosis, mitoses and nuclear pleomorphism, MEC can often be graded as low-grade and high-grade in FNA samples. While low- and intermediate-grade MEC can often be adequately treated by complete surgical excision, high-grade MEC may require nodal dissection and adjuvant therapy in addition to surgery. The 10-year survival rates for low-, intermediate-, and high-grade tumors are approximately 90%, 70%, and 25%, respectively.

Cytologic Criteria

MEC is a malignant glandular epithelial neoplasm characterized by epidermoid, intermediate, and goblet-type mucus cells which vary in proportion depending upon the histologic grade. In addition, MEC can have columnar, clear, and oncocytic features.

- Cellularity is variable depending on the grade of the tumor
- Admixture of goblet-type mucus cells, intermediate, and epidermoid cells: (Fig. 7.25) low-grade tumors contain more mucinous cells (Figs. 7.26, 7.27, and 7.28) and high-grade tumors have a predominance of epidermoid cells
- Variable nuclear atypia from mild (low-grade) to markedly atypical (high-grade)
- Variable presence of oncocytic cells, clear cells, and columnar cells
- Cystic background with abundant extracellular mucin in low and intermediate grade tumors
- Lymphocytes are present in approximately 20% of cases
- Keratinization is *not* a feature of MEC

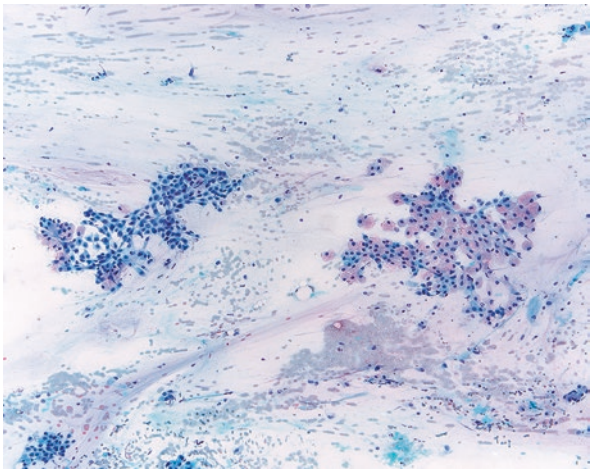

Fig. 7.25 Malignant. FNA of low-grade mucoepidermoid carcinoma. The aspirate contains abundant mucin in the background and loose sheets of bland epidermoid and mucinous cells (smear, Papanicolaou stain). (Courtesy of William Geddie, MD, Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada)

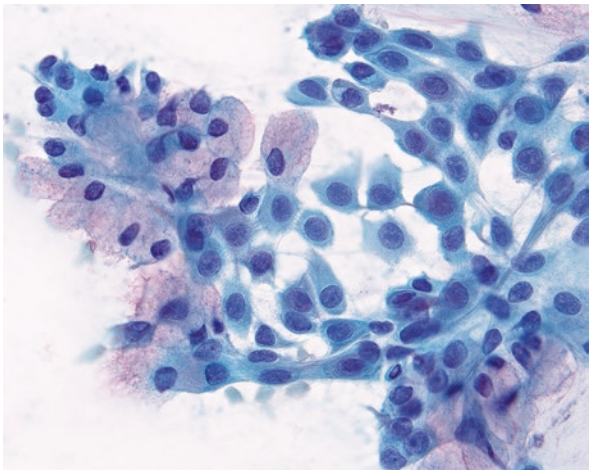

Fig. 7.26 Malignant. This aspirate of low-grade mucoepidermoid carcinoma contains bland epidermoid cells with moderate amounts of dense cytoplasm and well-defined cell borders, while mucus cells contain abundant delicate pink mucinous cytoplasm (smear, Papanicolaou stain). (Courtesy of William Geddie, MD, Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada)

Explanatory Notes

The cytologic features of MEC are dependent on the grade of the tumor. Low-grade MEC usually show abundant background mucin, cyst debris, and few scattered bland epidermoid cells. Low-grade MEC are among the most common cause of a false negative salivary gland FNA, with most cases being diagnosed as a mucocele

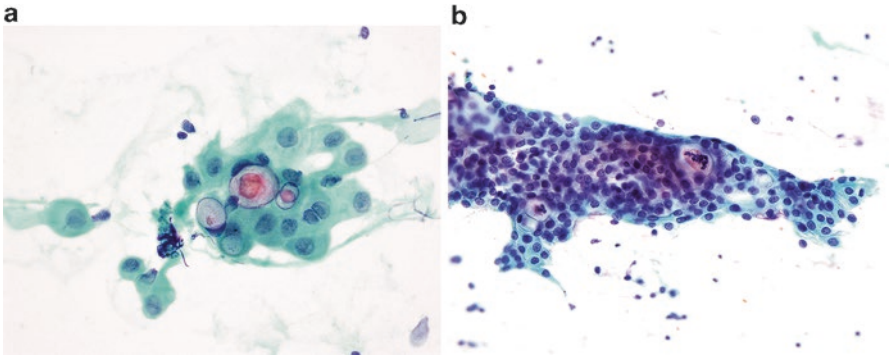

Fig. 7.27 Malignant. (**a**) This aspirate of low-grade mucoepidermoid carcinoma has occasional mucus cells with a large cytoplasmic vacuole indenting the nucleus and occupying the majority of cytoplasm with a central pink mucin droplet; (**b**) FNA of mucoepidermoid carcinoma, low- to intermediate-grade. The aspirate shows a solid sheet of tumor cells, predominantly composed of epidermoid and intermediate cells with occasional interspersed mucus cells (smears, Papanicolaou stain)

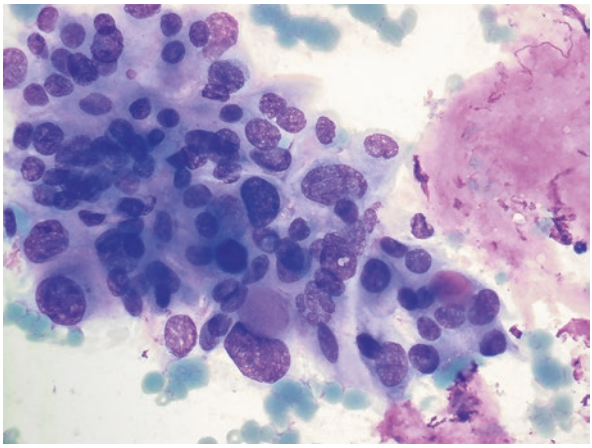

Fig. 7.28 Malignant. FNA of high-grade mucoepidermoid carcinoma showing a cluster of pleomorphic cells with dense cytoplasm and rare interspersed glandular cells with intracytoplasmic mucin. Pink material farthest to the right of the image likely represents thick mucin (smear, Romanowsky stain)

or cyst contents. If only cyst fluid is obtained, the aspirate should be classified as AUS. An effort should be made to aspirate any solid areas of a cystic salivary gland lesion. Any salivary gland aspirate with abundant background mucin should be evaluated with caution, to exclude a low-grade MEC (see Chaps. 2 and 4).

The epidermoid cells of low- and intermediate-grade MEC occur in bland cohesive but crowded sheets with well-defined intercellular borders and dense waxy cytoplasm. Intermediate cells are columnar to polygonal, occur in flat cohesive sheets, and have a higher N: C ratio than epidermoid cells. Goblet-type mucus cells have abundant vacuolated cytoplasm, low N: C ratio, indented eccentrically placed nucleus, and can occur singly, admixed within a sheet of epidermoid cells,

or in clusters. The presence of other cell types including oncocytic cells, clear cells, and columnar cells can cause a diagnostic challenge. Obtaining an adequate sample, combined with ancillary studies, can help. Keratinization is not a feature of MEC and if present, should raise suspicion of SCC (which is usually metastatic) or an adenosquamous carcinoma. Approximately 20% of MEC have abundant background lymphocytes, which together with oncocytic cells and cystic debris can be misinterpreted as Warthin tumor. This is particularly challenging since a subset of Warthin tumors can exhibit squamous metaplasia and/or have a mucoid background.

High-grade MEC yields cellular aspirates with a predominance of markedly atypical epidermoid cells in crowded sheets and clusters. Overt malignant nuclear features are present resembling a high-grade SCC. The presence of rare interspersed goblet cells is a clue to the diagnosis of high-grade MEC (Fig. 7.29; see also Fig. 7.28). The differential diagnosis includes other high-grade carcinomas such as SDC, Ca-ex-PA, primary SCC, and metastatic carcinomas. SDC can be distinguished immunohistochemically using a panel that includes AR, GATA-3, and p63. MEC are positive for p63 and negative for AR and GATA-3. The most common differential diagnoses of high-grade MEC is with either primary or more commonly secondary SCC. Primary SCC of the salivary gland is very rare; most of cases of salivary gland SCC represent metastases to intra or periglandular lymph nodes from a cutaneous head and neck primary. A preceding history of head and neck cutaneous SCC and the lack of mucin-positive epithelial cells can help in the differential diagnosis. The rare adenosquamous carcinoma cannot be reliably differentiated from high-grade MEC based on cytologic criteria alone. However most adenosquamous carcinomas arise in the upper aerodigestive tract and do not affect the major salivary glands.

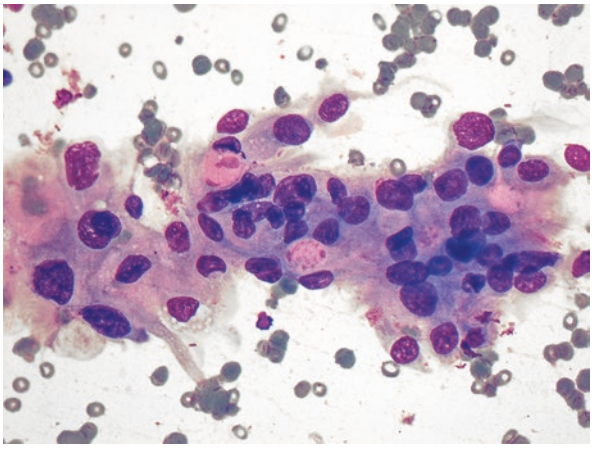

Fig. 7.29 Malignant. FNA of high-grade mucoepidermoid carcinoma with markedly atypical epidermoid cells and occasional interspersed mucinous cells (smear, Romanowsky stain)

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma (AdCC) is a primary salivary gland malignancy, accounting for <10% of all salivary gland tumors [7]. It is a disease of the adult population with a peak incidence in the 4th to 6th decades of life and a slight female predominance. It usually presents as a slow growing, firm mass which can be circumscribed or less well defined. Given the tendency of the tumor to invade nerves, patients often present with facial nerve palsy or pain. AdCC are characterized by a protracted clinical course with slow progression, multiple recurrences, and late metastasis. Three major histologic subtypes of AdCC are recognized: tubular, cribriform, and solid (>30% solid area). Similar to ACC, AdCC can undergo high-grade transformation [25, 27].

Cytologic Criteria

AdCC is a malignant basaloid tumor consisting of epithelial and myoepithelial cells in various morphologic configurations including tubular, cribriform, and solid patterns and a propensity for perineural invasion (Fig. 7.30). Aspirates of AdCC are characterized by:

- Variably cellular aspirate
- Cohesive groups of basaloid cells arranged in small syncytial sheets with irregular borders, sometimes showing microcystic sieve-like spaces, clusters, "cylinders," and tubules (see Fig. 7.30)
- Uniform, small basaloid tumor cells with high N:C ratio (Fig. 7.31)
- Scant, indistinct cytoplasm
- Bland, oval to angulated hyperchromatic nuclei with indistinct nucleoli
- Mitoses and necrosis are uncommon in the absence of high-grade transformation
- Acellular homogenous matrix with sharp borders, best seen on Romanowsky-type stains (magenta); matrix is translucent and therefore less well visualized in Papanicolaou stained smears, and may be absent or sparse in the solid variant (Figs. 7.32, 7.33, and 7.34)

Explanatory Notes

The cribriform type of AdCC is the most common and easiest to recognize in salivary gland aspirates. The cells are monotonous, small, and basaloid and are arranged most often in a sheet or tubular pattern. Nuclei are dark and angulated, and the cells have scant indistinct cytoplasm. Mitoses, necrosis and significant pleomorphism are typically absent. The most important cytologic feature for recognizing an aspirate as AdCC

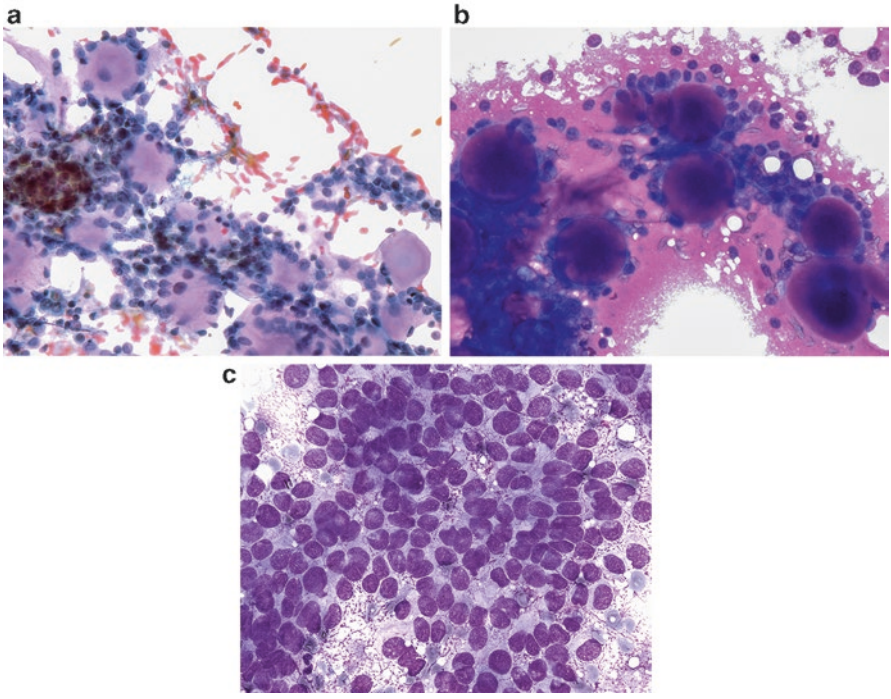

Fig. 7.30 Malignant. Adenoid cystic carcinoma. Aspirates show small high N:C ratio basaloid tumor cells surrounding acellular matrix with: a *cribriform pattern* (**a**) (smear, Papanicolaou stain) and (**b**) (smear, Romanowsky stain); or with a *matrix-poor solid pattern* (**c**) (smear, Romanowsky stain)

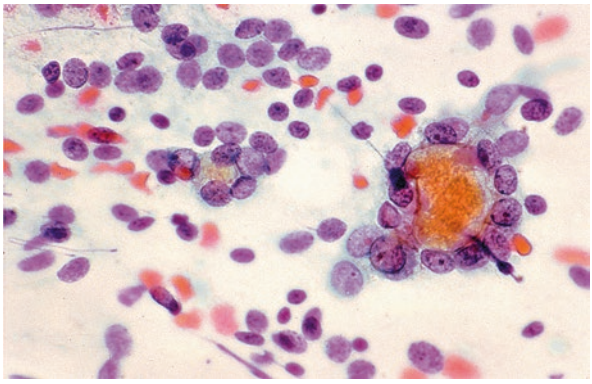

Fig. 7.31 Malignant. This aspirate of adenoid cystic carcinoma shows monotonous basaloid tumor cells, with high N:C ratio, some of which are surrounding pale-staining basement membrane-like material (smear, Papanicolaou stain)

is its characteristic homogenous, acellular, non-fibrillary, and intensely metachromatic matrix, which appears magenta-colored in Romanowsky-type stains. The matrix takes the form of variably sized spheres, cylinders, and branching tubules with sharp edges with or without basaloid cells at their border. The matrix is pale green and translucent and is often difficult to visualize using Papanicolaou-stained preparations.

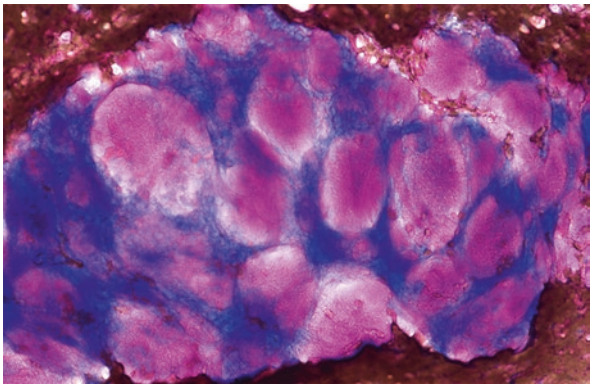

Fig. 7.32 Malignant. This FNA of adenoid cystic carcinoma shows abundant acellular homogeneous matrix with sharp borders. Basaloid tumor cells often form a syncytial smear surrounding the matrix material (smear, Romanowsky stain)

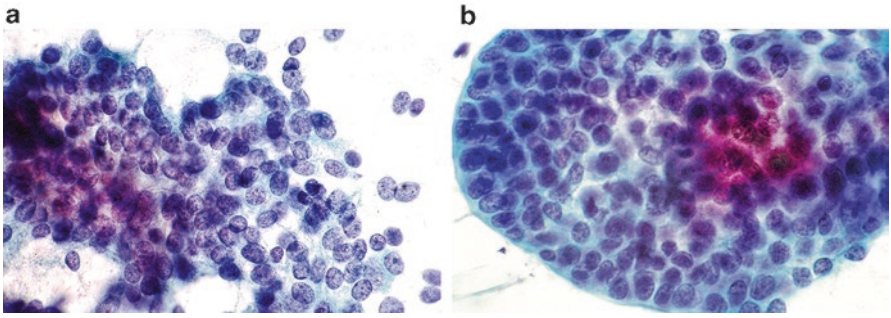

Fig. 7.33 Malignant. (**a**, **b**) FNA of the solid subtype of adenoid cystic carcinoma containing sheets of basaloid tumor cells with no matrix and large and monotonous nuclei with scant cytoplasm (smear, Papanicolaou stain)

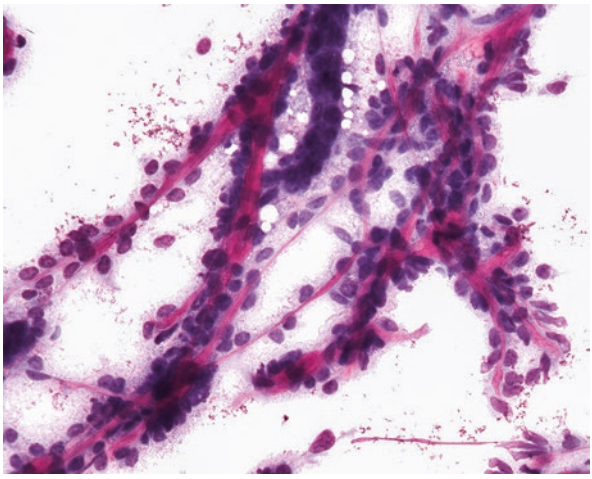

Fig. 7.34 Malignant. This FNA of adenoid cystic carcinoma shows the acellular tubular matrix pattern (smear, Romanowsky stain)

The differential diagnosis of AdCC includes several benign and malignant entities. In contrast to PA, in which the neoplastic cells are embedded in the matrix, the basaloid cells of AdCC surround the matrix with a sharp interface between the cells and matrix. In addition, the matrix of AdCC typically lacks the fibrillary qualities and frayed edges seen in PA. The solid subtype of AdCC is the most difficult to recognize in cytologic specimens. It is composed of sheets of basaloid cells with little or no matrix. This variant may have larger and less monotonous nuclei, overlapping nuclei, visible nucleoli, occasional mitoses, apoptotic bodies and focal necrosis. These features make the diagnosis of the solid subtype of AdCC very challenging. Ancillary studies and a careful search for telltale signs of acellular matrix globules can sometimes be useful. Hyaline globules are not specific for the diagnosis of AdCC and can be encountered in several other entities including polymorphous adenocarcinoma, basal cell adenoma, basal cell adenocarcinoma, epithelial-myoepithelial carcinoma, and even basaloid SCC. AdCC lacks squamous differentiation which is usually present at least focally in basaloid SCC. In addition, basaloid SCC characteristically has higher grade features than AdCC including apoptotic bodies, mitotic figures, conspicuous necrosis, and severe cytologic atypia. Polymorphous adenocarcinoma enters into the differential diagnosis of AdCC for aspirates of minor salivary gland lesions, especially in the palate. In contrast to AdCC, the cells of polymorphous adenocarcinoma are not basaloid. They are a uniform population of polygonal and medium-size cells with moderate amounts of cytoplasm, nuclei with open chromatin, and small distinct nucleoli (Fig. 7.35) [25]. The differential diagnosis of AdCC with basal cell adenomas and adenocarcinomas is among the most challenging in salivary gland cytology (see also Chap. 5). The arrangement of the extracellular basement membrane-like material in some basal cell tumors can be an important clue distinguishing them from AdCC. This is especially true for the membranous subtype of basal cell tumor. However, the overlapping cytomorphologic features in some cases will lead to a diagnosis of Neoplasm: SUMP, or SM. Epithelial-myoepithelial carcinoma is recognized based primarily by its abundance of large clear myoepithelial cells which are not a feature seen in AdCC [15].

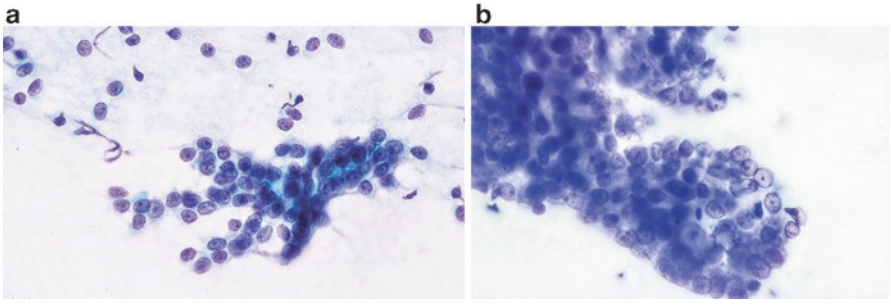

Fig. 7.35 Malignant. (**a**, **b**) FNA of polymorphous adenocarcinoma containing bland tumor cells with moderate amounts of cytoplasm, open chromatin, and pseudopapillary structures with minimal matrix material (smear, Papanicolaou stain)

Obtaining material for ancillary studies can be very helpful for confirming the diagnosis of AdCC. A majority of AdCC are strongly positive for CD117 (c-kit, cytoplasmic), but no mutation in the CD117 gene has been identified. In addition, most AdCC show overexpression of MYB and NOTCH, and a majority of AdCC have a signature chromosomal translocation t(6;9)(q22–23;p23–24) resulting in a fusion involving the v-myb myeloblastosis viral oncogene homolog (MYB) oncogene and the transcription factor gene NFIB (see Chap. 8).

Myoepithelial Carcinoma

Myoepithelial carcinoma (MC) is rare, accounting for <1% of all salivary gland carcinomas. There is no age or gender predilection. A majority of MC occur in the parotid gland where they can arise de novo or as a component of Ca-ex-PA [7, 28]. Patients usually present with a painless enlarging mass of variable duration. MC can range from low- to high-grade, and hence can have variable clinical outcomes; distant metastasis is relatively common.

Cytologic Criteria

MC by definition is composed of cells with myoepithelial differentiation, and represents the malignant counterpart of myoepithelioma. Aspirates of MC show the following:

- Cellular aspirate
- Neoplastic cells arranged singly, in small clusters, sheets, and crowded groups (Fig. 7.36)
- Metachromatic stromal material is variably present as small globules, bands, and spheres (Fig. 7.37)
- Variable nuclear atypia (pleomorphism, nucleoli, mitoses, hyperchromasia), depending upon grade (Fig. 7.38)
- Several cell morphologies including plasmacytoid, spindled, clear, and epithelioid
- Intranuclear pseudoinclusions
- Moderate amounts of glycogen-rich cytoplasm

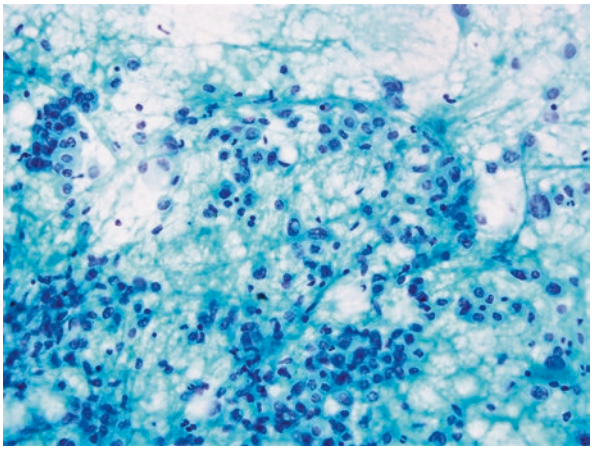

Fig. 7.36 Malignant. FNA of myoepithelial carcinoma. The aspirate is cellular and contains loosely cohesive highly atypical cells with plasmacytoid morphology, nuclear pleomorphism, and distinct nucleoli. Note the presence of delicate stroma in the background (smear, Papanicolaou stain)

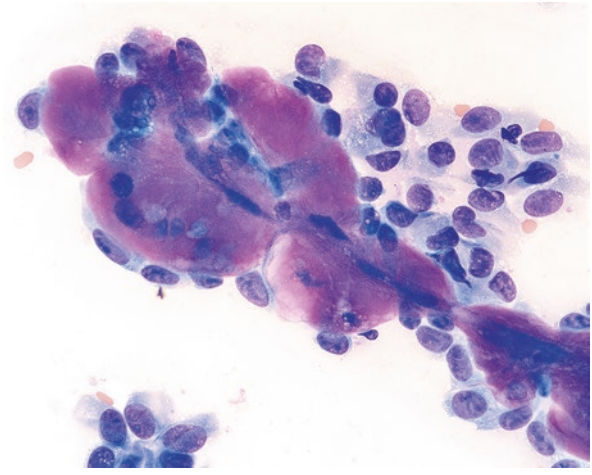

Fig. 7.37 Malignant. FNA of myoepithelial carcinoma. The aspirate contains atypical plamacytoid tumor cells with moderate amounts of cytoplasm, oval nuclei, and acellular matrix material (smear, Romanowsky stain)

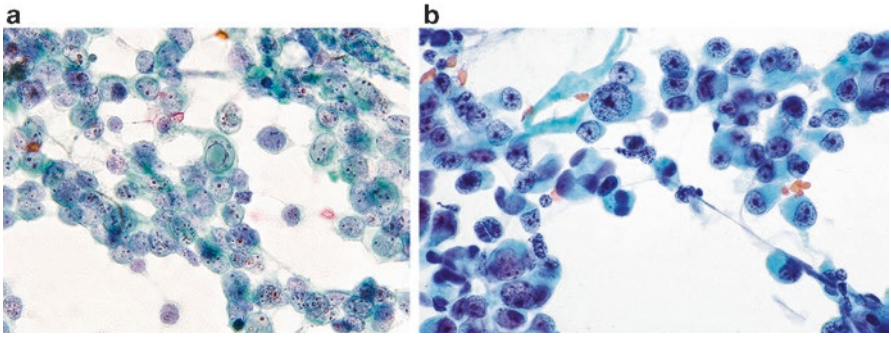

Fig. 7.38 Malignant. (**a**, **b**) FNA of high-grade myoepithelial carcinoma showing pleomorphic plasmacytoid and epithelioid cells with large round to oval nuclei and prominent nucleoli (smear, Papanicolaou stain)

Explanatory Notes

MC is the malignant counterpart of myoepithelioma. Because of this, lower grade forms of MC will be difficult to distinguish from myoepithelioma (or myoepithelial-predominant PA) by FNA, and most will be diagnosed as "Neoplasm: SUMP" (see Chap. 5). Aspirates of MC classified as "Malignant" will be high-grade forms displaying significant nuclear atypia. Material for ancillary studies should be collected to document the homogenous myoepithelial differentiation of the tumor. Since myoepithelial cells are a component of several benign and malignant salivary gland tumors, the differential diagnosis is broad; however, as mentioned above, low- to intermediate-grade forms of MC are generally not recognizable by FNA (see Chap. 5). As described previously for EMC, MC is distinguished by its lack of the characteristic biphasic pattern and laminated stromal component of EMC. Rarely, oncocytic neoplasms may be entertained in the differential diagnosis of MC. Oncocytic tumors have centrally placed nuclei with prominent nucleoli, which are not characteristic of MC. For difficult cases, immunochemistry using a panel of myoepithelial markers can be helpful.

Carcinoma ex Pleomorphic Adenoma

Carcinoma ex pleomorphic adenomas (Ca-ex-PA) comprise 3.6% of all salivary gland tumors, and about 12% of all salivary gland malignancies. They usually occur in the 6th to 7th decade, about a decade later than PA, and are slightly more common in women. Most Ca-ex-PA occur in the parotid gland. Patients typically present with a history of a long-standing mass with recent rapid growth [13]. Most Ca-ex-PA are high-grade carcinomas, and as such, patients may have facial nerve palsy or skin involvement at presentation. The average tumor size can vary from 2 to 4 cm, but may be much larger. Ca-ex-PA are often widely infiltrative, although in situ (non-invasive) forms have also been described [9]. The carcinomatous component of Ca-ex-PA is most often SDC or high-grade adenocarcinoma, NOS, although a wide range of carcinomas can occur in the setting of Ca-ex-PA.

Cytologic Criteria

Defined as an epithelial or myoepithelial malignancy developing from primary or recurrent PA [7, 9]. FNA of Ca-ex-PA shows the following characteristics:

- Cellular aspirate
- Often high-grade carcinoma, usually SDC (Fig. 7.39)
- Focal component of classical PA (Fig. 7.40)

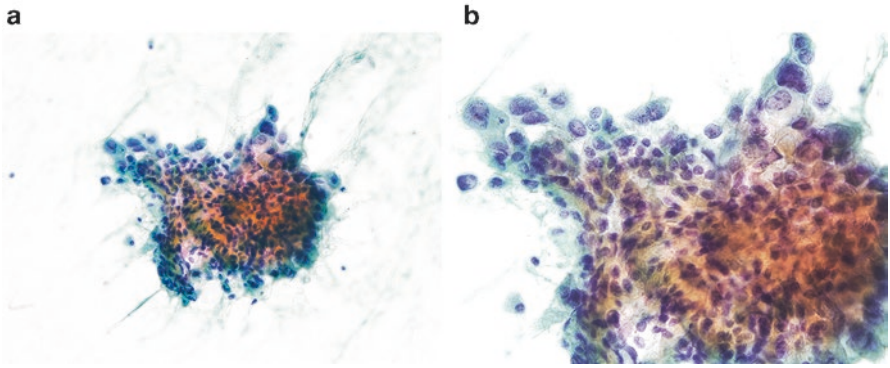

Fig. 7.39 Malignant. (**a**, **b**) FNA of high-grade carcinoma ex pleomorphic adenoma; only the carcinomatous component is seen since in most instances the carcinomatous component overgrows and masks the presence of an underlying pleomorphic adenoma (smear, Papanicolaou stain)

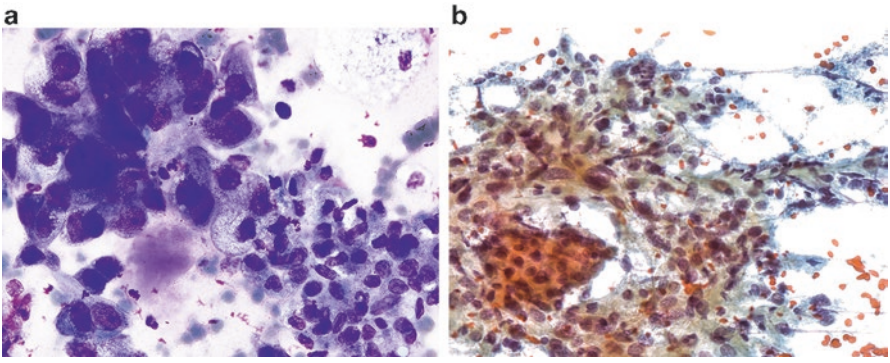

Fig. 7.40 Malignant. FNA of high-grade carcinoma ex pleomorphic adenoma. The aspirates show loose groups of high-grade carcinoma cells. Scant background metachromatic material likely represents residual pleomorphic adenoma. (**a**) (smear, Romanowsky stain) and (**b**) (smear, Papanicolaou stain)

Explanatory Notes

Because the carcinomatous component of Ca-ex-PA usually dominates, the PA component is often not represented in the FNA. In such cases, a diagnosis of high-grade carcinoma, NOS or SDC will usually be made. Therefore, a diagnosis of Ca-ex-PA can only be made with confidence if both the high-grade carcinoma and features of classic PA are present. In addition, FNA cannot distinguish between widely invasive, minimally invasive, and non-invasive forms of Ca-ex-PA; clinical and radiologic correlations are needed.

Hematolymphoid Tumors

Primary salivary gland non-Hodgkin lymphomas constitute 1.7–6% of salivary gland neoplasms and 6–26% of all extranodal lymphomas in the head and neck region [7]. The distinction between primary lymphoma of the salivary gland versus secondary involvement of a periparotid or intraparotid lymph node can be difficult based solely upon cytologic findings [29]. Most primary salivary gland lymphomas are B-cell non-Hodgkin lymphoma. Extranodal marginal zone B-cell lymphoma (EMZBCL) of MALT type is the most common subtype of primary salivary gland lymphomas, and is frequently associated with Sjögren's syndrome. Diffuse large B-cell lymphoma (DLBCL) accounts for 7–27% of all salivary gland lymphomas. The parotid gland is most commonly affected (70%) followed by the submandibular gland (20%). The average age of presentation is in the 6th decade. Up to 10% of cases present with bilateral involvement.

Cytologic Criteria

EMZBCL: A low-grade B-cell lymphoma arising in mucosa-associated lymphoid tissue (MALT). FNA of EMZBCL shows the following features:

- Cellular aspirate
- Polymorphous population of predominantly small to intermediate size lymphocytes, monocytoid B-cells, immunoblasts, lymphoplasmacytic cells, plasma cells (Fig. 7.41)
- Lymphohistiocytic aggregates and tingible body macrophages (see Fig. 7.41)
- Immunocytochemistry shows CD20+, CD5−, CD10−, CD23−, CD43±, Ki67 low
- Flow cytometry shows: CD5−/CD19+, CD19+/FMC7−, CD19+/CD23−, CD19+/CD10−, Bcl1−, Bcl6−, Bcl2+, κ or λ light chain restriction

DLBCL: A high-grade lymphoma composed of large B-cells (i.e., cells with nuclei >2x the size of normal lymphocyte) that has a diffuse growth pattern. Aspirates show the following:

- Cellular aspirate
- Large atypical lymphoid cells (> 2 times the size of mature lymphocytes) (Fig. 7.42)
- Distinct to large nucleoli often present
- Lymphoglandular bodies in the background
- Tingible body macrophages may be present
- Immunocytochemistry shows CD20+, CD45+, PAX5+, CD79a+, Ki67-high
- Flow cytometry: CD5−/CD19+, CD19+/FMC7−, CD19+/CD23−, CD10∓, κ or λ light chain restriction

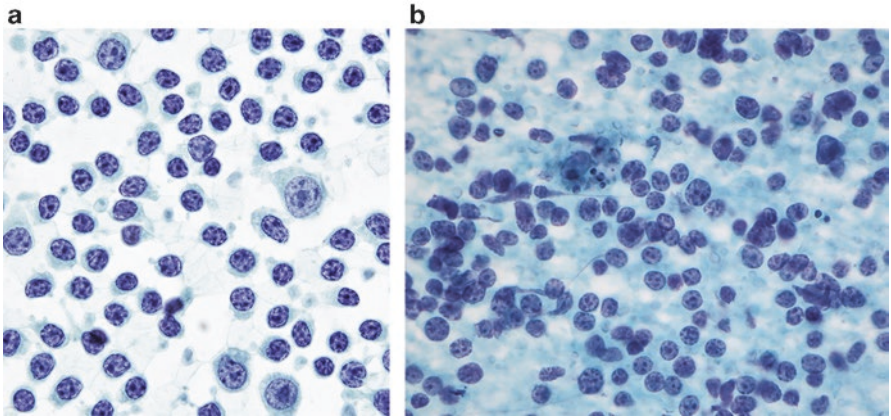

Fig. 7.41 Malignant. (**a**, **b**) FNA of extranodal marginal zone lymphoma. The aspirates contain a dispersed mixed population of small- to intermediate-sized lymphocytes with small amounts of preserved cytoplasm, coarse chromatin, and round to irregular nuclei. Scattered larger lymphocytes and tingible body macrophages are also seen (smear, Papanicolaou stain)

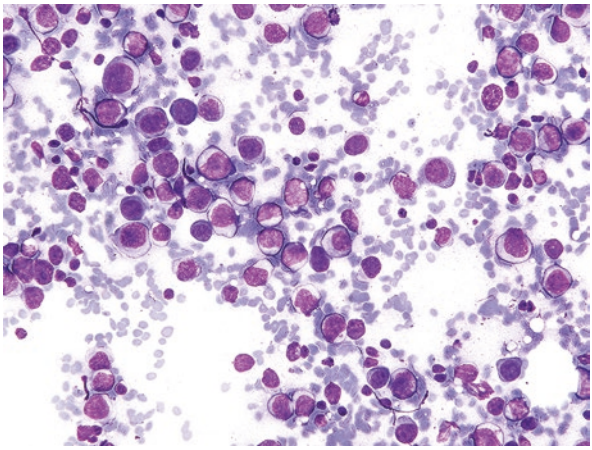

Fig. 7.42 Malignant. FNA of diffuse large B-cell lymphoma containing a dispersed population of large atypical lymphocytes >3 times the size of a small mature lymphocyte (smear, Romanowsky stain)

Explanatory Notes

The differential diagnosis of lymphoproliferative lesions includes both reactive and neoplastic conditions of salivary glands accompanied by a prominent lymphoid reaction. The list of reactive conditions includes chronic sialadenitis, lymphoepithelial sialadenitis (LESA), HIV-associated lymphoepithelial cysts, and, most importantly, reactive lymph nodes. Chronic sialadenitis usually yields a paucicellular aspirate with rare groups of ductal cells and few small mature-appearing B- and T-lymphocytes that are polyclonal by flow cytometry. In contrast, aspirates of lymphomas are typically cellular and include an abundance of background lymphoglandular bodies. In addition to EMZBCL and DLBCL, periparotid and intraparotid lymph nodes can occasionally be involved by other lymphomas such as mantle cell

lymphoma and follicular lymphoma (Fig. 7.43). Aspirates of the latter are usually suggestive of lymphoma, but ancillary studies are required for accurate subclassification. One of the most difficult diagnostic problems when evaluating a lymphoid lesion of the salivary gland is the cytologic distinction between LESA and a low-grade lymphoma such as EMZBCL (see also Chap. 3). The two entities have overlapping cytomorphologic features that include a heterogeneous population of cells composed of polymorphous but predominantly small lymphocytes, tingible body macrophages, follicular dendritic cells, plasma cells, and lymphohistiocytic aggregates. Cytomorphology alone cannot reliably distinguish between these two entities, and flow cytometry or some other means of immunophenotypic analysis is necessary to make the distinction, and would be needed prior to classifying an aspirate as lymphoma. It is therefore essential to collect material for ancillary studies including flow cytometry. Consultation with a pathologist having subspecialty experience in hematopathology can also be very useful.

Aspirates of DLBCL are usually readily recognized cytomorphologically as malignant, but in some cases, the differential diagnosis will also include other malignant neoplasms composed of small cells such as melanoma, small cell carcinoma, and certain sarcomas. The recognition of lymphoglandular bodies in the background can provide a helpful clue to the diagnosis. Obtaining material for ancillary studies, including a directed immunochemical panel for cytokeratin, CD45, CD20, and S100, among others, can be used in difficult cases to resolve the differential diagnosis. Flow cytometry can be informative as well; however, caution is warranted in the interpretation of flow cytometry since a significant subset of DLBCLs will yield a negative flow cytometry result.

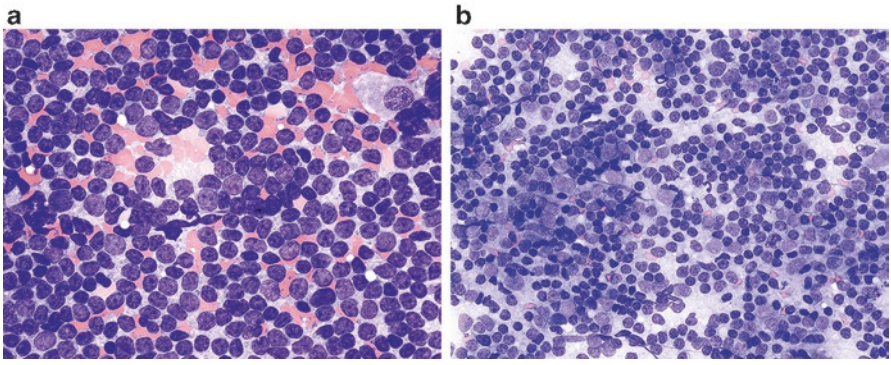

Fig. 7.43 Malignant. These FNAs of (**a**) mantle cell lymphoma and (**b**) follicular lymphoma show very atypical cytomorphologic features suggestive of lymphoma, but ancillary studies are needed for accurate subclassification of the lymphomas (smear, Romanowsky stain). (Courtesy of William Geddie, MD, Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada)

Secondary Malignant Tumors

Metastatic tumors constituted 7.5% of all non-hematolymphoid malignant salivary gland neoplasms in an Armed Forces Institute of Pathology (US) series, and most cases presented as a solitary salivary gland mass [7]. In the majority of cases, there was a known history of a non-salivary gland primary cancer. The parotid gland, in particular intraparotid and periparotid lymph nodes, is involved 20 times more often than the submandibular gland. The peak incidence of a secondary malignant salivary gland tumor is in the 7th to 8th decade with almost 70% occurring in men. Eighty percent of the metastatic tumors to the parotid gland are from head and neck sites, especially cutaneous carcinomas of the face and scalp, while 85% of metastatic tumors in the submandibular gland are from distant sites [20, 30]. Cutaneous SCC is the most commonly diagnosed secondary tumor of the parotid gland, followed by melanoma. Secondary salivary gland tumors from distant sites include those from lung, breast, and kidney.

Cytologic Criteria

Aspirates of secondary cancers involving the salivary gland exhibit the following cytologic features:

- Cellular aspirate
- Usually high-grade nuclear features
- The cytomorphologic characteristics depend upon the tumor type; most common metastases are: SCC, melanoma, or cancers from distant sites (lung, breast, kidney) (Figs. 7.44 and 7.45)

Explanatory Notes

Squamous cell carcinoma (SCC) is the most common tumor metastatic to salivary glands. Aspirates are usually cellular and include atypical squamous cells and keratin debris in a necrotic background. In some cases, there may be a cystic background. In contrast to MEC, metastatic SCC lack evidence of intracellular mucin and are usually keratinizing. Most cases occur in older patients who have a known history of a cutaneous SCC; primary SCC of the salivary glands is very rare. Aspirates of metastatic melanoma can have a wide range of cytomorphologic appearances. The classic FNA of metastatic melanoma shows a population of dyshesive pleomorphic cells with eccentric nuclei, prominent nucleoli, and granular pigment in the cytoplasm. Intranuclear inclusions are also a common finding. Amelanotic melanoma or spindle cell melanomas in

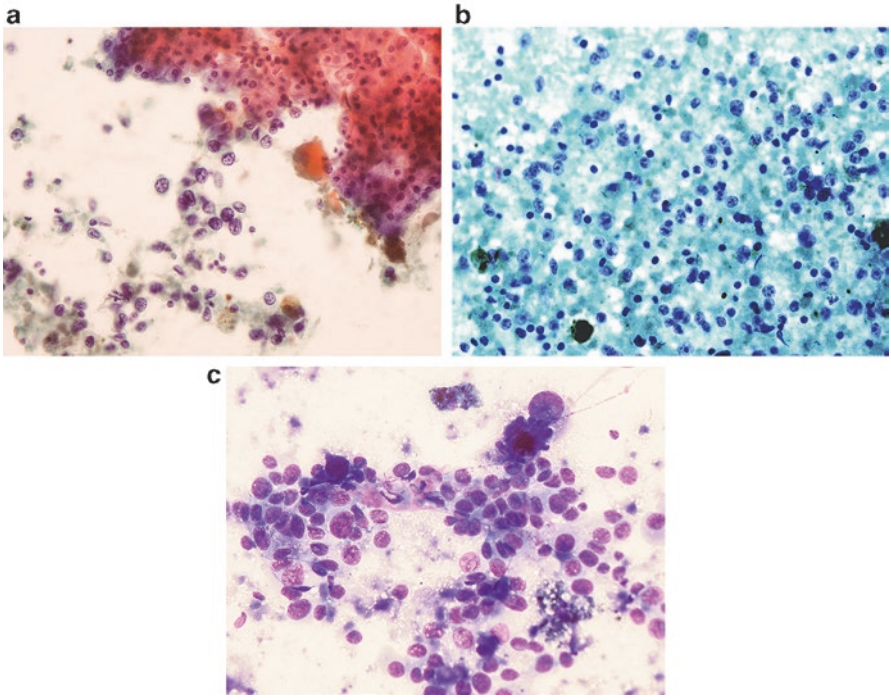

Fig. 7.44 Malignant. These aspirates of metastatic melanoma show the characteristic dyshesive FNA pattern of pleomorphic cells as well as background melanophages with fine brown melanin pigment. (**a**, **b**) (smear, Papanicoloau stain) and (**c**) (smear, Romanowsky stain)

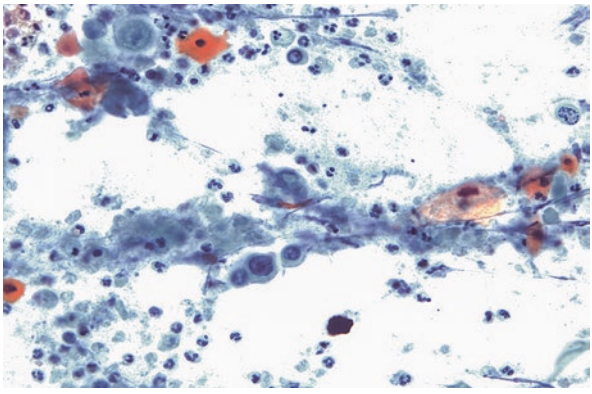

Fig. 7.45 Malignant. Metastatic squamous cell carcinoma. The cellular aspirate shows high N:C ratio cells as well as dyskeratotic orangeophilic cells in a background of necrotic debris (smear, Papanicolaou stain)

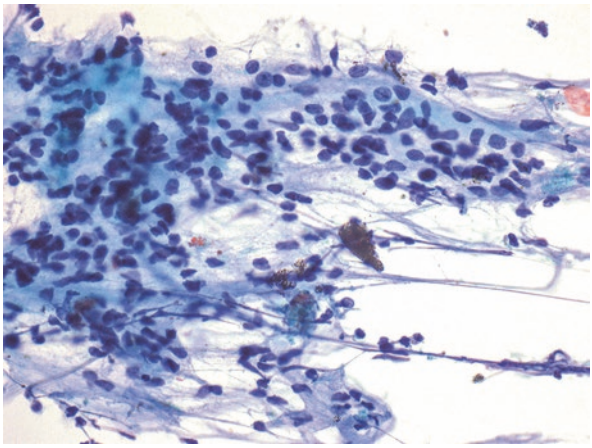

Fig. 7.46 Malignant. FNA of a malignant spindle cell neoplasm (myxoid sarcoma) of the parotid gland (smear, Papanicolaou stain)

the absence of a corroborating history can be misdiagnosed as poorly differentiated carcinoma or sarcoma. Material for ancillary studies can be useful for any case where the cytomorphology does not match that of a primary salivary gland tumor, or for cases where there is a history of a non-salivary gland primary malignancy. This is especially true for cases where the patient has a history of melanoma.

Malignant Mesenchymal Tumors

Primary salivary gland soft tissue tumors are rare; benign tumors are more common than malignant ones. Of the wide variety of soft tissue tumors that involve the parotid gland, benign vascular neoplasms (hemangiomas) are the most frequent. The reader is referred to other sources for a detailed description of soft tissue tumor cytology (Fig. 7.46) [31].

Clinical Management

A definitive classification of a specific malignant salivary gland tumor including its grade provides important information for clinical decision making (see Chap. 9). The grade of the cancer will often be useful to the clinician in determining the extent of surgery. This may include the need to perform a neck dissection, and the potential need to sacrifice a large nerve. For high-grade salivary gland cancers involving the deep lobe of the parotid, a total parotidectomy would be necessary. In addition, identifying a cancer as primary versus metastatic would also have implications for the managing clinician. Patients with metastatic disease to parotid gland lymph nodes often require a concurrent neck dissection. If a lesion is metastatic from a non-cutaneous source, PET-CT may be indicated to locate a primary site of origin.

Sample Reports

Example 1:
Satisfactory for evaluation
MALIGNANT
Keratinizing squamous cell carcinoma. See note.
Note: Since primary squamous cell carcinomas of salivary glands are exceedingly rare, a comprehensive clinical examination including a detailed history and skin examination should be performed to rule out a metastasis from a cutaneous or mucosal head and neck primary.

Example 2:
Satisfactory for evaluation
MALIGNANT
High-grade carcinoma, consistent with salivary duct carcinoma. See note.
Note: The aspirate is cellular and shows high-grade pleomorphic cells arranged in cribriform and papillary groupings with prominent nucleoli and background necrosis. Immunostains performed on the corresponding cell block sections are positive for androgen receptor, GATA-3, and Her2/neu.

Example 3:
Satisfactory for evaluation
MALIGNANT
High-grade carcinoma. See note.
Note: The aspirate is cellular and shows pleomorphic cells arranged in cribriform and papillary groupings with prominent nucleoli and background necrosis. The cytomorphologic findings are suggestive of salivary duct carcinoma; however, ancillary testing could not be performed due to a paucity of tumor cells in the corresponding cell block sections.

Example 4:
Satisfactory for evaluation
MALIGNANT
Adenoid cystic carcinoma. See note.
Note: The aspirate is cellular and shows basaloid cells with scant cytoplasm, and angulated dark nuclei arranged around homogenous, magenta-colored matrix spheres. A FISH study showed *MYB* (6q23) rearrangement supporting the diagnosis of adenoid cystic carcinoma.

Example 5:
Satisfactory for evaluation
MALIGNANT
High-grade carcinoma-ex-pleomorphic adenoma (Ca-ex-PA). See note.
Note: The aspirate shows high-grade pleomorphic tumor cells with prominent nucleoli, anisonucleosis, and rare mitotic figures; separate foci of bland cells embedded within chondromyxoid matrix are also present. Ancillary studies for PLAG1 are positive by immunohistochemistry. The overall findings are consistent with a high grade Ca-ex-PA.

Example 6:
Satisfactory for evaluation
MALIGNANT
High-grade carcinoma. See note.
Note: The aspirate shows high-grade pleomorphic tumor cells with prominent nucleoli, anisonucleosis, and rare mitotic figures with scant chondromyxoid matrix in one slide. In the context of the patient's history of a longstanding mass with recent rapid increase in size, a diagnosis of carcinoma-ex-pleomorphic adenoma (Ca-ex-PA) is favored.

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Chapter 8
Ancillary Studies for Salivary Gland Cytology

Marc Pusztaszeri, Jorge S. Reis-Filho, Fernando Carlos de Lander Schmitt, and Marcia Edelweiss

General Background

A precise cytologic classification of salivary gland tumors based on cytomorphology alone is possible for many of the commonly encountered lesions; however, there are challenges for the cytologic diagnosis of some entities. Ancillary tests have become invaluable tools that assist in refining our cytologic diagnoses, and recent advances have improved the diagnostic accuracy of salivary gland fine-needle aspiration (FNA), leading to better patient management. A subset of tumors has been characterized cytogenetically by the presence of specific and recurrent translocations (see Table 10.3, Chap. 10, Histologic Considerations and Salivary Gland Tumor Classification in Surgical Pathology) [1–5]. These translocations and their resulting fusion oncogenes and oncoproteins can be used as diagnostic markers in salivary gland FNA [3–12]. In this chapter, we describe the ancillary techniques and several currently available ancillary markers for salivary gland FNA with a practical approach covering the most common diagnostically challenging scenarios.

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_8

Ancillary Techniques on Cytology

Different methods, including special histochemical stains, immunochemistry (IC), fluorescent in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), next generation sequencing (NGS), and flow cytometry (FC), can be successfully applied to FNA material to improve the diagnostic accuracy for many salivary gland tumors (SGT) [3–14]. Most of these methods can be readily integrated into the diagnostic workflow, particularly as they become more widely available, cost-effective, and efficient with shorter turnaround times [3, 4]. While many of the immunocytochemical and molecular techniques can be applied to a variety of cytologic preparations, including alcohol-fixed or air-dried smears, cytospins, and liquid-based preparations, their application to formalin-fixed, paraffin-embedded (FFPE) cell block material is considered the most reliable [3, 4, 8]. This is because most biomarkers have been validated using FFPE tissue blocks, and most of the primary studies involving the use of cytological samples for molecular analysis have relied on FFPE cell blocks. Cell blocks have the advantage of being analogous to paraffin tissue blocks, with minimal need of standardization and with reliable results. In addition, cell blocks have the advantage over other cytologic preparations by being able to produce a number of nearly identical samples for cases where a panel of IC stains will be anticipated. In contrast, cytologic preparations are generally superior to FFPE cell block sections or FFPE tissue sections for FISH because the issue of section based nuclear truncation that can lead to inaccuracy in signal visualization and counting is avoided by having the probe hybridize directly to intact cells on a glass slide.

For practical purposes, FISH is most useful for confirming a specific diagnosis that is strongly favored by the clinical, cytomorphologic, and/or immunophenotypic features of the tumor, but it has limited ability to definitively exclude a diagnosis in many cases [3, 4, 9, 10]. When positive, FISH analysis can confirm a diagnosis, even on samples with few cells: a suspected malignancy from a FNA can be confirmed using FISH to assess for a specific gene rearrangement (see sample reports). The overexpression of translocation-associated proteins and/or downstream target proteins can be assessed using IC and can serve as a diagnostic surrogate for the molecular alterations discussed above [3–8, 11, 12]. Since IC for the fusion protein is usually more sensitive but less specific than FISH analysis, it can be used as a triage tool before FISH testing (see sample reports).

Special Stains

Histochemical stains are often used to highlight stromal or cytoplasmic components. Periodic acid-Schiff (PAS) and PAS with diastase (PAS-D) can be used to highlight the zymogen granules in the granular cytoplasm of acinic cell carcinoma (ACC). These stains also detect intracytoplasmic mucin, which can be found in a

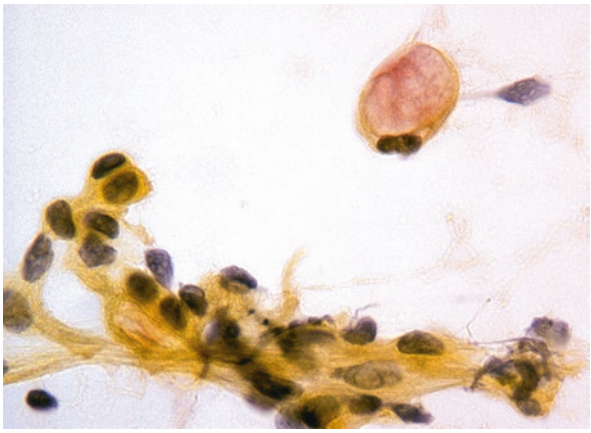

Fig. 8.1 Mucoepidermoid carcinoma**.** Mucicarmine histochemical stain highlighting a mucin-positive goblet cell

variety of SGT, most notably mucoepidermoid carcinoma (MEC) and secretory carcinoma (SC) (mammary analogue secretory carcinoma [MASC]). Other mucin stains include mucicarmine stain for neutral mucin (Fig. 8.1) and alcian blue pH 2.5 for acid mucin. Oil Red O remains one of the best stains to confirm sebaceous differentiation by highlighting lipid droplets in unfixed cells.

Immunochemistry

IC can be used to help narrow the differential diagnosis in challenging cases. IC on FNA material should be interpreted with caution and in the context of cytomorphological features. In addition, a panel approach to the use of IC in salivary gland cytology is recommended as opposed to using a single immunostain.

Immunochemistry for Basaloid Neoplasms

Aspirates of basaloid neoplasms present a very broad and challenging differential diagnosis (see Chap. 5). IC can be useful in narrowing the differential diagnostic possibilities. Table 8.1 summarizes the most common IC profiles for selected basaloid SGT. Among them, the distinction between pleomorphic adenoma (PA) and adenoid cystic carcinoma (AdCC) is probably the most critical given the significant clinical and prognostic implications.

While myoepithelial cell markers are not specific for a particular diagnosis, they can be useful in a variety of circumstances to demonstrate the presence of a minor or predominant myoepithelial component in a SGT. SGT containing a myoepithelial component include several benign and malignant tumors: PA, myoepithelioma, myoepithelial carcinoma, basal cell adenoma (BCA), basal cell adenocarcinoma

Table 8.1 Most common immunoprofiles for selected basaloid salivary gland tumors

	Immunostains									
Diagnosis	P63	P40	SMA	Calponin	S100	C-kit (CD117)	LEF-1	PLAG1	HMGA2	MYB
Pleomorphic adenoma	+[a]	+[a]	+[a]	+[a]	+[a]	±	±	+	±	−
Basal cell adenoma/basal cell adenocarcinoma	+[a]	+[a]	+[a]	+[a]	−[b]	±	+	−	−	−
Adenoid cystic carcinoma	±	±	+[a]	+[a]	+[a]	+	−	−	−	+
Myoepithelioma/myoepithelial carcinoma	+	+	+	+	+	−	−	±	±	−
Epithelial myoepithelial carcinoma	+	+	+	+	+	−	−	−	−	−
Polymorphous adenocarcinoma	+	−	−	−	+	±	−	±	−	−

SMA smooth muscle actin, *LEF-1* lymphoid enhancer-binding factor, *PLAG1* pleomorphic adenoma gene 1, *HMGA2* high-mobility group AT-hook 2

[a]Abluminal cells

[b]Stromal cells can be S100 positive

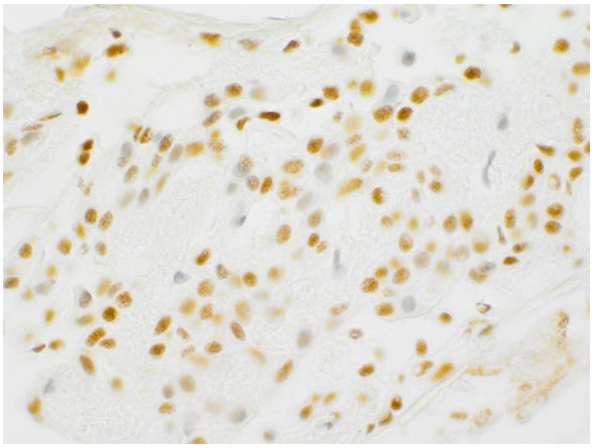

Fig. 8.2 Pleomorphic adenoma. PLAG1 immunostain showing strong nuclear expression in the tumor cells in a cell block (Courtesy of Jeffrey F. Krane, MD, PhD, Brigham and Women's Hospital, Boston MA, USA)

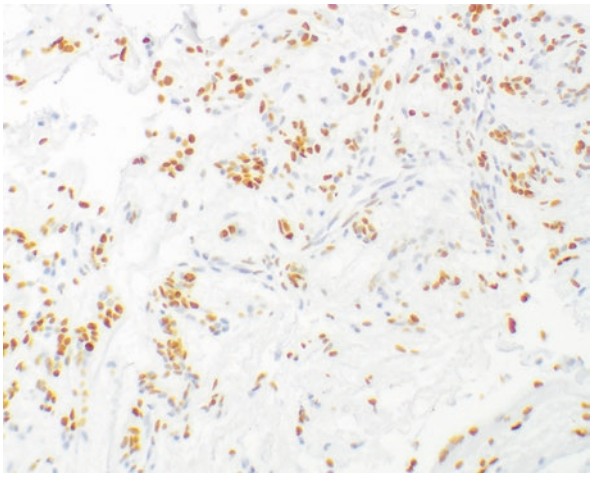

Fig. 8.3 Pleomorphic adenoma. HMGA-2 immunostain showing moderate nuclear expression in the tumor cells in a cell block (Courtesy of Jeffrey F. Krane, MD, PhD, Brigham and Women's Hospital, Boston MA, USA)

(BCAdc), AdCC, epithelial-myoepithelial carcinoma, and—to a limited extent—polymorphous (low-grade) adenocarcinoma (PACA). Generally, a panel of immunochemical stains is used to demonstrate myoepithelial cells, including p63, p40, keratin 5/6, glial fibrillary acidic protein (GFAP), and S100, as well as more specific myoid markers such as smooth muscle actin (SMA) and calponin.

Several more specific immunomarkers are useful for addressing the differential diagnosis of basaloid SGT. In order to increase the sensitivity and specificity in distinguishing various basaloid neoplasms, an IC panel consisting of MYB, CD117 (c-KIT), pleomorphic adenoma gene 1 (PLAG1), HMGA-2, β-catenin, and lymphoid enhancer-binding factor 1 (LEF-1) can be helpful (Figs. 8.2, 8.3, 8.4, and 8.5) [3, 4, 6, 8, 11, 12, 15, 16]. Most PAs with or without a *PLAG1* gene rearrangement are immunoreactive for PLAG1 (see Fig. 8.2) [8]. PLAG1 is also positive in myoepitheliomas, which is considered a myoepithelial-predominant variant of PA by

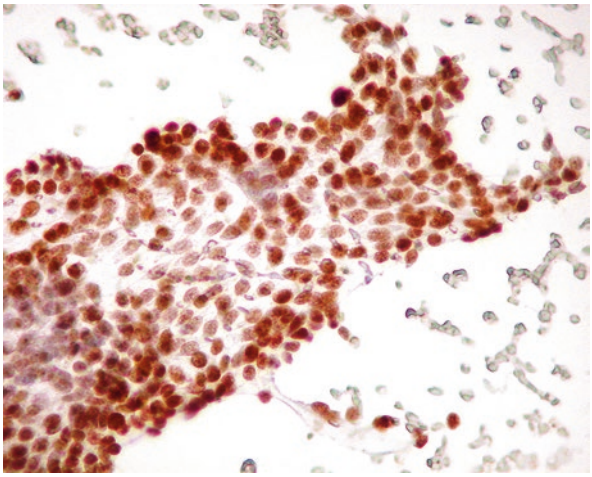

Fig. 8.4 Adenoid cystic carcinoma. MYB immunostain showing strong nuclear expression in the tumor cells in a cytologic smear

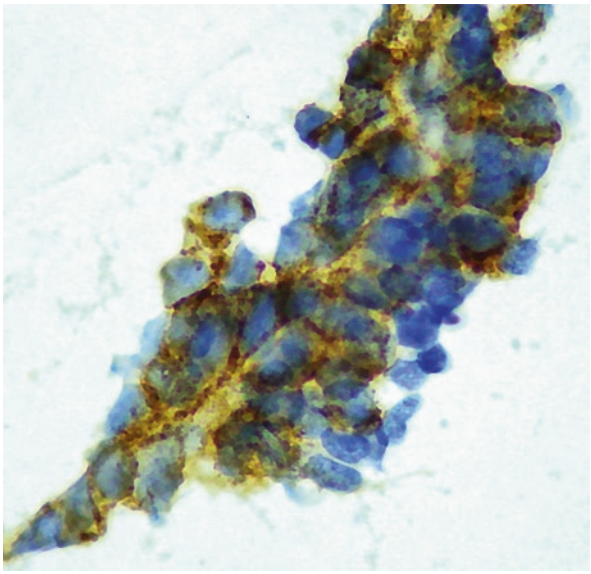

Fig. 8.5 Adenoid cystic carcinoma. CD117 immunostain showing strong cytoplasmic expression in the tumor cells in a cytologic smear

some authors, and in a subset of PACA [8]. In contrast, PLAG1 is usually negative in other SGT, including AdCC, MEC, and ACC. HMGA2 is positive in about 20% PA (see Fig. 8.3), and is usually negative in other SGT, although myoepitheliomas may be positive [8]. MYB is a useful marker for AdCC, since most AdCC, with or without the *MYB-NFIB* fusion transcript, are positive. In cytologic preparations, a majority of AdCC shows strong immunoreactivity for MYB (see Fig. 8.4), while other SGT are usually negative or only focally positive [8, 11, 12]. In addition to MYB, over 90% of AdCC are strongly and diffusely positive for CD117 (c-KIT) (see Fig. 8.5) [17].

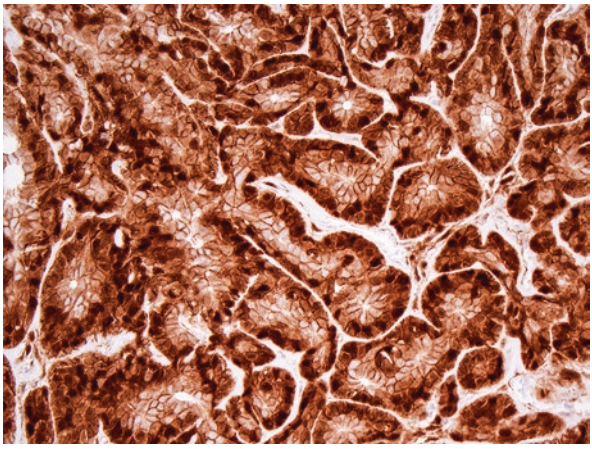

Fig. 8.6 Basal cell adenoma. β-catenin immunostain showing strong nuclear expression in the tumor cells (Courtesy of Vickie Y. Jo, MD, Brigham and Women's Hospital, Boston MA, USA)

The main discriminating feature for BCA and BCAdc is the presence or absence of invasion as detected by histologic evaluation. Therefore, it is difficult to distinguish these two entities by FNA. In addition, the immunohistochemical phenotypes for BCA and BCAdc are relatively similar [6, 15]. BCA and BCAdc are both positive for nuclear β-catenin and for its coactivator LEF-1 (i.e., coexpression) in approximately 40% to 80% of cases, depending upon the study (i.e., various cutoffs and antibodies used) [15, 16]. Nuclear β-catenin expression in BCA is commonly strong and diffuse, and predominant in the basal component (Fig. 8.6), while it is generally more moderate and focal in BCAdc [15]. In terms of specificity, nuclear β-catenin and LEF-1 expression are also common in certain non-SGT such as cutaneous basal cell carcinoma, pilomatrixoma, and some odontogenic tumors, and LEF-1 expression has also been reported in some cases of squamous cell carcinoma (SCC) [6]. At the molecular level, 30% to 80% of BCA have *CTNNB1* mutations, while BCAdc show a different and sometimes more complex genomic profile, including activating mutations in *PIK3CA,* usually without *CTNNB1* mutation despite β-catenin expression [15, 18]. The uncommon membranous subtype of BCA/BCAdc, which is associated with *CYLD1* gene alterations, is also less likely to show β-catenin or LEF-1 expression.

Immunochemistry for Oncocytic/Oncocytoid Neoplasms

Oncocytic SGT detected by FNA have a broad differential diagnosis (see Chap. 5). IC can be used to narrow the diagnostic possibilities. Table 8.2 summarizes the most common IC in SGT with oncocytic features. A limited IC panel consisting of DOG1, SOX10, and p63 is recommended for separating ACC from Warthin tumor (WT), MEC, and oncocytoma [19–21]. DOG1 and SOX10 are markers of acinar and

Table 8.2 Most common immunoprofiles in salivary gland tumors with oncocytic features

Diagnosis	Immunostains							
	P63	P40	S100	MGB	SOX10	DOG1	GATA3	AR
Warthin tumor/oncocytoma	+	–	–	–	–	–	–	–
Acinic cell carcinoma	–	–	–	–	+	+	–	–
Secretory carcinoma	–	–	+	+	+	–	+	–
Mucoepidermoid carcinoma	+	+	–	–	±	±	–	–
Salivary duct carcinoma	–	–	–	±	–	–	+	+

MGB mammaglobin, *DOG1* discovered on GIST1, *GATA3* GATA binding protein 3, *AR* androgen receptor

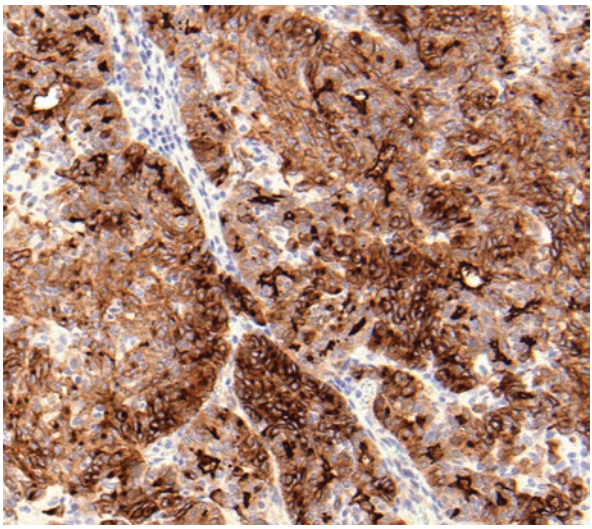

Fig. 8.7 Acinic cell carcinoma. DOG1 immunostain showing strong cytoplasmic expression in the tumor cells in a cell block.

intercalated duct differentiation in SGT, and both are characteristically strongly and diffusely positive in ACC (Figs. 8.7 and 8.8). In isolation, SOX10 is also a marker of myoepithelial cells. DOG1 and SOX10 are predominantly negative in WT, oncocytoma, oncocytic carcinoma, SC, and MEC. Conversely, p63 typically shows diffuse expression in MEC, including its oncocytic variant, and is negative in ACC. While WT and MEC both show reactivity for p63 & p40, the distribution of positive cells differs—a single basal layer of cells in WT and a more diffuse pattern in MEC. S100, GATA-3, and mammaglobin are useful to support the diagnosis of SC, since other oncocytic neoplasms in the differential diagnosis are usually negative for those IC markers [19, 22, 23]. Recently, overexpression of STAT5a, which may be related to the *ETV6-NTRK3* translocation, has also been shown to be positive in SC, and can be assessed on cytological material using IC [24]. For difficult

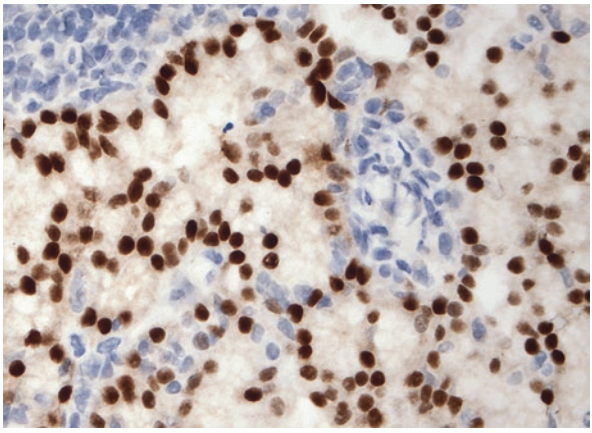

Fig. 8.8 Acinic cell carcinoma. SOX10 immunostain showing strong nuclear expression in the tumor cells in a cell block

Table 8.3 Most common immunoprofiles for salivary gland tumors with clear cell features

	Immunostains				
Diagnosis	P63	P40	S100	SOX10	DOG1
Myoepithelioma/myoepithelial carcinoma	+	+	+	−	−
Epithelial myoepithelial carcinoma	+	+	+	−	−
Acinic cell carcinoma	−	−	−	+	+
Mucoepidermoid carcinoma	+	+	−	±	±

or indeterminate cases, the most definitive diagnostic markers of SC and MEC (including its oncocytic variant) are the presence of specific translocations [9, 23].

Immunochemistry Markers in Clear Cell Neoplasms

Several SGTs, in particular, several of the SGTs with oncocytic features above, can also have clear cell morphology (see Chap. 5). The use of IC with the same panel of markers described for oncocytic SGT can be useful; Table 8.3 summarizes the most common IC results in SGT with clear cell features. In addition, clear cell carcinoma is a rare low-grade salivary gland carcinoma that is often positive for p63, but lacks myoepithelial differentiation and also lacks intracellular mucin [25]. Epithelial-myoepithelial carcinoma is characterized by a predominant population of myoepithelial cells displaying an unusually large amount of clear cytoplasm. A panel of IC to demonstrate the myoepithelial nature of the clear cells combined with a marker for ductal cells such as keratin AE1.3 or EMA is helpful to demonstrate the biphasic pattern of the tumor (Fig. 8.9).

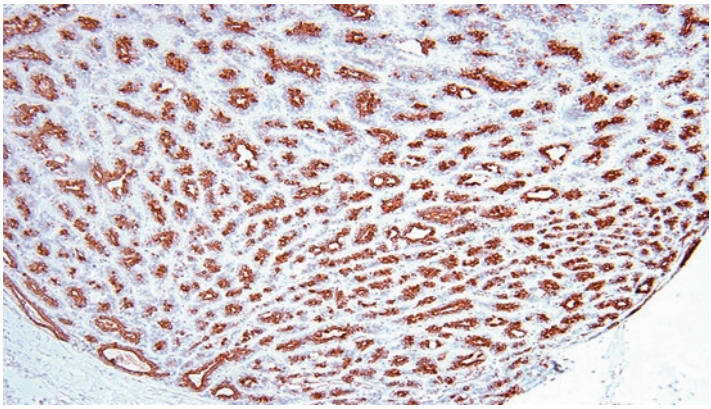

Fig. 8.9 Epithelial-myoepithelial carcinoma. Pancytokeratin immunostain showing the biphasic pattern of the tumor

Immunochemistry for Primary vs. Secondary Salivary Gland Tumors

High-grade carcinomas in the salivary glands are usually easily recognized as malignant; however, the distinction between primary and secondary malignancy can occasionally be problematic yet clinically important. A limited panel of immunostains can be very helpful to distinguish a primary SGT from a metastasis (Table 8.4). Most patients with a secondary malignancy of the salivary gland have a clinical history. SCC is the most common secondary metastasis, frequently from a cutaneous source. Histochemical staining for intracellular mucin is used to distinguish metastatic SCC from MEC that is positive. In addition, androgen receptor, GATA-3, and p63 are very helpful to distinguish salivary duct carcinoma from its cytomorphologic mimics, especially metastatic nonkeratinizing SCC. More than 95% of salivary duct carcinoma are positive for androgen receptor and GATA-3, but negative for p63 [26]. A focused IC panel depending upon the known primary site should be used. The most common distant metastases to the salivary gland are lung, breast, and kidney (see Table 8.4).

Translocations and Fusion Oncogenes in Salivary Gland Tumors

SGT currently known to harbor recurrent genetic alterations are summarized in Chap. 10, Table 10.3. With ongoing advances in molecular diagnostics, other SGT as well as additional molecular alterations are likely to join this list. Although some of these genetic alterations can be found in various tumors, including SGT

Table 8.4 Common immunomarkers to suggest a site of origin for salivary gland metastatic carcinomas[a]

Immunomarker	Probable site of origin[a]
CDX-2 and SATB-2	Enteric
TTF-1	Lung, thyroid
Napsin A	Lung
ER and PR	Breast, Müllerian[b]
PAX-8	Kidney, Müllerian, thyroid
CD10 and RCC	Kidney
PSA and PHAP	Prostate[c]
Thyroglobulin	Thyroid
Hep Par-1 and glypican 3	Hepatocellular
GATA-3	Breast, urothelial, others[d]
p63, p40 and cytokeratin 5/6	Squamous or urothelial
GCDFP15 and MGB	Breast[e]

Transcription factors are in bold (nuclear staining)
TTF-1 thyroid transcription factor-1, *ER* estrogen receptor, *PR* progesterone receptor, *RCC* renal cell carcinoma, *PSA* prostate specific antigen, *PHAP prostatic acid phosphatase, GATA3* GATA binding protein 3, *GCDFP15* gross cystic disease fluid protein 15, *MGB* mammaglobin
[a]Immunomarkers are best used as a panel and in conjunction with clinicoradiological data; several of these markers can also be expressed in primary salivary gland carcinomas.
[b]Can be expressed in a wide variety of other carcinomas
[c]A subset of salivary duct carcinomas and oncocytomas are also PSA positive
[d]A subset of carcinomas from the skin and a subset from the salivary gland are also positive
[e]A subset of primary salivary gland carcinomas (e.g., mammary analogue secretory carcinoma are also positive)

analogues from other organs, they are highly specific in the spectrum of primary SGT, representing powerful diagnostic markers in histological specimens as well as in FNA material [1–4]. The absence of a given genetic rearrangement, however, may not exclude a particular SGT, as its prevalence may vary significantly between different SGT. In addition to their diagnostic role, in some cases these genetic translocations can also represent prognostic markers and therapeutic targets [1, 2].

Salivary Gland Tumors with Specific Molecular Features

A specific translocation t(3;8)(p21;q12) involving *PLAG1* and one of several other fusion partners, the most common being *CTNNB1*, the gene encoding β-catenin can be found in 50%–60% of PA [1, 2]. Within SGT, the *PLAG1* and *HMGA2* gene rearrangements are present only in PA and carcinoma ex pleomorphic adenoma (Ca-ex-PA) and have not been found in any other SGT. A specific translocation

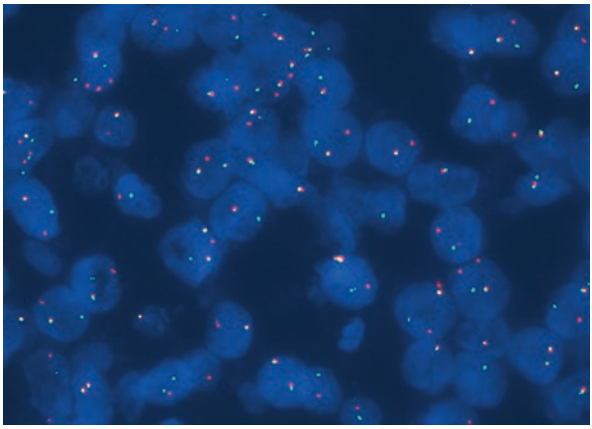

Fig. 8.10 Secretory carcinoma of salivary glands. Fluorescent in situ hybridization (FISH) showing rearrangement of the *ETV6* locus (separation of red and green signals) (Courtesy of Joaquin J. Garcia, MD Mayo Clinic, Rochester MN, USA)

t(11;19) (q14–21;p12–13), involving the *CRTC1* (*MECT1*) gene at 19p13 and the *MAML2* gene at 11q21, has been reported in approximately 60%–70% of MEC [1, 2]. The translocation is more often found in low- to intermediate-grade MEC. The presence of this translocation is also associated with fewer recurrences, metastases, and tumor-related mortality. The translocation is considered to be a reliable diagnostic and prognostic biomarker for MEC [1, 2]. Detection of a *MAML2* rearrangement by FISH has the potential to be useful in cytologic samples and small biopsies to confirm a diagnosis of MEC [9]. One limitation, however, is that hypocellular FNA specimens may lack sufficient cellularity for testing. The specific translocation t(6;9)(q21–24;p13–23), involving *MYB* and *NFIB* genes, is found in up to 86% (range: 28%–86%) of AdCC [1, 2]. Furthermore, both MYB and NFIB overexpression occur in most AdCCs, including those without the *MYB-NFIB* fusion, suggesting that other molecular regulatory mechanisms are likely involved. SC is characterized by the specific translocation t(12;15)(p13;q25), leading to a fusion between *ETV6* and *NTRK3* (Fig. 8.10) [1, 2]. The latter is a hallmark of SC since it is found in nearly 100% of cases, and has not been reported in any other primary SGT. Of note, a subset of SC shows ETV6 rearrangements with an unknown partner.

Hyalinizing clear cell carcinoma (HCCC) is a rare SGT that can be difficult to classify by FNA [25]. However, given its well established low-grade nature, correct classification and distinction from other primary SGT is important. HCCC is characterized by the specific translocation t(12;22)(q13;q12) generating an *EWSR1-ATF1* fusion gene, which is present in approximately 85% of cases [1, 2]. A definite diagnosis of HCCC relies on the demonstration of the specific *EWSR-1* rearrangement that is not present in other clear cell SGT, except for a subset (35%) of clear cell myoepithelial carcinomas and rare epithelial-myoepithelial carcinoma (9%). The latter two entities can be distinguished in part by their IC profiles.

PACA is a SGT primarily arising in minor salivary glands of the oral cavity, particularly the palate. Because of its low-grade behavior, it is very important to distinguish it from AdCC [27]. The majority of PACA harbor a *PRKD1* E710D

mutation or one of the PRKD gene family (*PRKD1*, *PRKD2*, or *PRKD3)* rearrangements, which have not been found in other SGT [28]. The presence of the *PRKD1* mutation has been significantly associated with metastasis-free survival.

Fluorescent in Situ Hybridization (FISH)

In contrast to other molecular methods, in situ-based detection of nucleic acids has the advantage of providing useful diagnostic information within the context of the cytomorphology rather than histology. Currently there are two major ways of assessing DNA copy number/rearrangement status in situ—fluorescent-based methods (FISH) and bright-field-based methods—chromogenic in situ hybridization (CISH). Since most of the clinically relevant genetic alterations in SGT are rearrangements generating gene fusions, and FISH is superior to the other ISH techniques for demonstrating rearrangements, this section will focus only on the detection of DNA by FISH. The success of the FISH technique lies with proper execution of the assay and interpretation of the results. The use of cytological material has the advantage of not having truncated nuclei due to sectioning, but cell-blocks can also be used with the same adaptations used for histological sections. Dual-observer scoring is recommended due to the intraobserver and interobserver variations, and use of internal and external quality controls are strongly advised.

Polymerase Chain Reaction (PCR)

The core principle of PCR is the amplification of a DNA region of interest. Material from different cytological preparations is an excellent source for PCR analysis, and 50 to 100 cells are adequate to obtain good PCR results. One of the most used applications of PCR is the study of gene expression, including the production of fusion transcripts based upon the ability of PCR to amplify RNA. PCR is much more sensitive than FISH for detecting different translocations; however, it is not able to detect unknown molecular variants, which can be detected by FISH analysis.

Flow Cytometry (FC)

FC is a technique that measures the physical and immunological properties of intact cells in suspension. In salivary gland FNA, FC is primarily used to characterize lymphoproliferative lesions (see Chaps. 3 and 7) [13, 14]. Unfixed FNA material can be processed for FC after the filtration of small cell aggregates. A morphological assessment of the FNA material before processing it for FC, using a cytospin, for example, is highly desirable. If the number of cells is limited, tailored antibody

panels should be designed based upon clinical features, patient history, and specimen source.

Given that the diagnosis of lymphoid lesions in aspirates of the salivary gland has significant limitations using cytomorphology alone, FC can be extremely useful in distinguishing reactive conditions from lymphoma [13, 14]. For B-cell lymphomas, the demonstration of a clonal population based upon the presence of kappa or lambda light chain restriction as well expression of Bcl2 is diagnostic. The presence of an altered T-cell immunophenotype also can be used to suggest a possible T-cell lymphoma. In a series of 61 cases, Stacchini et al. showed that a combination of cytology and FC could diagnose and classify lymphoid proliferations in salivary gland FNA with a sensitivity of 100% and specificity of 83% [14]. FC is also able to detect the presence of non-lymphoid neoplastic cells in an FNA.

Sample Reports

Example 1:
Satisfactory for evaluation
NEOPLASM: SALIVARY GLAND NEOPLASM OF UNCERTAIN MALIGNANT POTENTIAL (SUMP)
Basaloid neoplasm. See note
Note: Immunochemical staining is positive for PLAG-1, and negative for β catenin, MYB, and CD117 (focal weak) with low Ki-67. This immunoprofile combined with the cytomorphologic findings favors a diagnosis of pleomorphic adenoma. FISH for PLAG-1 rearrangement could be useful for further evaluation if clinically warranted.

Example 2:
Satisfactory for evaluation
SUSPICIOUS FOR MALIGNANCY
Basaloid neoplasm suspicious for carcinoma. See note.
Note: Immunostains are positive in tumor cells for MYB and CD117, and negative for PLAG1 and β-catenin. Combined with the cytomorphologic findings, the features are suspicious for adenoid cystic carcinoma. FISH for MYB rearrangement could be helpful to confirm the diagnosis, if clinically indicated.

Example 3:
Satisfactory for evaluation
MALIGNANT
Oncocytoid neoplasm consistent with acinic cell carcinoma. See note.
Note: Immunochemical staining is positive in tumor cells for both DOG1 and SOX10, and negative for mammaglobin and p63. Combined with the cytomorphologic findings, the overall features are consistent with acinic cell carcinoma.

Example 4:
Satisfactory for evaluation
MALIGNANT
Secretory carcinoma. See note.
Note: The presence of the specific t(12;15) translocation, demonstrated by FISH analysis, supports the diagnosis of secretory carcinoma.

Example 5:
Satisfactory for evaluation
NON-NEOPLASTIC
Reactive lymph node. See note.
Note: The combined cytomorphologic findings and benign flow cytometry favor a reactive lymph node. If lymphadenopathy persists, repeat sampling would be indicated for further evaluation.

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Chapter 9
Clinical Management

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General Background

The heterogeneity of salivary gland disease presents unique challenges for the pathologist, radiologist, and treating clinician in their pursuit of optimal patient care. Clinical history, physical exam, and information provided by imaging studies such as ultrasound, contrast-enhanced computed tomography (CT) Fig. 9.1 [1], or magnetic resonance imaging (MRI) with contrast as well as fine-needle aspiration (FNA) all contribute to the development of a management plan that can range from observation to limited or extensive surgical resection and possible adjuvant therapy [2–6]. FNA has a well-established role in salivary gland diagnostics. Cytomorphology is able to provide valuable information regarding the nature of the salivary gland lesion. FNA is quick, and well tolerated with very few complications. It also lends itself to rapid on-site evaluation (ROSE) when used in conjunction with clinical assessment and imaging studies, and can significantly improve triage of the patient for definitive therapy [3].

An understanding of the diagnostic challenges that cytopathologists face when assessing a salivary FNA can be extrapolated from the World Health Organization (WHO) classification of salivary neoplasms, which has over 40 different entities based on histological features [7]. Because of significant morphologic overlap of some entities, it is unavoidable that at times only a morphological description of the

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_9

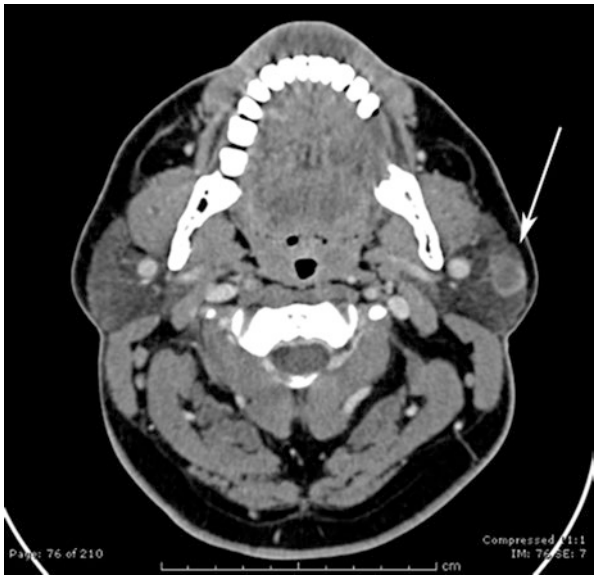

Fig. 9.1 Axial CT with intravenous contrast of a superficial left parotid gland tumor. The mass measures 1.2 cm, has sharp margins, and shows slight enhancement. Fine needle aspiration of the mass showed a pleomorphic adenoma (From Faquin and Powers [1], with permission)

FNA will be provided to the treating clinician without a specific diagnosis [2]. This mandates that a clear line of communication exists between cytopathologist and the treating clinician to ensure that the patient receives the correct management. It is in this context that a uniform reporting system for salivary gland cytology is most beneficial. The clinical utility of *The Milan System for Reporting Salivary Gland Cytopathology* to surgical specialists can be summarized as follows:

- Standardizes reporting and clarity of communication
- Correlates and stratifies the cytologic diagnosis with a risk of malignancy (ROM)
- Facilitates the use of a management algorithm
- Is relevant, transferable, and practical for institutions with variable experience and expertise in salivary gland cytology
- Facilitates quality assurance review and clinical audits by setting standards (e.g., the proportion of inadequate samples less than 10%) as well as providing a potential outcome measure for further research

Clinical Management Considerations: Overview for the Parotid and Submandibular Glands

There are several key questions that the clinician should address when developing a clinical management strategy for salivary gland lesions:

- Do I need any additional information, clarification, or staging radiologic scans prior to formulating a definitive treatment plan?

  - For masses involving the parotid gland, nearly all patients should have cross-sectional imaging performed preoperatively (CT or MRI with contrast). This is done to determine the extent of the lesion (superficial and/or deep lobe involvement) and the probability of complete resection of the primary tumor with facial nerve preservation in cases where this is possible. In a few patients with small (1 cm or less), well-defined lesions that are lateral in the parotid gland and with a benign cytologic diagnosis (i.e., "Neoplasm: Benign"), cross-sectional imaging may not be necessary. Patients with clinical scenarios that indicate the possibility of nerve involvement by tumor should undergo specific assessment for cranial nerve involvement (using MRI and/or CT). Patients with malignant disease should also have imaging that assesses the regional lymph node groups (CT or MRI with contrast), and the most likely sites of distant metastasis should be studied (CT of chest with contrast or skull base to mid-thigh positron emission tomography [PET]/CT).

- Does this case need to be discussed in a multidisciplinary setting with early involvement of the medical or radiation oncologist for treatment planning?
  - In both small and large institutions, the use of a multidisciplinary discussion should be considered for any salivary gland lesion that is not unequivocally benign.
- Does the lesion need to be surgically removed or can it be safely monitored clinically?
  - In certain scenarios, asymptomatic benign lesions with a low risk of malignant transformation, such as a Warthin tumor or a deep lobe pleomorphic adenoma in an elderly patient, may be managed by clinical observation. This can include selected cases when the patient wishes to avoid the possible risk of facial nerve injury.
- If I am considering monitoring the lesion, do I need any further investigations to be sure that this is a safe option?
  - Some lesions may require serial imaging or repeat FNA. This will vary, depending on the individual patient scenario. Tumors in locations not easily assessed on physical exam could be imaged serially until a "growth rate" is determined, at which time the interval between studies may be lengthened. Tumors with indeterminate cytology (e.g., "Salivary Gland Neoplasm of Uncertain Malignant Potential [SUMP]") that appear to be benign based on their clinical presentation could undergo repeat FNA after a period of observation. Lastly, benign or indeterminate tumors under observation that show a change in their clinical status, such as rapid growth after a period of stability or the onset of new symptoms such as pain or facial nerve weakness, should undergo repeat FNA to help further define the evolving tumor.
- When surgical intervention is indicated, what is the minimal necessary procedure needed to adequately manage the tumor?

  - The presurgical evaluation should address the possibility of postoperative facial nerve dysfunction and contour defect that may be required to completely remove the tumor and leave the patient with the smallest possible risk of recurrence. In the case of parotid malignancies, the procedure may span the spectrum from superficial parotidectomy to subtotal or total parotidectomy. In all cases, the facial nerve is preserved unless it is impossible to separate it from the tumor without leaving gross disease behind. In cases of malignancy, when considering nerve sacrifice, a balance must be reached between the morbidity of resection and the possibility of eventual therapeutic failure and patient mortality if gross disease is left behind to be controlled with adjuvant radiation or chemoradiation.

- Do I need to consent the patient for an increased risk of nerve injury or sacrifice and the donor site morbidity of a nerve graft?
  - This topic is the centerpiece of the process of informed consent. For patients with large but clearly benign tumors, the low risk of permanent and significant nerve injury should be discussed. In any patient with the possibility of malignancy, the potential of nerve sacrifice, graft harvest, nerve defect reconstruction and nerve transfer should be discussed with the patient. The possibility of eyelid procedures as well as static procedures to maintain midface tone should also be discussed.

- Is a neck dissection indicated?
  - Patients with clinical evidence of cervical lymph node involvement will undergo therapeutic neck dissection in nearly all cases. Patients without known neck disease may undergo elective neck dissection, depending upon either the preoperative FNA evaluation, or the findings of intraoperative frozen section, or both. The authors accept that the use of frozen section is highly variable internationally and needs to be interpreted by an expert pathologist; however, it may facilitate the management decision. The best time to perform a neck dissection is at the time of primary site surgery. Alternatively, the decision of how to manage the neck and the treatment modality (neck dissection versus radiation) may be made after formal histological assessment of the primary lesion. Patients who do not have a diagnosis of malignancy prior to surgery, due either to an inaccessible site of lesion for FNA or an equivocal cytological diagnosis, may have the decision made to proceed with neck dissection based upon the intraoperative frozen section diagnosis rendered on the primary parotid lesion. Patients with low-grade malignancies such as low-grade mucoepidermoid carcinoma can be followed clinically without neck dissection if the clinical and radiological evaluations both indicate that the neck is free of metastatic disease. Patients with higher grade pathology (e.g., salivary duct carcinoma or high-grade mucoepidermoid carcinoma) are candidates for elective selective neck dissection.

- Will I require the use of intraoperative frozen section to address prior indeterminate cytology such as “Atypia of Undetermined Significance (AUS),” “Neoplasm: SUMP,” “Suspicious for Malignancy,” or “Non-Diagnostic” FNA?

– In some institutions intraoperative frozen section is used as an important adjunct to the preoperative cytological diagnosis. This involves sending a partial parotidectomy specimen containing the entire tumor to an expert pathologist. It is important not to breach the capsule by performing an incisional biopsy, as this risks tumor spillage and the associated increased risk of recurrence. When used, frozen section has a role in the assessment of the completeness of surgical resection margins and clearance of nerve margins in cases with nerve invasion. Frozen section can be helpful in clarifying what may have been an equivocal cytological diagnosis by defining the histologic classification, tumor grade, and extent of invasion. Clinicians are cautioned that frozen sections have their own sets of artifacts and limitations to consider. The impact on decision making on neck management is addressed in the prior section.

Management Options by Milan System Diagnostic Category

Non-Diagnostic

Management

- Repeat FNA. If the first FNA was by palpation, then consider ultrasound guidance (USG).
- If the second FNA is also Non-Diagnostic despite USG and adequate sample preparation, consider alternative investigations. First, perform cross-sectional imaging with contrast enhanced CT or MRI if not already obtained. Second, if the MRI or CT or clinical picture shows features concerning for malignancy or if there is still doubt as to the nature of the lesion, consider USG core needle biopsy (CNB), open biopsy (both controversial due to the inherent risk of tumor spillage), or formal surgical excision.
- If the sample is "cyst contents only," completely aspirate the cyst contents under USG. If a solid component remains, it should be resampled. If the lesion disappears completely, then repeat US +/− FNA in 3–4 months. The FNA would be repeated in cases where US shows a recurrent lesion.

Non-Neoplastic

The majority of "Non-Neoplastic" lesions are managed non-surgically.

Management

- Lesions that are clearly non-neoplastic on FNA may be followed with either serial physical examinations, cross-sectional imaging, or a combination of both to assure stability. Any change in either the clinical exam or imaging could warrant repeat sampling to confirm no change in cytological status.

- USG for the FNA is important for non-neoplastic cases to help avoid sampling errors, which are not uncommon in this diagnostic category. If the FNA findings do not provide sufficient diagnostic information to explain clinical and radiologic findings, repeat FNA; the possible use of CNB, open biopsy, or surgical resection could be considered.
- MRI or CT is useful to assess the lesion serially and to assess regional lymph nodes.

Atypia of Undetermined Significance (AUS)

Management

- Repeat FNA. If the first FNA was performed by palpation, then consider USG FNA.
- Regular clinical follow-up with duration interval to be determined based upon clinical suspicion; every 3–6 months is a general rule of thumb.
- Cross-sectional imaging with contrast enhanced MRI or CT.
- CNB, open biopsy, or surgical resection should be considered for this lesion when the clinical presentation is concerning for malignancy. Examples would be a painful mass that lacks signs of inflammation, a concurrent facial nerve weakness or paralysis, or a prior history of cutaneous malignancy.

Neoplasm

- Benign
- Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)

Management (General)

- Complete resection of the tumor with a narrow cuff of normal tissue as a margin (this can be as narrow as 1–2 cell layers thick). For tumors with an unequivocal preoperative FNA diagnosis of "Benign," no frozen section is necessary.
- Given the increased risk of a low-grade malignancy in the SUMP category, surgical resection is indicated. Intraoperative frozen section can be used for more definitive histologic classification and to help inform any decisions pertaining to possible neck dissection. For SUMP parotid lesions, the type of parotidectomy will depend upon the size and location of the tumor. However, a nerve dissecting parotidectomy with nerve preservation is the most oncologically safe option.
- For SUMP lesions involving the submandibular glands (SMG), excision should be performed removing the entire gland in a suprafascial plane. Frozen section can be performed to determine malignancy and to assist in the decision to

perform a selective neck dissection for intermediate and high-grade malignancies. Because SMGs have a higher proportion of malignant neoplasms, one should consider placing the skin incision low enough to facilitate a neck dissection if needed.

Benign Neoplasm Management Summary

Parotid gland lesions:

- Cross-sectional imaging (MRI or CT) in nearly all cases to determine extent of the lesion, reserving only very small lesions with clearly palpable borders to be managed without preoperative imaging.
- Complete excision of the lesion with either extracapsular dissection or nerve dissecting parotidectomy with nerve preservation. Lesions in the superficial or lateral lobe would undergo superficial parotidectomy; those in the deep lobe would require resection of the deep lobe lesion often with preservation of the superficial portion of the gland. Sparing the superficial portion of the gland helps to minimize the postoperative contour defect.
- A subset of patients who are medically inoperable or who are unable to accept the risk of nerve injury might be clinically followed without surgical management.

SMG lesions

- Cross-sectional imaging (MRI or CT) with SMG surgical resection in suprafascial plane.

SUMP Management Summary

Parotid gland lesions:

- Cross-sectional imaging (MRI or CT) to assess neck preoperatively and nerve-preserving parotidectomy.
- Nerve sparing surgical resection unless clinically not indicated (such as a medically inoperable patient).
- Consider performing frozen section to better define the histologic classification and determine if neck dissection is indicated.

SMG lesions:

- Cross-sectional imaging (MRI or CT) preoperatively with SMG resection in suprafascial plane.
- Ensure that neck incision is low enough to facilitate neck dissection.
- Consider frozen section to better define the histologic classification and determine if neck dissection is indicated in the primary setting.

Suspicious for Malignancy

Management (General)

- Salivary gland lesions in this diagnostic category have a high ROM and mandate cross-sectional imaging for the purposes of assessing the extent of the lesion and staging prior to surgical resection. Chest imaging should be performed to rule out metastatic disease.
- It is important to assess the need for elective neck dissection at the time of primary surgical resection versus adjuvant radiotherapy to address the primary site and upper cervical lymph nodes. Not all malignant tumors require an elective neck dissection. Based upon the classic works of Frankenthaler et al. [4] and Armstrong et al. [2] the indications for elective neck dissection are: tumor >4 cm; high-grade histology; extraglandular extension, and neurological deficit.
- Frozen section of the primary salivary gland tumor with a preoperative cytology "Suspicious for Malignancy" can be used to help inform the decision to perform an elective neck dissection for cases that are clinically and radiologically negative.
- A therapeutic neck dissection of levels II–IV should be planned for cases with clinical or radiographic evidence of neck disease preoperatively or when the preoperative cytology is "Suspicious for Malignancy." Intraoperative frozen section of the primary salivary gland tumor can be used to confirm that neck dissection is necessary in this setting.
- The extent of neck dissection is largely determined by the location and stage of neck disease. Dissection of levels II-IV is almost always required.

Suspicious for Malignancy Management Summary

- Preoperative staging contrast-enhanced MRI or CT of the neck and imaging of the chest
- Parotid gland lesions:
  - Nerve-preserving parotidectomy with complete excision of the lesion.
  - Consent patient for the increased risk of nerve dysfunction and possibility that nerve cannot be separated from tumor. The surgeon may choose to use intraoperative frozen section to confirm malignancy before sacrificing the facial nerve.
  - Consent patient that nerve may need to be sacrificed in exceptional circumstances and reanimation procedures performed.
  - If imaging suggests a malignant process, nerve sparing parotidectomy with complete tumor excision should be performed. Some institutions use frozen section evaluation. If the frozen section is positive for malignancy and pathological nodes are identified, then concurrent comprehensive neck dissection is performed, sparing nonlymphatic structures (internal jugular vein,

sternocleidomastoid muscle, spinal accessory nerve) if possible. For tumors >4 cm in greatest dimension, high-grade features on frozen section of the primary site, extraglandular extension on imaging or noted intraoperatively, or preoperative facial weakness, perform elective selective neck dissection for the clinically and radiographically N0 neck.

- For institutions that do not routinely use frozen section, the decision to manage the neck is made once formal histological assessment of the primary site has been performed. In cases of malignancy, the decision to offer radiation therapy or further surgery (i.e., neck dissection) is made by an informed patient in a multidisciplinary setting. If indicated, neck dissection is performed as a second procedure.
- SMG lesions
  - If clinical and contrast enhanced MRI or CT features appear benign without possible nodal disease, consider removal of the gland in a suprafascial plane with a low neck incision to facilitate neck dissection. Frozen section should be performed. If findings are consistent with an intermediate or high-grade malignancy, selective neck dissection may be performed.
  - If contrast enhanced MRI or CT indicates a malignant process, the frozen section shows primary submandibular gland malignancy, and pathological nodes are present, perform selective neck dissection.

Malignant

In the clinical management of clearly "Malignant" salivary gland lesions, a definitive classification of a specific malignant histologic tumor type, including grade (low- versus high-grade), provides important information for clinical decision making. When a definitive classification is not possible, information about tumor grade is still useful. Low- versus intermediate- versus high-grade classification may be useful to the clinician in determining the extent of surgery required at the primary site and the likelihood that a neck dissection would be needed. For high-grade malignancies involving the deep lobe, a total parotidectomy is necessary. For lateral lesions, controversy exists regarding the extent of surgery with some surgeons electing to perform a total parotidectomy to optimize surgical clearance and others performing a superficial parotidectomy with the knowledge that the patient will be receiving postoperative radiotherapy. In addition, a subcategory of "metastatic" would also be informative for the managing clinician. Parotid gland lymph nodes are a common site for metastases from cutaneous primaries, and these patients often require a concurrent neck dissection. If a lesion is metastatic from a non-cutaneous source, PET-CT may be indicated to locate a primary site of origin.

Management Summary

- Presurgical staging MRI or CT neck plus CT neck and chest
- Parotid gland lesions:
  - For low-grade with no clinical or radiographic evidence of involved neck nodes and no other indicators for neck dissection (as mentioned above), perform nerve sparing parotidectomy with complete tumor excision.
  - For intermediate- or high-grade and negative for involved neck nodes, perform nerve-preserving total parotidectomy and elective selective neck dissection.
  - For intermediate- or high-grade and evidence of involved neck nodes, perform nerve-preserving total parotidectomy and selective neck dissection.
  - Consent patient for the increased risk of nerve dysfunction and possibility that nerve cannot be separated from tumor. The surgeon may choose to use intraoperative frozen section to confirm malignancy before sacrificing the facial nerve.
  - Consent patient that nerve may need to be sacrificed and reanimation procedures performed.
- SMG lesions
  - For low-grade with no clinical or radiographic neck nodes and no other indicators for neck dissection present, perform suprafascial SMG resection.
  - For intermediate- or high-grade tumors, perform suprafascial SMG, and if no clinical or radiographic evidence of involved neck lymph nodes, perform elective neck dissection.
  - For intermediate- or high-grade histology, perform suprafascial SMG resection and if neck shows clinical or radiographic evidence of involved neck lymph nodes, perform selective neck dissection.
- Metastatic
  - Known primary site—management based on primary tumor

    For cutaneous squamous cell carcinoma, consider nerve-preserving parotidectomy and selective neck dissection if clinically N0.
  - Unknown primary site—consider PET-CT to identify the primary site. If identified, management would be based upon specific aspects of the primary cancer. If no primary site is identified and the salivary gland lesion is isolated, it can be managed as a high-grade primary lesion in order to avoid issues related to uncontrolled head and neck malignancy. In such a setting, avoiding facial nerve injury is a priority.

Table 9.1 lists the main indications for clinical observation versus operative management; Table 9.2, the indications for neck dissection and the extent of dissection; Table 9.3, the degrees of parotidectomy required; Table 9.4, management of the facial nerve.

Table 9.1 Indications for clinical observation versus operative management

1. Unequivocal diagnosis of benign cytology of a lesion with very low risk of malignant transformation in an asymptomatic patient
2. Resection of a benign tumor would result in significant morbidity (e.g., Warthin tumor in a patient not interested in surgical resection or a facial nerve schwannoma, where resection will result in complete facial paralysis; such lesions are observed and in some cases, such as the schwannoma, irradiated when symptoms develop)
3. Lesions classified as "Atypia of Undetermined Significance (AUS)" with two FNA that support the diagnosis with no worrisome symptoms or examination findings concerning for malignancy

Table 9.2 Indications for neck dissection and extent of dissection

1. When there is clinical or radiographic evidence of nodal disease, comprehensive dissection should be performed, sparing any non-lymphatic structures that can be spared (internal jugular vein, spinal accessory nerve, or sternocleidomastoid muscle)
2. Clinically and radiographically N0 necks with high risk primary site cytology (tumor >4 cm, high-grade features on frozen section of the primary site, extraglandular extension on imaging or noted intraoperatively, or preoperative facial weakness) should undergo selective neck dissection

Table 9.3 Degree of parotidectomy required

1. Benign neoplasm cytology: Nerve-preserving tumor resection with small cuff of normal parotid tissue, may be less than complete lateral lobectomy or superficial parotidectomy
2. "Atypia of Undetermined Significance (AUS)" and "Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)": Nerve-preserving tumor resection with cuff of normal parotid tissue with frozen section. If findings consistent with low-grade malignancy, consider completion superficial parotidectomy to encompass intraparotid lymph nodes. If found to be high-grade by frozen section, consider nerve-preserving subtotal parotidectomy
3. Malignant cytology: Superficial parotidectomy for low-grade lesions, total or subtotal parotidectomy for higher grade lesions, both with facial nerve preservation whenever possible

Table 9.4 Management of the facial nerve

1. Never sacrifice a major nerve branch when removing benign disease unless the nerve branch is completely encased, and even in that circumstance consider debulking
2. Do not sacrifice a functioning nerve without first establishing a diagnosis of malignancy (unequivocal cytology or frozen section) and determining that the nerve cannot be separated from tumor with microscopic residual disease
3. A non-functional nerve in the setting of proven malignancy should be resected and rehabilitated with the appropriate method based on available donor and recipient nerve for grafts and transfers and by static techniques

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Chapter 10
Histologic Considerations and Salivary Gland Tumor Classification in Surgical Pathology

Bruce M. Wenig

The classification of salivary gland neoplasms is dynamic and continues to evolve (Table 10.1 [1] and Table 10.2 [2]), as reflected in the World Health Organization Classification (WHO) of Head and Neck Tumours [2]. This includes recently identified and defined tumor types such as intraductal carcinoma, cribriform adenocarcinoma of minor salivary glands, and new nomenclature for well-established tumors. Notable changes include polymorphous adenocarcinoma (PAd) for polymorphous low-grade adenocarcinoma (PLGA), and secretory carcinoma (SC) for mammary analogue secretory carcinoma (MASC). The WHO classification separates neoplastic entities primarily on tumor morphology and attempts to predict biologic behavior [3]. Newly identified and growing numbers of specific molecular alterations in salivary gland tumors support the morphologic-based classification (Table 10.3) [1, 4–12].

The surgical pathology diagnosis of salivary gland tumors is generally accomplished by light microscopy alone or with histochemical and immunohistochemical (IHC) staining. The most common salivary gland neoplasm in adult and pediatric age groups is pleomorphic adenoma (PA). PA has a characteristic admixture of epithelial cells in tubules and glands with myoepithelial cells as the peripheral cell layer, and myoepithelial cell containing chondromyxoid stroma. Special stains are usually unnecessary in the diagnosis of PA. Warthin tumor (WT), another common parotid gland neoplasm, has a unique diagnostic combination of bilayered oncocytic cyst lining cells with underlying mature lymphocytes and plasma cells. The most common malignant neoplasm in adults and pediatrics is mucoepidermoid carcinoma (MEC). The majority of MECs are low-grade and composed of an admixture of epidermoid cells, mucin-containing epithelial cells, and intermediate cells. Most MECs can also be diagnosed by light microscopy alone. As there is no benign counterpart to MEC, the identification of these three cell types is diagnostic for MEC, even in a tumor without invasion. Similarly, acinic cell carcinoma (ACC) has

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7_10

Table 10.1 World Health Organization classification of nonneoplastic salivary gland lesions (Adapted from Wenig [1], with permission)

Developmental lesions
Heterotopias
Hyperplasia and metaplasia
Adenomatoid hyperplasia
Squamous metaplasia
Necrotizing sialometaplasia
Oncocytic changes (oncocytic metaplasia, oncocytosis)
Intercalated duct hyperplasia
True cysts
Lymphoepithelial cyst
Salivary duct cyst
Polycystic (dysgenetic) disease
Non-developmental cysts
Mucus extravasation phenomenon
Mucus retention cyst
Ranulas
Infectious, inflammatory and autoimmune disease
Bacterial sialadenitis
Mumps
HIV salivary gland disease
Chronic sialadenitis
Nonobstructive Infectious Noninfectious
Obstructive Sialolithiasis Sialadenosis
IgG4-related sialadenitis
Lymphoepithelial sialadenitis, including Sjögren syndrome

a pathognomonic cell type with basophilic granular cytoplasm that is not identified in other tumors. Given these unique identifying features, most PA, MEC, and ACC can be diagnosed by fine-needle aspiration (FNA) or by core needle biopsy (CNB).

The marked heterogeneity of salivary gland neoplasms (SGN) in growth patterns, cellularity, and cell type—within the same tumor, as well as between tumor types—creates diagnostic challenges in limited tissue samples, including FNA and CNB, as exemplified by:

- Benign neoplasms, such as cellular variants of PA and basal cell adenoma (BCA), can share a basaloid cell type and can show similar growth patterns, including: tubular/glandular, microcystic/cribriform, and solid. Some malignant SGN, including but not limited to polymorphous adenocarcinoma, adenoid cystic carcinoma (AdCC), basal cell adenocarcinoma, and cribriform adenocarcinoma of minor salivary glands, can show similar patterns [13].

Table 10.2 Classification of salivary gland tumors (Adapted from El-Naggar [2], with permission of the World Health Organization [WHO], International Agency for Research on Cancer)

Benign epithelial tumors
Pleomorphic adenoma
Basal cell adenoma
Canalicular adenoma
Warthin tumor
Myoepithelioma
Oncocytoma
Sclerosing polycystic adenosis
Cystadenoma
Ductal papillomas
Sialadenoma papilliferum
Inverted ductal papilloma
Intraductal papilloma
Other uncommon adenomas
Striated duct adenoma
Intercalated duct adenoma
Lymphadenoma (nonsebaceous)
Keratocystoma
Lipoadenoma
Apocrine adenoma
Adenofibroma
Tumors with sebaceous differentiation
Sebaceous adenoma
Sebaceous lymphadenoma
Salivary gland anlage tumor
Benign non-epithelial tumors
Hemangioma
Neurilemmoma/neurofibroma
Lipoma
Others
Malignant epithelial tumors
Mucoepidermoid carcinoma
Acinic cell adenocarcinoma
Adenoid cystic carcinoma
Mammary analogue secretory carcinoma (WHO: Secretory carcinoma)
Adenocarcinoma, NOS
Polymorphous low-grade adenocarcinoma (WHO: Polymorphous adenocarcinoma)
Cribriform adenocarcinoma of minor salivary glands (CAMSG)
Carcinoma ex pleomorphic adenoma
Invasive
Intracapsular
Carcinosarcoma
Metastasizing pleomorphic adenoma

(continued)

Table 10.2 (continued)

Salivary duct carcinoma
Intraductal carcinoma (low-grade cribriform cystadenocarcinoma; low-grade salivary duct carcinoma)
Basal cell adenocarcinoma
Epithelial-myoepithelial carcinoma
Clear cell adenocarcinoma
Cystadenocarcinoma
Myoepithelial carcinoma
Squamous cell carcinoma
Adenosquamous carcinoma
Lymphoepithelial carcinoma
Neuroendocrine carcinomas
Undifferentiated (large cell) carcinoma
Oncocytic carcinoma
Mucinous adenocarcinoma
Sebaceous carcinoma/lymphadenocarcinoma
Sialoblastoma
Non-epithelial malignant tumors
Hematolymphoid
Non-Hodgkin lymphoma
Hodgkin lymphoma
Sarcomas

- A tumor with cribriform growth composed of cells with basaloid nuclei suggests adenoid cystic carcinoma, but a cribriform pattern and basaloid cells can be seen in benign tumors, in particular BCA [14, 15].
- Epithelial cells and myoepithelial cells are present in a variety of salivary gland neoplasms, including PA, AdCC, polymorphous adenocarcinoma, and epithelial-myoepithelial carcinoma so that IHC staining showing epithelial and myoepithelial cell differentiation is not unique to any specific tumor (Table 10.4) [1].
- Oncocytic cell salivary gland lesions include oncocytosis, oncocytoma, oncocytic variant of MEC, and oncocytic carcinoma. The oncocytic cells in the latter two lesions often are bland, lacking malignant cytomorphologic findings that would differentiate them from benign oncocytic lesions.
- Differentiating a benign salivary gland tumor from a low-grade carcinoma often relies on the presence of invasion, requiring inspection of the tumor-to-stromal interface, which often is not present in a core biopsy and is typically absent in FNA.
- "Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)" will often be used with a recommendation for conservative management, but with complete tumor resection, which will provide the tumor's entire margin. It should be noted

Table 10.3 Genetic profile of select salivary gland neoplasms (Adapted from Wenig [1], with permission)

Tumor type	Chromosomal translocation	Gene fusion
Pleomorphic adenoma	Rearrangement of 8q12:	*PLAG1*
	t(3;8)(p21;112)	
	t(5;8)(p13;q12)	
	Rearrangement of 12q13–15:	*HMGA2*
	t(3;12)(p14.2;q14–5)	
	Ins(9;12)(p23;q12–15)	
Mucoepidermoid carcinoma	t(11;19)(q21;p13)	*CRTC1-MAML2*
	t(11;15)(q21;q26)	*CRTC3-MAML2*
Adenoid cystic carcinoma	t(6;9)(q22–23;p23–24)	*MYB-NFIB*
	Rarely t(8;9)	
SC	t(12;15)(p13q25)	*ETV6*
HCCC; myoepithelial carcinoma, clear cell variant	t(12;22)(q13;q12)	*EWSR1-ATF*
CAMSG/PAd family[a]		
CAMSG, "classic" type	t(1;14)(p36.11;q12)	*ARID1A-PRKD1*
	t(x;14)(p11.4;q12)	*DDX3X-PRKD1*
PAd, "classic" type	PRKD1 E710D mutation	Not known

SC secretory carcinoma, *HCCC* hyalinizing clear cell carcinoma, *MASC* aka mammary analogue secretory carcinoma, *CAMSG* cribriform adenocarcinoma of minor salivary glands, *PAd* polymorphous (low-grade) adenocarcinoma

[a]Associated with rearrangements of PRKD1 gene family including *PRKD1, PRKD2, PRKD3*

that the treatment of low-grade salivary gland carcinoma is likewise usually conservative [13], similar to benign neoplasms, with complete resection to include tumor-free margins and without the need for lymph node dissection unless there is clinical evidence of neck disease [14] (see Chap. 9).

Diagnostic problems occur less frequently in high-grade tumors, such as salivary duct carcinoma, high-grade MEC, and high-grade carcinoma ex pleomorphic adenoma. These tumors have overtly malignant cytomorphologic features, including marked nuclear pleomorphism, necrosis, and increased mitotic activity. There may be atypical cellular forms, even in small amounts of FNA and CNB material. Once a diagnosis of a high-grade salivary gland tumor is established, specific tumor classification is largely irrelevant, as this diagnosis irrespective of the specific tumor type results in similar treatment. Management is usually radical excision, which may necessitate facial nerve resection and neck dissection with postoperative adjunctive therapy [14].

FNA remains the recommended initial diagnostic modality for both major and minor salivary gland masses despite any limitations. In the hands of an experienced cytopathologist, it is a reliable, minimally invasive diagnostic modality with a high sensitivity for the diagnosis of salivary gland lesions [16, 17]. It is an excellent tool

Table 10.4 Immunohistochemistry of select salivary gland neoplasms (Adapted from Wenig [1], with permission)

	PanK	LMWK	HMWK	p63 and p40	S100	DOG1	MGB	AR	GATA3	CD117	PLAG1
Tumor											
PA	+	+	+	+	+	−	−	−	v	v	+
BCA/ BCAdC	+	+	+	+	+	−	−	−	v	v	v
MYO	+	+	+	+	+	−	−	−	v	v	−
MEC	+	+	+	+	−	−	−	−	v	v	−
ACC	+	+	+	−	−	+[a]	−	−	−	−	−
SC	+	+	+	−	+	−	+	−	+ (n)	−	−
AdCC	+	+	+	+	+	−	−	−	−	+ (lum)	−
PAd	+	+	+	+[b]	+	−	−	−	−	v	v
SDC	+	+	+	−	−	−	−	+ (n)	+ (n)	−	−
EMC	+	+	+	+	+	−	−	−	−	−	−
CCC	+/+	+/+	+/+	+	−/−	−/−	−/−	−/−	−/−	−/−	−/−

NOTE: Staining characteristics vary widely among tumor types and even within the same tumor type. This table details ideal staining characteristics per tumor type and while these staining patterns generally remain consistent, any given tumor listed may defy "convention" and show reactivity for a marker usually not associated with that tumor or may lack a marker associated with that tumor

PanK pancytokeratin (e.g., AE1/AE3; CAM5.2), *LMWK* low molecular weight cytokeratin (e.g., CK7, CK8, CK19), *HMWK* high molecular weight cytokeratin (e.g., CK5/6, CK14), *DOG1* discovered on GIST1, *MGB* mammaglobin, *AR* androgen receptor, *GATA3* GATA binding protein 3, *PLAG1* pleomorphic adenoma gene 1, *PA* pleomorphic adenoma, *BCA* basal cell adenoma, *BCAdC* basal cell adenocarcinoma, *MYO* myoepithelioma, *MEC* mucoepidermoid carcinoma, *ACC* acinic cell carcinoma, *HCCC* hyalinizing clear cell carcinoma, *SC* secretory carcinoma, *MASC* aka mammary analogue secretory carcinoma, *AdCC* adenoid cystic carcinoma, *PAd* polymorphous (low-grade) adenocarcinoma, *SDC* salivary duct carcinoma, *EMC* epithelial-myoepithelial carcinoma, *CCC* clear cell carcinoma including hyalinizing type, *n* nuclear, *lum* strong staining luminal cells

[a]Specific staining characteristics: DOG1: should be admixture of strong apical membranous, cytoplasmic and complete membranous staining; Mammaglobin: should be strong and diffuse cytoplasmic staining

v variably positive

PLAG1 immunohistochemical staining may not be confirmed by fluorescent in situ hybridization (FISH) analysis even for PA

[b]Differential staining may be present including presence of p63 but absence of p40

to allow the cytopathologist to assist in guiding treatment. FNA can identify a non-neoplastic process or high-grade malignancy to help select the proper management. Between the extremes there is a subset of salivary gland lesions where a definitive diagnosis cannot be rendered, and in these cases a conservative diagnosis, such as "Neoplasm: SUMP," can be used.

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W.C. Faquin et al. (eds.), *The Milan System for Reporting Salivary Gland Cytopathology*, https://doi.org/10.1007/978-3-319-71285-7