Respiratory Exfoliative Cytology

6

Introduction

The respiratory tract is comprised of upper and lower airways. The upper airway extends from the sinonasal tract to the larynx, and the lower tract extends from the trachea to the lungs. Although all sites are amenable to cytological sampling, the lower tract is usually the target for the detection of infections, benign lesions, and neoplastic processes. Various sampling techniques are utilized, occasionally with the use of concurrent needle core biopsies (NCB). In this chapter only the lower respiratory tract will be addressed.

Lung carcinoma is common and is the leading cause of death in men and women. According to the American Cancer Society, there will be approximately 225,000 new cases of lung cancer and about 158,080 deaths from the disease (accounting for one of four cancer deaths) in 2016. Only 20 % of lung cancers are diagnosed at an early stage when the disease is still localized within the lungs. At the time of diagnosis, 25 % of patients have regional metastasis, and 55 % of patients have distant spread of disease. Thus, early detection remains the major cornerstone for the successful treatment of pulmonary malignancies.

Carcinomas, both of the small cell and non-small cell types, are by far the most common malignancy of the respiratory tract. Non-small cell lung cancer (NSCLC) accounts for ~85 % of all lung cancers. Histologically, NSCLC is divided into adenocarcinoma, squamous cell carcinoma, and large cell neuroendocrine carcinoma. Patients with NSCLC require a complete staging workup to evaluate the extent of disease because stage plays a major role in determining the choice of treatment.

The accuracy of differentiating between small cell carcinoma and NSCLC on cytology specimens ranges from 94 to 100 % when compared with resection or autopsy specimens. The accuracy of subclassifying NSCLC into adenocarcinoma, squamous cell carcinoma, and large cell neuroendocrine carcinoma ranges from 66 to 91 %. Concordance between bronchoscopically obtained cytology and biopsy specimens is >95 % in recent studies. The overall sensitivity increases with a combined use of different sampling modalities.

R.S. Hoda et al., *Diagnostic Liquid-Based Cytology*,
DOI 10.1007/978-3-662-53905-7_6, © Springer-Verlag GmbH Germany 2017

Metastatic malignant neoplasms, from almost any site, can metastasize to the lung. Generally, lung metastases are identified in 30–55 % of all cancer patients. Tumors that commonly metastasize to the lung include carcinomas from the colon, breast, prostate, and urinary bladder, sarcomas, and melanomas.

Cytological Reporting Guidelines

The new Papanicolaou Society of Cytology guidelines for standardized terminology and nomenclature for respiratory cytology are designed to stratify the risk of malignancy with diagnostic categories for appropriate patient management [1]. The current guidelines provide diagnostic categories and criteria and also describe techniques for obtaining specimens, ancillary testing, and patient follow-up and management. A six-tiered system is recommended as the standardized nomenclature for reporting respiratory cytology diagnoses. The categories proposed are nondiagnostic, negative (for malignancy), atypical, neoplastic (benign and low-grade malignancy), suspicious for malignancy, and malignant. A multidisciplinary diagnostic approach is recommended. Patient management should be determined by correlating the clinical findings in concert with imaging features, cytological findings, and result of molecular analysis, if pertinent.

Indications, Collection, and Laboratory Processing of Exfoliative Respiratory Tract Samples

The examination of exfoliative respiratory cytology is an efficient and cost-effective method for diagnosing respiratory tract lesions. An accurate diagnosis relies on receiving an adequate sample, optimal specimen preparation using LBP, cell block, and expertise in interpretation of LBP.

The principal indications for exfoliative cytology of the respiratory tract are as follows:

1. Workup of a solitary pulmonary nodule detected on diagnostic or screening CT
2. Workup of a pulmonary nodule detected on CT in patients with a prior malignancy
3. Workup of pulmonary infiltrates to exclude infectious etiology

Methods of Collection for Exfoliative Cytological Samples

- Sputum
- Bronchial brush and bronchial wash
- Bronchoalveolar lavage (BAL)

Sputum

The cytological examination of a spontaneously expectorated sputum is a non-invasive technique and can be used for the detection of infectious lesions and large centrally located tumors. Diagnostic sensitivity of sputum cytology is directly proportional to the number of samples examined, and its accuracy approaches 95 % when five (5) sequential samples are examined. However, sputum cytology is not recommended for screening lung cancer. A post-bronchoscopic sputum may be examined when the expectorated sputum is negative.

Bronchial Brush and Bronchial Wash Specimens

Bronchial brushing and bronchial wash specimens are obtained via flexible fiber-optic bronchoscope (FOB). FOB can also obtain fine-needle aspiration (FNA) biopsies and transbronchial forceps biopsies. The advantages of FOB include an increased visual range, especially in the upper lobes, minimal discomfort to the patient, and sampling of previously inaccessible lesions such as peripheral nodules.

Both bronchial brushing and bronchial wash can detect infectious and neoplastic lesions. If indicated, microbiological cultures can also be performed concurrently. The diagnostic yield for bronchial brushing depends upon an adequate bronchoscopic sampling as well as the size and location of the lesion. For central tumors, the diagnostic sensitivity of bronchial brushing and bronchial wash is lower than that of transbronchial biopsy (TBBx). The sensitivity of bronchial brushing and bronchial wash is further decreased for peripheral lesions. The concordance rate of bronchial brushing and TBBx is ~97 %. The highest diagnostic yield for bronchial brushing and bronchial wash is for squamous cell carcinoma, adenocarcinoma, and small cell carcinoma (in that order).

Bronchoalveolar Lavage (BAL) Specimens

BAL also performed using FOB is a safe technique with a diagnostic accuracy comparable to TBBx. It is valuable for the detection of opportunistic infections, and since it samples multiple bronchi, it is also suitable for sampling diffuse lesions such as adenocarcinoma with a lepidic growth pattern. The diagnostic sensitivity of BAL for organisms in immunosuppressed people is 82 % for *Pneumocystis jirovecii*, 83 % for cytomegalovirus (CMV) and fungal pneumonia, and 80 % for mycobacterial disease. The overall reported diagnostic yield of BAL for malignancy is about 50 % with a lower yield for peripheral lesions. The diagnostic yield is improved with the addition of other modalities such as bronchial brushing, bronchial wash, and TBBx.

Laboratory Processing of LBP Specimens

Exfoliative respiratory tract specimens are usually processed as one Papanicolaou (Pap)-stained LBP. The collection specimen is rinsed in a preservative medium for LBP. Direct smears from brushings can also be made with a quick rolling motion of the brush on glass slides. The slides can be fixed in 95 % ethanol for Pap staining or air-dried for Romanowsky staining (e.g., Diff-Quik stain). Residual material can be rinsed in collection medium and used to process additional LBP for special stains, immunostains, or cell block preparation, especially if tissue fragments are noted. Please see Chap. 1 also.

Advantages of Cytological Specimens for Respiratory Tract over Needle Core Biopsy (NCB)

- Allows sampling of larger area of concern
- Allows sampling of narrow areas by brushings
- Shorter turnaround time
- Provides high quality of DNA for molecular testing

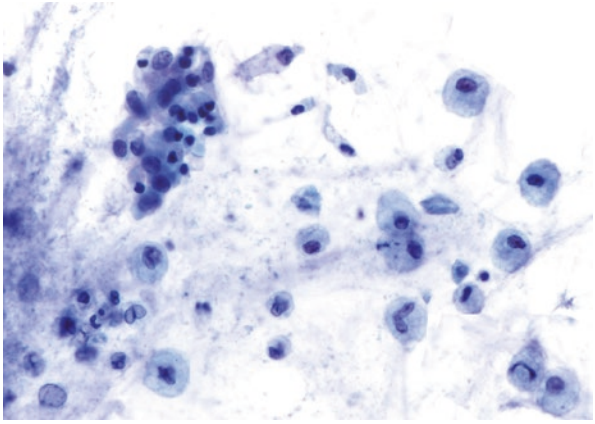

Fig. 6.1 Benign bronchial wash. This field shows a mucoid background with embedded benign bronchial cells and scattered macrophages. Note the well-preserved benign bronchial cells which are uniform with minimal overlapping. Terminal bar and cilia are visible in some cells. The macrophages retain finely vacuolated cytoplasm and pale kidney-shaped or oval nuclei. The mucin stains a purplish color and is of moderate thickness (TP). Excessive mucus, as well as blood and inflammatory cells, can be problematic in the processing and screening of LBP [2]. TP may show a loss of cellularity with large areas of the filters showing nearly complete absence of cells. This appears to be the result of excess mucin covering or obstructing the filtration membrane which diminishes cell retrieval, potentially impacting the detection of disease. In SP, cell enrichment process better manages excess mucus and does not affect cell recovery

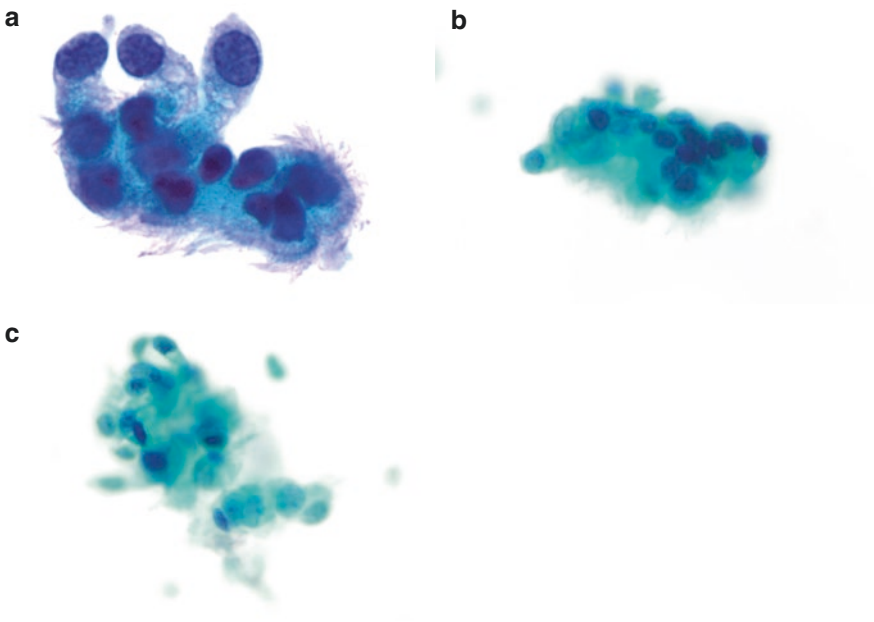

Fig. 6.2 Benign bronchial cells from bronchial wash. (**a**) The cells are elongated with oval apically placed nuclei with condensed chromatin with cytoplasmic terminal bars and eosinophilic-staining cilia (TP). (**b**) Benign bronchial cells in SP show features similar to TP (SP). (**c**) In reactive conditions, benign bronchial cells with cilia may form clusters with relatively high nuclear-to-cytoplasmic ratio and subtle nuclear atypia. Such reactive groups are termed "Creola bodies" and are in the differential diagnosis of well-differentiated adenocarcinoma. Cilia are the most salient distinguishing feature (SP)

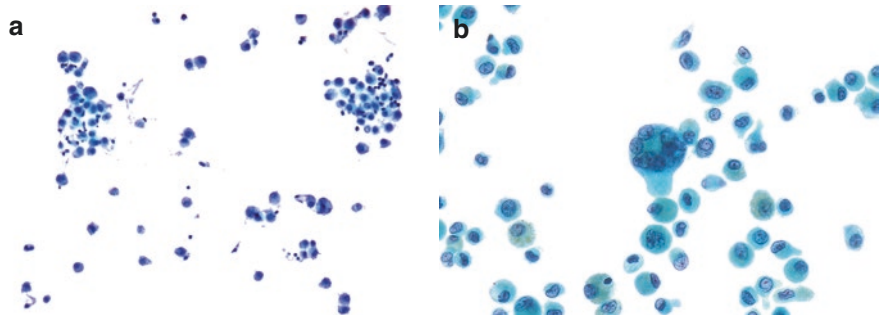

Fig. 6.3 Benign bronchoalveolar lavage. **(a)** An adequate BAL specimen must sample the alveolar spaces, as determined by the presence of pulmonary alveolar macrophages, as seen here intermixed with lymphocytes and small amounts of mucin. In LBP, the background is clean. Note the pulmonary alveolar macrophages clustering together and found singly with mucin attached to cell clusters (TP). (**b**) Pulmonary alveolar macrophages may contain hemosiderin pigment and have greenish-yellow staining cytoplasm on Pap stain. Binucleated and multinucleated cells are common and not suggestive of any specific disease process. The cells have abundant, foamy cytoplasm with an eccentrically placed, hyphen-shaped nucleus. Small nucleoli and chromocenters may be seen (TP)

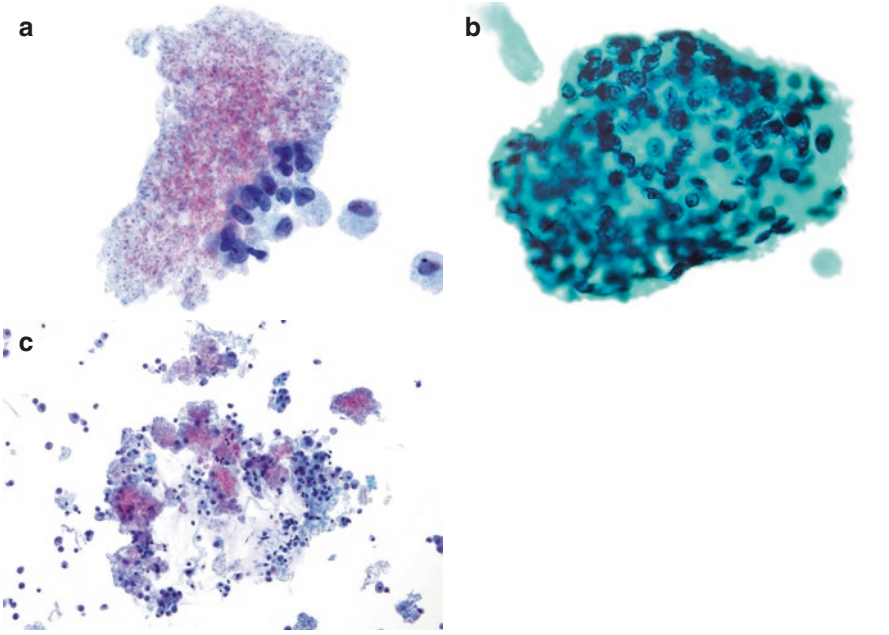

Fig. 6.4 Bronchoalveolar lavage with *Pneumocystis jirovecii*. (**a**) This specimen taken from a 40-year-old HIV-positive patient shows the characteristic foamy alveolar cast of *Pneumocystis* organisms. The casts have a bubble-like appearance and comprise of clusters of organisms that are spherical to ovoid with a smooth contour on Papanicolaou stain. The central eosinophilic and peripheral basophilic staining and trophozoites appearing as microdots within the organism's shell are characteristics for *Pneumocystis*. The organisms are also readily identified on Giemsa, Wright, Gram, and H&E stains. Ultrastructurally, the frothy appearance to the cast is due to filopodia connecting the organisms (TP). (**b**) Cup-shaped *Pneumocystis jirovecii* cysts (Grocott methenamine silver [GMS] stain). GMS may be helpful in distinguishing various fungi. *Pneumocystis* is more likely found in bronchial wash specimens. The latter sample has distal bronchial and alveolar spaces and has a diagnostic yield of 82–94 %. The background in BAL specimens with *Pneumocystis* is typically clean. Alveolar proteinosis is a potential mimic of *Pneumocystis jirovecii*. The central eosinophilic and peripheral basophilic staining and trophozoite microdots of *Pneumocystis* are lacking in alveolar proteinosis (Fig. 6.5). (**c**) Numerous intact alveolar casts of *Pneumocystis* organisms are present despite the multiple vigorous steps involved in TP processing (TP)

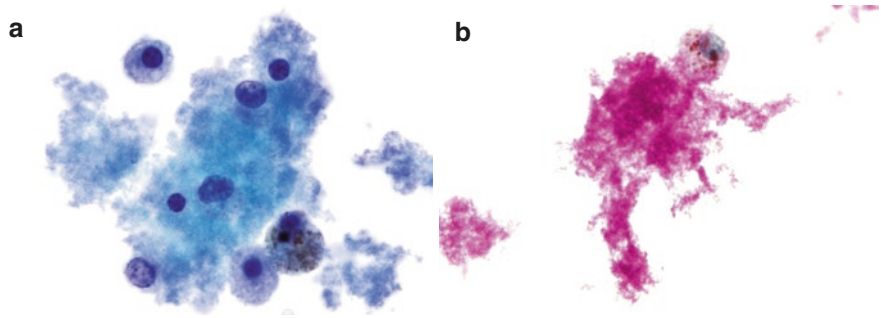

Fig. 6.5 Bronchoalveolar lavage with pulmonary alveolar proteinosis. **(a)** This entity is in the differential diagnosis of *Pneumocystis* organisms. A frothy, bubbly alveolar cast is seen surrounded by alveolar macrophages, some containing a suggestion of proteinaceous material and hemosiderin. No epithelial cells are noted. Unlike *Pneumocystis*, neither the ghosts of organisms nor microdots are visible. The background is clean (TP). (**b**) Special stains performed on additional TP slides showed the material to be diastase-resistant periodic acid-Schiff (PAS-D) positive, but negative for Alcian blue, GMS, and Congo red (PAS-D stain on TP). Pulmonary alveolar proteinosis is a rare disease characterized by abnormal intra-alveolar accumulation of extracellular surfactant-like material, which is composed of proteins and lipids [3]. Grossly, BAL may be somewhat milky. Electron microscopy shows type II pneumocytes containing concentrically laminated structures, some of which contain dense osmophilic cores, amid background proteinaceous debris

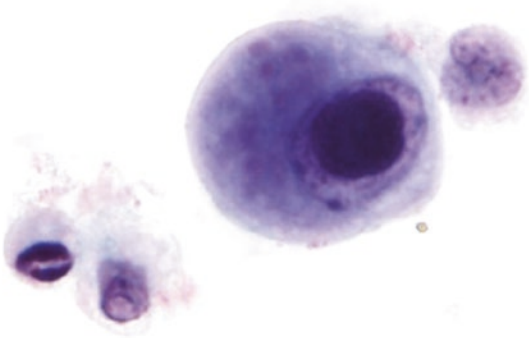

Fig. 6.6 Bronchoalveolar lavage with cytomegalovirus (CMV). CMV pneumonitis in an immunocompetent patient shows the virus in a glandular cell with cellular enlargement (compare its size with that of the inflammatory cells), nuclear enlargement, and large intranuclear basophilic inclusion surrounded by a halo ("owl's eye") (TP). Occasionally, smaller nuclear or cytoplasmic inclusions may also be seen. Multinucleation is uncommon in CMV-infected cells. BAL is a major diagnostic tool in lung diseases, including the detection of viral respiratory infections with herpes simplex virus (HSV), CMV, and Epstein-Barr virus (EBV) among others. Rapid detection of CMV pneumonitis is reliable using PCR testing of BAL cells. Immunocytochemistry and histologic evaluation of lung parenchyma obtained by TBBx may also be used to increase detection

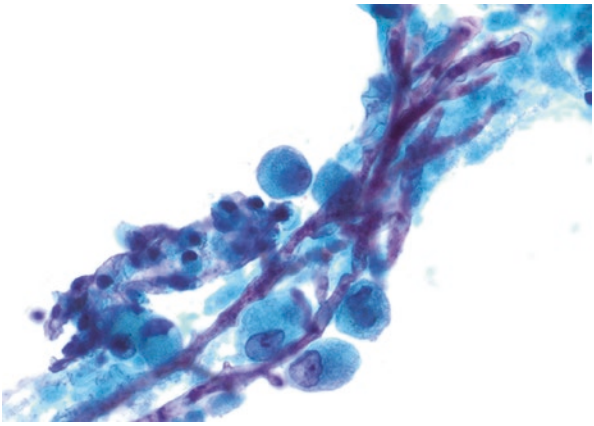

Fig. 6.7 Bronchial wash with aspergillus. The field shows septate hyphae, 3–6 μm in size and with a 45° angle branching pattern (TP). The background shows mucus and macrophages. Aspergillus is a common fungus and is transmitted by airborne conidia. It can occur in immunocompetent and immunosuppressed individuals. *Aspergillus fumigatus* is the most frequent human pathogen. The fungi form conidial head or "fruiting bodies" that produce spores. These spores can easily be airborne. *Aspergillus spp*. cannot be morphologically distinguished from its mimics such as Zygomycetes and *Candida spp*. unless it is accompanied by fruiting bodies. The latter are important in the identification of particular *Aspergillus* species and in differentiating *Aspergillus* from its mimics

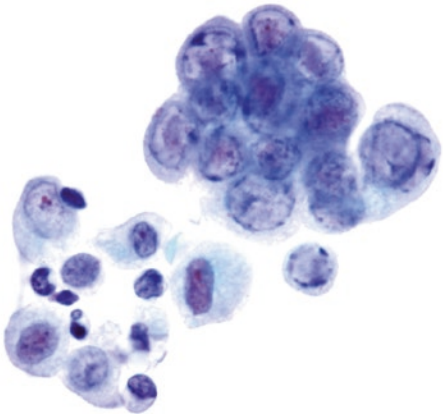

Fig. 6.8 Bronchoalveolar lavage with herpes simplex virus: HSV-infected cells have enlarged nuclei with a homogenous "ground glass" appearance. The nuclei mold against one another, and individual cells may be multinucleated. Note also the dark condensation seen along the nuclear rim which contrasts with the pale nucleus. Inflammatory cells are present adjacent to the infected cells (TP)

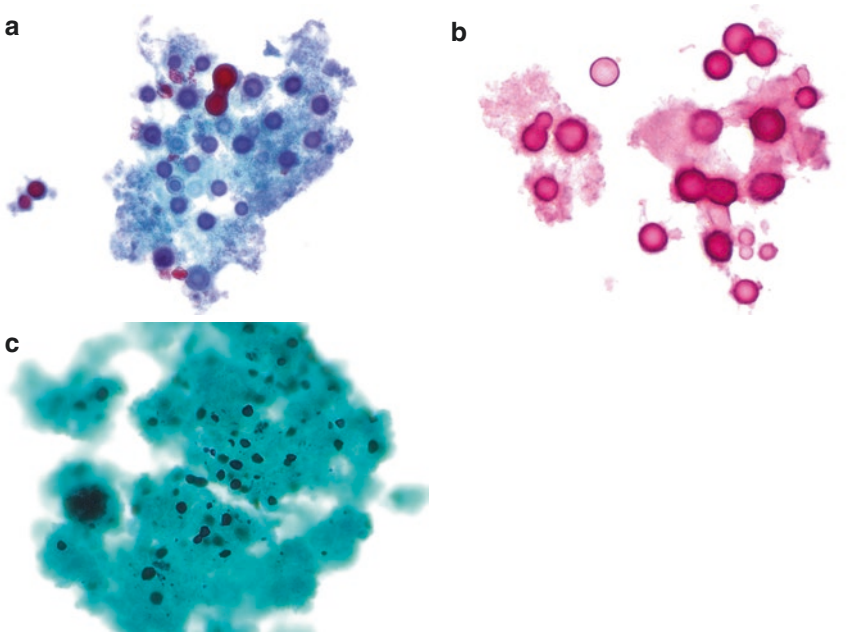

Fig. 6.9 Bronchoalveolar lavage with cryptococcus. **(a)** A single spherical organism surrounded by a thick capsule is noted with pulmonary macrophages (TP). (**b**, **c**) Narrow-based budding is prominent on mucin (mucicarmine) stain and GMS; the former stains the organisms bright red and the latter black. Note the translucent capsule seen on mucicarmine stain (**b**, mucicarmine stain on TP, C, GMS stain on TP). A multiplex real-time PCR technique has been developed for the detection of the three most common causes of fungal opportunistic pneumonia in AIDS patients: *Pneumocystis*, *Histoplasma capsulatum*, and *Cryptococcus*

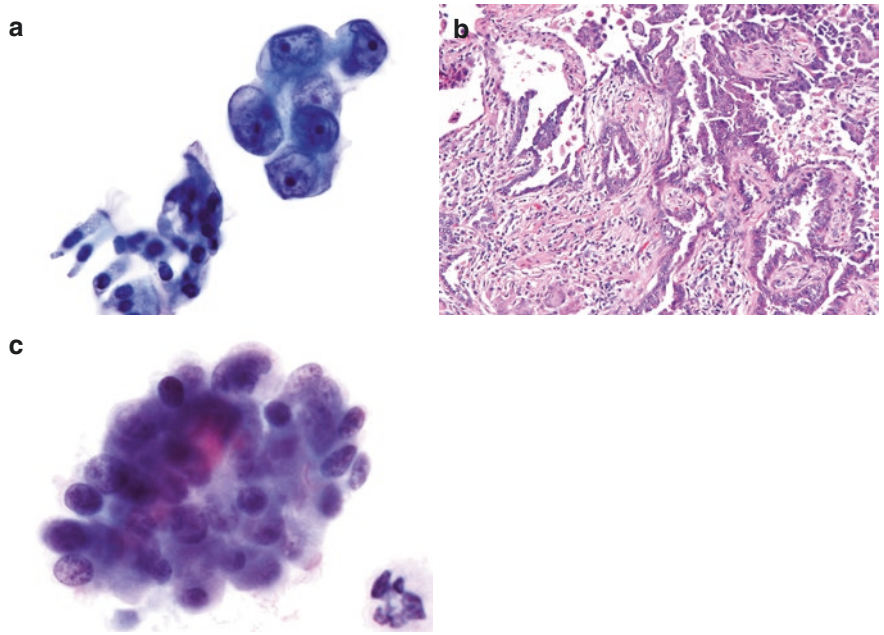

Fig. 6.10 Bronchial wash with adenocarcinoma. **(a)** Cluster of malignant cells is evident. The nuclear features of malignancy can be appreciated, with enlarged nuclei with irregular borders, thick membranes and macronucleoli. The nuclear-to-cytoplasmic ratio is high. Compare these cells to benign respiratory epithelial cells seen in the bottom left. Cluster formations are retained in TP with better preservation of their nuclear features as compared to conventional smear (TP). (**b**) TBBx shows adenocarcinoma. Tumor cells were immunoreactive for TTF-1 and Napsin-A, both indicative of lung origin (H&E). (**c**) Creola body on TP. Compare with SP in Fig. 6.2c. Note cilia faintly visible in some cells and terminal bars without distinct cilia in other cells (TP)

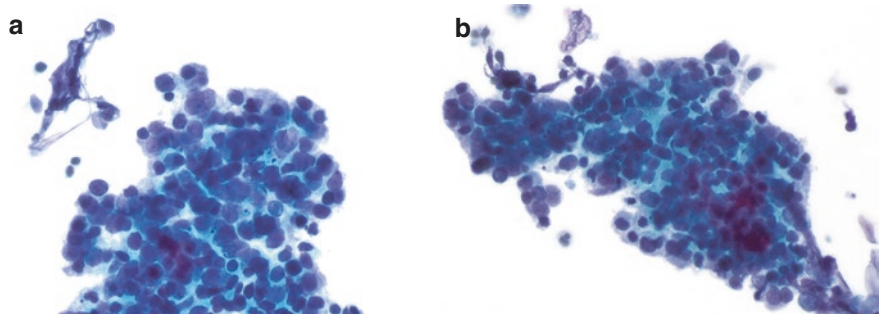

Fig. 6.11 Bronchial wash with small cell carcinoma. **(a, b)** Group of small cell carcinoma cells with high nuclear-to-cytoplasmic ratios and apoptotic bodies. Nuclei show molding, irregularity, condensed and coarse chromatin, occasional small nucleoli, and minimally discernible cytoplasm (TP). Background shows crush artifact. Some granular diathesis clings to tumor cells. Kim et al. [4] examined a series of small cell carcinoma on TP and found that the cells could demonstrate shrinkage artifact and display small nucleoli and nuclei with odd shapes (dumbbell and pencillike). Nuclear molding and nuclear streaks were rarely seen; but apoptotic cells and granular necrotic debris could be faintly discerned in the background. In small cell carcinoma, nuclear chromatin details were better observed on TP; however, there was loss of spindling and nuclear molding observed in direct smears

Suggested Readings

1. Layfield LJ, Baloch Z, Elsheikh T. Standardized terminology and nomenclature for respiratory cytology: the Papanicolaou society of cytopathology guidelines. Diagn Cytopathol. 2016;44:399–409.
2. Kenyon S, Sweeney BJ, Happel J. Comparison of BD Surepath and ThinPrep Pap systems in the processing of mucus-rich specimens. Cancer Cytopathol. 2010;118:244–9.
3. Narine N, Rana DN, Santhanakrishnan K. Cytological and electron microscopic findings in a bronchoalveolar lavage sample from a case of pulmonary alveolar proteinosis with radiological correlation. Cytopathology. 2015; doi:10.1111/cyt.12301.
4. Kim S, Owens CL. Analysis of ThinPrep cytology in establishing the diagnosis of small cell carcinoma of lung. Cancer. 2009;117:51–6.
5. Tyler KL, Selvaggi SM, Stewart J 3rd. *Pneumocystis jirovecii* in a bronchoalveolar lavage specimen on ThinPrep® cytology. Diagn Cytopathol. 2011;39:675–6.
6. Rana DN, O'Donnell M, Malkin A. A comparative study: conventional preparation and ThinPrep 2000 in respiratory cytology. Cytopathology. 2001;12:390–8.
7. Choi YD, Han CW, Kim JH. Effectiveness of sputum cytology using ThinPrep method for evaluation of lung cancer. Diagn Cytopathol. 2008;36:167–71.
8. Elsheikh TM, Kirkpatrick JL, Wu HH. Comparison of ThinPrep and cytospin preparations in the evaluation of exfoliative cytology specimens. Cancer. 2006;108:144–9.
9. Hoda RS, Colello C, Roddy M. "Fruiting body" of Aspergillus species in a routine cervicovaginal smear (Pap test). Diagn Cytopathol. 2005;33:244–5.
10. Collins GR, Thomas J, Joshi N. The diagnostic value of cell block as an adjunct to liquid-based cytology of bronchial washing specimens in diagnosis and subclassification of pulmonary neoplasms. Cancer Cytopathol. 2012;120:134–41.
11. Choi YD, Han CW, Kim JH. Effectiveness of sputum cytology using ThinPrep method for evaluation of lung cancer. Diagn Cytopathol. 2008;36:167–71.