Chapter 1

Exfoliative pulmonary cytology

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Types of specimens and adequacy

Exfoliative (ex- "out, from" + folium "leaf") cytology is the study of cells shedding from the lining of an organ into a bodily cavity. These cells can be collected by nonabrasive means after they have either spontaneously detached (spontaneous exfoliation) or by manually scraping/brushing them off from a bodily surface (mechanical exfoliation). From the respiratory system, several types of exfoliative cytological specimens can be prepared: sputum, bronchial washing, bronchial brushing (BBr), and broncho-alveolar lavage (BAL). The type, amount, and to some degree, morphology of the cells obtained vary with each type.

Sputum

Compared to other techniques, sputum is a less invasive, easy to repeat, and inexpensive technique that allows direct sampling from the lower respiratory tract. Although spontaneous production and expectoration of sputum is feasible for adults, children require sputum induction. Sputum induction with hypertonic saline is a safe and effective procedure, with a success rate ranging from 59% to 76%, and with minor adverse effects such as sore throat and a transient decrease in oxygen saturation. It is now a recommended method of specimen collection for investigating *Mycobacterium tuberculosis, Pneumocystis jiroveci*, and respiratory *Cryptosporidium* species infections among immunocompromised children, as well as in immunocompetent children hospitalized with severe pneumonia. Assessment of adequacy is essential in all exfoliative cytology specimens, as it conveys to the clinicians how well represented target cells are in the specimen. In sputum cytology, the criterion used is the presence of numerous alveolar macrophages in order to consider it representative of deep bronchiolar lesions. No reference range for number of macrophages is consistently reported in the literature, but an adequate specimen should be rich in macrophages, contain some neutrophils, and be poor in eosinophils, lymphocytes, and epithelial cells. Sputum is mainly used as a screening method, and patients with abnormal sputum cytology should undergo bronchoscopy, especially in the adult age group.

Broncho-alveolar lavage

Broncho-alveolar lavage is an excellent technique for detecting pathologic processes of the terminal airways, including alveoli, and is usually performed using a pediatric flexible fiberoptic bronchoscope (FFB). When a lesion is identified through imaging or bronchoscopy, BAL is collected from the most affected area, allowing adequate analysis of the localized process. In cases of diffuse lung disease, the right middle lobe is the preferred site because this area offers better fluid recovery. In neonates and young children, BAL is often used as a first-line diagnostic technique since sputum induction and collection is not feasible. In children beyond the neonatal period, an FFB with a 3.5–3.7 mm external diameter is commonly used. Patients beyond nine years of age usually tolerate a 4.6 ± 4.9 mm in diameter FFB. With a small-diameter FFB (2.2–2.8 mm) even neonates and premature infants can undergo the procedure. Using the same small size bronchoscope in patients of different age results in washing a greatly varying portion of lung volume. For instance, in a neonate, a 3.5 mm FFB may wage into a lobar bronchus, whereas in a four-year-old, it may wedge into a subsegmental bronchus.

Despite being a relatively non-invasive technique compared to tissue biopsy, BAL increases the duration of the bronchoscopic procedure by 2–3 min, and marginally increases the risk of hypercapnia and/or hypoxia due to retained saline. Because of such risks, pediatric patients undergoing BAL should be routinely monitored by continuous transcutaneous oximetry using a well calibrated pulse oximeter. Other minor complications may include stridor, minor bleeding, and fever.

Compared to sputum, BALs render more richly cellular and cohesive samples, with better preserved material, cleaner background, and less saliva contamination.

According to the European Respiratory Society, the BAL fluid can be technically acceptable when the volume recovered is more than 40% of the volume instilled. BAL fluid obtained from healthy individuals should contain, on average, a majority of alveolar macrophages (80–90%), some lymphocytes and respiratory epithelial cells, and very few neutrophils or eosinophils. For an adequate BAL, ciliated columnar cells, mucous

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goblet cells, and alveolar macrophages should be present. The percentage of bronchial epithelial cells in BAL specimens depends on the lavage technique and method of specimen processing. Compared to adults, children under the age of eight years have a significantly higher absolute granulocyte count (2.5%) and relatively higher proportion of lymphocytes (16%), independent of age. The presence of squamous epithelial cells in BAL suggests contamination by oropharyngeal secretions has occurred due to either poor technique in performing the BAL, or aspiration of upper airway secretions by the subject.

The liquid-based cytology using the ThinPrep® system (Cytyc Corporation, Marlborough, MA) is currently a board-accepted alternative to conventional cytopreparatory method. Papanicolaou stain (Pap) should be used routinely for its value to detect and define neoplastic cells. Diff Quik is preferred for rapid on-site evaluation and for suspected hematologic malignancies. In our hands, Wright-Giemsa staining offers excellent morphological detail. Preparation of cell block from the BAL fluid specimen can be beneficial for recognition of the histological patterns and immunohistochemical staining.

Ancillary studies, such as immunohistochemistry, flow cytometry, molecular pathology techniques, and electron microscopy can be performed on BAL or sputum samples for further characterization of abnormalities noted on routine Pap and/or Wright-Giemsa stain(s).

Lesions to consider in pediatric exfoliative pulmonary cytology

Many infective and reactive lung diseases of children show cytologic appearances no different from those seen in adults with such conditions. Primary epithelial lung tumors with the highest diagnostic yield on BAL specimen are exceptionally rare in children. However, some rare infections, inflammatory and metabolic conditions, as well as tumors, of the pediatric age require more detailed description.

Non-neoplastic diseases

- Respiratory infections
- Asthma and airway eosinophilia
- Pulmonary alveolar microlithiasis
- Pulmonary alveolar proteinosis
- Bronchopulmonary dysplasia
- Sarcoidosis
- Aspirational (lipoid) pneumonia
- Bone marrow embolism and acute chest syndrome
- Idiopathic pulmonary hemosiderosis

Neoplastic disease

- Langerhans cell histiocytosis
- Neuroendocrine tumors
- Granular cell tumor
- Hematopoietic malignancies
- Inflammatory myofibroblastic tumor

Special consideration

- Cystic fibrosis
- Lung transplant recipient
- Therapy-related changes

Respiratory infections

Most pulmonary diseases in the general pediatric population are infectious. Invasive diagnostic procedures are rarely indicated since self-limiting infections are treated based on clinical judgment. Diagnostic BAL or induced sputum can be obtained for culture in more severe cases.

Bacterial, viral, and fungal infections

BAL obtained for culture for a suspected infectious process should be examined microscopically to evaluate the relative distribution and number of different cell types. Acute respiratory viral infections occur with high frequency, especially in younger children. Not all viral infections produce cytopathic changes. However, BAL lymphocytosis – defined as more than 15% of lymphocytes in a sample – has been commonly noted in association with viral airway disease. Nevertheless, healthy children show a higher mean fraction of lymphocytes (16.2 ± 12.4% with a median of 12.5%) compared with the normal distribution in non-smoking adults (4–18%). It has been hypothesized that a relative increase of lymphocytes in children with no active respiratory disease might be indicative of a subclinical alveolitis or prior respiratory viral infection. Be that as it may and for diagnostic purposes, an increased number of lymphocytes in BAL is nonspecific for viral lung disease. Classically, neutrophilia ranging from 25% to 95% of the total cellularity (Fig. 1.1) is seen in the setting of active bacterial infections and interstitial pneumonia caused by Gram-positive and Gram-negative bacteria. This picture is lost in patients

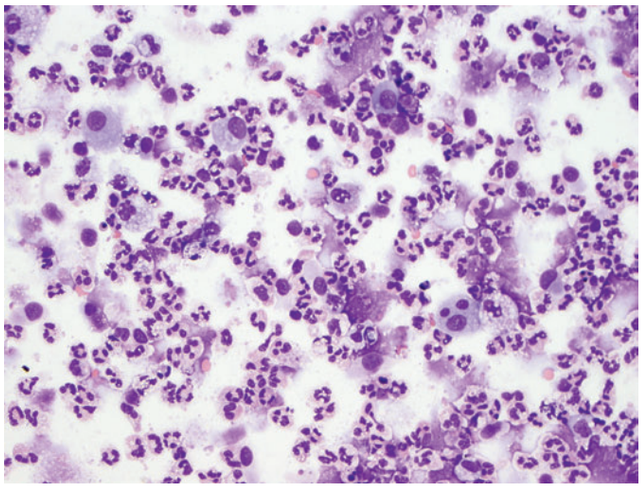

Fig. 1.1 BAL showing abundant neutrophils, representing the vast majority of the obtained material in a patient with acute bacterial pneumonia (Wright-Giemsa stain).

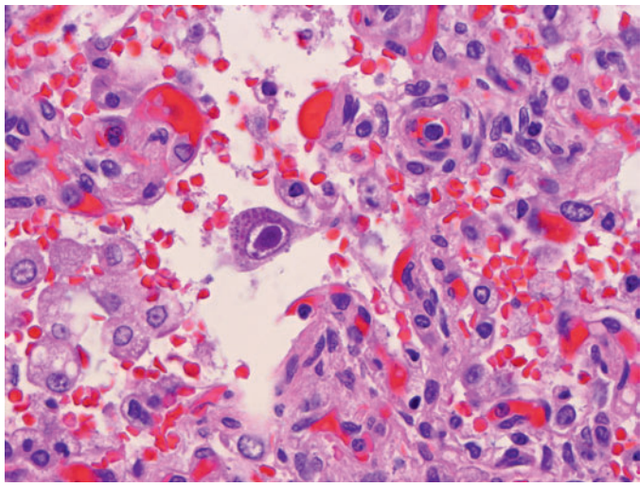

Fig. 1.2 Lung section showing intranuclear and intracytoplasmic inclusions in a patient with congenital CMV infection.

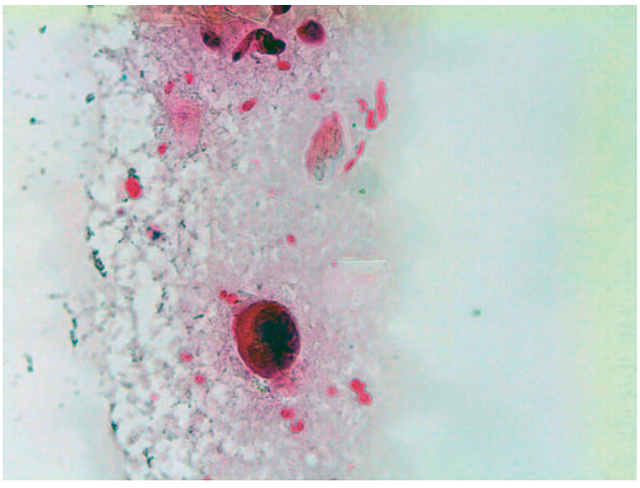

Fig. 1.3 Tzank preparation from the skin of a patient with herpes infection showing a Cowdry A inclusion (Papanicolaou stain; courtesy of Dr. Kumala Pillay).

with neutropenia secondary to antineoplastic therapy. The diagnosis of bacterial pneumonia on BAL is one of exclusion, requiring quantitative culture results for differentiation between infective and non-infective etiologies. Thus, increased cellularity in conjunction with a positive culture will strongly support the diagnosis of bacterial lung disease, as opposed to contamination from the upper respiratory tract.

Identification of specific morphologic and cytopathic changes on a BAL specimen is possible for some viral and fungal organisms.

Cytomegalovirus (CMV) is a common cause of opportunistic disease in immunocompromised patients with primary or secondary immunodeficiency, such as transplant recipients, children with Hodgkin lymphoma, or those with congenital immunodeficiency like Di George syndrome. The diagnostic yield for CMV in BAL is similar to open lung biopsy (Fig. 1.2). In cytology, CMV is characterized by cytomegaly, an eosinophilic nuclear inclusion, perinuclear halo, cytoplasmic granules or inclusions, and ciliocytophthoria, a prominent detachment of cilia (from cilio, cyto and *phthora* = corruption, decay), a reactive process occurring in viral pneumonia, but also an artifact induced by specimen processing. Inclusions are usually noted involving individual cells rather than clusters of cells within the same focus. CMV usually induces a prominent plasma-cell response with variable inflammatory cellular reaction. The differential diagnosis includes other viruses, particularly herpes simplex virus (HSV), adenovirus, and respiratory syncytial virus (RSV). In addition, other nonviral reactive cells such as reactive macrophages with inclusions or large nucleoli, and reactive epithelial cells can pose a morphological overlap.

HSV (both type 1 and type 2) may be seen in immunosuppressed patients and in neonates presenting with sepsis and severe respiratory failure. HSV infection is characterized by extensive necrotizing pneumonitis showing in BAL fragmented epithelial bronchial cells and debris. A dense inflammatory background with neutrophil predominance is usually present, accompanied by prominent giant cells. HSV cytopathic changes are identical to those seen in other sites and in tissues: multinucleation of the epithelial cells, nuclear molding, chromatin margination, and large eosinophilic nuclear (Cowdry A) inclusions (Fig. 1.3). Note that chicken pox infection by varicella zoster virus (VZV) causes a similar cytopathic effect.

RSV is an infection seen in the immunocompromised host and usually involves the upper respiratory tract. RSV infection in infants frequently causes bronchiolitis and pneumonia. The cytopathic effect includes giant cell transformation with multinucleation and inconspicuous cytoplasmic and nuclear viral inclusions. These findings are similar to the changes caused by the measles virus. The diagnosis is made by antigen detection in BAL specimens, since the typical cells are rarely present.

Adenoviral pneumonia may occur in non-immunocompromised patients, and can lead to a potentially fatal infection in immunosuppressed children. Classic findings in adenovirus bronchopneumonia include epithelial cells containing nuclear inclusions with radiated strands ("rosette" cells), large homogeneously staining nuclei ("smudge" cells) (Fig. 1.4), and nuclei with a "honeycomb" appearance. In addition, adenovirus also produces ciliocytophthoria.

Fungi are readily diagnosed by transthoracic fine needle aspiration (FNA), but occasionally may be obtained by BAL or BBr, allowing characterization by their size and unique morphology (Fig. 1.5). Despite an apparently specific morphological impression, it is advisable to correlate cytologic findings with culture results, particularly in cases of rare pathogens.

Although the lungs are the portal of entry for *Cryptococcus neoformans*, pulmonary disease is relatively uncommon, but should be considered when dealing with patients suffering meningeal infection. However, this fungus may cause pneumonia or a lung mass (cryptococcoma), particularly in immunocompromised patients. On Pap stain, *Cryptococcus* yeasts are of variable size (5–20 μm) with narrow budding and a clear zone resulting from the unstained fungal capsule, which may be highlighted by special stains such as mucicarmine, periodic acid-Schiff (PAS), or Grocott's silver methenamine.

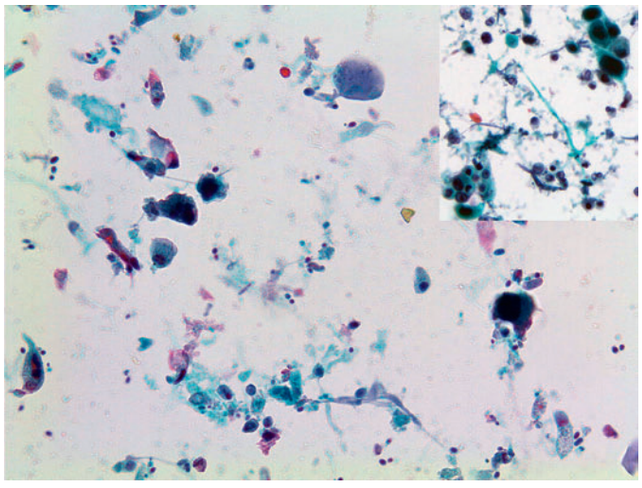

Fig. 1.4 BAL showing "smudge" nuclei, characteristic of adenoviral infection (Papanicolaou stain).

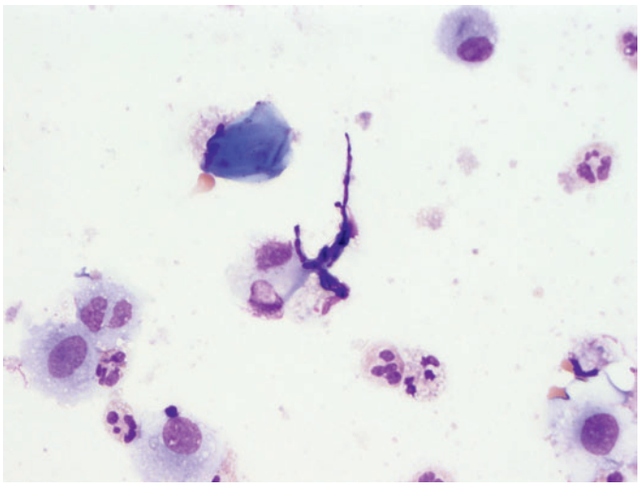

Fig. 1.5 BAL from a patient with cystic fibrosis and infection with *Candida* sp. Note the incomplete budding of yeasts forming pseudohyphae (Wright-Giemsa).

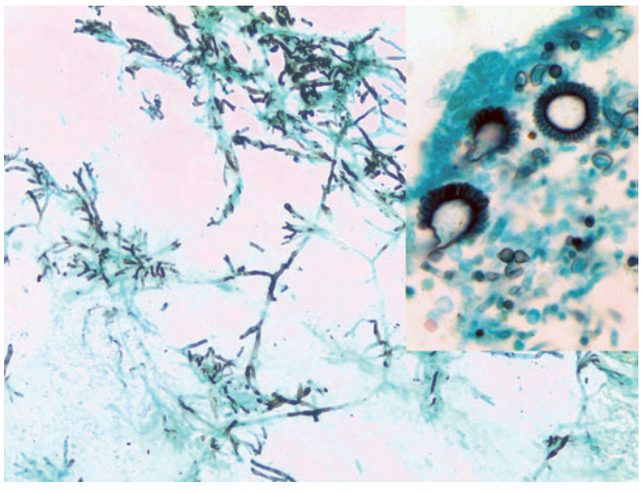

Fig. 1.6 *Aspergillus* septate hyphae with the characteristic acute angle branching (left). Inset shows conidia in *Aspergillus* culture (Grocott).

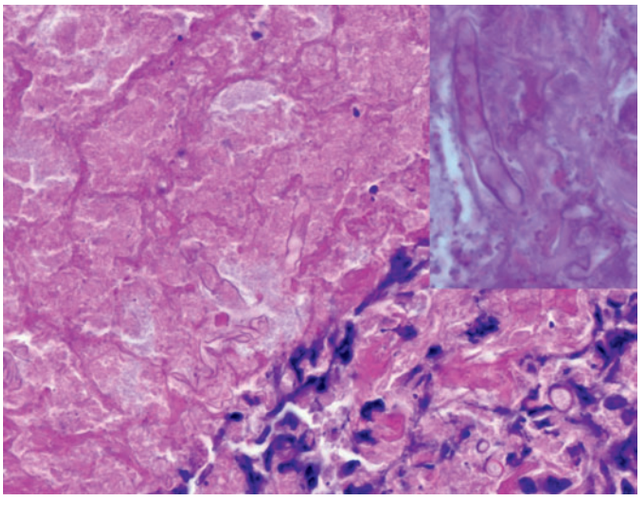

Fig. 1.7 Mucor organisms forming non-septate hyphae in a patient with necrotizing pneumonia. Inset shows a wide hypha (PAS-D stain).

Histoplasma capsulatum shows small (3–5 μm) intracellular budding yeasts with a narrow "neck." Since it may overlap morphologically with *Candida* these two fungi (*Candida* and *Histoplasma*) should be definitively differentiated by culture.

Coccidioides immitis is identified in BAL featuring numerous endospores (2–5 μm) packed within spherules (10–80 μm), which are better highlighted using silver impregnation techniques.

In BAL, *Aspergillus* species are characterized by thick-walled septate uniform hyphae branching at 45° (Fig. 1.6). Pulmonary aspergillosis is often accompanied by eosinophilia, Charcot-Leyden, and calcium oxalate crystals. The morphologic differential diagnosis includes other fungal elements, particularly Zygomyces (including *Mucor* and *Rhizopus*) (Fig. 1.7) and contamination with exogenous fibers that can superficially resemble *Aspergillus* hyphae.

Mycobacterium tuberculosis and *Mycobacterium avium intracellulare* complex infection

BAL in patients with pulmonary tuberculosis might reveal mixed inflammatory cells, epithelioid histiocytes, and Langhans giant cells. Neutrophils, eosinophils, and lymphocytes, particularly CD4+ T cells are increased in BALs of children with tuberculosis compared with controls. Identification of necrotizing granulomata, which are characteristic but not specific for tuberculosis, is possible on samples obtained by FNA, but rarely encountered in BrB or BAL samples. The usefulness of Ziehl-Neelsen, rhodamin-auramine, and other special stains for morphological diagnosis of *Mycobacterium tuberculosis* is low due to paucity of the organisms.

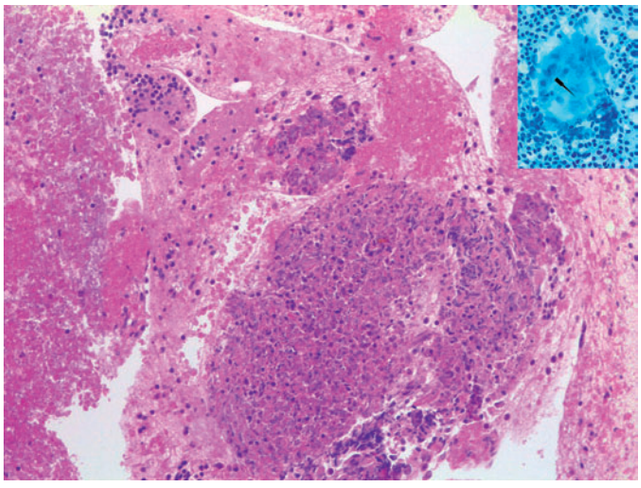

Fig. 1.8 Mycobacterial infection showing large masses of caseating material (H&E). Inset shows an acid fast bacillus (arrow) in a granulomatous lesion (courtesy of Dr. Kumala Pillay).

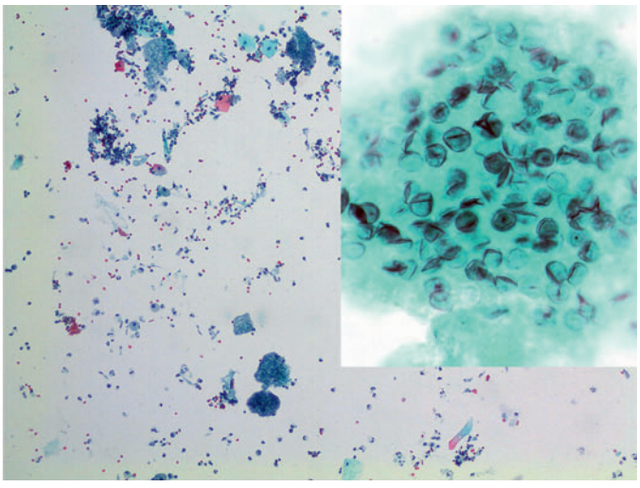

Fig. 1.9 BAL showing alveolar casts with *Pneumocystis jiroveci* organisms present (Papanicolaou; courtesy of Dr. Kumala Pillay). Inset shows *Pneumocystis* organisms within an alveolar cast (Grocott).

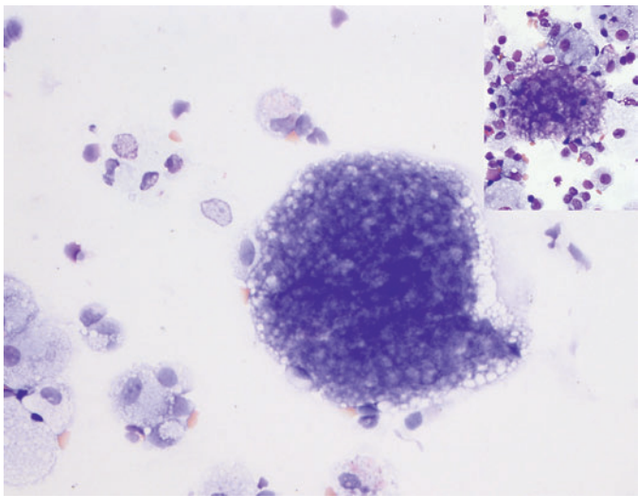

Fig. 1.10 Alveolar cast in a BAL. Note the bubbly appearance caused by *Pneumocystis jiroveci* organisms (Wright-Giemsa).

When identified, the isolated organisms are 1–4 μm long, slender, bent acid fast rods (Fig. 1.8). It is recommended to culture the material and submit it for additional molecular work-up and sensitivity testing. In contrast, several studies have documented the utility of BAL in diagnosing *Mycobacterium avium intracellulare*, which are abundant in samples from immunocompromised patients. The negative image of extracellular mycobacteria may also be useful.

Pneumocystis jiroveci

The sensitivity of BAL for detecting *Pneumocystis jiroveci* approaches or exceeds that of traditional biopsy procedure. Sputum induction can be attempted in older children. *P. jiroveci* trophozoites (1–5 μm) and/or cysts (5–8 μm) may be identified. On Pap-stained cytospins, characteristic foamy casts of proteinaceous material with scanty inflammatory response are usually present, but the individual organisms are not visible. Identification of the microorganism is achieved with PAS, Grocott's methamine silver, and other special stains. The collapsed cysts are cup-shaped and tend to cluster rather than appear individually. Many of the cysts display a central dark zone that allows confirmation of the organism and its discrimination from non-budding yeasts which can overlap morphologically (Figs 1.9, 1.10). Direct immunofluorescence is sensitive to detect the organism in a limited sample.

Asthma and airway eosinophilia

Eosinophils are rarely seen in BALs from healthy individuals and account for less than 1% of the differential count. Eosinophilia in BAL is always significant and may be seen in asthma, reaction to drugs, allergic bronchopulmonary aspergillosis/bronchocentric granulomatosis, and eosinophilic syndromes such as Churg-Strauss or idiopathic hypereosinophilic syndrome, chronic eosinophilic pneumonia, hypersensitivity pneumonitis, allergic angiitis, and even in rare cases as a manifestation of Hodgkin lymphoma. Asthma usually first presents in early childhood and can persist into adulthood. In infants and very young children, the diagnosis, assessment of severity, and prognosis remain difficult. Studies reveal that the number of eosinophils in BAL fluid is higher in children with atopic asthma than in children with transient wheezing. Such difference in number is consistently seen even in those with a normal or low serum immunoglobulin E (IgE) and no peripheral eosinophilia. Eosinophil counts in induced sputum from patients with asthma correlate with those in bronchial wash, BAL, and, to a lesser extent, with the counts in bronchial biopsies. Thus, induced sputum can be used in older children to monitor the presence and severity of airway inflammation.

Charcot-Leyden crystals (CLC) are lysophospholipase crystals of variable sizes and shapes (rhomboid or slender), with pointed ends, measuring up to 50 μm in length; they develop from breakdown of eosinophils. CLC can be seen in sputum and

BAL of patients with airway hypereosinophilia. They appear purple-red with Masson trichrome staining. Creola bodies are another important finding seen in up to 60% of induced sputum or BAL fluid samples from asthmatic patients. Creola bodies are indicative of acute exacerbation of asthma, and their presence correlates positively with epithelial damage and increased production of the specific neutrophil-mobilizing cytokine IL-8 and neutrophilic elastase (NE), a specific neutrophil activation product. In wheezing neonates and in infants, finding Creola bodies predicts development of clinical asthma. Cytologically, Creola bodies are three-dimensional papillary formations or clumps of benign ciliated respiratory epithelium where the columnar cells retain their terminal bodies and show bland nuclei with smooth nuclear membranes.

Parasitic infections can also produce airway and peripheral eosinophilia. *Toxoplasma gondii* is one of the numerous parasitic organisms with pulmonary involvement. It occurs in neonates as a result of disseminated infection from vertical (transplacental) transmission. BAL shows extra- and intracellular crescent-shaped trophozoides (5–7 μm) with large central nuclei. Immunohistochemical staining for *Toxoplasma* is available.

Pulmonary alveolar microlithiasis

Pulmonary alveolar microlithiasis (PAM) is a rare, autosomal recessive pneumopathy that occurs in the absence of any known disorder of calcium metabolism and is characterized by widely spread intra-alveolar accumulation of tiny, roundish calcified deposits called "microliths." The name "alveolar microlithiasis" was first used by Puhr in 1933 and the entity was described in detail by Sosman in 1957; since then over 576 individuals with this condition have been reported, 35.8% below 20 years of age. It takes decades for the disease to progress to an advanced stage, where pulmonary function deteriorates, leading to end-stage respiratory failure. However, cases with rapid clinical course are on record. This condition might present a diagnostic challenge, with 88 out of 576 patients initially misdiagnosed with sarcoidosis and pulmonary tuberculosis. Recently, mutation in gene SLC34A2 encoding a type IIb sodium-dependent phosphate transporter has been identified as responsible for the PAM phenotype (www.sciencedirect.com/science/article/pii/S095461111200399X#). Characteristic chest computed tomography (CT) findings in patients with PAM correlate well with specific pathological findings.

In BAL, extra- and intracellular concentrically laminated, purple-brown, round-to-oval microliths are characteristic. Cyanophilic PAS positive intracytoplasmic amorphous material is also frequently seen in alveolar macrophages. Chemically, microliths consist of large amounts of calcium and phosphorus and, in reference to histology, they consist of calcareous concentric lamellas which are placed around an amorphous or granular central nucleus.

Pulmonary alveolar proteinosis, congenital surfactant deficiencies

Pulmonary alveolar proteinosis (PAP) is a chronic disorder of surfactant clearance from the alveoli. Its prevalence is rare, especially in the pediatric population. It is a heterogeneous disease with immediate-onset forms leading to early and fatal respiratory failure, possibly related to Surfactant Protein B deficiency. Postnatal-onset PAP may be associated with various diseases, and has a polymorphic progression from asymptomatic to uncontrollable respiratory failure. It is characterized by intra-alveolar accumulation of material of lipoprotein origin, similar to surfactant.

This resemblance suggests defective resorption, or excessive production, of surfactant by alveolar macrophages or by type II pneumocytes, respectively. Recently, patients with adenosine deaminase (ADA) were described as having a clinical picture similar to PAP, suggesting possible enzyme involvement in the developmental pathway. In PAP, BAL fluid is described as milky or opaque and demonstrates PAS positive surfactant-like material with macrophages engulfing degenerating lamellar bodies. On lung biopsy, it corresponds to alveolar spaces filled with homogeneous granular eosinophilic material and large macrophages. Cellular analyses of BAL fluid do not reveal a specific pattern, although hypercellularity and monocytosis were observed. The BAL is as much a diagnostic as it is a therapeutic procedure, and symptoms of PAP are managed most effectively through whole-lung lavage (WLL) in adult and pediatric patients.

Bronchopulmonary dysplasia

Survival of extremely premature infants with very low birth weight has improved considerably over the last decade. However, many of them remain chronically dependent on respiratory support for many weeks and about one-quarter of infants under 1500 g develop bronchopulmonary dysplasia (BPD).

The classic ("old type") form of BPD, with severe pathologic changes, was described before the improved ventilatory strategies, exogenous surfactant, and prenatal steroids were used as treatment modalities in these infants. The pathophysiologic changes were mostly due to the effect of elevated oxygen and ventilator-induced injury on a relatively immature and surfactant-deficient lung, yielding histopathological changes indicative of severe airway epithelial injury and smooth muscle hyperplasia, alternating sites of overinflation and atelectasis, extensive fibrosis, and severe vascular hypertensive lesions.

BAL changes seen in this type of BPD were divided according to cytomorphology and duration of ventilation into three classes, with Classes I and II occurring in the first ten days. Minimal degenerative or regenerative changes were seen in Class I, with additional polymorphonuclear leukocyte infiltrate noted in Class II. Class III, which occurs at the tenth day, shows prominent epithelial cell regeneration, well-developed squamous metaplasia, and the presence of macrophages, multinucleated giant cells, and neutrophils.

New treatment strategies led to improvement of the BPD, but also to the survival of smaller and more immature infants. The pathophysiology of the "new type" of BPD also changed, leading to less airway epithelial disease, less airway-associated muscular hyperplasia, less severe vascular disease, varying

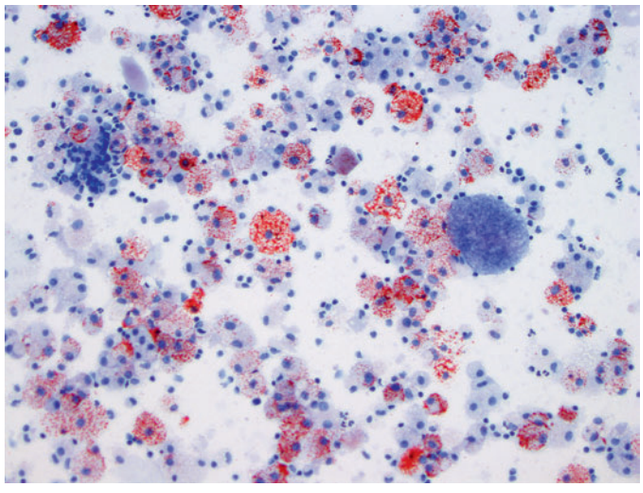

Fig. 1.11 Abundant lipid-laden macrophages associated with an alveolar cast in a patient with aspiration and *Pneumocystis* infection (oil red O).

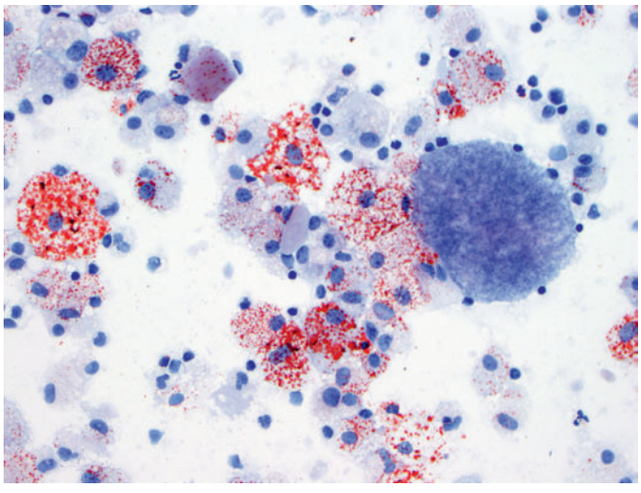

Fig. 1.12 Detail from Fig. 1.11, showing a variable amount of individual lipid droplets within alveolar macrophages. The *Pneumocystis*-containing alveolar cast is also shown on the right (oil red O).

degrees of interstitial fibrosis, increased elastic fiber deposition in alveolar walls, and an abundance of large, simplified air spaces yielding reduced numbers of alveoli and decreased internal surface area. This alveolar simplification and enlargement results from impaired postnatal alveolization in an extremely immature lung following preterm birth.

Sarcoidosis

Sarcoidosis is a chronic multisystemic disorder with almost universal lung involvement. It is very rare in a pediatric group, but cases of children as young as five years old are on record. The diagnostic modalities for sarcoidosis rarely involve bronchoscopy and BAL. When performed, BAL fluid shows lymphocytosis of 20% to 50% of cell counts. However, relatively high BAL lymphocytosis is not specific to sarcoidosis. On flow cytometry, accumulation of T lymphocytes with shifted ratio toward helper CD4+ T cells is suggestive of sarcoidosis, in contrast with other clinically and morphologically overlapping diseases such as hypersensitivity pneumonitis, where the shift is toward suppressor T cells. Characteristic non-necrotizing granulomas are only observed on transbronchial biopsy specimens.

Aspiration (lipoid) pneumonia

Children with chronic gastroesophageal reflux or poor swallowing mechanisms can be prone to tracheal aspiration. Quantitation of lipid-laden alveolar macrophages (LLAMs) was proposed as an indicator of milk inhalation in infants and of aspiration in children with gastroesophageal reflux. A LLAM was defined as a cell with ten or more ingested fat globules visible on oil red O stain (Figs 1.11, 1.12).

In a study by Colombo and Hallberg, macrophages were graded by the amount of lipid in the cytoplasm of individual macrophages with a score of 0–4 (0 = not opacified; 1 = up to ¼ opacified; 2 = ¼–½ opacified; 3 = ½–¾ opacified; and 4 = totally opacified). A total of 100 macrophages were evaluated with a score ranging from 0 to 400. The extracellular fat globules were excluded from the count. LLAMs' scores of 86 or higher were seen in the aspiration group (mean ± SD, 139 ± 46), whereas none of the nonaspirators had an index exceeding 72 (mean ± SD, 21 ± 20). However, further studies challenged the specificity of LLAMs to aspiration pneumonia as they were abundant in neonates receiving only intravenous lipids. Currently, enumeration of LLAMs in BAL and tracheal aspirates is considered sensitive, but not a totally specific modality for diagnosis of aspiration. This test provides clinically important information relatively rapidly and is commonly requested. In our practice, we use a simplified semiquantitative technique similar to Geisinger *et al.*, counting the number of positive macrophages on each slide without evaluation of individual macrophages for the amount of phagocytized lipid vacuoles. We consider a small number of lipid-laden macrophages as negative for clinically important aspiration. The finding of moderate to large numbers of lipid-laden macrophages is interpreted as highly sensitive for aspiration, but it is not specific as it does not discriminate other causes of chronic lung disease.

In addition to its diagnostic value, repeating BAL has been described as a valid treatment in cases of lipoid pneumonia secondary to mineral oil aspiration, as it provides mechanical removal of cells and prevention of interstitial fibrosis.

Bone marrow embolism and acute chest syndrome

LLAMs are an indicator of bone marrow embolism in trauma patients, or in those with sickle cell hemoglobinopathy presenting with acute chest syndrome (ACS). Godeau *et al.* set a cut-off value of >5% of LLAM (median value 46.5%) in 12 cases of ACS. In 11 cases, fat embolism was associated with proven (n = 8) or probable (n = 3) bone marrow infarction.

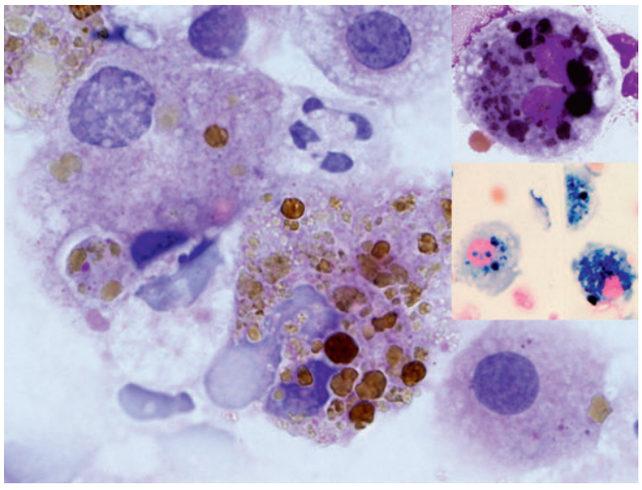

Fig. 1.13 Iron-containing macrophages. Note the dark brown granules within the histiocytic cytoplasm. Upper inset shows abundant hemosiderin granules (Wright-Giemsa). Lower inset shows a Perls stain highlighting hemosiderin in blue.

Chastre *et al.* studied 18 trauma patients and found that while the percentage of fat-laden macrophages obtained from bronchial washings ranged from 31% to 82% in patients with the clinical syndrome of fat embolism, the percentage of LLAMs from trauma patients without fat embolism was less than 2%.

Although BAL seems to be sensitive for the diagnosis of marrow embolism, its specificity is questionable. Stanley *et al.* evaluated 34 patients with pulmonary disease and found the calculated specificity to be only 26.5% when a cut-off value of 5% is used. Thus, interpretation of LLAM as an indicator of bone marrow embolism should be considered only in appropriate clinical settings.

Idiopathic pulmonary hemosiderosis

Idiopathic pulmonary hemosiderosis (IPH) is a rare disorder with variable course that can occur at any age and is characterized by the triad of hemoptysis, iron deficiency anemia, and diffuse pulmonary infiltrates. The average survival of IPH patients is improved using long-term immunosuppression. The cytologic examination of BAL, gastric aspirate, or sputum supports the diagnosis as it demonstrates numerous hemosiderin-laden macrophages (Fig. 1.13) confirmed by Perls Prussian blue stain.

Neoplastic diseases

In contrast with adult-age tumors, primary pediatric neoplastic pulmonary lesions are infrequent, and exfoliative cytology is not widely used to diagnose them. FNA remains the main diagnostic modality for the peripheral solitary tumors, with BAL reserved for disseminated or diffuse lesions. Higher diagnostic yield of BAL is achieved by careful consideration of the tumor location, cytological characteristics, imaging, and supplemental studies (flow cytometry, molecular techniques, and immunohistochemistry).

Langerhans cell histiocytosis

Langerhans cell histiocytosis (LCH) is characterized by uncontrolled proliferation and infiltration of various organs by Langerhans cells (Fig. 1.14). Although LCH is approximately three times more common in children than adults, pulmonary involvement is much more common in adults with LCH, in whom it frequently occurs as the sole organ involved. Cytologic examination of BAL fluid does not reveal remarkable abnormalities, but some reports indicate an increase in CD1a+ cells above 5% (normal is less than 1%). Active smokers without LCH also may have mildly elevated CD1a+ cell counts in BAL. Langerhans cells in the lavage fluid resemble pulmonary macrophages but have coffee-bean-shaped, grooved nuclei. Although finding more than 5% CD1a+ cells in the BAL may raise suspicion for LCH, the unequivocal identification requires supplemental techniques and is better performed on a biopsy specimen.

Neuroendocrine neoplasms

According to the WHO 2004 classification, pulmonary neuroendocrine neoplasms are subdivided as follows: typical carcinoid (low grade), atypical carcinoid (intermediate grade), and neuroendocrine carcinoma (small cell and large cell type). Pulmonary carcinoid tumors (PCTs) are relatively rare within the pediatric and young adult populations, and are rarely suspected before tumor resection. Pulmonary atypical carcinoid can be aggressive, and some of them cause significant morbidity and mortality. Small cell lung cancer is exceptional in children and adolescents, with only a few cases reported in the English literature.

Neuroendocrine tumors of the lung arise from Kulchitzky cells, which are normally present in the bronchial mucosa and share the usual morphologic features of neuroendocrine tumors: organoid nesting, palisading, rosettes, or a trabecular growth pattern. PCT are uncommonly diagnosed by exfoliative cytology due to their submucosal location beneath an intact bronchial epithelium, although occasionally bronchial brushing may disrupt the epithelium and yield diagnostic cells. Elements of a typical carcinoid tumor appear as single cells or loose cell clusters. They are strikingly uniform, with scanty homogeneous to finely granular cytoplasm that may be stripped from the nucleus. There is a moderate to high N/C ratio. The nuclei are small, round to oval, and tend to be central or slightly eccentric, with smooth nuclear membranes (plasmacytoid appearance). In the spindle cell variant, nuclei are elongated, somewhat hyperchromatic, with limited cytoplasm. The nuclear features include finely stippled to granular salt-and-pepper chromatin, with inconspicuous nucleoli. Nuclear molding, mitoses, and necrosis are absent. Pitfalls in diagnosis of PCT include confusion with reserve cell hyperplasia (RCH), bronchial epithelial cell hyperplasia, lymphocytosis, and small cell carcinoma.

Reserve cells are located at the bottom of the columnar respiratory epithelium and proliferate, replacing exfoliated columnar cells. With chronic irritation these cells may become prominent and mimic a neuroendocrine neoplasm.

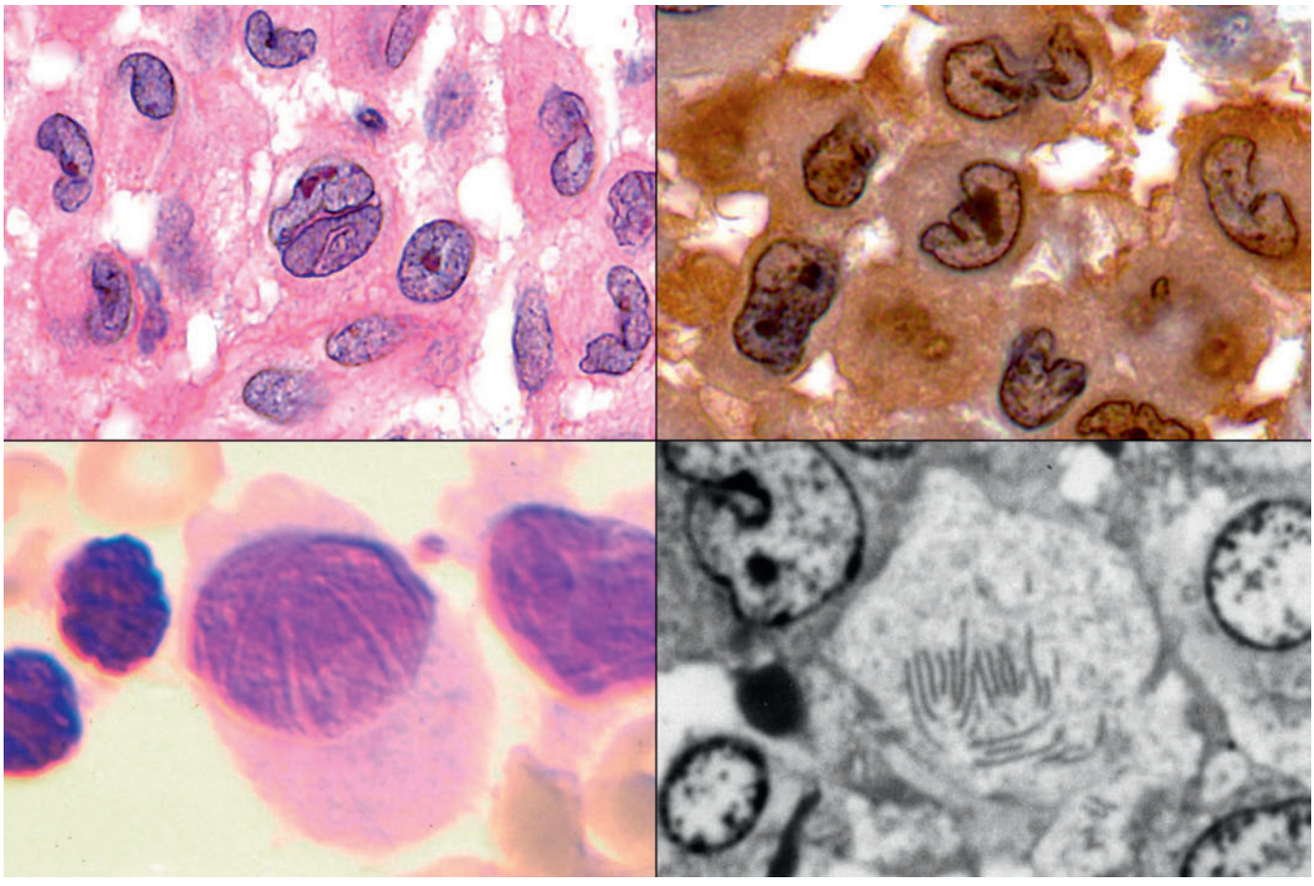

Fig. 1.14 Langerhans cell histiocytosis. Upper left: note the highly convoluted nature of the nuclei of Langerhans cells. Upper right: CD1a immunohistochemical staining of Langerhans cells. Lower left: Langerhans cells seen under Nomarski interference contrast microscopy highlighting the grooved surface of their nuclei. Lower right: black and white high-resolution photomicrograph (light microscopy) of Birbeck granules within the cytoplasm of a Langerhans cell (courtesy of Dr. Joaquín Carrillo-Farga).

Cytomorphologically, RCH shows tight, cohesive, uniform but hyperchromatic cells of scanty and dense cytoplasm, sometimes with cilia, approximately the size of lymphocytes, somewhat smaller than small cell carcinoma cells. Their nuclei are round to oval, with high N/C ratios, a smooth nuclear membrane, hyperchromatic chromatin, and sometimes prominent nucleoli. The tightness of these clusters, their uniformity, smooth nuclear membranes, hyperchromatic nuclei and association with epithelial cells are features helpful to discriminate between RCH and PCT.

Bronchial cell hyperplasia may be seen in the context of asthma, tuberculosis, viral infection, or instrumentation. Cytologically, it consists of tight papillary clusters with occasional molding and variable numbers of mucus-producing cells (Creola bodies; *vide supra*). The cells have uniform, small, round to oval nuclei with bland finely granular chromatin and inconspicuous nucleoli. Typically, cilia and/or terminal bars are identifiable. Depending on the etiology, the background may be clean or show eosinophils. There may be increased cellularity, and the clusters may sometimes contain atypical cells mimicking adenocarcinoma. Clues to the benign nature of these clusters, in addition to taking into consideration the clinical history and imaging findings, include the presence of cilia, the bland nuclei, finely granular chromatin, and the tightness of the cluster.

In lymphoid hyperplasia, the cells are discohesive and monomorphic, with scant basophilic cytoplasm. Nuclei are usually round and chromatin smooth, with small indistinct nucleoli. Lymphoglandular bodies are characteristic; however, it must be pointed out that pseudolymphoglandular bodies may rarely be seen in small cell carcinoma. In well-preserved samples, the distinction should be relatively easy. However, in poor preparations, cells may be crushed in the smears, mimicking the DNA artifact seen in small cell carcinoma. Furthermore, there may be artifactual clustering of cells simulating molding. Awareness of this pitfall is crucial in avoiding a misdiagnosis.

In the case of small cell carcinoma, mitotic activity, nuclei irregularity with molding and smearing, prominent nucleoli and necrosis are helpful to distinguish it from benign tumors.

Hematopoietic malignancies

The primary purpose of BAL in patients with a history of acute myeloid leukemia who have been treated with

chemotherapy is to look for specific infectious agents. Uncommonly, the washing may contain neoplastic cells. Using Pap stain, leukemic cells display atypical features denoting malignancy, but their hematopoietic nature is not apparent. Diff Quik or Romanosky (Giemsa) stains are generally superior for the morphologic analysis, since the myeloid nature of the cells is readily evident, with vesicular chromatin, prominent nuclei, basophilia, and granularity of the cytoplasm.

The diagnostic yield of BAL is good in secondary, diffuse indolent B cell lymphomas, and in primary B cell lymphomas of the mucosa-associated lymphoid tissue (MALT) type, but low in Hodgkin disease. A lymphocytosis picture is evident in the majority of cases. Neoplastic cells in indolent B cell lymphomas may have morphological features not easily distinguished from normal lymphocytes present in bronchial specimens of patients with follicular bronchitis/bronchiolitis, lymphocytic interstitial pneumonia, or diffuse Castleman disease. In cases of high-grade lymphomas, frankly malignant cells with cleaved, noncleaved, or convoluted nuclei are apparent.

Flow cytometry assay is mandatory to define the lineage.

In cases of pulmonary involvement in Hodgkin disease, the diagnostic yield is estimated at 33%, mainly because Reed–Sternberg cells or Hodgkin cells are scattered in an inflammatory background. Binucleate alveolar macrophages and CMV-infected cells can sometimes resemble Reed–Sternberg cells, making immunohistochemical staining for CD30 and CD15 helpful to rule-out the mimickers.

Granular cell tumor

Granular cell tumor is generally a benign tumor of Schwann-cell origin, usually endobronchial, although it may be located in the lung parenchyma. Given the predominant location, it is often sampled by FNA, but may be obtained by bronchial brushing. Cytologically, the tumor consists of variably large, thick syncytial clusters or sheets, with ill-defined, coarsely granular cytoplasm, oval nuclei with smooth nuclear membranes, finely granular chromatin, inconspicuous nucleoli, and no mitoses. The cytoplasm is fragile and often stripped off, creating a vacuolated and granular background. This tumor is positive for S-100.

Inflammatory myofibroblastic tumor

Inflammatory myofibroblastic tumor (inflammatory pseudotumor) is a rare neoplastic lesion with a high incidence in children and young people. The tumor may occur in the respiratory airways and is commonly presented as an asymptomatic solitary lung nodule. Rare case reports describe exfoliative cytology showing a mixture of various cell types, including inflammatory cells with predominance of plasma cells and lymphocytes, as well as cohesive clusters of myofibroblasts. The myofibroblasts have enlarged, oval, vesicular nuclei, finely granular chromatin and small nucleoli (Fig. 1.15). This picture can prompt an erroneous diagnosis of granulomatous inflammation.

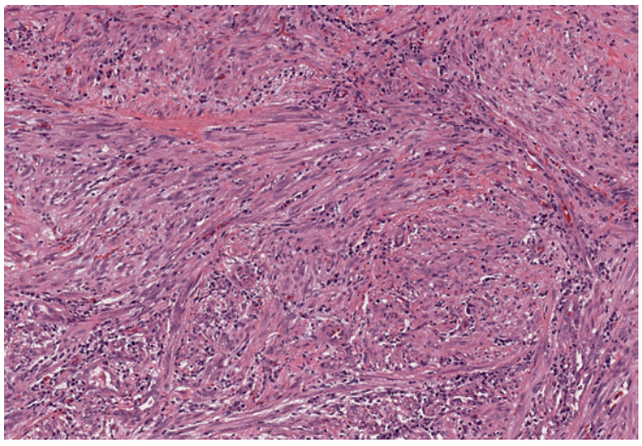

Fig. 1.15 Inflammatory myofibroblastic tumor of the lung. Note the spindly appearance of the myofibroblasts, associated with a sparse inflammatory component represented predominantly by plasma cells (H&E).

Special consideration

Cystic fibrosis

BAL is increasingly used for surveillance of children with cystic fibrosis for the early detection of microorganisms, to monitor the inflammatory response, and for the evaluation of therapeutic strategies.

In BAL, fluid of children and adolescents with cystic fibrosis shows rich cellularity, proportional to the increased number of neutrophils and alveolar macrophages, compared to the control groups.

Children with cystic fibrosis with positive cultures for *Pseudomonas aeruginosa*, especially mucoid strains, present with significant higher total cell counts and percentages of neutrophils than do individuals with negative cultures, or with cultures positive for other pathogenic agents.

A high percentage of eosinophils has been found in children diagnosed with allergic bronchopulmonary aspergillosis, a condition that affects approximately 5% of the children with cystic fibrosis. However, the presence of raised serum eosinophil cationic protein levels has been described in patients with cystic fibrosis with exacerbation of infection in the absence of allergic bronchopulmonary aspergillosis, militating against allergic bronchopulmonary aspergillosis as the sole explanation of high eosinophils in these patients.

Lung transplant

In the pediatric age group, indications for lung transplant vary with age. In early childhood (1–5 years of age), primary pulmonary hypertension is the most frequent transplant-related disease, followed by interstitial pneumonitis, congenital heart disease, and idiopathic pulmonary fibrosis. In late childhood and adolescence, cystic fibrosis is the most common indication for transplant (over half of transplant recipients), followed by pulmonary hypertension. The risk of increased transplant failure includes preoperative infection with *Burkholderia cepacia*