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Introduction

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Henryk A. Domanski and Fredrik Mertens

The Role and Evaluation of Fine-Needle Aspiration Cytology in Clinical Examination

Fine-needle aspiration cytology (FNAC) has been used as a tool to obtain specimens for the morphological diagnosis of numerous lesions in a variety of locations for more than 80 years. Despite early attempts to use thin-needle aspiration for diagnosis of neoplasm and inflammatory conditions [1, 2], first large-scale studies published in the early 1920s and 1930s by Martin, Ellis, and Steward [3, 4] are considered to be the beginning of the modern era of FNAC. In 1950s–1960s, FNAC became a widely used diagnostic tool particularly in Europe, pioneered by some physicians and pathologists in Sweden [5, 6]. Today, this diagnostic modality is more powerful than ever in making rapid preliminary diagnoses in neoplastic and nonneoplastic conditions, guiding further work-up of the patient or even allowing the initiation of definitive treatment. In many clinical situations, FNAC can render a definitive diagnosis either from aspiration smears alone using well-defined cytological criteria (see Fig. 1.1) or from aspiration smears combined with clinical data, radiological findings, and the results of ancillary studies (see Figs. 1.2 and 1.3). The use of ever more sophisticated ancillary methods on aspiration specimens, such as molecular/genetic analysis and immunocytochemistry, allows a diagnosis of tumors that can be used for predicting prognosis and tailoring individualized "targeted" oncological therapy [7–11].

Nevertheless, considerable differences exist between various diagnostic centers with regard to the role that aspiration cytology plays in the work-up of patients. Many physicians are skeptical about the use of cytological diagnosis due simply to the small amount of diagnostic material obtained. Consequently, in some centers, cytological material is not collected or processed in an optimal or standardized way. In such places, there has never been an understanding of the potential of cytological diagnosis, and clinicians have had to rely on other diagnostic modalities.

In diagnostic centers where there is a tradition of diagnostic procedures using reliable FNAC, the technique of aspiration has been optimized and taught to generations of cytologists, radiologists, and clinicians. Careful attention has been paid to specimen handling, and strict morphological criteria have been applied to the microscopical examination.

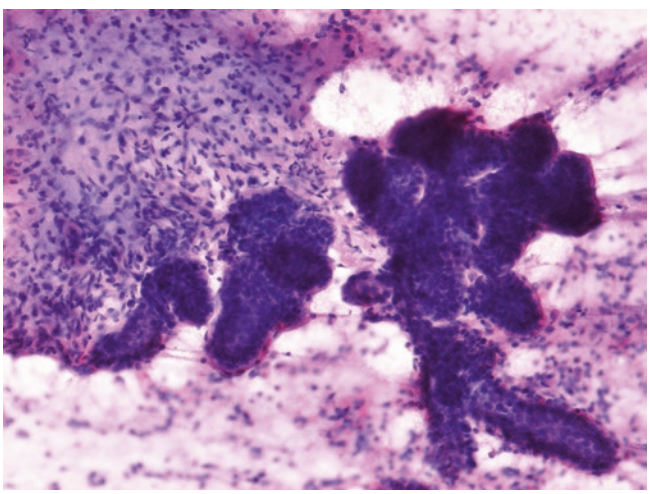

Fig. 1.1 FNA of breast mass: cellular smears with a mixture of benign, branching cell clusters with fragments of myxoid matrix and myoepithelial cells, indicative of fibroadenoma

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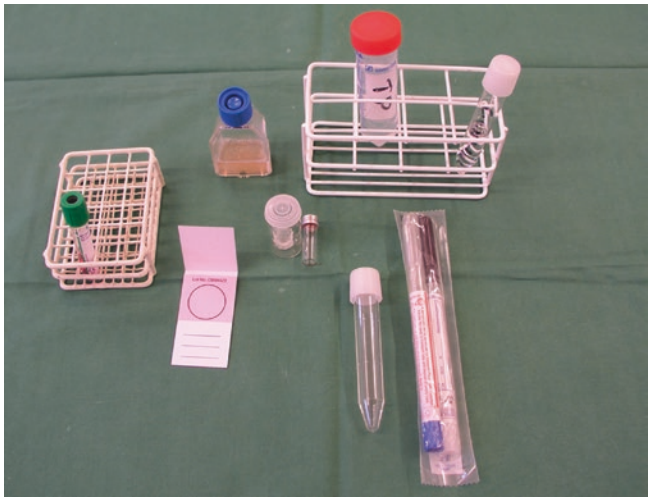

Fig. 1.2 Combination to preserve FNA specimen for ancillary techniques

Immunocytochemistry has been a special problem as this technique has proven to be more difficult to standardize than immunohistochemistry. Great efforts have to be made in the laboratory to ensure the usefulness of immunological stains in cytology [12–16].

In many institutions, aspirations are routinely performed by a clinician or radiologist without the assistance of a cytopathologist or cytotechnologist. Many non-cytopathologists are well experienced in the method and obtain a material that is perfectly adequate for diagnosis [17–19]. It must be noted, however, that the results of aspiration biopsy are often better when it is performed by an experienced cytopathologist in a puncture cytology clinic (FNA clinic) [16, 20]. In this setting, it is possible to do a rapid, preliminary evaluation (rapid on-site evaluation, ROSE) of the aspirate while the patient waits and then immediately perform additional aspirations if the material is found to be inadequate or

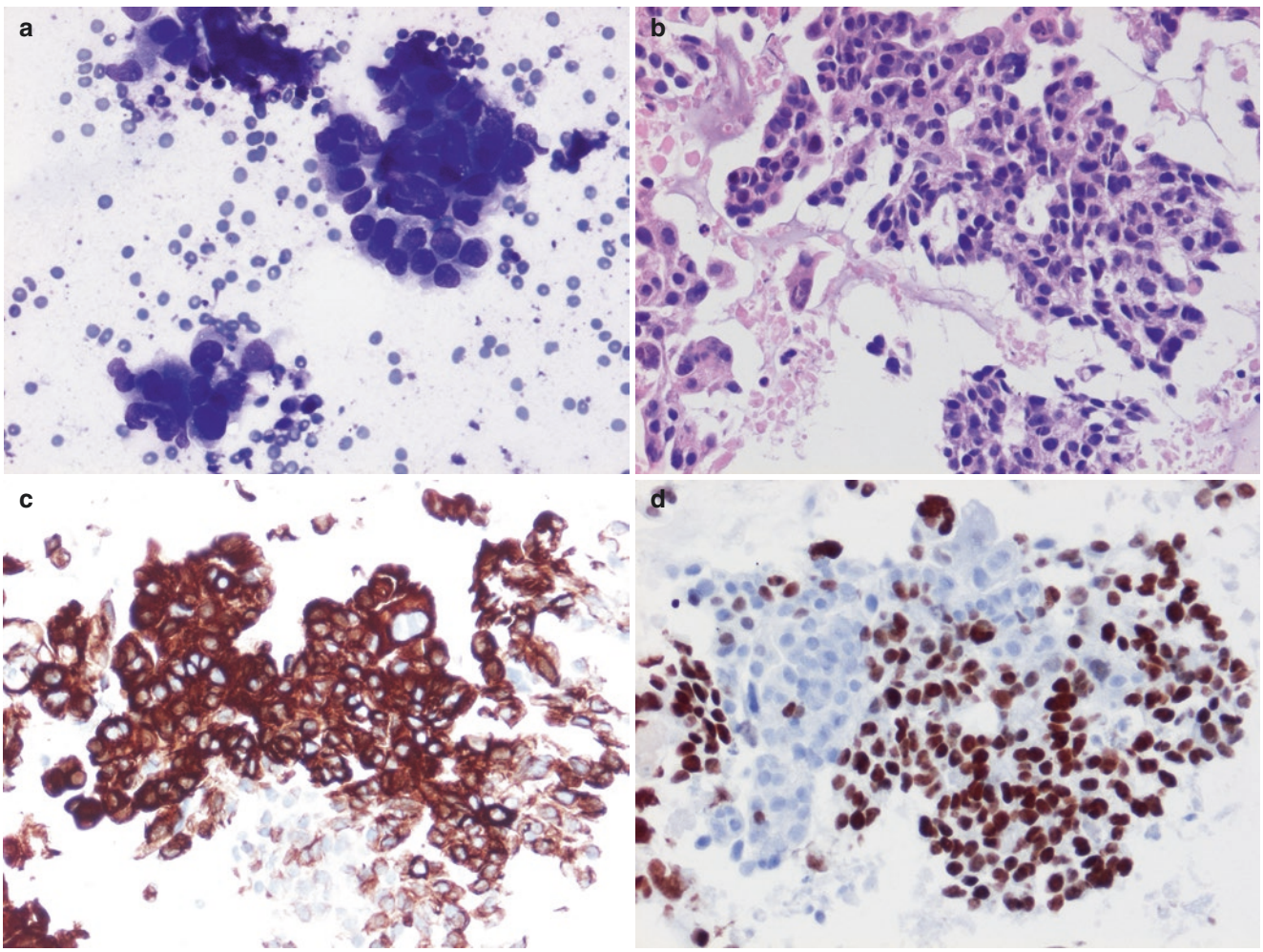

Fig. 1.3 Rapid on-site evaluation (ROSE) in the puncture cytology clinic (FNA clinic). (**a**) FNA of a defect in the acromion in a previously healthy 49-year-old man. ROSE: sheets of carcinoma cells (Diff-Quik staining). (**b**) Cell block preparation from aspiration specimen. Note preserved architecture of the tumor tissue consistent with adenocarcinoma (H&E). (**c**) Cytokeratin positivity in a cell block. (**d**) Thyroid transcription factor (TTF-1) positivity of tumor cells on cell block section indicates lung origin. Subsequent investigation disclosed a mass in the lung

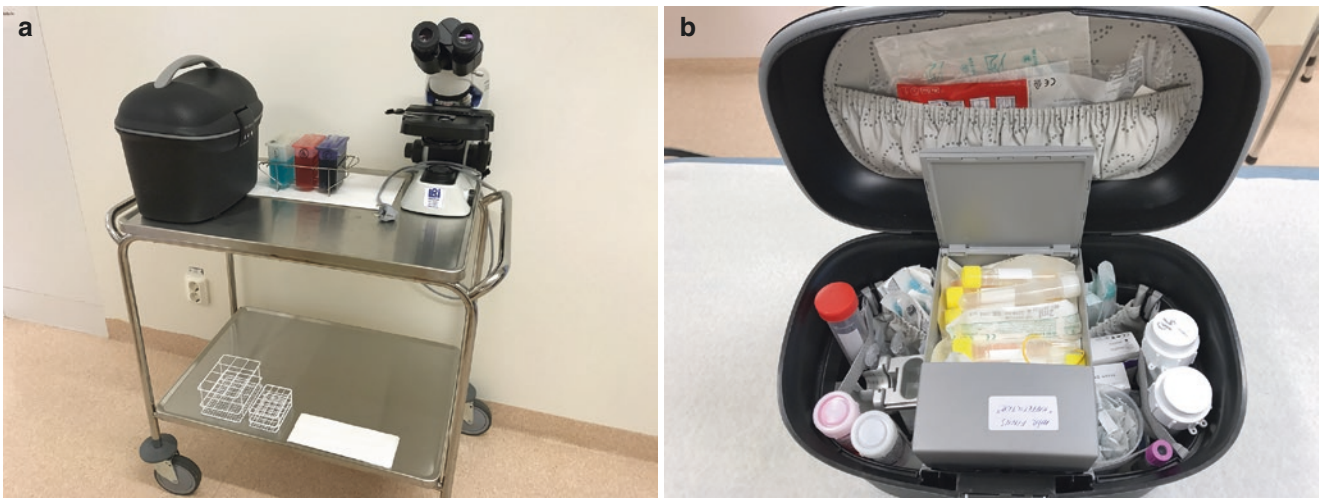

Fig. 1.4 Equipment for ROSE outside of the puncture cytology clinic. (**a**) A portable microscope and arrangement for rapid Diff-Quik staining. (**b**) A box containing FNA equipment, such as syringe holder, syringes, needles, slides, fixative, and Cytolyt to collect FNA specimen for further liquid-based and cell block preparations and combination to preserve specimen for ancillary studies

if more material for ancillary diagnostic examination are needed (see Fig. 1.3).

An expanding role of cytopathology staff members such as pathologists/cytopathologists and cytotechnologists in FNAC performed outside of the dedicated FNA clinic depends mostly on the growing popularity of the ROSE service among radiologists and clinicians performing FNA procedures (see Fig. 1.4) [21–35]. ROSE for FNA samples and touch preparation from core biopsy can be performed in many clinical circumstances and from many body sites (see Fig. 1.5) regardless of palpable or non-palpable lesions [36–40].

In the coming age of telepathology/telecytology, there may be potential for using this technique to ensure FNA specimen adequacy (ROSE) and reliable FNAC diagnosis [41–44].

As FNAC does not always provide adequate cellular or architectural details in certain types of lesions and the FNA specimen may not be sufficient to prepare a cell block, the combined use of FNAC and core needle biopsy (CNB) can improve obtaining satisfactory amount of material for both routine microscopic examination and ancillary tests [45, 46]. As FNAC and CNB are complementary techniques and both can be performed in the outpatient setting, the procedure of CNB in conjunction with FNAC in the same séance is patient-friendly and cost-effective approach which improves diagnostic accuracy of the FNAC alone (see Figs. 1.6 and 1.7) [46, 47]. The complementary nature of FNAC and CNB in the outpatient setting has been underlined in some serial reports of musculoskeletal neoplasms sampled by those two modalities [48–53]. In one study from Lund, Sweden, a series of 130 consecutive patients with musculoskeletal lesions were evaluated by FNAC and CNB performed simultaneously by the cytopathologist showing a high diagnostic accuracy and speed of diagnosis using this double approach [47].

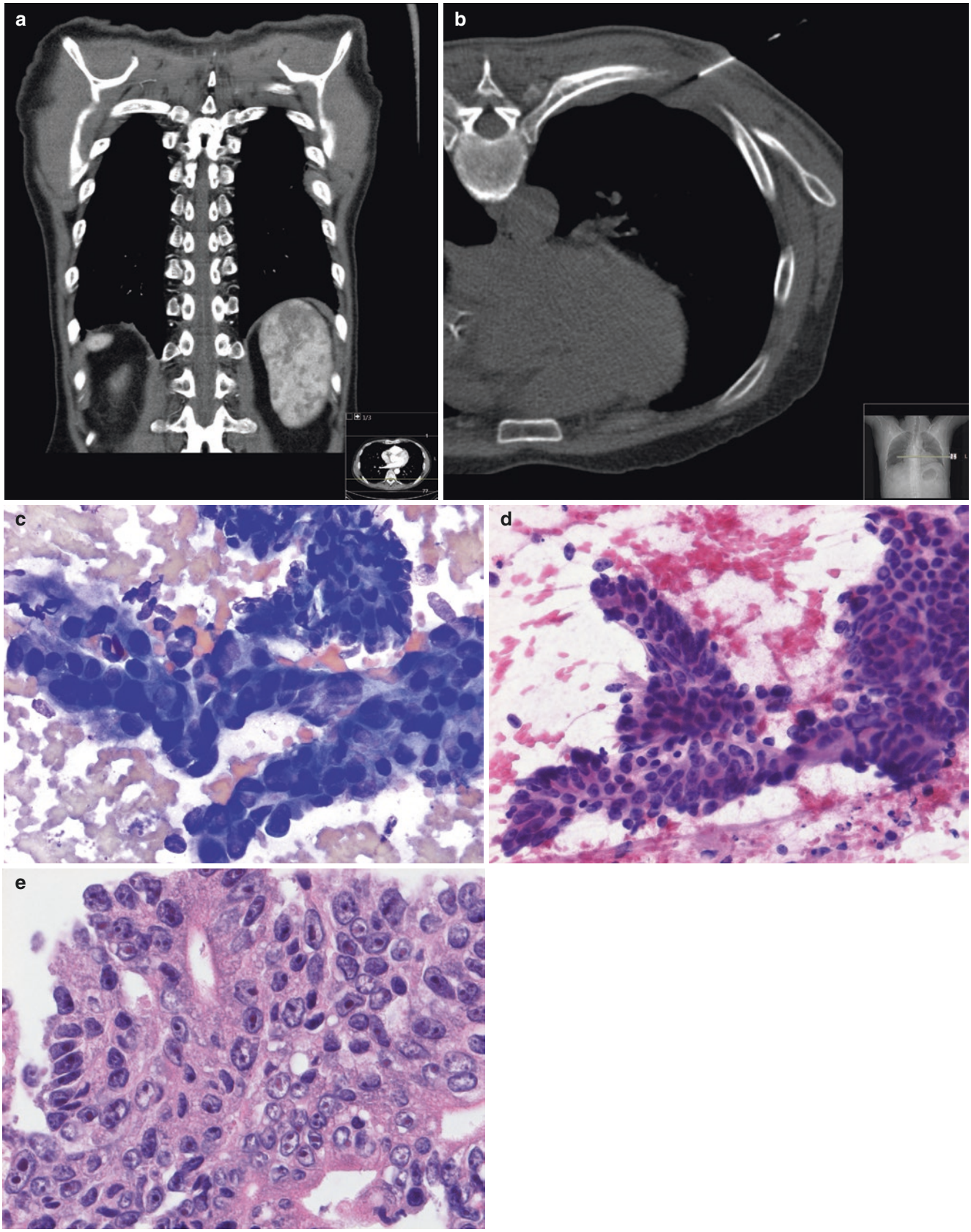

Fig. 1.5 ROSE in the department of radiology. (**a**) A 54-year-old man with a history of colonic adenocarcinoma 4 years ago. A single suspect metastatic lesion was detected during routine checkup. Diagnostic CT before FNAC showing a small mass surrounding the rib. (**b**) In the prone position, the lesion was aspirated through a trocar. (**c**) First FNA smears were air dried and Diff-Quik stained for immediate microscopic examination. Additional two FNA passes were performed to obtain material for (**d**) alcohol fixed smears (H&E) and (**e**) cell block preparation confirming diagnosis: metastasis of colonic adenocarcinoma (cell block; H&E)

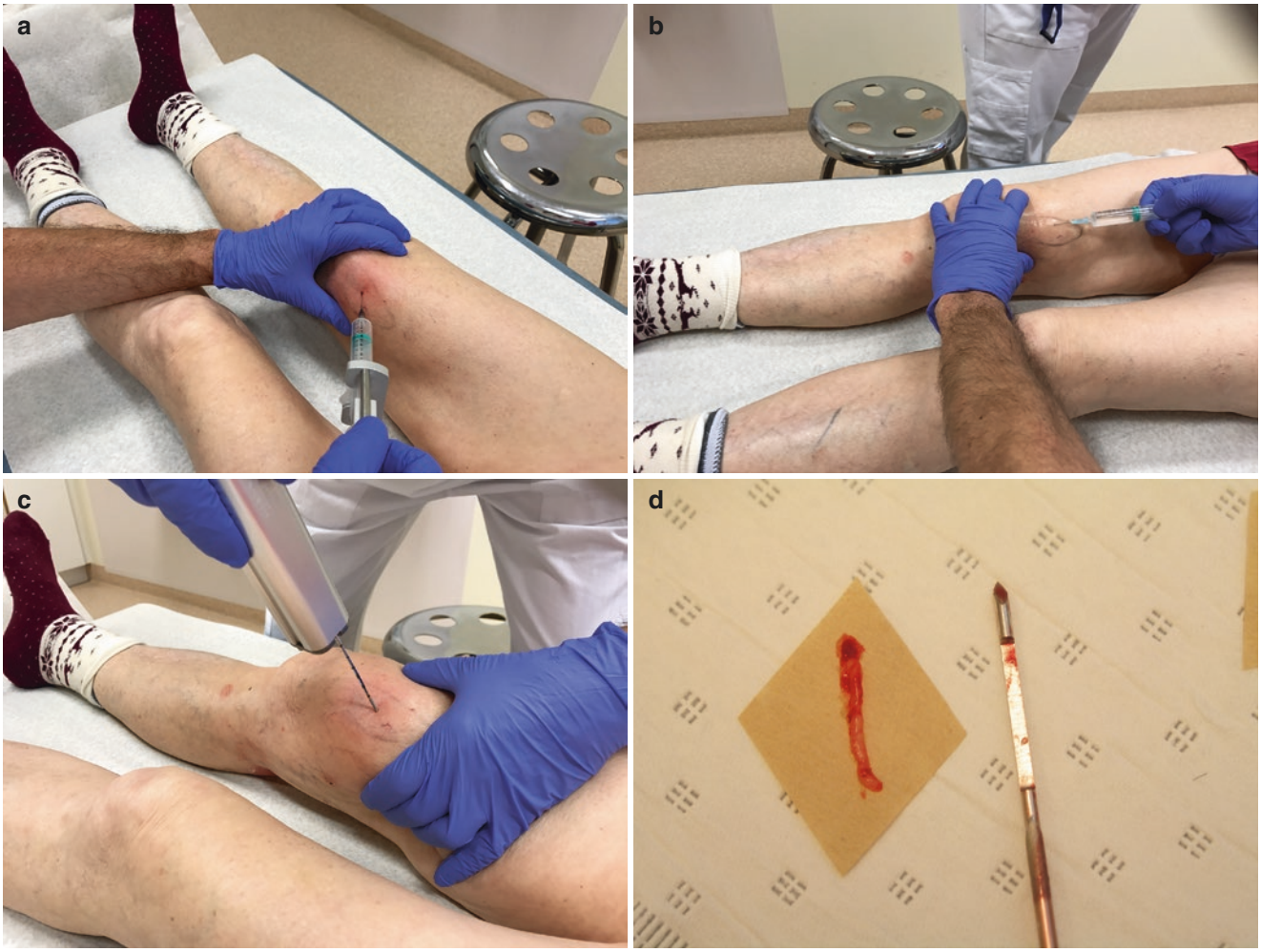

Fig. 1.6 The procedure of CNB in conjunction with FNAC in the same séance in the puncture cytology clinic. (**a**) FNA of soft tissue mass with clinical suspicious of sarcoma. Smears from two FNA passes stained for immediately microscopic examination disclosed scanty and paucicellular specimen containing scattered pleomorphic spindle cells and fragment of collagenous matrix. (**b**) Local anesthesia was administrated immediately after aspiration, and (**c**) four CNB passes were performed providing (**d**) cores of tumor tissue satisfactory for diagnosis. The combination of those both sampling techniques provided a precise diagnosis of high-grade myxofibrosarcoma

Terminology and Reporting

Two similar designations, FNAC and fine-needle aspiration biopsy (FNAB), have been used to describe the technique of aspirating cells and tissue fragments through a thin needle (up to 0.8 mm OD). Aspiration can be achieved using the capillary pressure of the needle itself or by applying a partial vacuum using a syringe [54–57].

Diagnostic results can be reported in a manner similar to other types of cytological examination: (1) unsatisfactory (inadequate), (2) negative for malignancy (benign), (3) atypical, probably benign (atypical/undetermined), (4) suspicious of malignancy (probably malignant), and (5) positive for malignancy (malignant). For example, these different levels of diagnoses can be applied to mammary FNAC (see Figs. 1.7, 1.8, 1.9, and 1.10) [58, 59]. Uncertain diagnosis of malignancy is often reported as "cannot rule out malignancy" or "suspicious of malignancy." The diagnosis of a specific pathological entity can also be given in many cases by FNAC. In some cases, only a descriptive diagnosis can be reached. Even such a diagnosis can be clinically relevant, guiding the subsequent work-up of the patient [60] (see Figs. 1.11 and 1.12). Recently, stanardized cytology reporting concerning numerousm areas of FNA cytology such as FNA of the thyroid, salivary glands and breast have been elaborated and published. These cytology reporting systems provide a uniform diagnostic terminology, guidance for appropriate clinical management and optimize communication between pathologist and clinician and a cytologic and histologic correlation of cases.

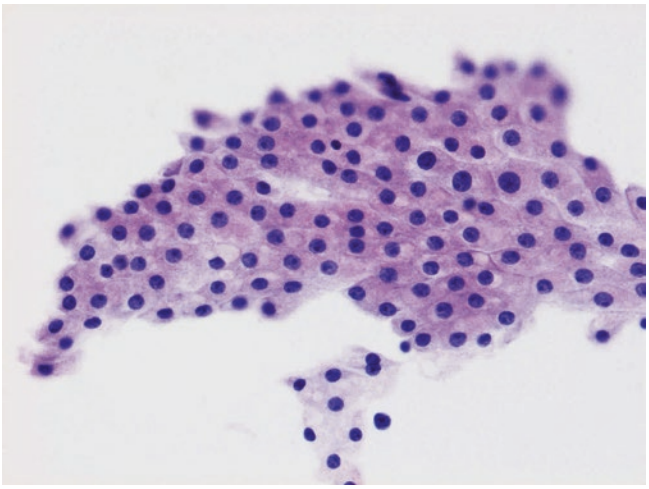

Fig. 1.7 FNA from fibroadenosis with a sheet of apocrine cells: C2 category, benign (negative for malignancy) (H&E)

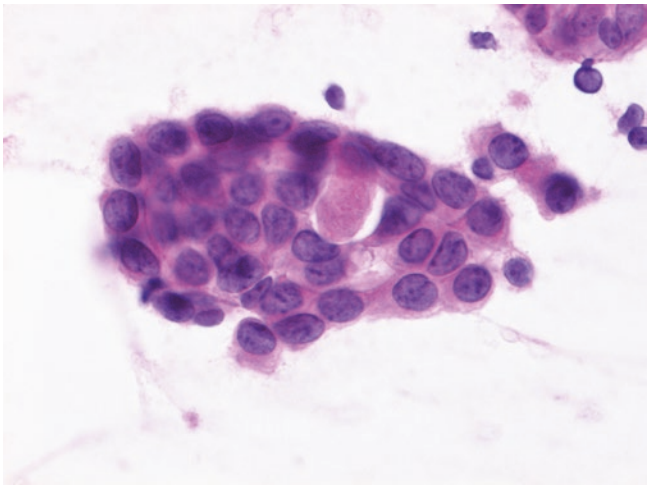

Fig. 1.8 FNA smears from fibroadenoma: C3 category, atypical, probably benign (H&E)

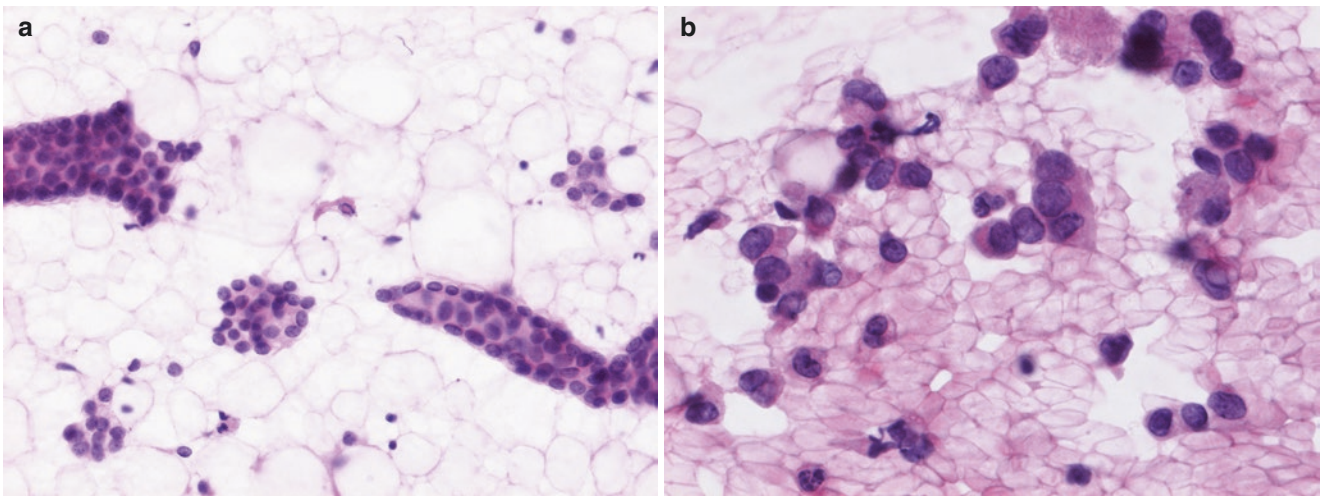

Fig. 1.9 (**a**) FNA smears from tubular carcinoma: C4 category, suspicious of malignancy. (**b**) FNA smears from lobular carcinoma: C4 category, suspicious of malignancy (H&E)

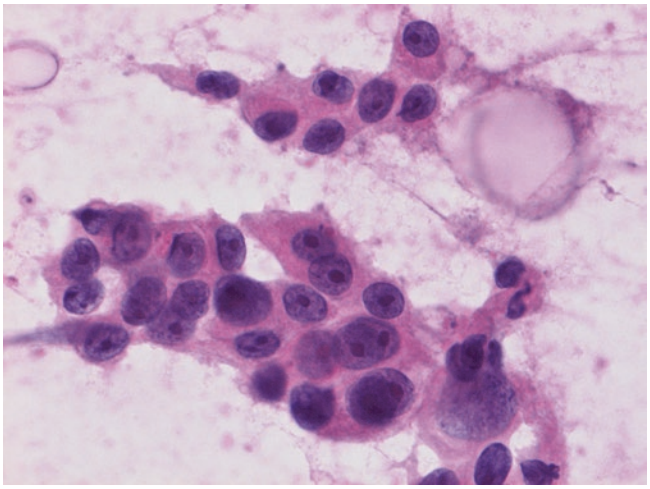

Fig. 1.10 FNA smears from ductal breast carcinoma: C5 category, malignant (positive for malignancy) (H&E)

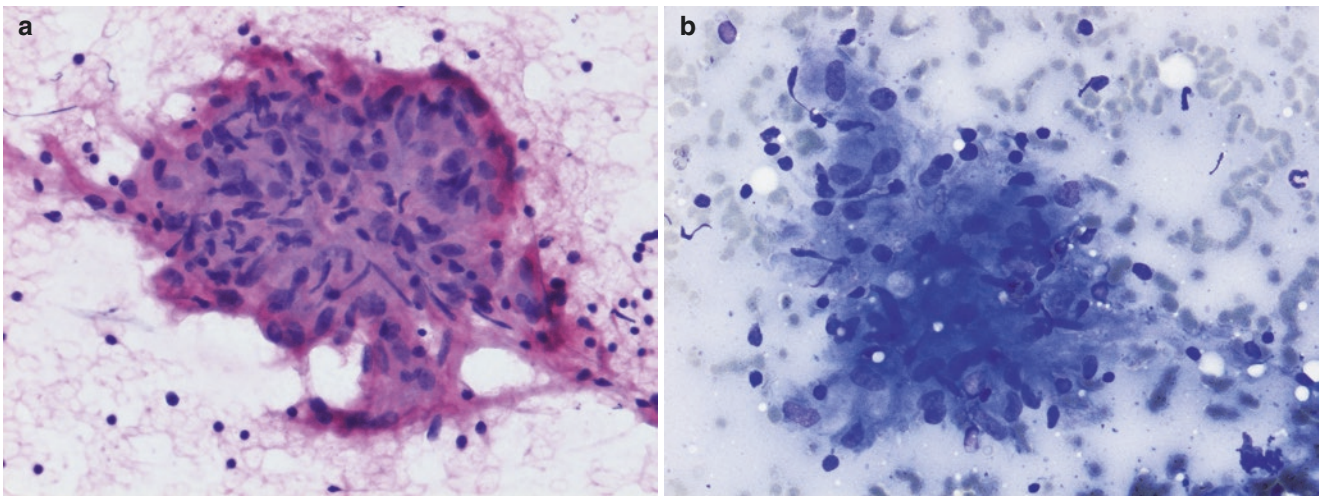

Fig. 1.11 Alcohol-fixed (**a**), (H&E) and air-dried (**b**), (MGG) FNA smears from an enlarged lymph node of a previously healthy 25-year-old man. Morphology of granulomatous lymphadenitis. Although the pathogenesis of granuloma is not apparent on the smear, a descriptive report excludes malignancy and guides further work-up

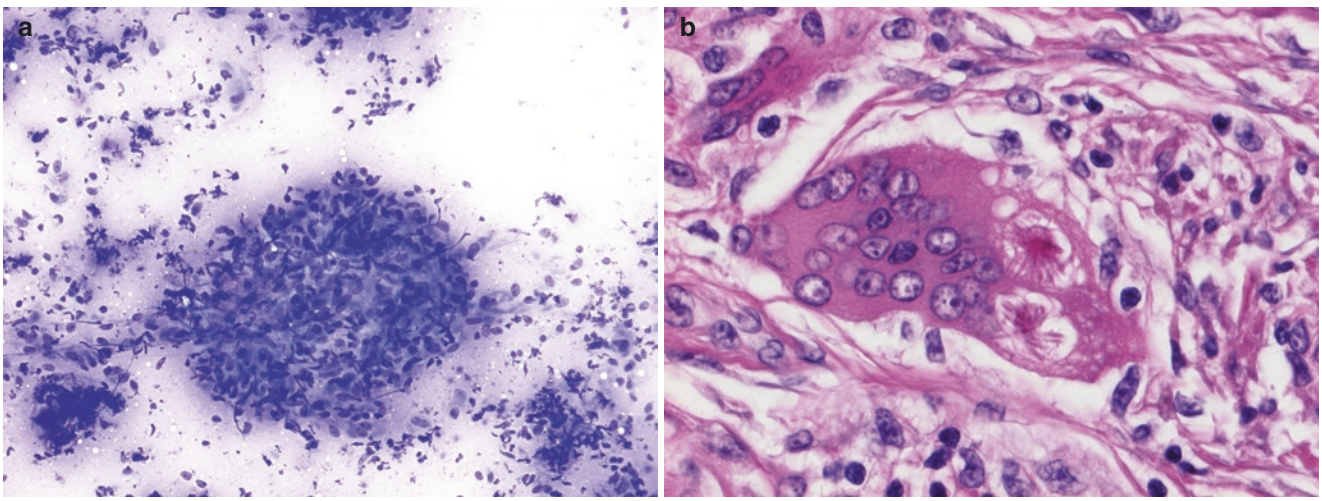

Fig. 1.12 FNA smears from a 5 cm subcutaneous swelling in the foot of a 40-year-old man. Granulomatous inflammation indicative of an inflammatory/reactive process (**a**). (MGG) Further investigation with incisional biopsy of the foot lesion from the previous figure disclosed sarcoidosis (**b**) (H&E)

FNAC Techniques and Preparation of Samples

FNAC Techniques

Depending on the clinical situation, the cytopathologist, surgeon, or radiologist may perform the FNA. The FNA procedure should be clearly explained to the patient to assure patient's cooperation during FNA procedure. The patient should be placed in a comfortable position that allows easy access to the lesion that needs to be aspirated. For FNA of palpable, superficial lesions, the skin over the area of the procedure is cleaned with an alcohol or with another antiseptic solution. Preparations as for minor surgical procedures are necessary for guided FNA of deeper, non-palpable lesions.

For palpable lesions a 27- to 22-gauge (0.4–0.7 mm) needles are suitable, either using a capillary technique without aspiration [56, 57] or a plastic disposable 10- or 20-ml syringe attached to a plastic or metal syringe holder (FNA with aspiration) (see Fig. 1.13). For guided FNAs of non-palpable, deeper lesions, longer needles are utilized. A large variety of specialist needle types and sizes are available.

The nodule is immobilized between the fingers, and the needle tip is rapidly directed through the skin into the nodule.

Once the needle enters the mass, the needle is continuously aspirated, while the needle is rapidly moved back and forth to obtain the sample. Suction is then relieved and the needle is withdrawn and detached from the syringe. Air is then aspirated into the syringe, the needle is replaced onto the syringe, and the material is expelled from the needle onto the glass slides (see Fig. 1.14a). The material is gently but rapidly smeared on the slides (see Fig. 1.14b) and immediately dipped in fixative (see Fig. 1.14c) or left to air dry. For liquid based preparation the aspirate is collected directly in specially developed liquid fixative (see Fig. 1.14d).

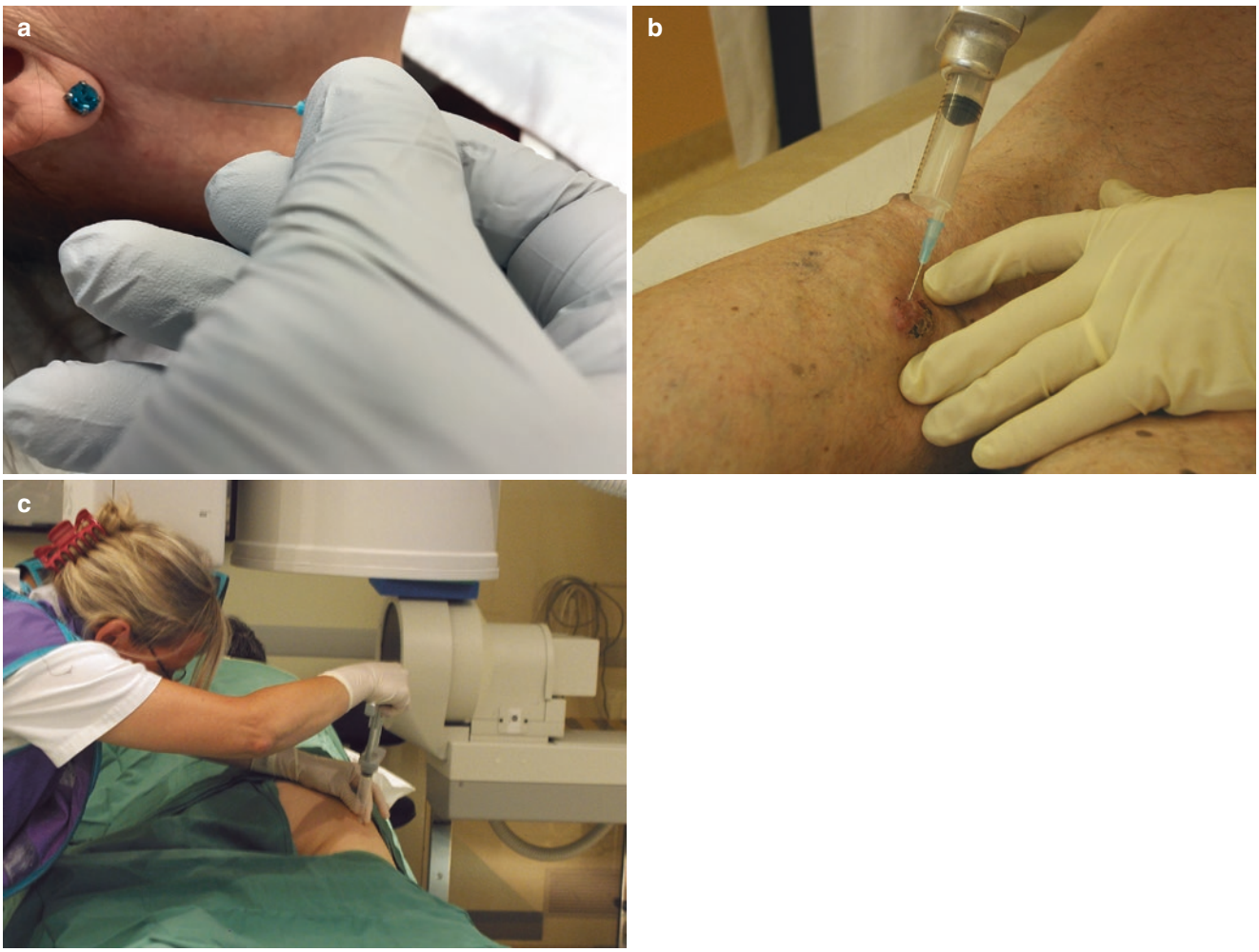

Fig. 1.13 (**a**) FNA procedure without aspiration (capillary technique): The needle tip is passed through the skin, inserted into the lesion, and moved back and forth to dislodge cells. (**b**) FNA of cutaneous basal cell carcinoma performed by a pathologist. Syringe attached to a Cameco syringe holder: vacuum in the syringe helps to dislodge cells and to obtain a cellular specimen. (**c**) Fluoroscopy-aided FNA of skeletal chondrosarcoma. The equipment and aspiration technique is entirely the same as in the aspiration of palpable masses

Fig. 1.14 (**a**) Air is aspirated and the material is expelled on glass slides. (**b**) The material is gently but rapidly smeared on the slides. (**c**) Slides with smears are immediately dipped in fixative or left to air dry. (**d**) For liquid based preparation the aspirate is collected directly in specially developed liquid fixative

Conventional Preparation of FNA

The preparation of aspiration specimens has been well described in many textbooks. The principle is to spread a suitable amount of aspirate (usually about one drop) thinly and evenly over a microscopic slide that is then stained and mounted. The result should be comparable to a sectioned histological slide with regard to specimen thickness and evenness.

With regard to fixation, there are two common methods: air drying or wet fixing using either 95% ethanol or ethanol-based Cytospray as a fixative. Other liquid fixatives such as methanol, Saccomanno's fixative, acetone, isopropyl alcohol, and acetone/methanol or other combinations of fixative are less frequently used in the routine preparation of aspiration smears. Where both air-dried and wet-fixed slides have been shown to provide comparable results, each of these techniques has characteristic advantages and limitations. Although wet fixation usually better demonstrates such details as nuclear pattern, chromatin structure, and nucleoli (see Figs. 1.15 and 1.16), air-dried specimens give better information on cytoplasmic details (see Fig. 1.17), as well as the extracellular matrix (see Fig. 1.18) and the background material (see Fig. 1.19). Despite the general rules with regard to which diagnostic features and morphological details can best be appreciated in either air-dried or wet-fixed specimens, it needs to be pointed out that results of fixation and staining may vary among laboratories (see Fig. 1.20). There is a relative lack of standardization in handling of FNA specimens, compared to the routines followed for histopathological specimens. The choice of the fixation and staining determines largely by the tradition of a particular laboratory and by local practice pattern. The standardization of sampling, fixation, and staining procedures in aspiration cytology, as well as of immunocytochemical methods, is one of the important challenges to be overcome if the role of FNAC in morphologic evaluation of tumors is to expand.

Air-dried smears can be stained with Diff-Quik or May-Grünwald-Giemsa (MGG) and wet-fixed smears with hematoxylin and eosin (H&E) or Papanicolaou (Pap). Diff-Quik staining is often used for rapid on-site evaluation (ROSE) (see Fig. 1.21) of smears but in many centers has replaced MGG as the routine stain for air-dried smears. MGG staining gives sometimes a slightly better micromorphology compared to Diff-Quik (see Fig. 1.22).

According to our experience, the best results are obtained when microscopic evaluation is based on both wet-fixed and air-dried smears.

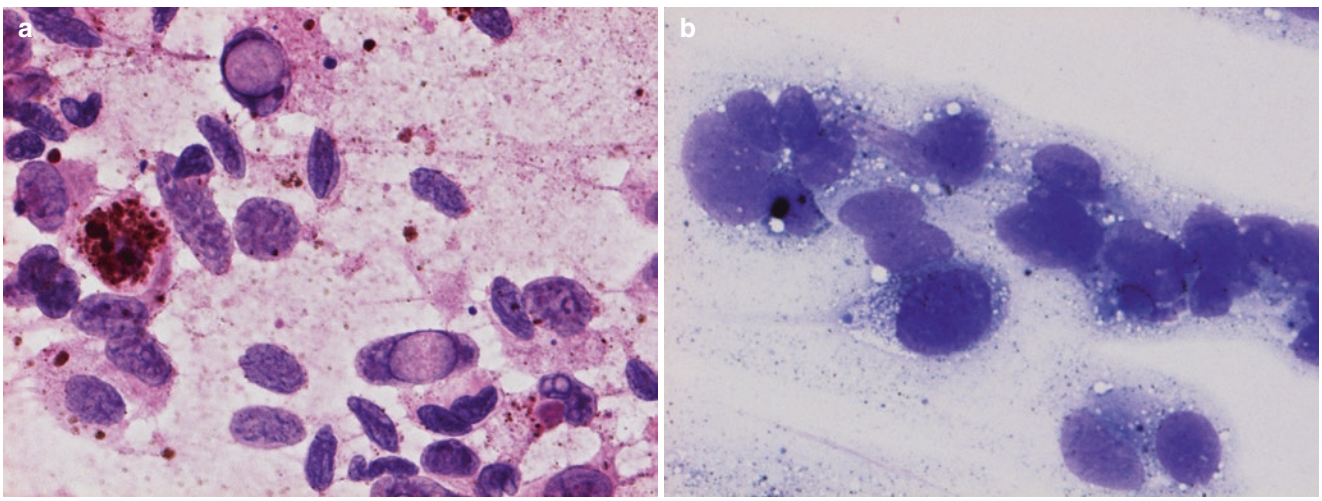

Fig. 1.15 FNA smears from metastatic malignant melanoma. Wet-fixed and H&E-stained smears (**a**) better appreciate nuclear details, such as inclusions, compared to air-dried and MGG-stained smears (**b**)

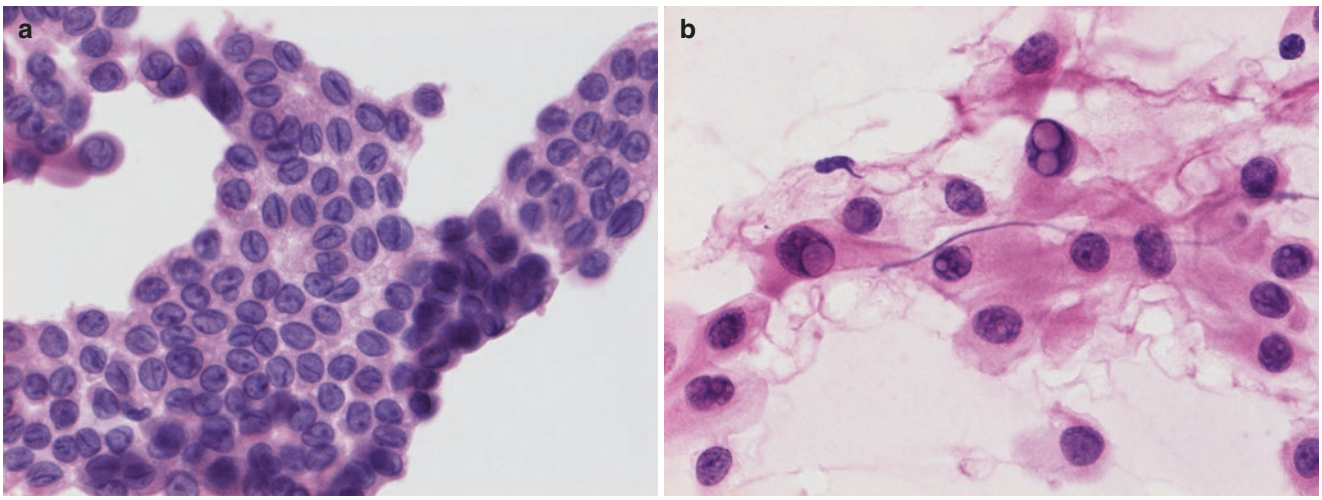

Fig. 1.16 FNA smears from thyroid papillary carcinoma. Nuclear details such as nuclear grooves (**a**) and nuclear inclusions (**b**) are better appreciated in wet-fixed and H&E-stained slides

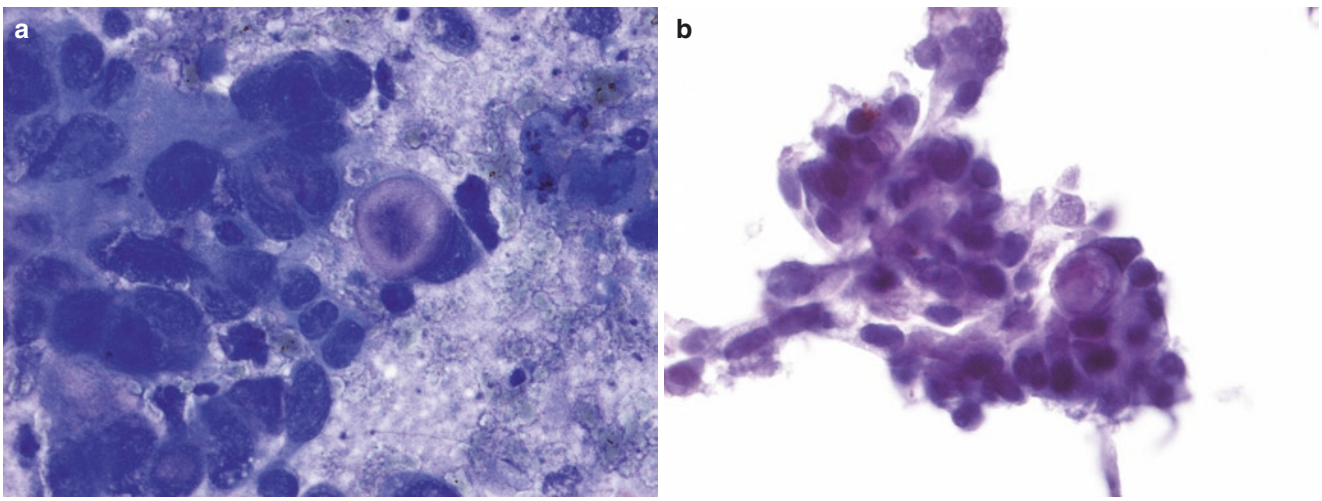

Fig. 1.17 FNA smears from metastatic pancreatic adenocarcinoma. Note mucin vacuoles in the tumor cells, better appreciated in air-dried and MGG-stained smears (**a**) than in liquid-based preparations and H&E-stained smears (**b**)

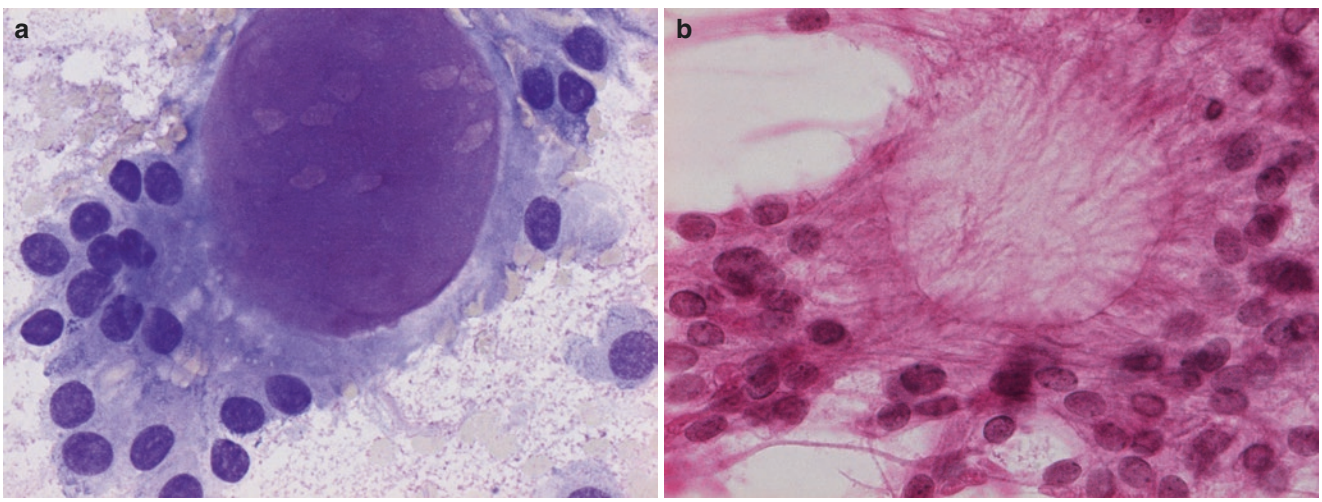

Fig. 1.18 FNA smears from myxopapillary ependymoma. Note distinctive hyaline globules in the MGG staining (**a**) compared to shadows appearing at the same locale in H&E staining (**b**)

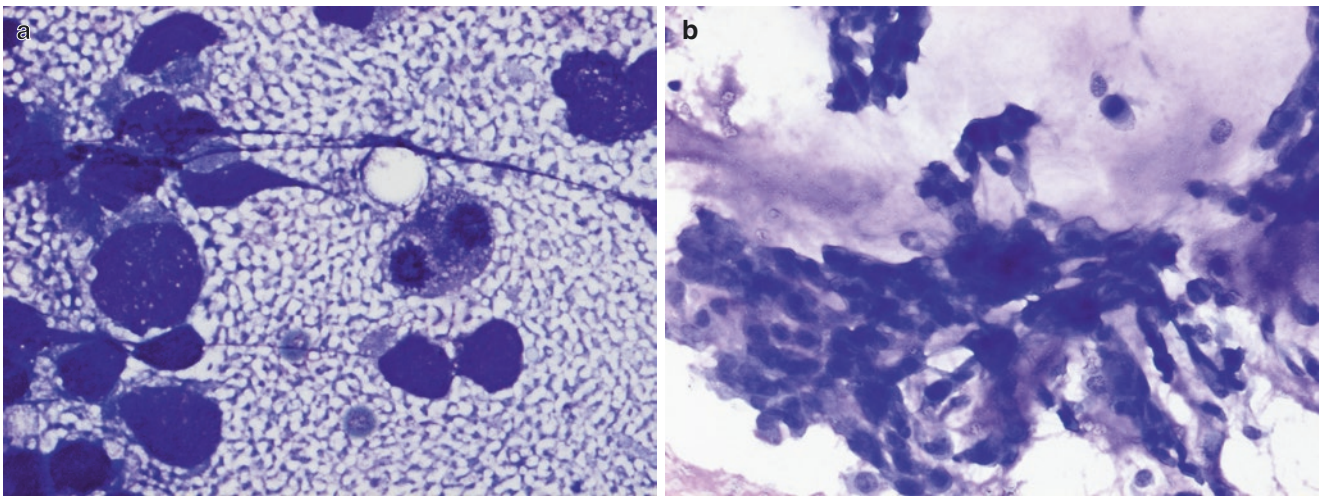

Fig. 1.19 Distinctive "tigroid" background of retroperitoneal metastatic seminoma in the air-dried and MGG-stained smears (**a**). Myxoid background matrix in the air-dried and MGG-stained smears from metastatic extraskeletal myxoid chondrosarcoma (**b**)

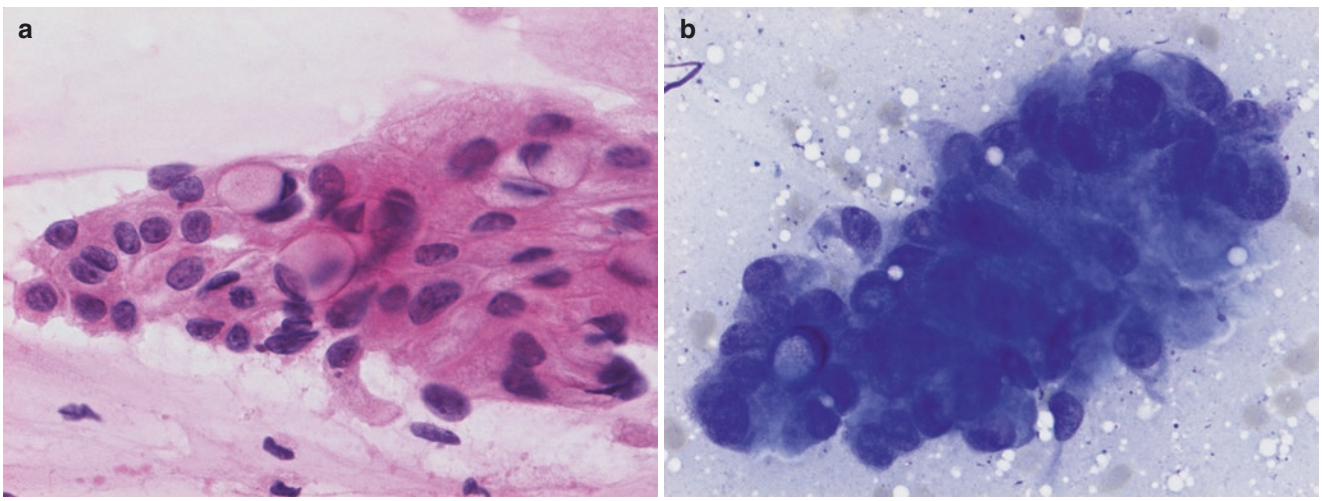

Fig. 1.20 FNA smears from metastatic gastric adenocarcinoma. Compared to Fig. 1.17, mucin vacuoles in the tumor cells are better appreciated in H&E (**a**) than in MGG staining (**b**)

Fig. 1.21 Arrangement for rapid Diff-Quik staining

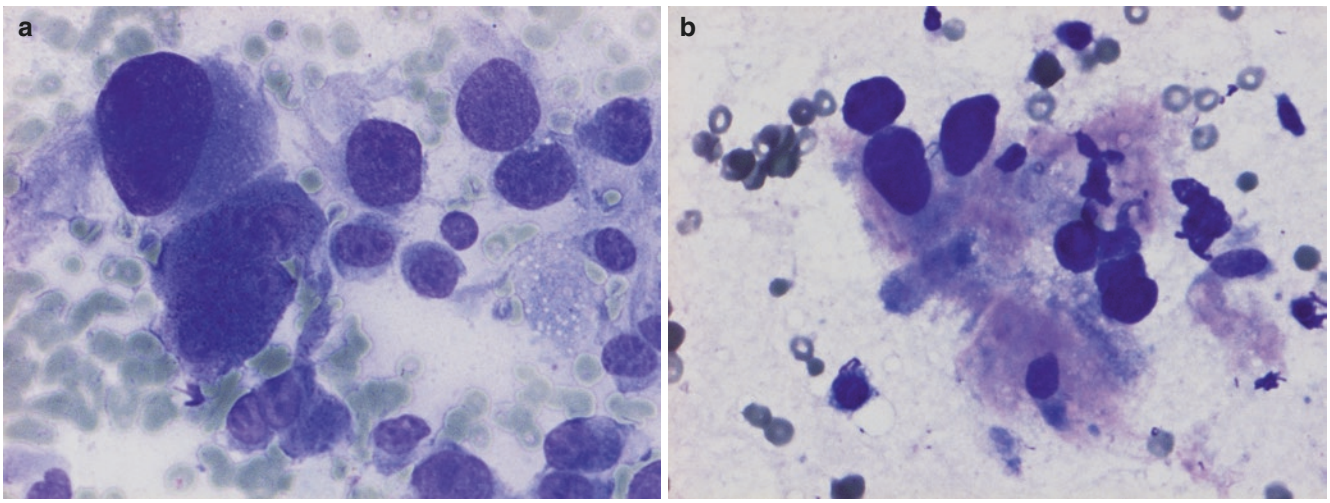

Fig. 1.22 FNA smears from high-grade osteosarcoma. MGG staining (**a**) provides slightly better micromorphology compared to the rapid Diff-Quik staining (**b**)

Cell Blocks

Smears can be difficult to prepare reliably as a result of the nature of the aspirate (cystic components, blood contamination) or due to inexperience in the person making the smear. Of the various techniques devised to make best use of aspirates, we and others have found that the preparation of cell blocks (CB) can be very helpful [61–63].There is usually adequate material for many sections from the resulting paraffin block, and the microbiopsies show a preserved tissue architecture, which can aid diagnosis. Since Koss described the plasma-thrombin method [64], several other techniques of preparing CB have been reported. CB preparation advantages are twofold: (1) better visualization of the tumor tissue pattern than is possible in FNA smear (see Figs. 1.23, 1.24, and 1.25) and (2) an opportunity of performing immunocytochemical and other special stains on several slides of comparable quality. It should be pointed out that CB quality derived from aspiration specimens is not exclusively a function of the quantity and quality of the cell material obtained. Some techniques for preparing CBs include a centrifugation of the specimen that can partly damage the cells, resulting occasionally in poor morphology, compared to traditional preparing techniques. A processor recently developed by the Hologic company, in which centrifugation has been replaced by filtration, seems to better preserve cells and tissue fragments, resulting in slightly better morphology (see Fig. 1.26).

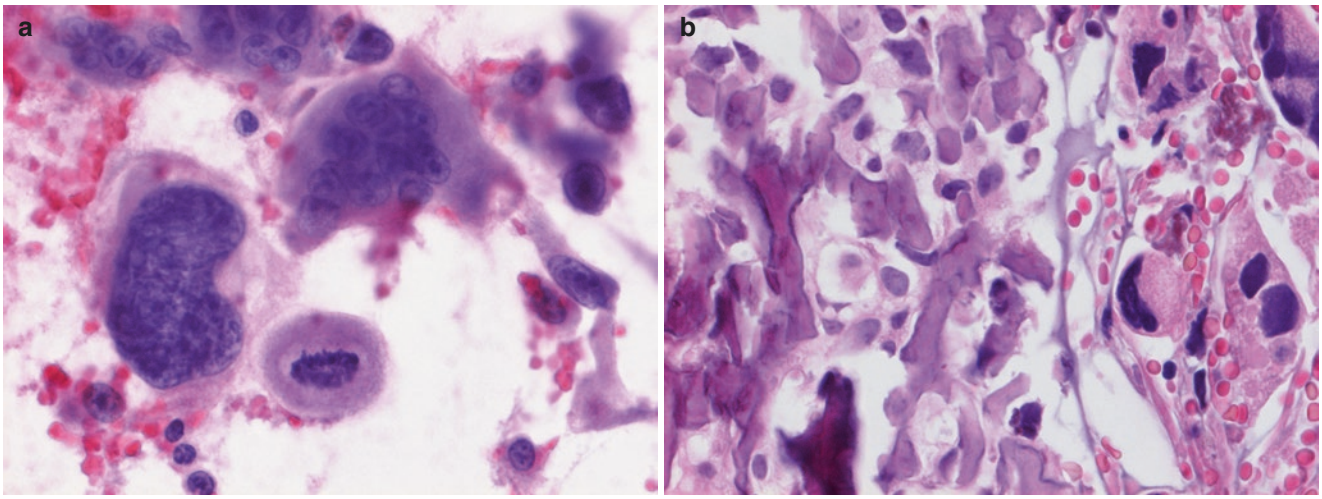

Fig. 1.23 FNA smears from high-grade osteosarcoma (**a**) (H&E) showing obvious features of high-grade sarcoma. Cell block prepared from the aspiration smears facilitating examination of tumor tissue architecture and occurrence of osteoid (**b**) (H&E)

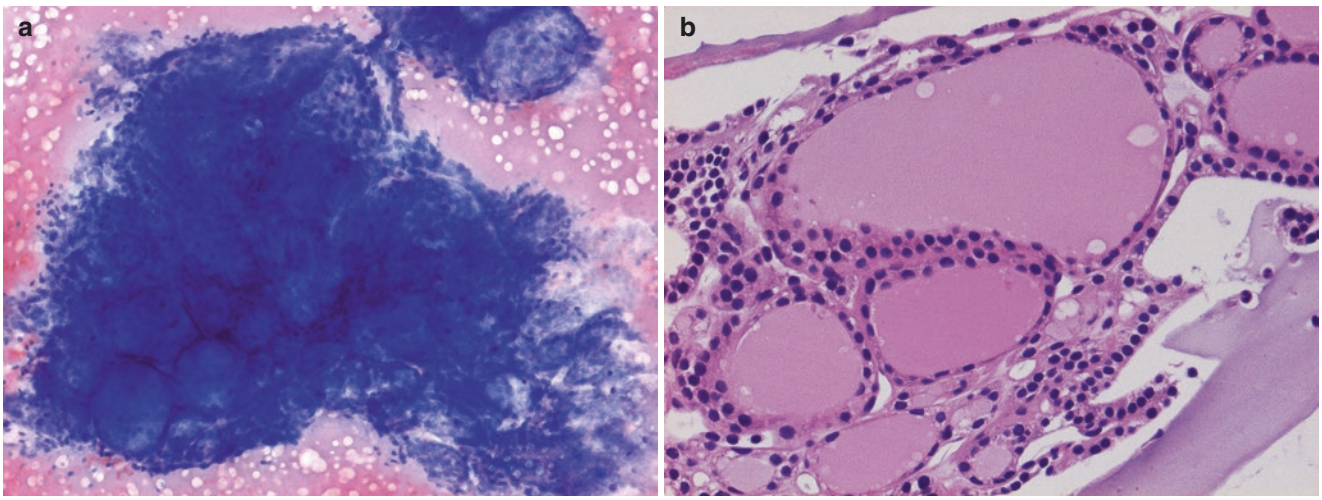

Fig. 1.24 FNA smears from colloid goiter (**a**) (MGG). Cell block prepared from the aspiration smears facilitating a diagnosis of benign goiter (**b**) (H&E)

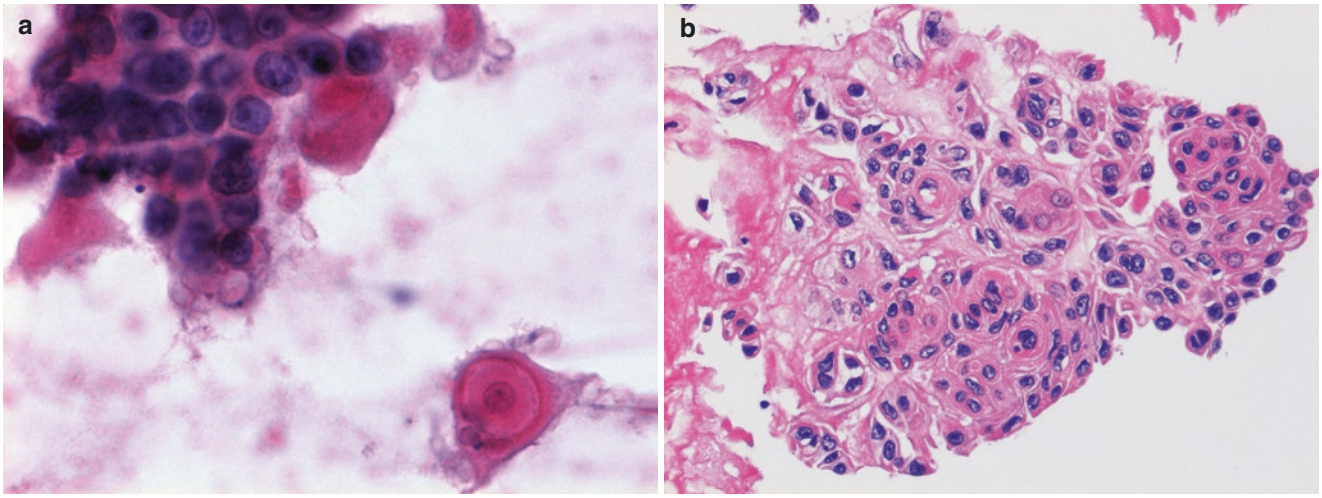

Fig. 1.25 FNA smears from a cystic metastasis of squamous carcinoma to the neck (**a**) (H&E). Cell block prepared from the aspiration smears confirming keratinizing squamous carcinoma (**b**) (H&E)

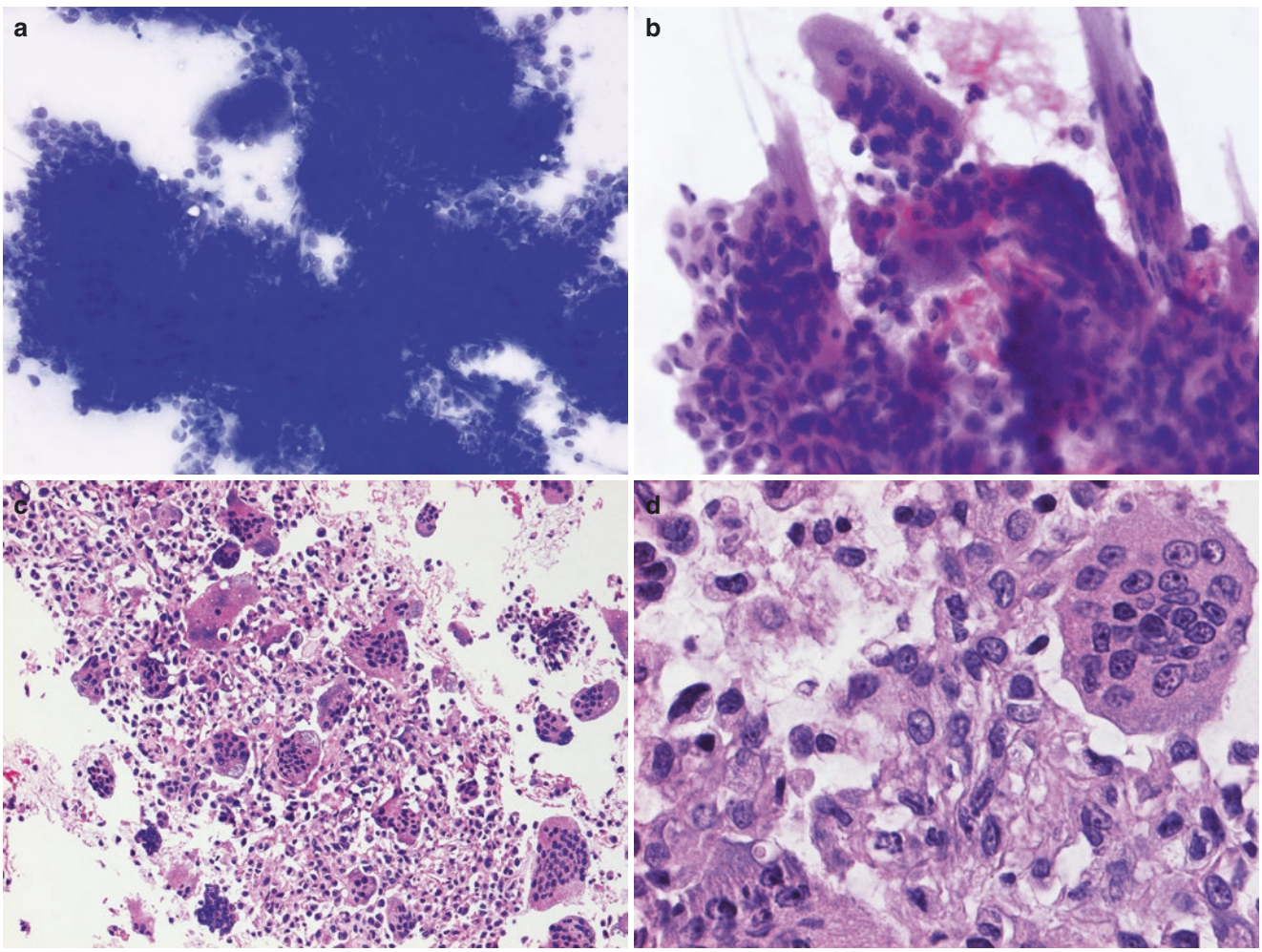

Fig. 1.26 FNA smears from a giant cell tumor of the fibula. MGG staining (**a**) and H&E staining (**b**): cell block prepared from the aspiration using filter technique shows good histomorphology with well-preserved histo- and cytomorphological details. Low-power (**c**) and high-power view (**d**) (H&E; Cellient; Hologic; Bedford, MA, USA)

Liquid-Based Preparations

The preparation of exudates, urine, and bronchial and bladder washings has always been "liquid based," and results in these situations have been satisfactory. It has become popular to prepare even cervix cytology and aspirates through a liquid medium. The liquid-based ThinPrep (TP; Hologic, Marlborough, Mass., USA) and SurePath (SP; BD TriPath, Burlington, N.C., USA) methods have become widely used for both gynecological and non-gynecological specimens including FNAC. Liquid-based preparation (LBP) was designed to improve traditional preparations techniques [65–67], but the result for FNAC can be occasionally problematic. Diagnostic criteria may be different in liquid-based specimens compared to conventional smears because of somewhat altered morphology, and because the smear background and extracellular elements, which is sometimes an important part of the diagnosis, is frequently lost after processing through Cytolyt [68]. Architectural changes such as discohesion of cells, smaller cell clusters and sheets, breakage of papillae, as well as generally smaller and occasionally spindled cells with attenuated chromatin detail and more prominent nucleoli are all common findings in LBP aspirates [66, 67, 69–72]. Gerhard et al. [73] reviewed the published literature of LBP applied to breast FNAC. They concluded that LBP has similar accuracy to conventional smears for the FNAC diagnosis of breast lesions and can be safely used in the preparation of breast aspirates. This technique is easier for collection of samples and can provide appropriate ancillary tests. Characteristic cytologic features of breast aspirates prepared using LBP require appropriate training, however, to prevent misinterpretation [73]. In our experience in Lund, cells and tissues obtained from epithelial neoplasms are usually better preserved than those of mesenchymal origin after liquid-based processing (see Figs. 1.27 and 1.28). Nevertheless, this method can be very useful, especially in the area of immunocytochemistry [68] (see Fig. 1.29) and molecular biological analyses [65, 74–79]. Cell blocks can be prepared with residual specimens preserved in a liquid-based cytology medium and immunocytochemical stains, and molecular testing can be successfully performed. These are important adjuncts in order to reach a definitive diagnosis.

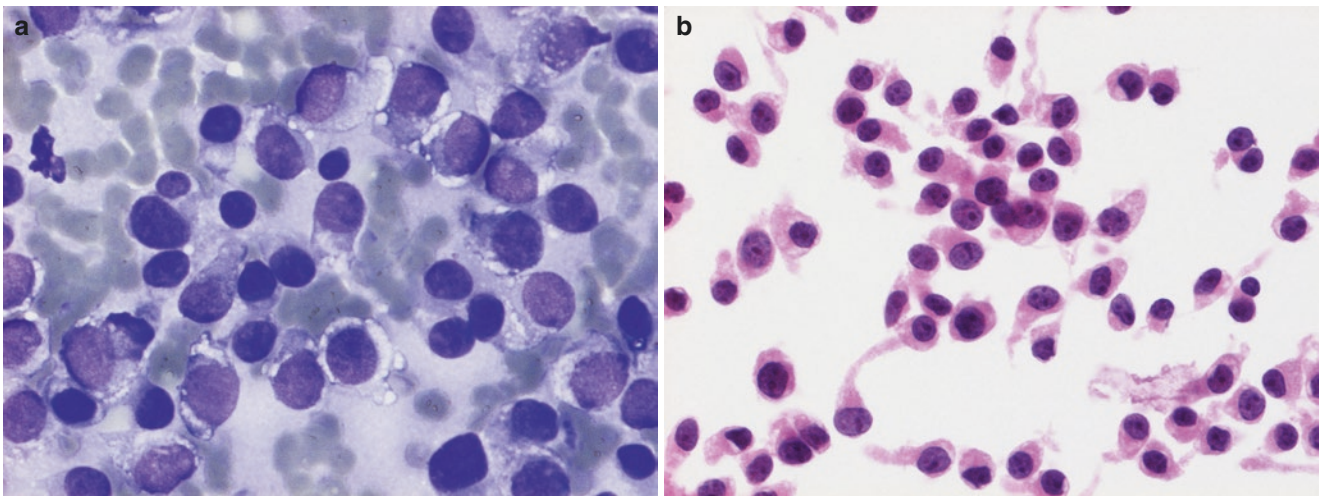

Fig. 1.27 FNA smears from Ewing's sarcoma. Air-dried and MGG-stained smears (**a**). Note double cell population and apparent vacuolization of cytoplasm in the larger light cells, indicative of glycogen deposition. The same cell specimen prepared by ThinPrep (**b**) (H&E). The double cell population and cytoplasm vacuolization are not apparent

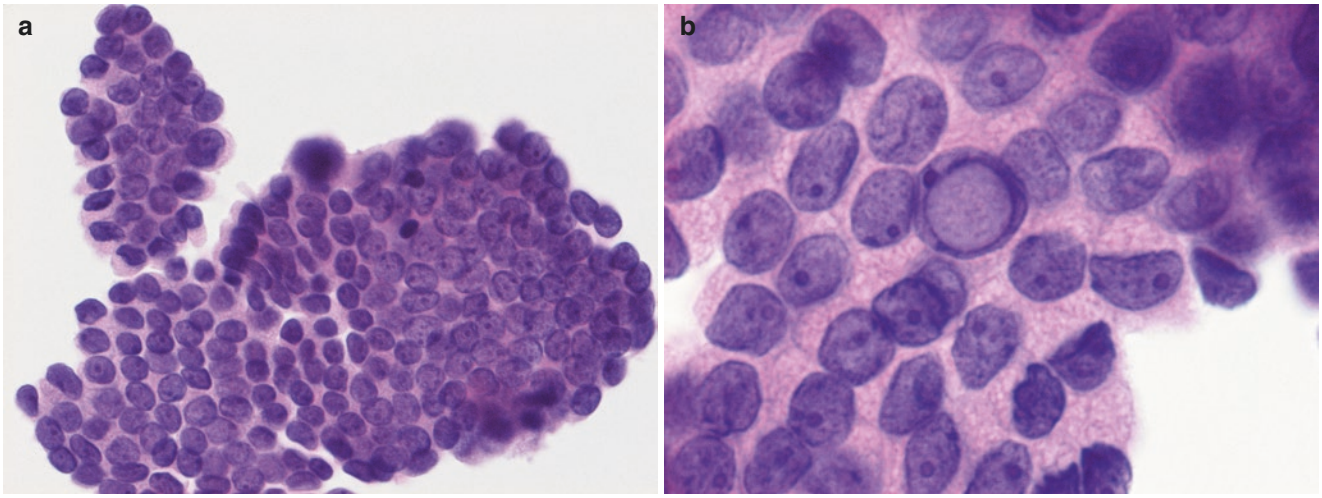

Fig. 1.28 Smears from papillary thyroid carcinoma prepared as ThinPrep. Note well-preserved monolayer morphology (**a**) (H&E) and typical intranuclear inclusion (**b**) (H&E)

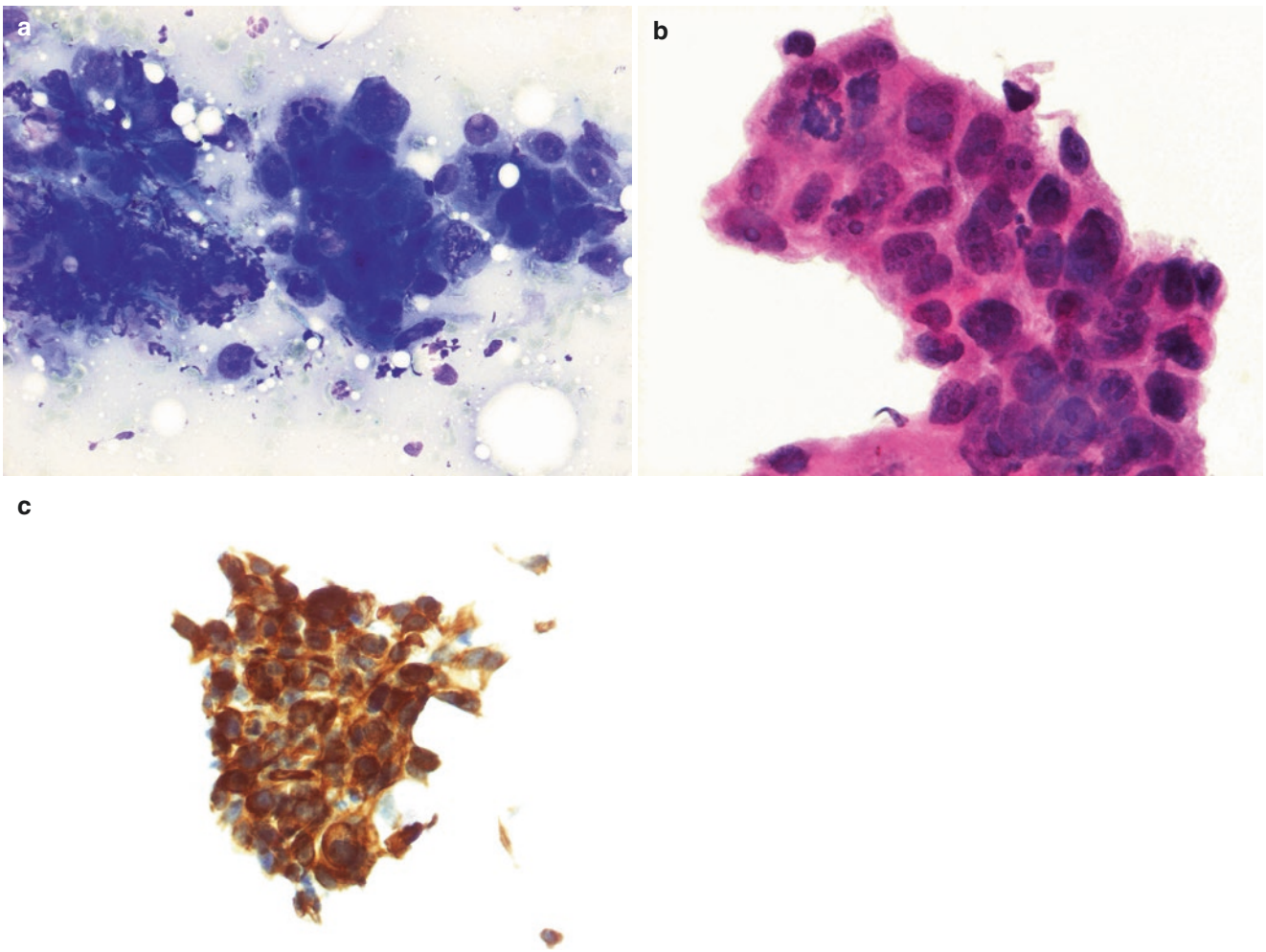

Fig. 1.29 Sister Mary Joseph nodule. FNA smears of umbilical nodule in a patient with a history of urothelial carcinoma of the bladder. MGG-stained, air-dried smears (**a**), and ThinPrep slides stained with H&E indicative of the metastatic urothelial carcinoma (**b**). Immunostains for keratin performed on ThinPrep preparation of residual specimen showing tumor cells with distinctive keratin positivity (**c**)

Ancillary Techniques in FNAC

Ancillary techniques are used extensively today as a diagnostic help in FNAC. Essentially all the methods used for surgical biopsy specimens can be applied to cytological material. Of these methods, immunocytochemistry (IC) is the one most often applied to FNA specimens. Molecular genetic methods are becoming increasingly important to verify or rule out a specific diagnosis for prognostic information and therapeutic drug selection [9]. Particular genetic changes have been described in many types of tumor, and these are steadily increasing. Several genetic and cytogenetic molecular methods, including cytogenetics (chromosome banding), fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), single nucleotide polymorphism (SNP) array, and next-generation sequencing (NGS), can be applied to cytological material. Experience has shown that chromosome banding analysis of cells from FNA specimens is difficult; it is not always possible to aspirate a sufficient number of tumor cells for successful culture [80, 81]. Molecular cytogenetic or molecular genetic techniques have proved to be better suitable for fine-needle aspirates as they do not require cell culturing and thus fewer cells. A number of reports on the diagnostic usefulness of molecular genetic techniques applied to FNA aspirates have been published, and the importance of molecular genetic examination of FNA samples from a variety of neoplasm constantly increases [8, 9, 81–92].

Electron microscopy (EM) applied to cytological specimens can reveal certain differentiated structural features and clarify the origin of cells that lack definite signs of differentiation by light microscopy [93–96] (see Fig. 1.30).

Flow cytometry is one of the techniques established in the area of cytology, playing an important role as a diagnostic adjunct in the examination of FNAC specimen from hematopoietic organs, mainly in the diagnosis and classification of leukemia and lymphoma [97–104].

One cannot forget that ancillary studies play a supportive and complementary role in the diagnostic process and must always be subordinate to the routine microscopic examination of smears and the clinical information regarding the case.

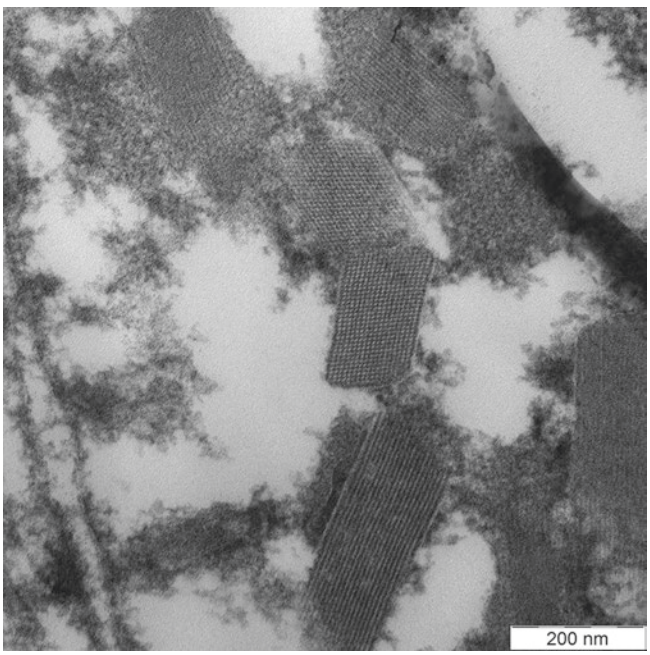

Fig. 1.30 Ultrastructure of alveolar soft part sarcoma. Tumor cells contain cytoplasmic crystals that reveal periodic linear, lamellar structure. (Image courtesy of Ms. Catarina Crammert, Department of Pathology, Skåne University Hospital, Lund, Sweden)

Immunocytochemistry

The most common ancillary technique used to complement routine microscopic examination of aspirated material is immunocytochemistry (IC). IC was designed to improve routine cytology to aid pathologist/cytopathologist in the differential diagnosis of neoplasm and to render confident and accurate diagnoses on limited tissue samples. As the technique evolved, it has become increasingly important for selection of therapy in some tumor type and to provide prognostic and predictive information [12, 105–107]. IC may be applied to any of the several types of preparations: to direct smears, cell-transferred direct smears [108, 109], cytospin preparations [110], liquid-based preparations, or cell blocks. Compared to histology, the results of immunostains of cytological material, however, can be difficult to standardize and can vary depending on the type of specimen fixation and preparation. In addition, IC controls are difficult to prepare, control, and maintain [16]. In our experience, the results of IC examinations of aspirates are dependent not only on the type of specimen preparation but also on the quality and quantity of the material, background material, and the presence of necrosis.

Direct smears and slides from cytocentrifugation have heretofore been commonly used techniques to prepare FNA material for IC. IC performed on direct smears can occasionally be difficult to evaluate due to trauma inherent in the technique. Nuclei can be stripped of their cytoplasm, and the background material can contain the fragments and remnants of many different cells (see Figs. 1.31 and 1.32). One problem occurring occasionally in the interpretation of IC results on direct smears and slides from cytocentrifugation is misinterpreting background/cytoplasmic staining as nuclear staining (see Figs. 1.31, 1.32, and 1.33).

Liquid-based cytological preparations have recently become a popular technique for preparing FNA material for immunostaining as well (see Fig. 1.34) [111–113]. In the author's experience, results of such preparations are promising (see Figs. 1.35 and 1.36).

Cell block preparations using paraffin-embedded cells are becoming increasingly popular in many busy FNA clinics, allowing both examination of the tissue architecture and making immunostaining more reliable (see Figs. 1.37, 1.38, and 1.39). An advantage of the preparation of cell blocks is that the processing of these slides for immunostaining follows the same procedures as for histology specimens (see Fig. 1.39) [86, 113–123].

Advantages and disadvantages of preparation methods of FNA aspirates for IC are presented in Table 1.1.

It bears reemphasizing that IC must be used as a complement to the routine cytological examination of the aspirate. Traditional light microscopic examination of air-dried or alcohol-fixed and routine-stained smears or liquid-based prepared smears is still the basis of cytological diagnosis.

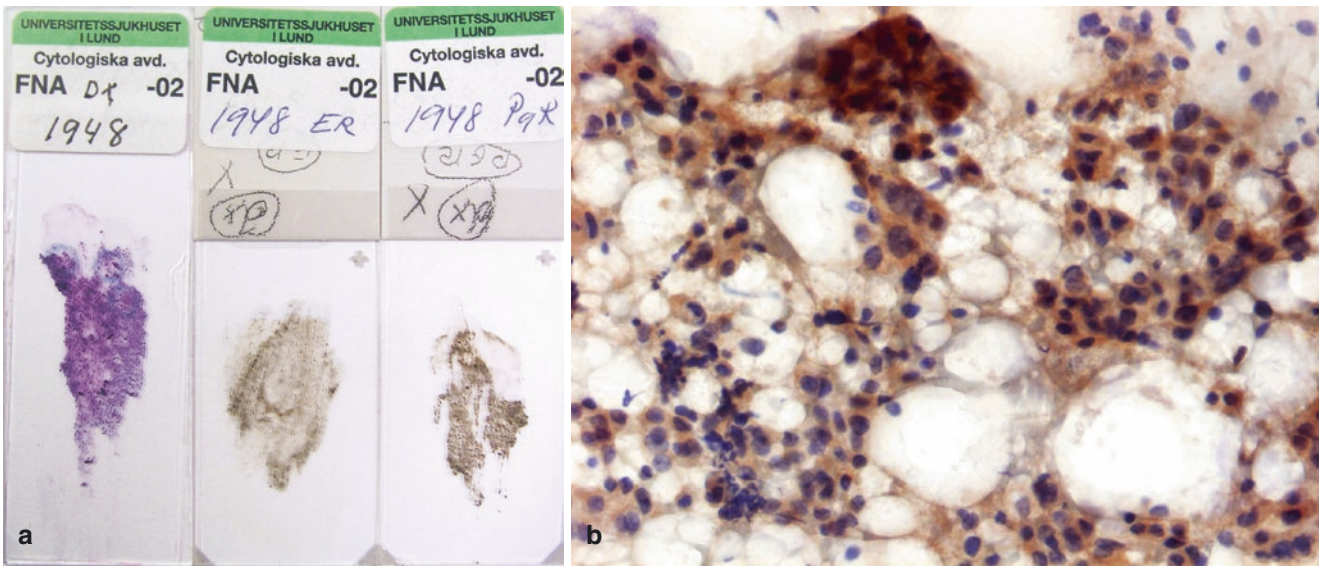

Fig. 1.31 Slides with direct FNA smears from breast carcinoma processed for estrogen IC (**a**). The results may be difficult to interpret due to suboptimal technique including heavy background staining (**b**)

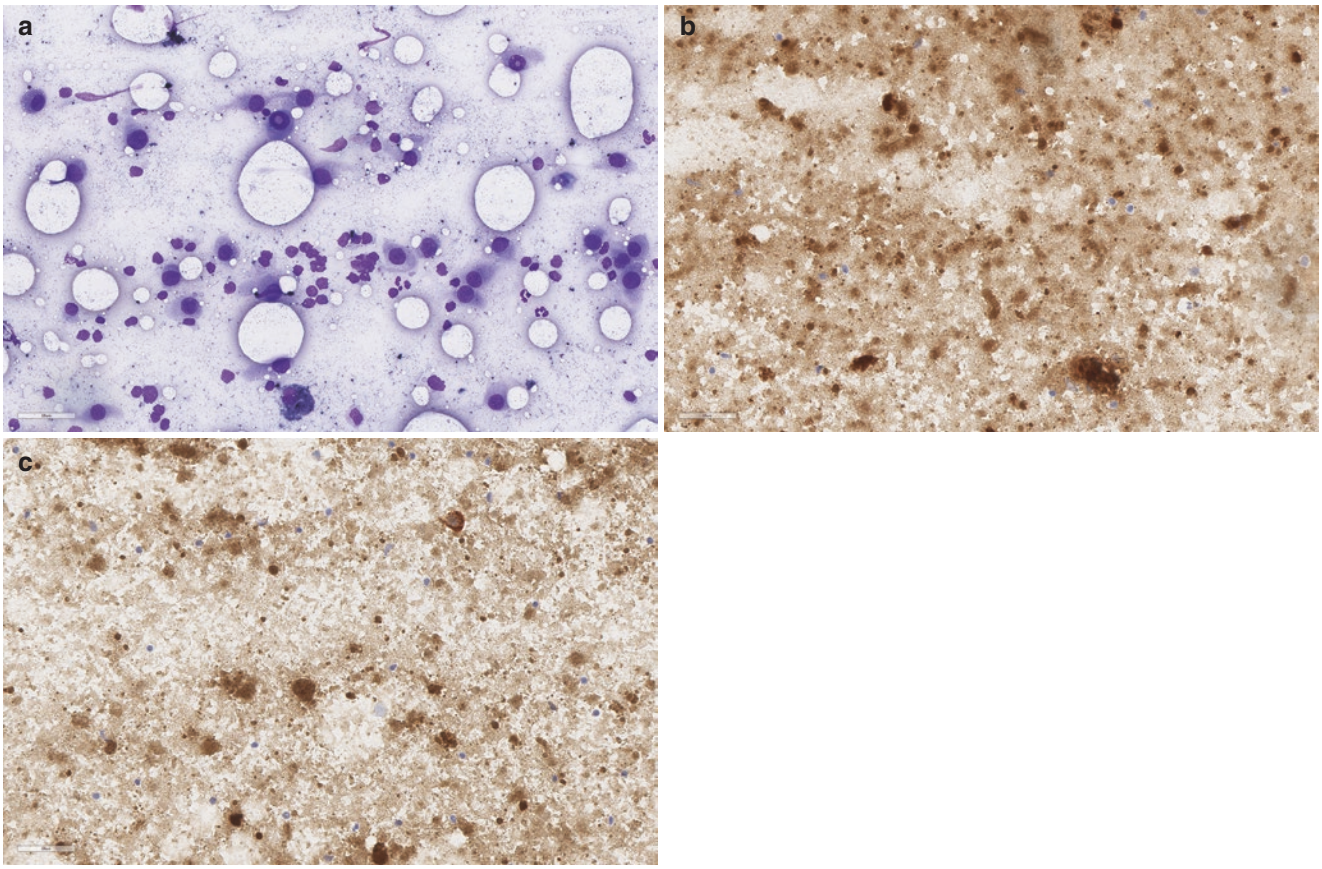

Fig. 1.32 Immunostains with S-100 and HMB45 on direct smears (**a**) from metastatic malignant melanoma with suboptimal results of both S-100 (**b**) and HMB45 (**c**): difficult to interpret due to technically suboptimal conditions

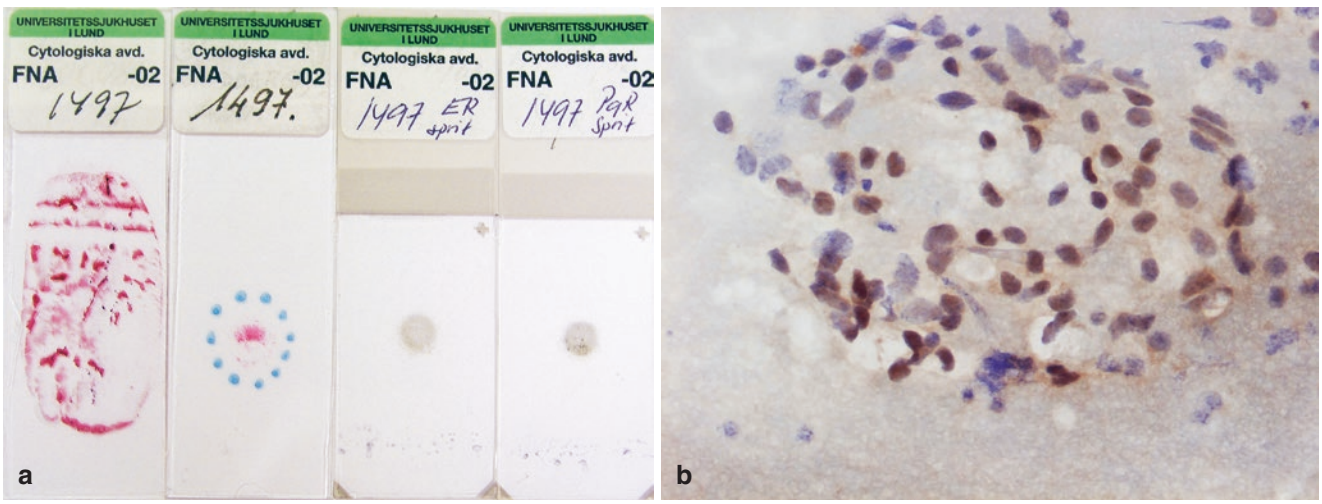

Fig. 1.33 Slides with cytocentrifuged smears (cytospin) from breast carcinoma processed for estrogen IC (**a**). As with direct smears, results may be difficult to interpret due to suboptimal technical conditions (**b**)

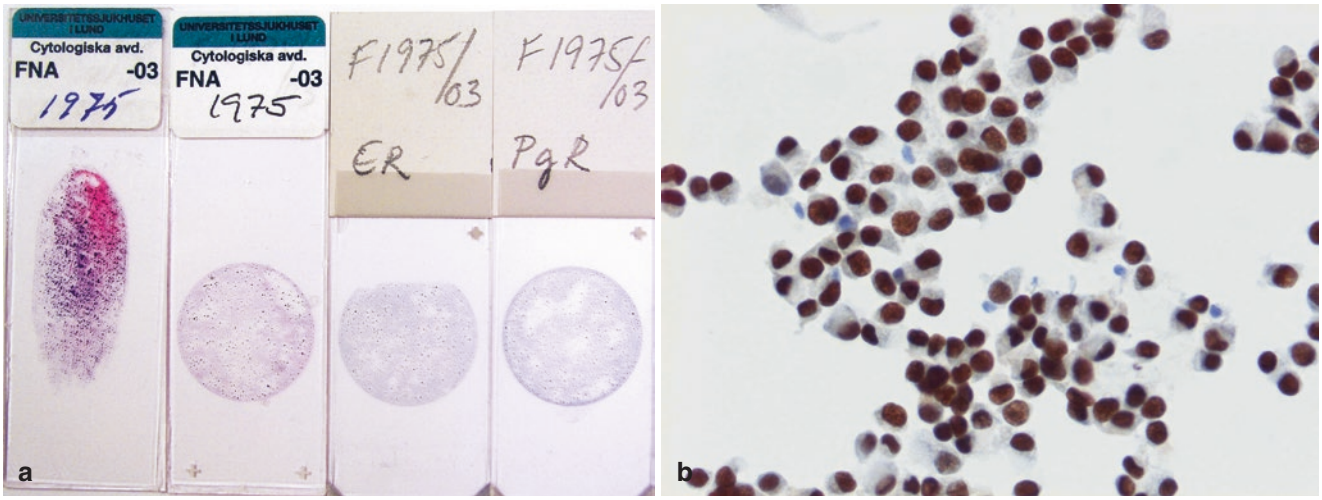

Fig. 1.34 Slides with ThinPrep-prepared smears from breast carcinoma processed for estrogen IC (**a**). The result of IC is comparable of that of histological specimen (**b**)

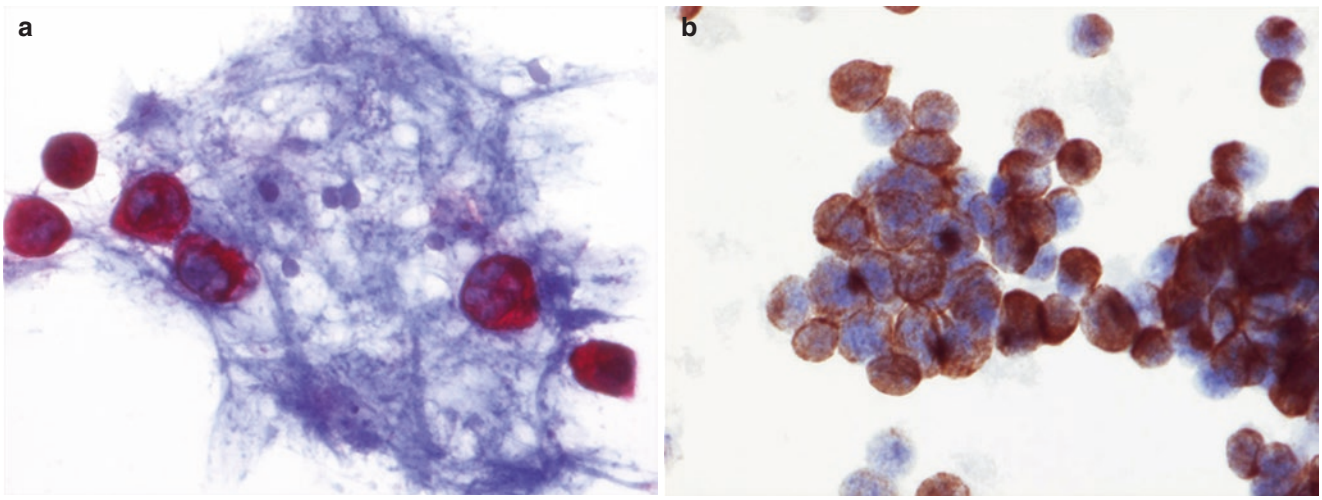

Fig. 1.35 The result of immunostains for desmin of the specimen aspirated from rhabdomyosarcoma prepared as centrifuged smears (**a**) and in ThinPrep processor (**b**)

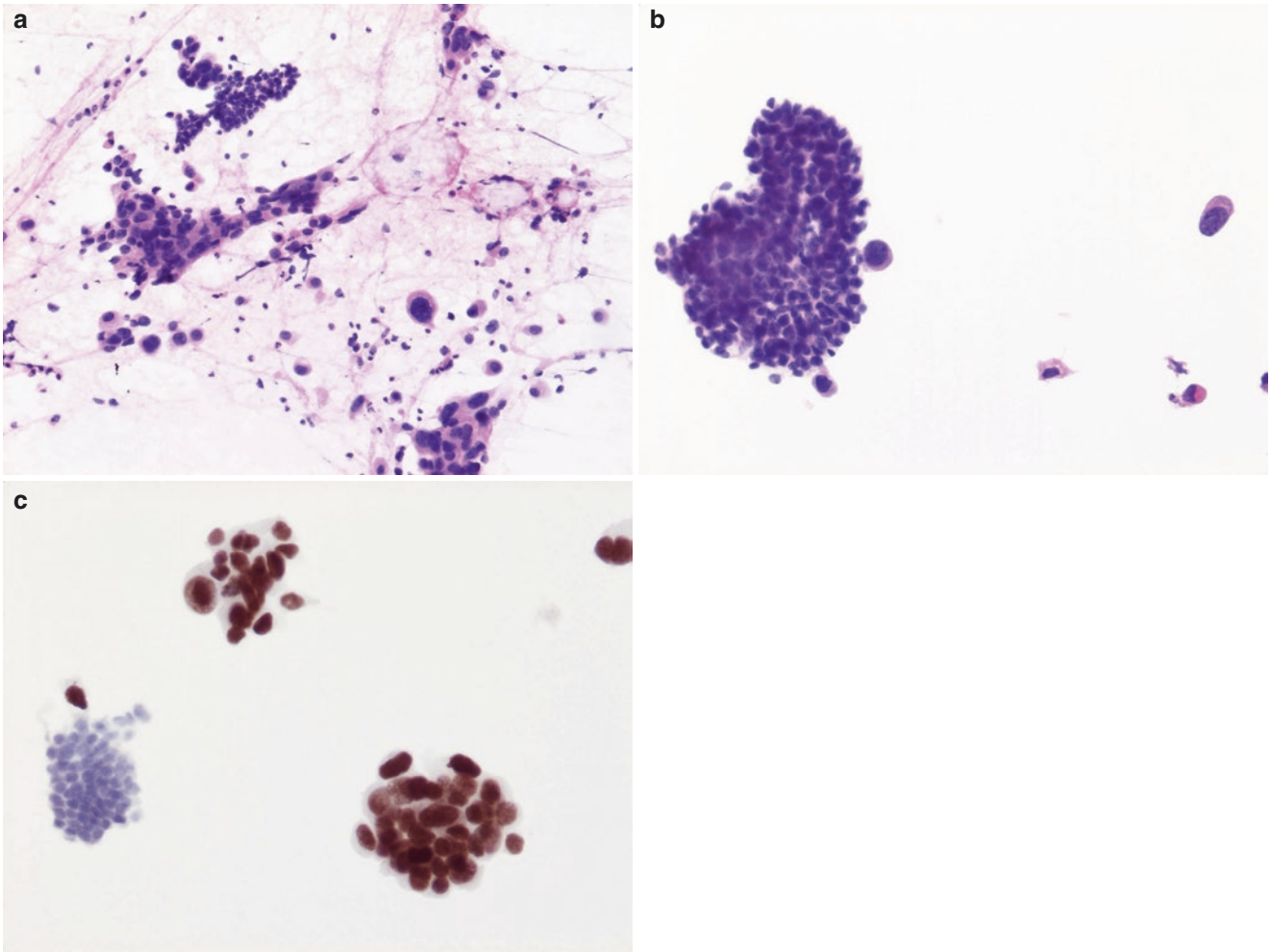

Fig. 1.36 Alcohol-fixed and H&E-stained FNA smears from a breast nodule in a patient with a history of lung carcinoma (**a**). Smears prepared in the ThinPrep processor showing a sheet of mammary epithelium with admixture of carcinoma cells (**b**). Immunostains performed on ThinPrep showing clear positivity for TTF-1, indicative of metastatic lung carcinoma (**c**)

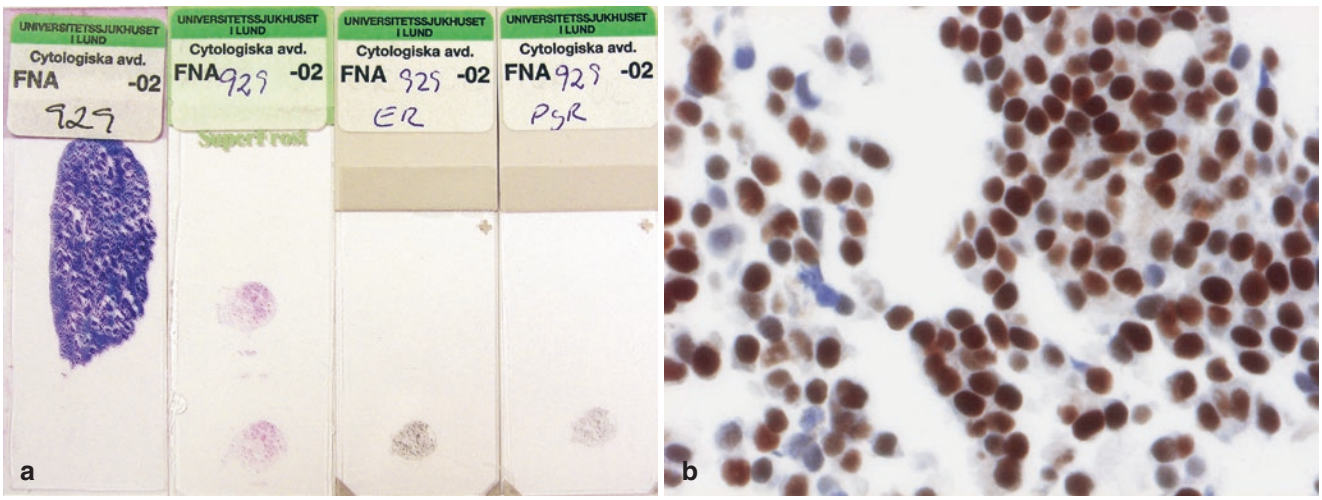

Fig. 1.37 Slides with cell block section from breast carcinoma processed for estrogen IC (**a**). The result is similar to immunohistochemical staining on histological sections from biopsy specimen (**b**)

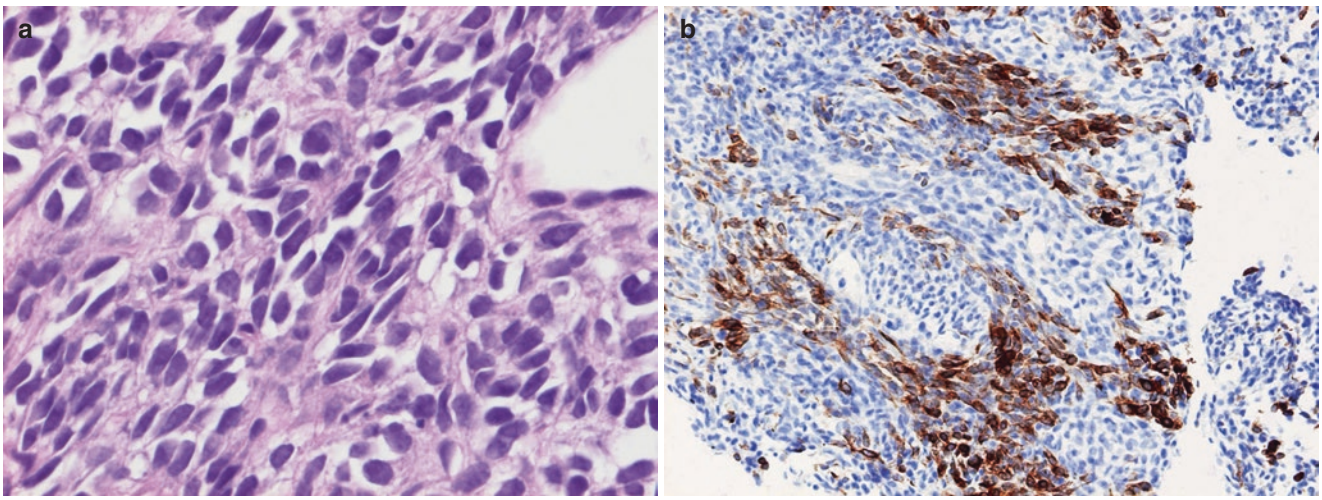

Fig. 1.38 Cell block section (**a**) and immunostains with EMA (**b**) on cell block section from monophasic synovial sarcoma. The result is similar to histological sections of biopsy specimen

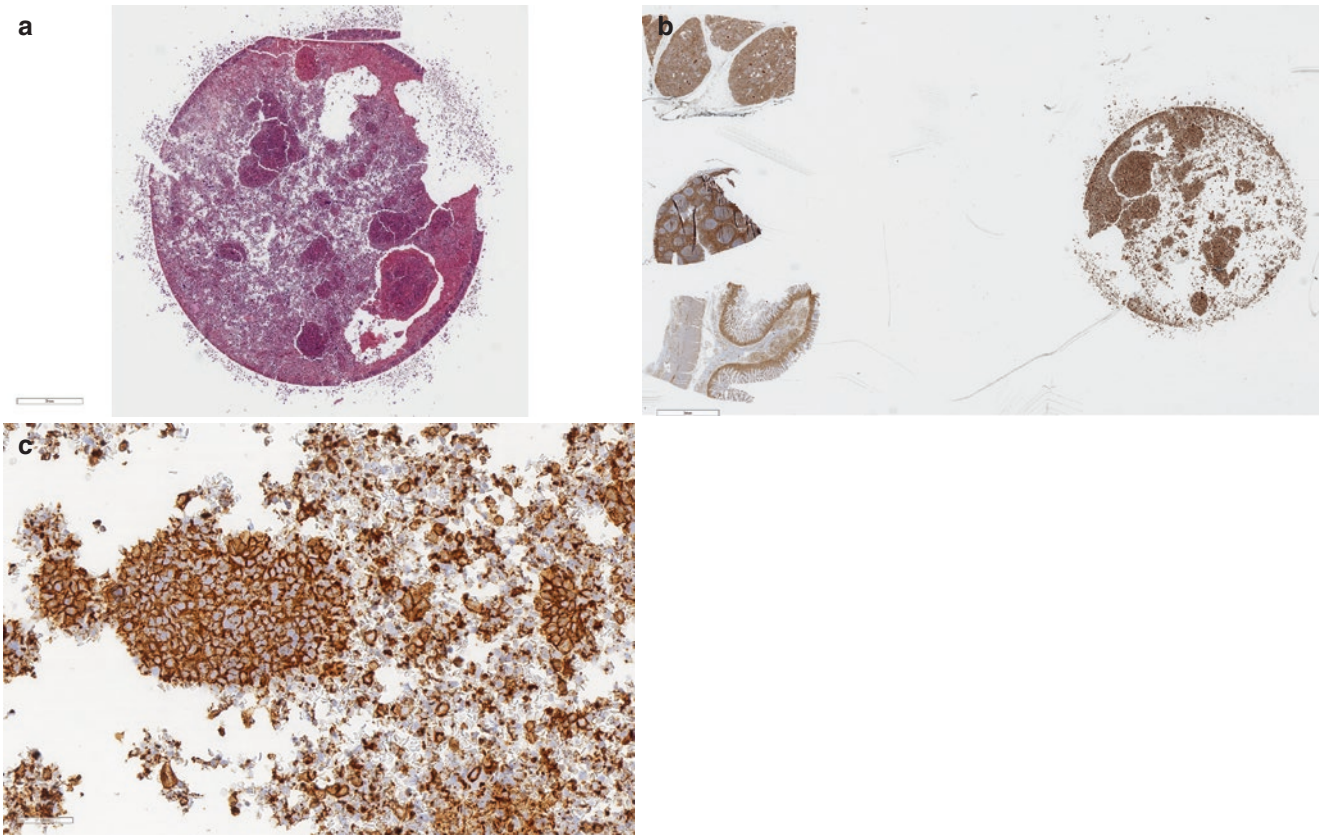

Fig. 1.39 Processing of the cell block slides (**a**) for immunostains follows the same procedure as for histology specimens with appropriate controls (**b**) and the result is similar to immunostains on biopsy specimen (Ewing sarcoma-cell block sections showing positive immunostains for CD99)

Table 1.1 Advantages and limitations of different preparation methods of fine-needle aspirates for immunocytochemistry

Preparation method	Advantages	Disadvantages
Direct smear	No specific preparation; universally accepted method for cytochemistry and immunocytochemistry	Can be difficult to evaluate due to cytoplasmic background and stripped nuclei. In our experience, nuclear antibodies suitable
Destaining of archive slides; cell-transferred direct smears	Can be used retrospectively	Not sufficiently evaluated; in our experience, gives a higher percentage of false-negative results
Cytospin preparation	Easy preparation. Universally accepted method for cytochemistry and immunocytochemistry	Risk for false-negative results due to focal expression of antibodies. Occasional difficulty in the interpretation of results
Cell block preparation: manually	Similar to a small histologic biopsy. Easy to perform controls and compare with subsequent IC on histological samples. Material can be saved for further evaluation	Time-consuming. Can be difficult to obtain sufficient specimen from lesions with abundant collagenous matrix
Cell block preparation: automatic	Standardized preparation procedure. Similar to a small histologic biopsy. Easy to perform controls and compare with subsequent IC on histological samples. Material can be saved for further evaluation	Expensive compared to other techniques. Can be difficult to obtain sufficient specimen from lesions with abundant collagenous matrix
Liquid-based cytology	"Clean" background. Monolayer of cells. Material can be saved for further evaluation	Not yet sufficiently evaluated with regard to all antibodies

Cytogenetic, Molecular Cytogenetic, and Molecular Genetic Analysis

Chromosome banding analysis of tumor cells has been performed for more than 40 years, and cytogenetic information is available on around 65,000 neoplasms [124]. From these studies, it has become apparent that most tumor types display nonrandom patterns of chromosome aberrations. Furthermore, it is also recognized that different chromosome aberrations play different roles during tumor development: some are primary, tumor-initiating events, whereas others are secondary aberrations, occurring later in tumor development and possibly influencing tumor progression. Particularly the primary aberrations are often strongly, sometimes specifically, associated with a certain morphologic entity (Table 1.2; see Figs. 1.40, 1.41, and 1.42) [125]. Thus, they may serve as excellent diagnostic markers. Many primary aberrations are balanced translocations, resulting in fusion genes that can be detected by reverse transcriptase PCR (RT-PCR) or FISH [126]. It is not only individual chromosome aberrations that may be of diagnostic importance, however. Simply finding clonal aberrations strongly suggests that a lesion under study is neoplastic rather than reactive, and the general correlation that exists between malignancy grade and genetic complexity makes it highly unlikely that a karyotype with gross aneuploidy and multiple structural aberrations should derive from a benign tumor [124].

Whereas the significance of individual chromosome aberrations as well as patterns of aberrations for diagnostic purposes is well recognized, the prognostic impact of acquired genetic changes remains less well investigated. With the development of novel therapeutic approaches that target specific proteins or pathways of cellular signalling, it is likely that many more studies in this area will be seen. Tests for a number of genetic aberrations that may occur in solid tumors are already routinely done to stratify patients with respect to further treatment. To mention one example, gastrointestinal stromal tumors (GIST) typically show activating mutations in *KIT* or *PDGFRA*, two genes encoding tyrosine kinases; type and location of the activating mutations have been shown to have a strong impact on the response to treatment with kinase inhibitors [127]. A detailed

Table 1.2 Examples of characteristic balanced chromosome rearrangements and their corresponding gene fusions in lymphomas and solid tumors

Chromosome rearrangement	Gene fusion	Tumor type
Lymphomas		
t(2;5)(p23;q35)	*NPM1/ALK*	Anaplastic large T-cell lymphoma
t(8;14)(q24;q32)	*IGH/MYC*	Burkitt lymphoma/leukemia
t(14;18)(q32;q21)	*IGH/MALT1*	Extranodal marginal zone B-cell lymphoma
t(14;18)(q32;q21)	*IGH/BCL2*	Follicular B-cell lymphoma, diffuse large B-cell lymphoma
Benign solid tumors		
t(1;2)(p13;q37)	*COL6A3/CSF1*	Tenosynovial giant cell tumor
t(2;3)(q13;p25)	*PAX8/PPARG*	Follicular thyroid adenoma
t(3;12)(q28;q14)	*HMGA2/LPP*	Conventional lipoma
Malignant solid tumors		
t(X;1)(p11;q23)	*PRCC/TFE3*	Papillary renal cell carcinoma in children and adolescents
t(X;18)(p11;q11)	*SS18/SSX1*,*SS18/SSX2*,or *SS18/SSX4*	Synovial sarcoma
t(2;3)(q13;p25)	*PAX8/PPARG*	Follicular thyroid carcinoma
t(7;16)(q33;p11)	*FUS/CREB3L2*	Low-grade fibromyxoid sarcoma
t(7;17)(p15;q11)	*JAZF1/SUZ12*	Endometrial stromal sarcoma of the uterus
t(9;22)(q31;q12)	*EWSR1/NR4A3*	Soft tissue chondrosarcoma with abundant myxoid matrix in adults
t(11;19)(q21;p13)	*CRTC1/MAML2*	Mucoepidermoid carcinoma of the salivary glands
t(11;22)(p13;q12)	*EWSR1/WT1*	Desmoplastic small round cell tumor
t(11;22)(q24;q12)	*EWSR1/FLI1*	Ewing family of tumors
t(15;19)(q14;p13)	*BRD4/NUTM1*	Poorly differentiated “midline” carcinoma

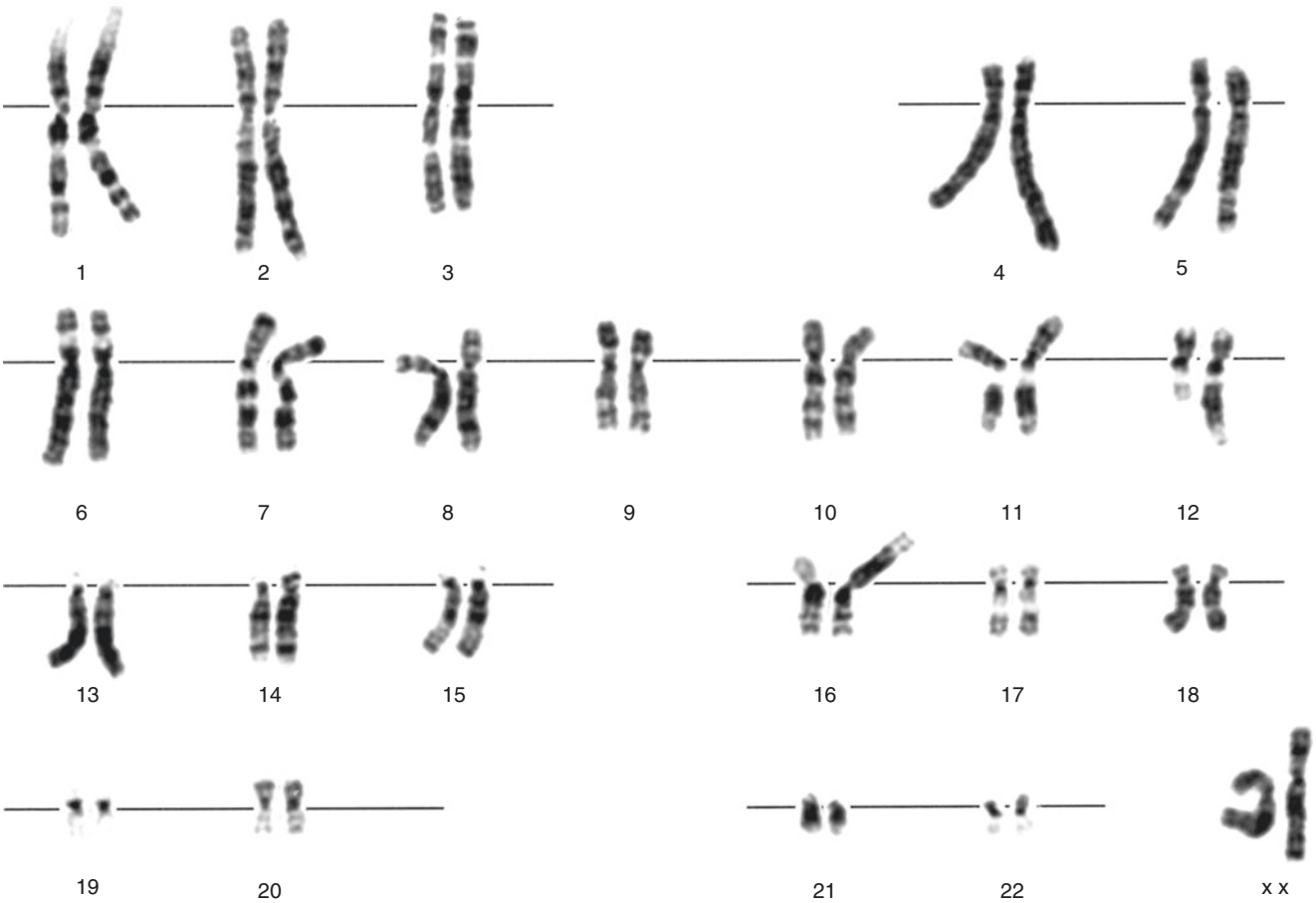

Fig. 1.40 Myxoid liposarcoma with the specific t(12;16)(q13;p11), which results in the *FUS/DDIT3* fusion gene

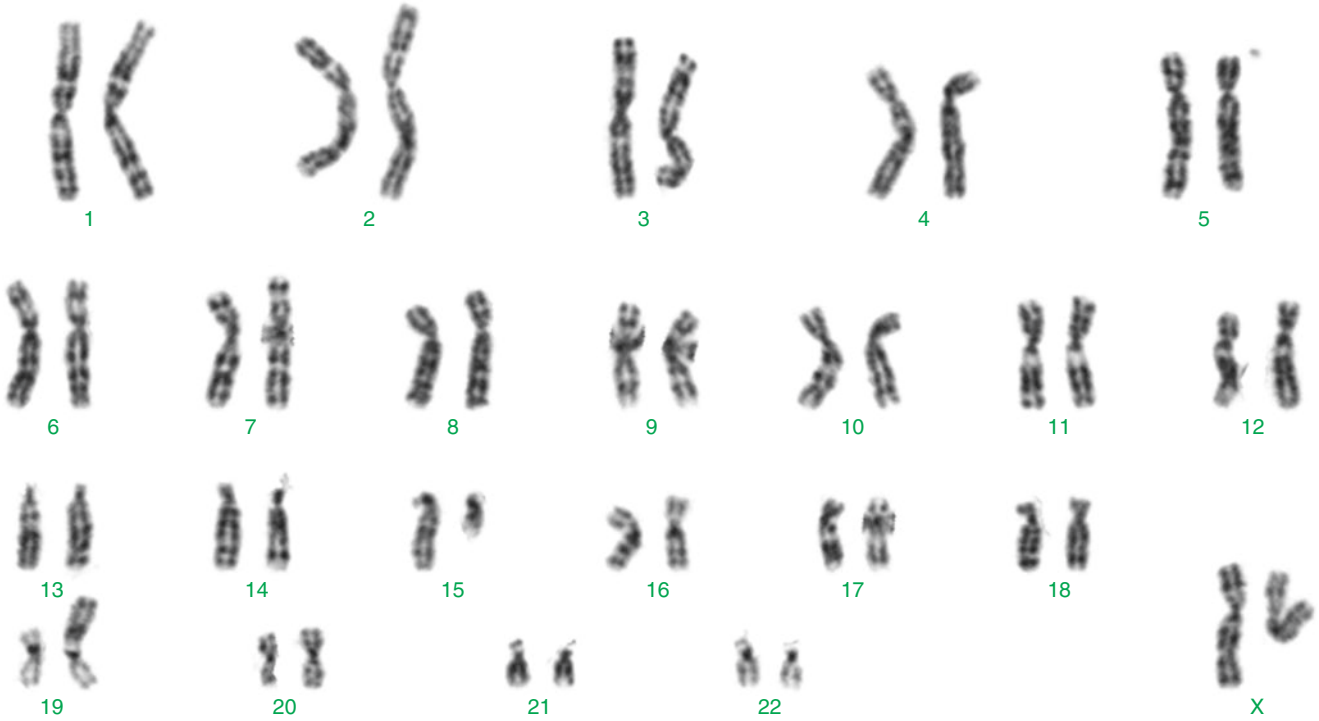

Fig. 1.41 Aggressive, undifferentiated midline carcinoma with the specific t(15;19)(q14;p13), which results in the *BRD4/NUT* fusion gene

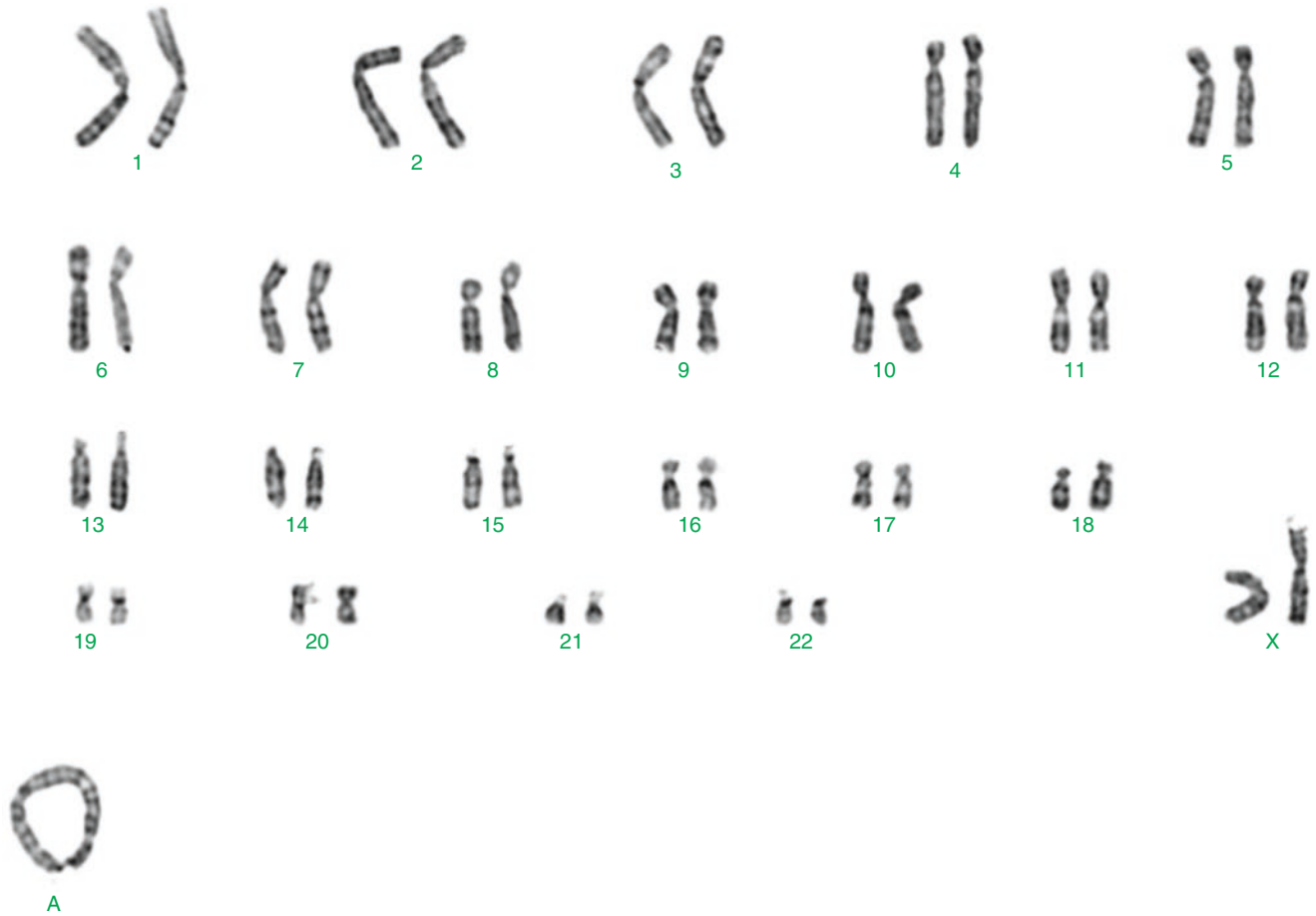

Fig. 1.42 Parosteal osteosarcoma with a supernumerary ring chromosome as the sole aberration. This karyotypic feature separates parosteal osteosarcomas from high-grade osteosarcomas. Supernumerary ring chromosomes may be found in a variety of other low-grade malignant mesenchymal tumors, such as atypical lipomatous tumor and dermatofibrosarcoma protuberans

description of the spectrum of chromosome aberrations occurring in human neoplasms, as well as a detailed account of their significance for diagnostic and prognostic purposes, can be found in [128].

The principles for handling FNA samples for cell culturing and cytogenetic analysis are similar to those for surgical biopsies. It is vital that the samples are handled under sterile conditions and that cell culturing is initiated as quickly as possible. Only a few large-scale attempts to obtain tumor karyotypes from FNA specimens have been made, and the general impression is that the results are clearly inferior to those on samples from surgical biopsies. For instance, in bone and soft tissue neoplasms, tumor-representative karyotypes were found in two-thirds of surgical biopsies but in only one-fourth of FNA specimens, strongly indicating that the number of cells obtained by FNA sampling is too small for reliable culturing [80]. Other drawbacks related to the fact that chromosome banding analysis requires living, dividing cells is that tumor cells of different lineages (e.g., epithelial and mesenchymal) require different culturing conditions and that some tumor cells must be cultured for many days before there is a sufficient number of mitotic cells to analyze [129].

None of the abovementioned shortcomings applies to directed genetic studies. For FISH analysis, which can be performed on smears as well as on cells that have been centrifuged and fixed on slides, typically around 100 interphase nuclei will suffice. By using locus-specific nucleotide probes, a variety of chromosome aberrations can be demonstrated: translocations and other structural rearrangements of specific genes, deletions, amplification, and aneuploidy (see Figs. 1.43, 1.44, 1.45, and 1.46) [130–132]. With few exceptions, the material should be sufficient for analyzing at least two to three different chromosome aberrations in a single-FNA specimen. A growing number of commercial probe sets for clinically relevant chromosome aberrations are now available and should be used whenever possible.

Various PCR approaches (e.g., for fusion genes, specific mutations, allelic imbalances, or gene expression levels) can also be used with good results on FNA specimens [133–136]. Compared to FISH, the PCR technologies are more sensitive, but this is also a potential drawback; high sensitivity also means that there is considerable risk of false-positive results due to contamination. Furthermore, when using RNA as the starting material for reverse transcriptase PCR (RT-PCR), it should be kept in mind that RNA is more sensitive than DNA/nuclei to degradation, making the time span from sampling to analysis more important for RT-PCR than for FISH or genomic PCR. Thus, both RT-PCR and genomic PCR protocols should always include negative and positive controls (see Fig. 1.47).

New genetic technologies that within the next few years may replace some of the current approaches include various array-based methods to identify chromosomal imbalances (see Fig. 1.48) and deep sequencing of DNA or RNA for mutation and gene fusion detection. The number of reported studies is still low, but preliminary data are promising [137, 138].

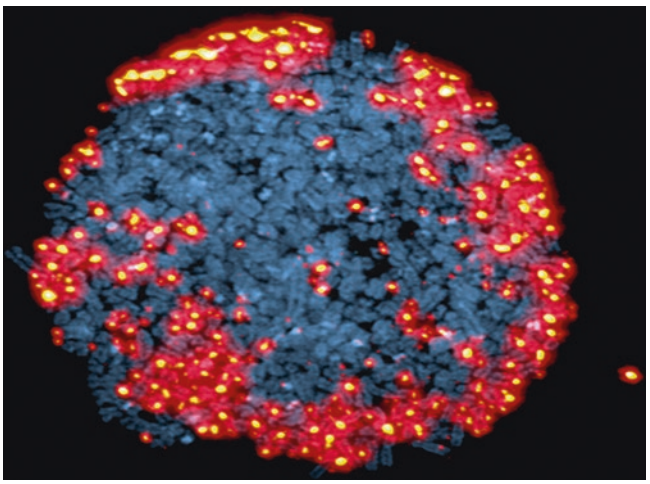

Fig. 1.43 FISH image showing amplification of the *COAS1* (*yellow*) and *COAS2* (*red*) genes in a high-grade leiomyosarcoma. (From Nilsson et al.; with permission) [125]

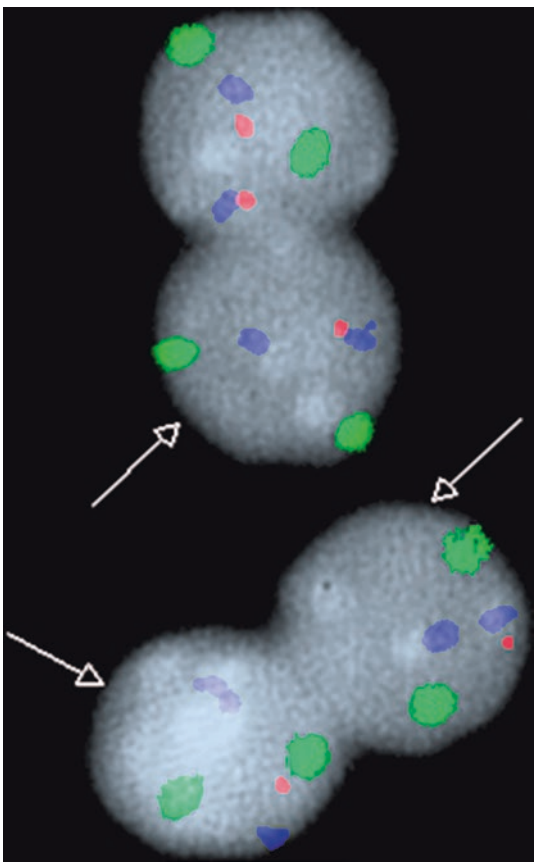

Fig. 1.44 Interphase FISH image showing hemizygous deletion of the *TP53* gene (*red*) in three nuclei (*arrows*) from a colorectal carcinoma. *Green* and *blue signals* represent control probes for chromosome 17

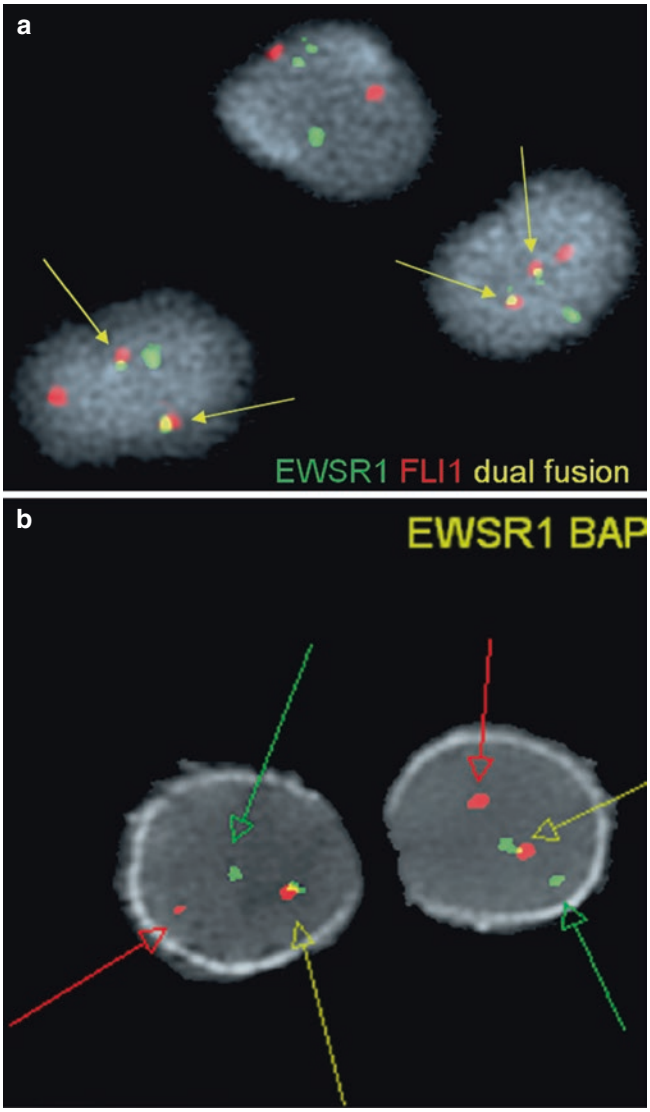

Fig. 1.45 Interphase FISH analysis of a Ewing sarcoma. Almost all Ewing sarcomas have structural chromosome rearrangements involving the *EWSR1* gene, the most common (85% of the cases) being the t(11;22)(q24;q12) resulting in an *EWSR1/FLI1* fusion gene. (**a**) The gene fusion can be detected in interphase nuclei using a dual fusion probe set, here with the probe for the *EWSR1* gene in *green* and the probe for the *FLI1* gene in *red*. The t(11;22) generates two fusion signals (*arrows*), one at each derivative chromosome. (**b**) By using a break-apart probe (*BAP*) only for the *EWSR1* gene, all possible translocations and fusions involving the *EWSR1* gene can be detected. The normal allele is seen as juxtaposed *red* and *green signals*, whereas the allele involved in the translocation is seen as split *red* and *green signals*. It should be emphasized that rearrangements of the *EWSR1* gene are not specific for the Ewing family of tumors

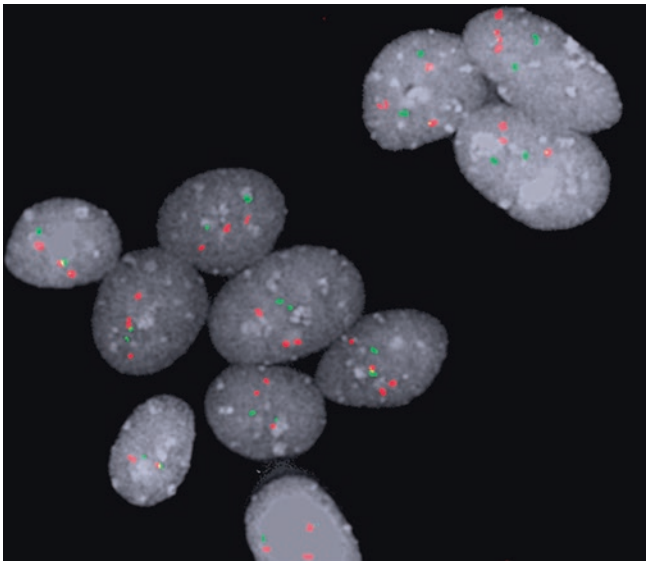

Fig. 1.46 Interphase FISH analysis of a neuroblastoma. Relative overrepresentation of distal 17q is a common feature of neuroblastomas, here illustrated by three to four copies of the 17q signal (*red*) compared to the two signals from the control locus at 17p (*green signal*)

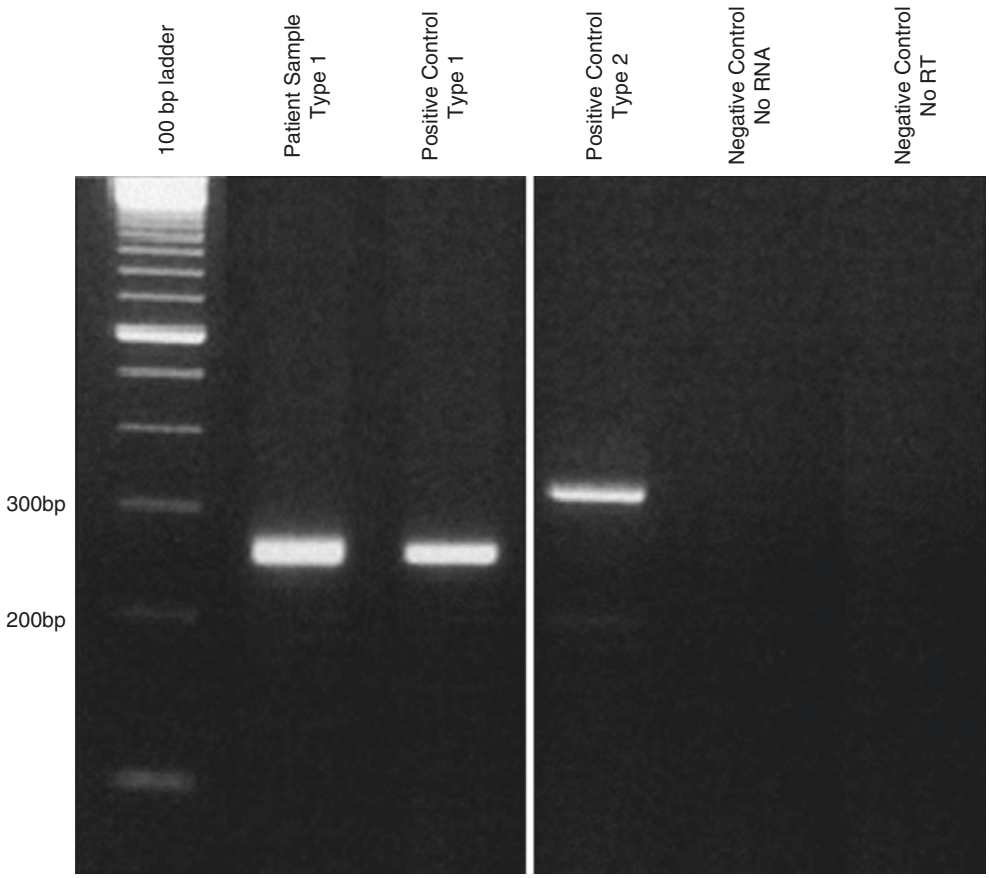

Fig. 1.47 RT-PCR using primers specific for the *EWSR1* and *FLI1* genes on RNA extracted from an FNA sample from a Ewing sarcoma. The analysis reveals a type 1 fusion transcript (exon 7 of *EWSR1* fused with exon 6 of *FLI1*) in the patient sample

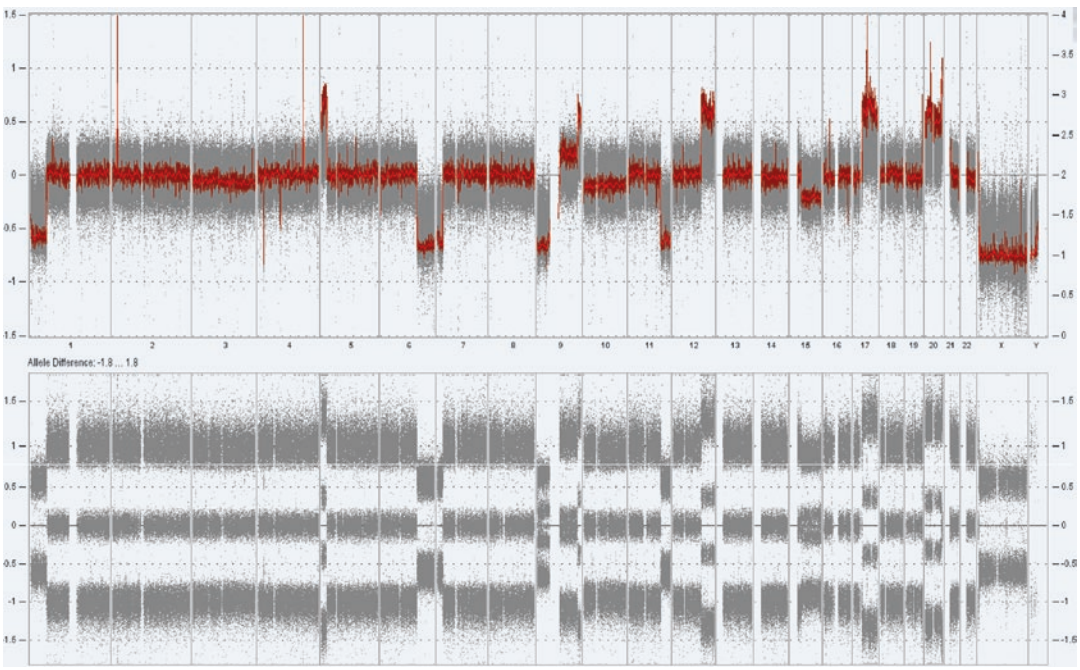

Fig. 1.48 Single nucleotide polymorphism (SNP) array analysis of a neuroblastoma. Copy number probes (*red, upper part*) show a near-diploid karyotype with chromosomal imbalances that are typical for aggressive neuroblastomas, e.g., loss of parts of chromosome arm 1p, amplification of the *MYCN* locus in 2p, and loss of distal 11q. The corresponding changes in allele status are shown below in grey

Electron Microscopy

Ultrastructural examination of aspiration smears in daily diagnostic work has been replaced to a large extent by other techniques, such as IC, and molecular biologic techniques. The diagnostic value of electron microscopy (EM) is still important [139–141] and is especially valuable in the differentiation of epithelial from mesenchymal neoplasms and for determining their specific histogenesis such as neuroendocrine, melanocytic, myogenic, neurogenic, or fibroblastic/myofibroblastic [142–145]. EM is also a valuable diagnostic adjunct in the evaluation of extracellular or intracellular deposits such as glycogen, amyloid [146], and osteoid [147].

Although electron microscopy cannot identify all cell types, it can reveal certain differentiated structural features and clarify the origin of cells that lack definite signs of differentiation by light microscopy. For example, specific ultrastructural hallmarks for a number of neoplasms (e.g., Birbeck granule in Langerhans cell histiocytosis; premelanosomes in melanoma and clear cell sarcoma; Weibel-Palade bodies in vascular tumors; lipoblasts in liposarcomas) can be of great diagnostic help. It is important to point out, however, that FNAC ultrastructural findings in routine diagnostic work should always be correlated to conventional smears and that interpretation must be made within the context of the overall morphologic pattern of the smears. Compared with the preparation of cytological material for other ancillary techniques, the processing of aspirates for EM is more time-consuming. The results of electron microscopy in aspirated cells and cell fragments compare favorably with results seen in biopsy material.

FCM Immunophenotyping

FCM immunophenotyping has been used for many years as a diagnostic complement of FNAC in cases of suspected non-Hodgkin's lymphoma. FCM is most important in the differential diagnosis of indolent B-cell lymphomas and reactive lymphadenitis as well as in rendering the exact lymphoma subtype from FNA material. More about this subject is presented in the chapter on lymph node FNAC (see Chap. 9).

Advantages and Limitations of FNAC

Fine-needle aspiration (FNA) is an outpatient procedure compared to most cases of incisional/excisional biopsy which are procedures that frequently require an operation room as well as medical staff and all the resources necessary to perform surgery. In addition, most open biopsies require general anesthesia, and there is a risk of complications connected to these procedures. Compared to open biopsy, the FNA procedure allows easy and quick sampling and provides adequate diagnostic specimens in most cases, using minimal resources with negligible risk of serious complications. General or local anesthesia is seldom necessary in sampling for FNA, and the procedure is well tolerated by patients. With thin needles and repeated aspirations, it is usually easy to sample material from different parts of large masses and thereby elucidate possible tumor heterogeneity.

In rapid on-site evaluation (ROSE) situations, rapid staining with Diff-Quik or rapid hematoxylin (H&E) makes it possible to assess the adequacy of aspiration smears while the patient waits. In such situations, additional aspirations can be performed to obtain diagnostic smears or more material from the lesion for ancillary studies.

In many settings, the accuracy of FNAC in distinguishing benign from malignant neoplasm and reactive/inflammatory conditions has been shown to be comparable to that of surgical biopsies, while its accuracy in establishing a specific subtype diagnosis is often inferior to surgical biopsies in some organs.

One major disadvantage of FNAC, however, is the occasional difficulty in obtaining sufficient material for ancillary studies. Another disadvantage is the inherent lack of histological architecture in most aspirates. Tumor tissue architecture is generally best evaluated in surgical biopsy or core needle biopsy (CNB) samples. In FNA samples, however, architecture can also be evaluated in the microbiopsies that can be seen in cell blocks (see Fig. 1.49).

Though rapid staining and preliminary reporting is more conveniently applied to aspiration smears, imprint preparations from CNB and cryostat sections from open biopsy specimen can be also evaluated quickly. Frozen section procedures are, however, more complicated, and it has to be pointed out that in technically satisfactory FNA smears, cytomorphology is usually superior to that seen in core needle and incisional biopsy specimens when these are prepared as frozen sections.

An additional advantage of FNA is that it is both easy to perform and easy to learn. A brief comparison of major advantages and disadvantages of different biopsy techniques is presented in Table 1.3.

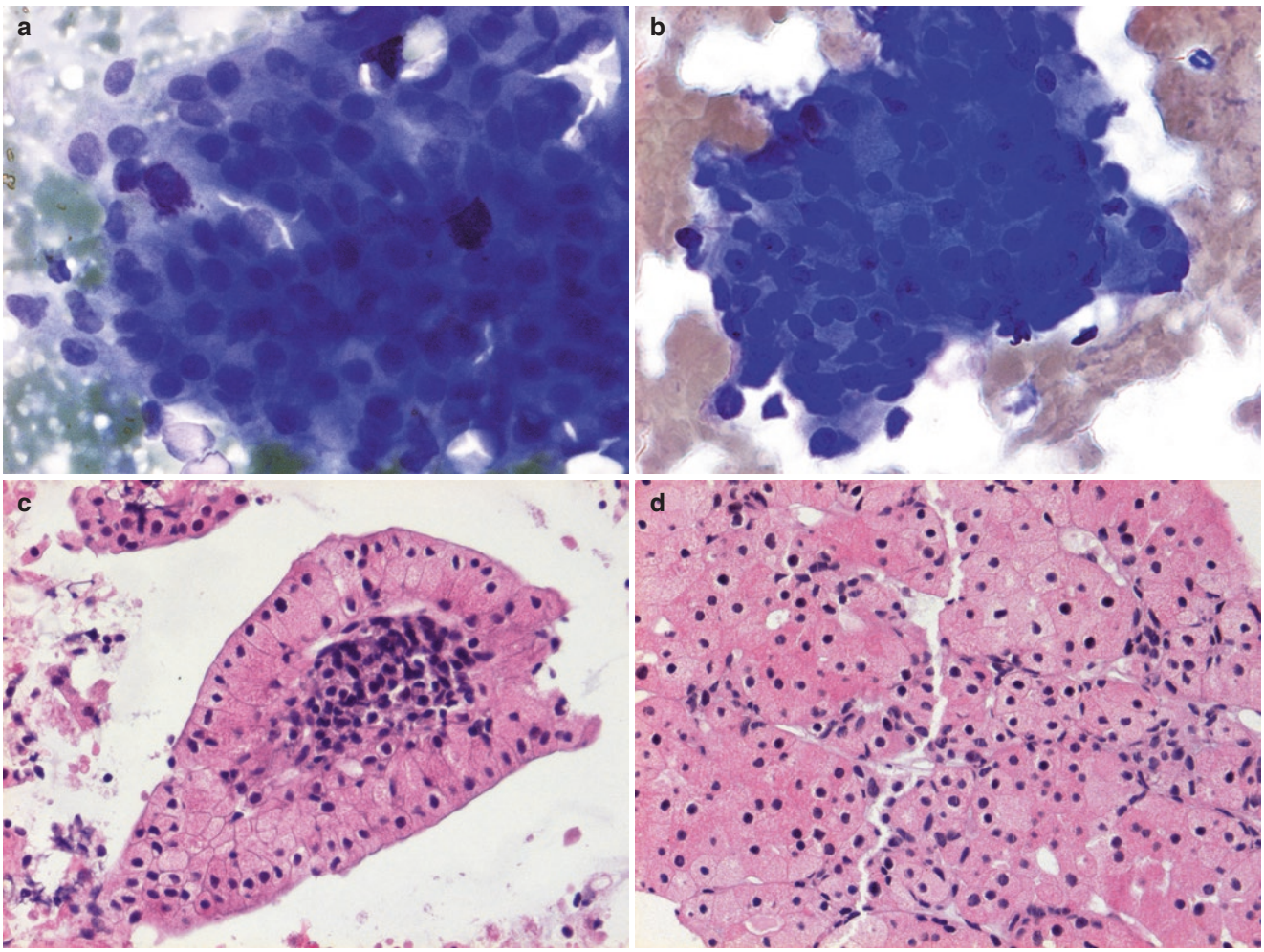

Fig. 1.49 FNA smears from Warthin's tumor (**a**) and oncocytoma (**b**) of the parotid gland (MGG). In the absence of lymphocytic infiltration in FNA smears from Warthin's tumor, it can be extremely difficult to distinguish these two neoplasms. Compared with cell block, this distinction is easy since the architecture of tissue fragments obtained by FNA is highly diagnostic for Warthin's tumor (**c**) (H&E) and oncocytoma (**d**) (H&E)

Table 1.3 Comparison of fine-needle aspiration cytology with core needle biopsy and open biopsy

Characteristics	Fine-needle aspiration cytology	Core needle biopsy	Open biopsy
Techniques	Easy procedure (to perform and learn)	Easy procedure (to perform and learn)	Complex procedure; most often requires general anesthesia and operating room/operating staff
Risk of complications	Very low	Low	Higher than FNAC and CNB
Interference with subsequent treatment	No	No	Yes
Speed of preparation	Fast	Slow[a]	Slow[a]
Availability of tissue architecture	Yes (cell block)[b]	Yes	Yes
Availability of ancillary techniques	Yes[b]	Yes	Yes
Accuracy in differentiating benign vs. malignant	High	High	High
Accuracy in malignancy grading	Low	High	High
Accuracy of histologic subtyping	30–75%[c]	75–90%[c]	>95%[c]
Cost	Low	Low	High

[a]Can be speeded by touch preparation and frozen section
[b]Depending on quality and quantity of specimen obtained
[c]See Kilpatrick [148]

Pitfalls, Risks, and Complications

FNAC in the routine evaluation of tumor masses requires an understanding of the potentials and limitations of the method. The final FNAC diagnosis should be correlated to the diagnostic level necessary for treatment initiation and/or planning of the further diagnostic work-up.

The technical problems of FNA techniques often relate to insufficient cytological specimens, which can result from different causes:

1. The lesion can be missed altogether by the aspirator, and cells can be aspirated from the tissue surrounding the lesion. Misinterpretation of reactive changes in the tissue surrounding a lesion may result in a wrong diagnosis. These problems occur most often when small and deep-seated lesions are needled. The person performing the aspiration should be experienced enough to evaluate whether the material obtained might be consistent with the lesion in question. This evaluation is often based, at least partly, on clinical data and on the findings of palpation. Ultrasound or CT guidance may be of help in the aspiration of small and deep-seated lesions.
2. If the aspirated mass is cystic, necrotic, or hemorrhagic, representative diagnostic areas may be difficult to sample adequately, and guided FNA may be required (see Fig. 1.50). Benign vascular neoplasms most often yield predominantly blood and only a few tissue cells.
3. Another difficulty is obtaining a sufficient number of cells from lesions with an abundance of collagenous or hyalinized matrix from which cells can be very hard to aspirate (see Fig. 1.51).

When the cytopathologist performs both the aspiration and examines the smears, the technical considerations and occasional difficulties are minimized. Non-palpable swellings should be aspirated by a radiologist with the aid of imaging techniques and in conjunction with a cytopathologist. In addition, radiologists and other specialists who perform aspirations should be trained in FNA clinics in the technical aspects and must receive immediate feedback on their performance. For optimal results, close cooperation between clinicians and the cytopathologist (ROSE) is necessary.

The histologic subtyping of neoplasms via FNA smears, in a manner similar to what is done in histopathology, is more reliable when dealing with well-differentiated tumors showing specific diagnostic cytomorphologic and immunocytochemical criteria. Definitive diagnosis can often be obtained from FNA smears complemented by ancillary techniques in lesions where cytological diagnostic criteria have been well characterized (see Fig. 1.52).

Conversely, poorly differentiated neoplasms usually represent a difficult diagnostic group due to a lack of distinctive morphological criteria. In such lesions, definitive diagnosis can be difficult to render from aspiration smears despite the use of ancillary techniques and access to clinical/radiographic data. Where definitive diagnosis is difficult to render from FNAC, differential diagnostic possibilities should be suggested and core biopsy/surgical biopsy considered as the next diagnostic procedure (see Figs. 1.53 and 1.54).

With regard to complications occurring during or after FNA procedures, the question of tumor cell spread in the needle tract is often brought up [149–155]. The incidence of this event is, in fact, exceedingly low [156–158]. Nevertheless, in cases where the risk for tumor cells spread exists, for example, in the aspiration of deep-seated masses suspected as being sarcomas, tattooing the skin at the aspiration site may be a good precaution so that the needle tract can be removed if surgery is required (see Figs. 1.55 and 1.56).

Serious complications such as major hemorrhage, septicemia, acute pancreatitis, rupture of cystic tumors, bile peritonitis, etc. are extremely rare [159–163]. Other complications of the aspiration procedure are minor and include hematomas and localized tenderness. Infection due to fine-needle aspiration can be easily avoided using elementary antiseptic methods such as cleansing the skin with alcohol or iodine swabs.

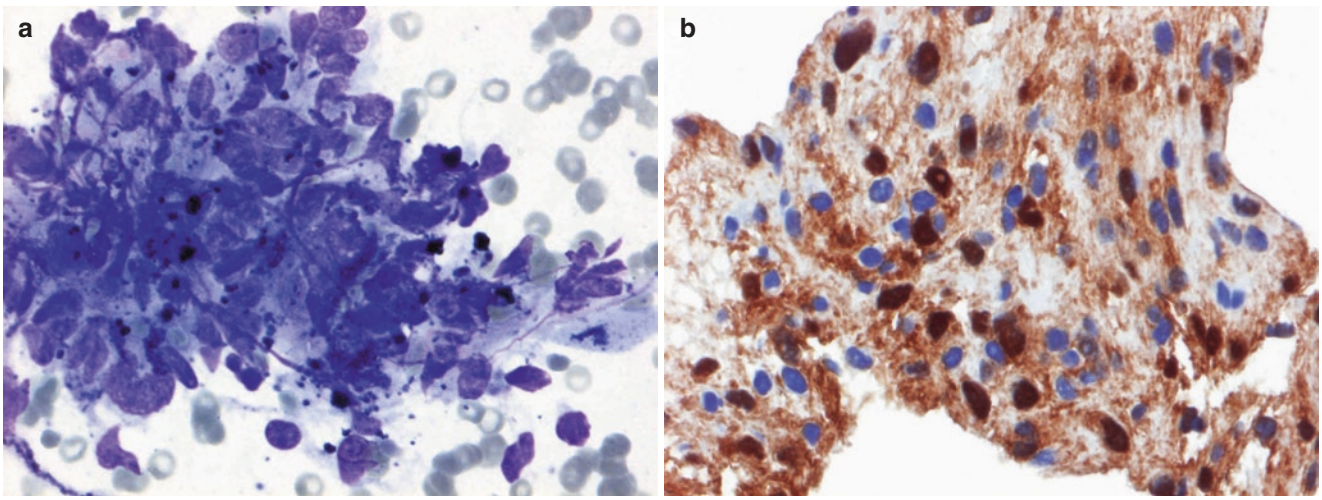

Fig. 1.50 FNA of cystic schwannoma showing clusters of epithelioid/histiocytoid cells with admixture of dark pigment. The FNA specimen does not allow any distinctive diagnosis (**a**) (MGG). Cell block of aspiration material (**b**). Fragment of the tumor tissue with spindle cells and epithelioid cells positive for S-100; these morphological features together with radiological findings allow correct diagnosis of schwannoma

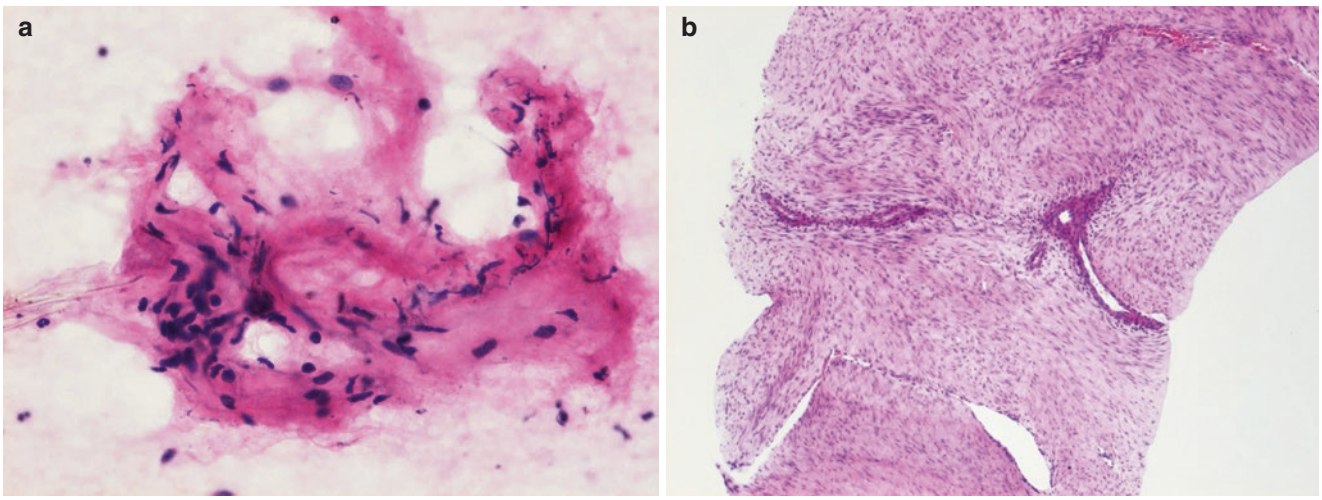

Fig. 1.51 Aspiration smears from an extra-abdominal desmoid showing hyalinized collagenous matrix and a few tumor cells (**a**) (H&E). In such cases, CNB sampling procedures can be superior to FNA (**b**) (H&E)

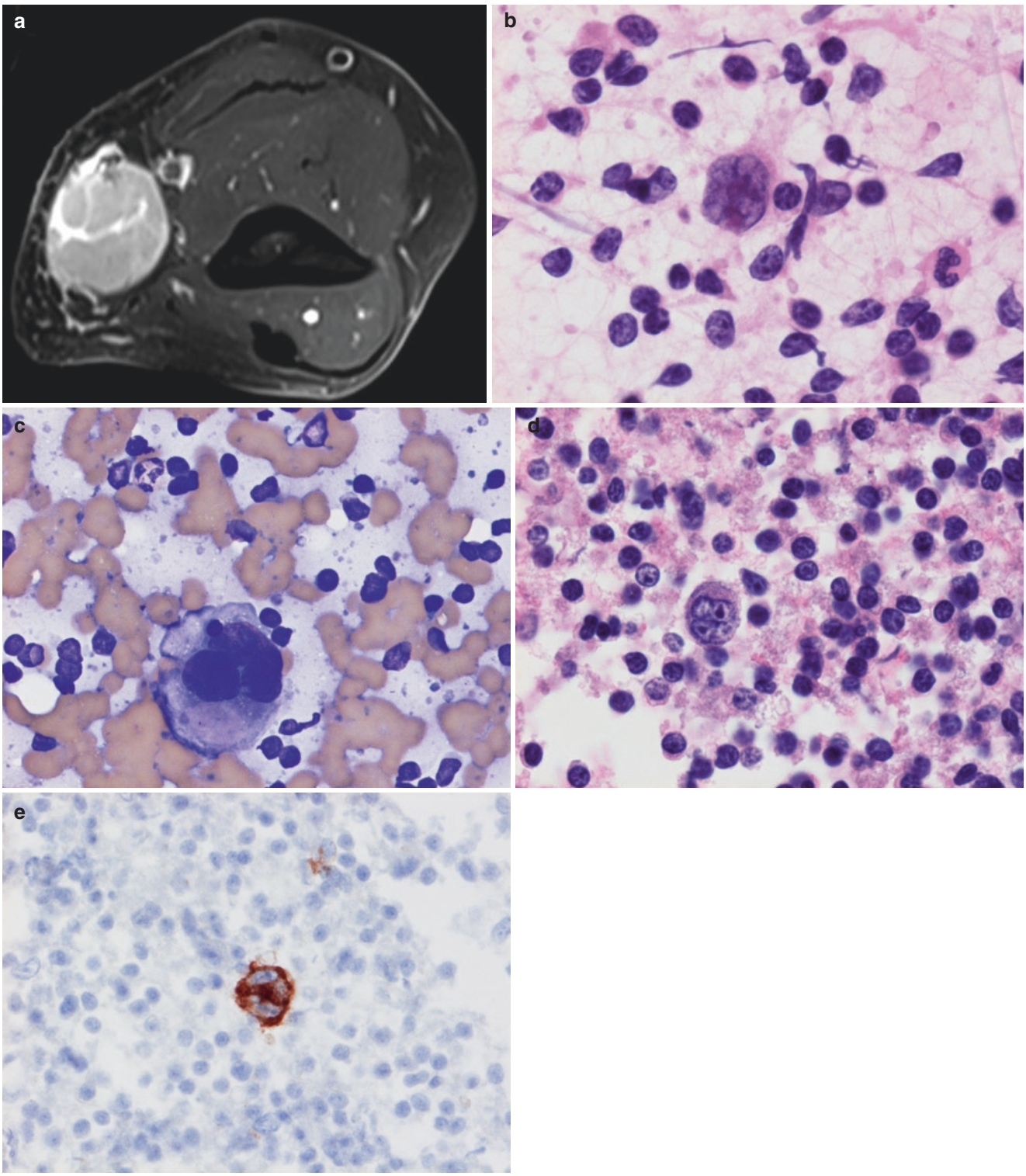

Fig. 1.52 MRI (**a**) showing soft tissue mass in the right elbow: FNA of the mass: alcohol-fixed, HTX-stained (**b**) and air-dried, MGG-stained (**c**) smears indicative of Hodgkin's lymphoma. HTX-stained (**d**) and immunostained for CD30 (**e**) sections from a cell block confirm diagnosis of Hodgkin's lymphoma

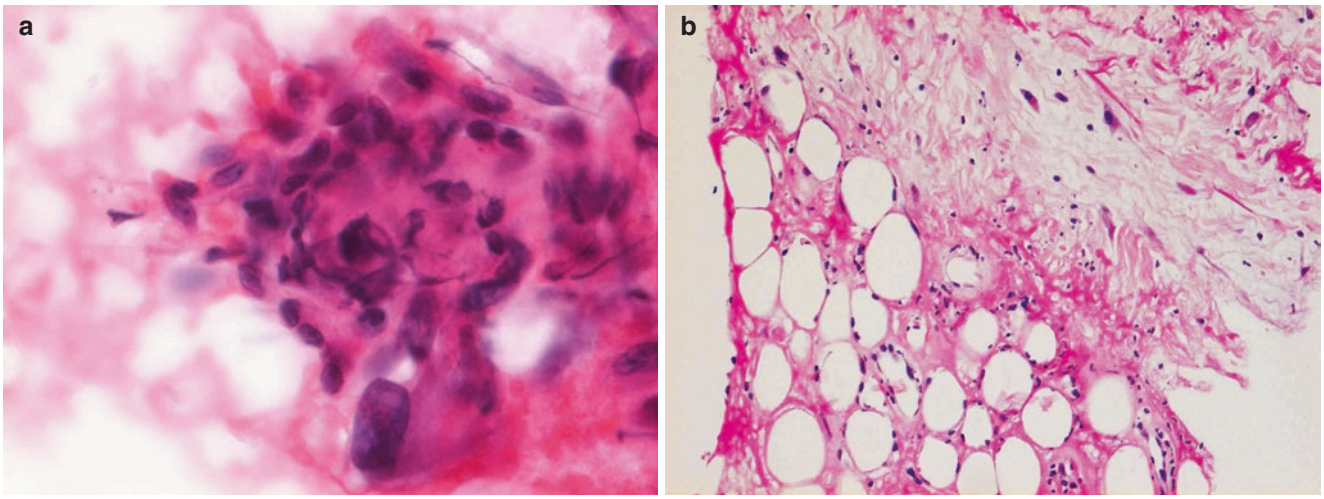

Fig. 1.53 FNA smears from ischemic fasciitis showing a cluster of spindle cells, some with pleomorphic hyperchromatic nuclei suggestive of malignancy (**a**) (H&E). Core needle biopsy performed simultaneously with FNA facilitated characteristic "zonal" architecture of ischemic fasciitis (**b**) (H&E)

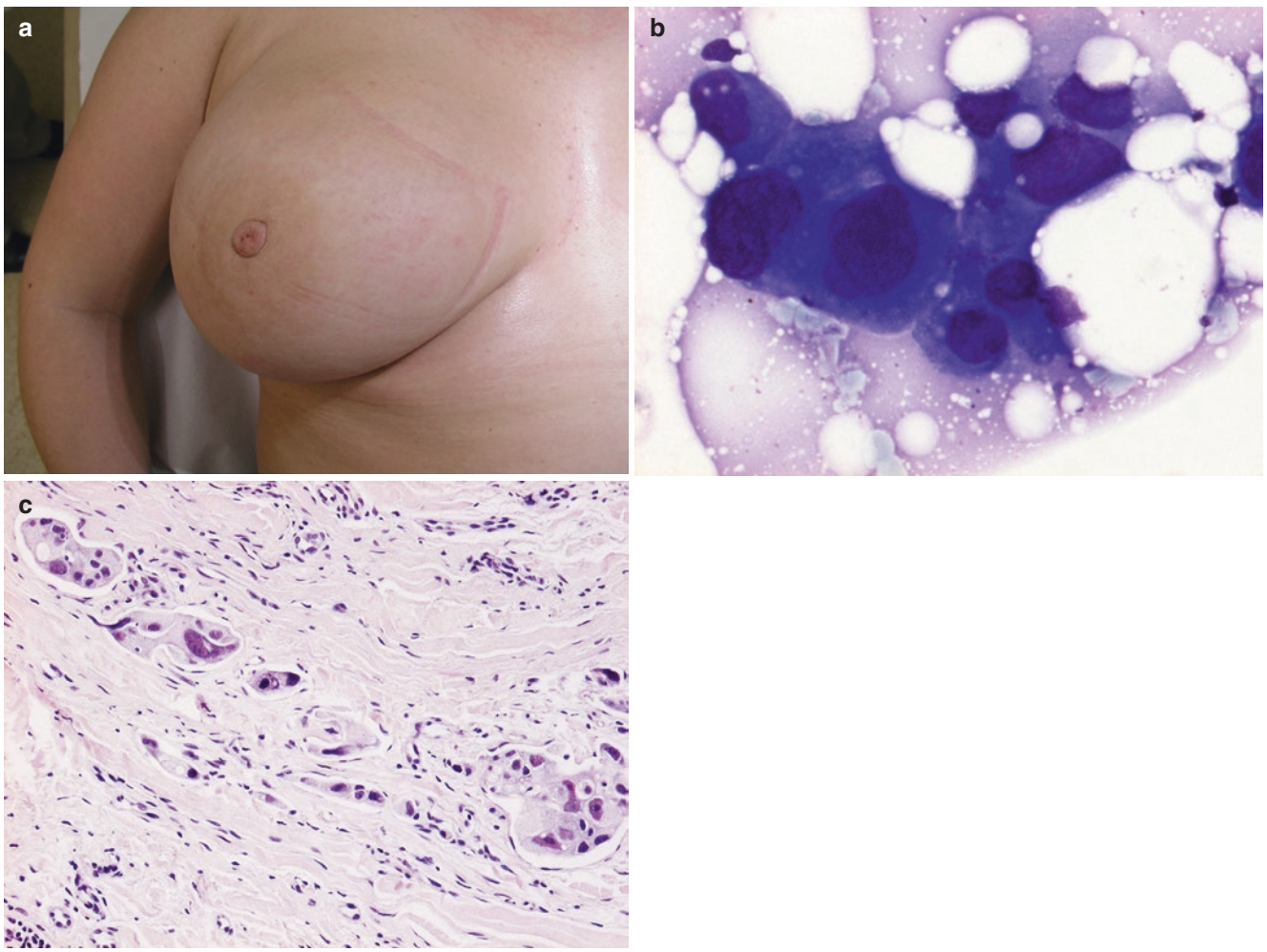

Fig. 1.54 Inflammatory mammary carcinoma (**a**). FNA specimen consists of some droplets of fluid containing a few highly atypical cells (**b**) (MGG). Subsequent core biopsy discloses pleomorphic carcinoma cells growing in the lymphatic vessels (**c**) (H&E)

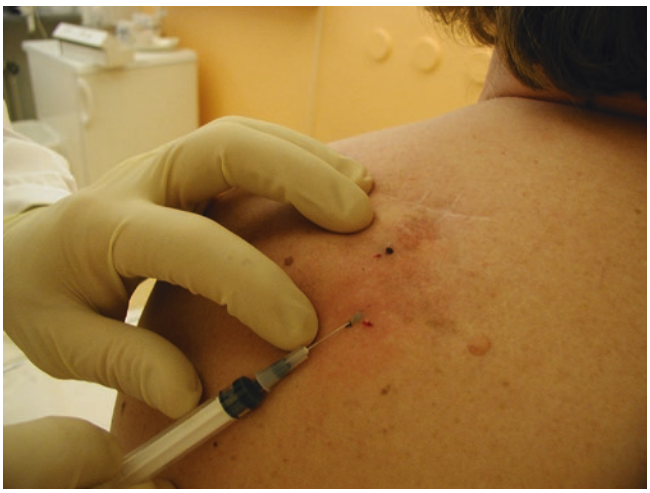

Fig. 1.55 Patient with a huge recurrence of dermatofibroma protuberans in the back. Tattoo with sterile ink of the needle insertion point helps an orthopedic surgeon remove the needle track

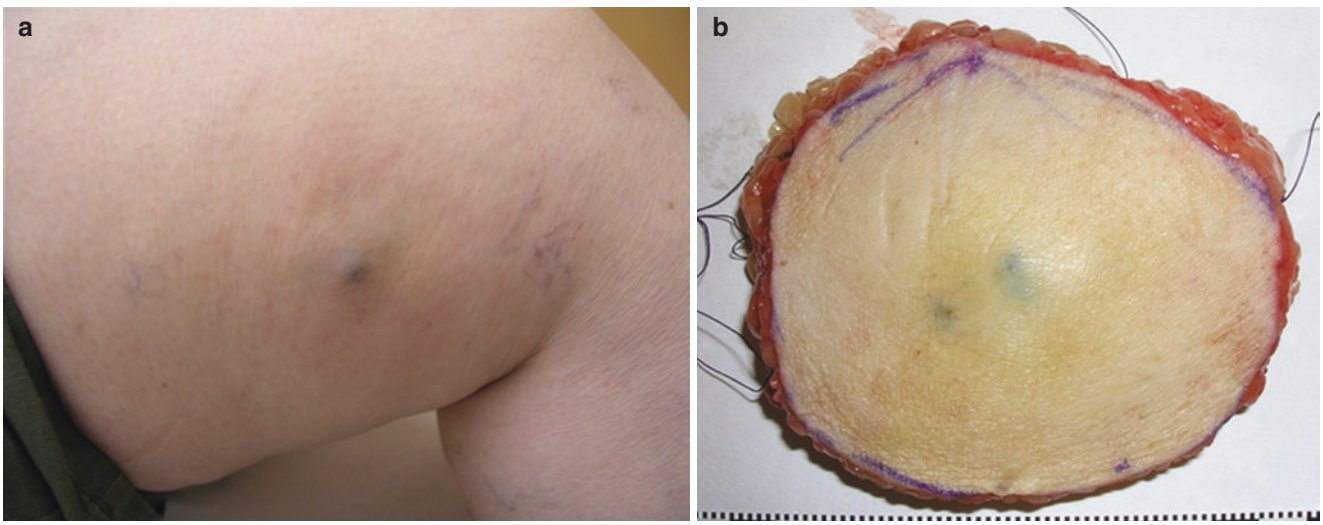

Fig. 1.56 Tattoo point after aspiration from a subcutaneous leiomyosarcoma (**a**). Operative specimen showing persistent tattoo in the central area of the specimen (**b**)

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