Chapter 11
Respiratory Tract

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Anatomy and Histology of the Low Respiratory System

The lower respiratory tract consists of the larynx, trachea, bronchi, and bronchioalveolar structures. The upper portion of larynx is lined by non-keratinizing squamous epithelium while the lower larynx, trachea, and bronchi are lined by respiratory epithelium. The respiratory epithelium is characteristically pseudostratified, ciliated columnar with admixed goblet cells (Fig. 11.1). Located at the base of the respiratory epithelium are basal or reserved cells. Mucinous and seromucinous glands are present in the subepithelial soft tissue of the tracheal and bronchial wall.

The lung parenchyma is primarily composed of roughly spherical thin-walled alveolar tissue, which is the functional unit of the lung (Fig. 11.2). It is lined by two types of epithelial cells—type I pneumocytes and type II pneumocytes. Type I cells are metabolically inactive, flattened, and with extremely attenuated cytoplasm surfacing approximately 90% of the alveolar wall. Type II cells are plump, cuboidal, and are capable of proliferation especially during chronic inflammation [1]. They synthesize alveolar surfactant that lines the inner alveolar surface to lower the surface tension and prevent pulmonary collapse.

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S. M. Gilani, G. Cai (eds.), *Non-Neoplastic Cytology*,
https://doi.org/10.1007/978-3-031-44289-6_11

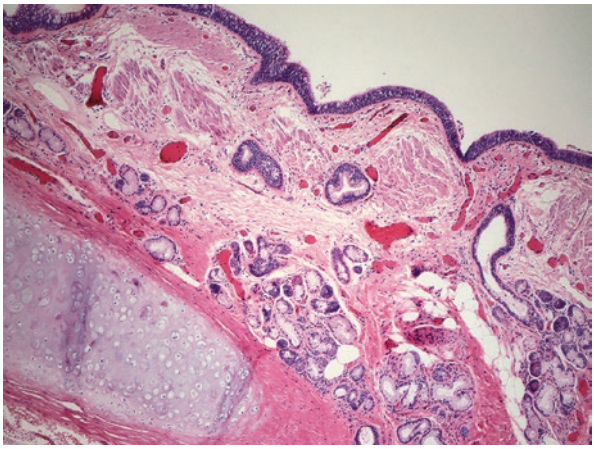

Fig. 11.1 Bronchial wall showing respiratory epithelial lining with subepithelial seromucinous glands (Hematoxylin-eosin stain)

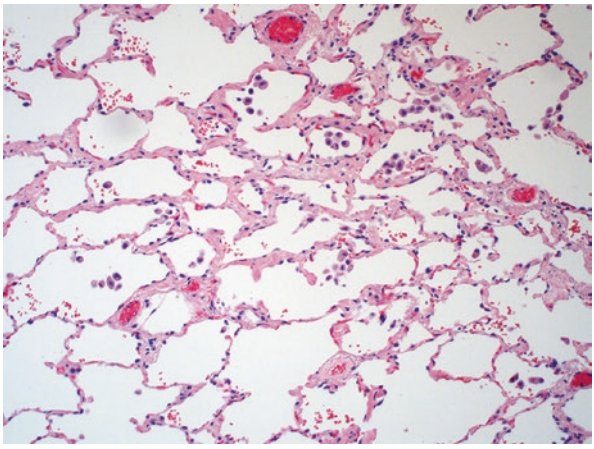

Fig. 11.2 Lung parenchyma showing thin alveolar septa with scattered intra-alveolar macrophages (Hematoxylin-eosin stain)

Specimen Types

The specimens from lower respiratory tract include sputum, bronchial brush, bronchial wash, bronchioalveolar lavage, and fine needle aspiration (Table 11.1).

Sputum

The simplest modality for studying respiratory tract pathology is collection of spontaneously or artificially produced sputum specimen. Patient should be instructed to follow certain precautions like clearing nasal passage and rinsing mouth before

Table 11.1 Sampling techniques for respiratory system disorders

	Method	Adequacy	Advantage	Disadvantage
Sputum	Expectorated spontaneously or induced	Presence of alveolar macrophages	Simple and low cost	Low sensitivity, oral contaminant may mask the findings
Bronchial brush	Direct sampling of bronchial abnormalities under bronchoscope	Whether the findings can explain the lesion of interest. Clinical correlation is required	Relatively simple. Can be combined with biopsy under bronchoscope	Difficult to differentiate reactive changes from dysplastic process and invasive tumor
Bronchial wash	Aspiration of washed saline through bronchoscope	Whether the findings can explain the lesion of interest. Clinical correlation is required	Detect both tumor and infectious lesions from relatively smaller area of lung tissue	Low sensitivity
Bronchioalveolar lavage	Aspiration of large volume of instilled saline through bronchoscope	Presence of alveolar macrophages	Detect mostly infectious lesions from larger area of lung tissue	Low sensitivity for identifying infectious agents
Fine needle aspiration	Direct sampling of the lesion under bronchoscopy or CT-guidance	Whether the findings can explain the lesion of interest. Clinical correlation is required	Direct sampling of the lesion helps to uncover the underlying etiologies	Sampling adequacy is a limiting factor. Rapid on-site evaluation may help improve diagnostic yield Cautious for contaminants such as bronchial cells and mesothelial cells

collecting the specimen, expelling directly into a wide mouth container with fixative and storing in the refrigerator. However, sometimes due to lack of instructions or other constraining factors the material expectorated might not be of diagnostic significance and consist entirely of mouth contents or saliva. Other drawbacks of the modality include contamination from mouth or nasopharynx and relatively low diagnostic yield due to non-productive cough. Sputum can be induced by inhaling heated aerosol of 20% propylene glycol in 10% hypertonic saline or water in salt restricted patients.

Bronchial Brush

The method exploits the utility of flexible bronchoscopes, which aids in the sampling of a visualized mucosal abnormality to confirm and localize occult in situ or early invasive carcinomas. Brushings can be supplemented by tissue biopsy or transbronchial aspiration biopsy. The limitation of the method is that the lesion of interest may not be within the reach of the bronchoscope such as distal peripheral bronchoalveolar region [2].

Bronchial Wash

Bronchial washing is less lesion-directed sampling technique, aiding in the sampling of more peripheral airway lesions, usually small bronchi beyond the reach of bronchoscopy brush. The lesional material is obtained by aspiration of small amount of sterile saline solution applied near the tip of the bronchoscope. Bronchial washing can be used to find tumors as well as infections.

Bronchioalveolar Lavage

Bronchioalveolar lavage was formerly introduced in clinical setting as a therapeutic procedure to remove accumulated secretions blocking the gaseous exchange in the lung. Subsequently, the technique has been widely used for diagnostic purposes particularly in patients with acquired immunodeficiency syndrome (AIDS). Under local anesthesia, the bronchoscope is passed to a secondary or tertiary bronchus and wedged to occlude the lumen. A total of 100–300 mL normal saline is instilled and re-aspirated. The specimen represents the epithelium of the bronchioles beyond the point of occlusion and alveolar contents. It is commonly used to identify various infectious agents—bacteria, fungal, parasitic, and sometimes viral organisms. The modality has replaced open lung biopsy for detection of opportunistic infections such as *Pneumocystis jirovecii pneumonia* [3]. The recent advancement has been detecting rejection and/or infection in post lung transplant patients.

Fine Needle Aspiration

Fine needle aspiration biopsy is an imaging-guided direct sampling of a pulmonary lesion. It can be performed via a percutaneous or transbronchial approach dependent on the location of the lesion. The former is used to sample peripheral lung

lesion that might not be within the reach of bronchoscopy, whereas the latter is applied to sample hilar and parahilar lymph nodes, or other near hilar masses which are difficult to access through percutaneous route [4, 5].

Normal Cytology—Cellular Elements

Respiratory Columnar Cells

The normal respiratory epithelium is identified as ciliated columnar cells. They do not desquamate easily, hence are uncommon in exfoliated material and typically seen in specimen obtained by bronchoscopy, bronchial brushing, bronchial wash, and fine needle aspiration. However, respiratory epithelial cells may also originate in the nasal cavity or nasopharynx; therefore, their presence cannot be reliable evidence for sampling of lower respiratory tract.

In brushings, columnar cells can be seen in clusters with cilia better appreciated at periphery, and sometimes even with attached reserve or basal epithelial cells (Fig. 11.3). While cilia may get damaged or lost, prominent linear thickening, the terminal plate or bar, at the luminal end of the epithelial cells is usually retained. The basal end of the cell tapers off representing the site of attachment to the basement membrane. The cytoplasm is lightly basophilic and homogenous, with occasional brown-lipochrome granules in the supranuclear area. The cells have oval-shaped nuclei with fine granular chromatin, and occasionally tiny nucleolus.

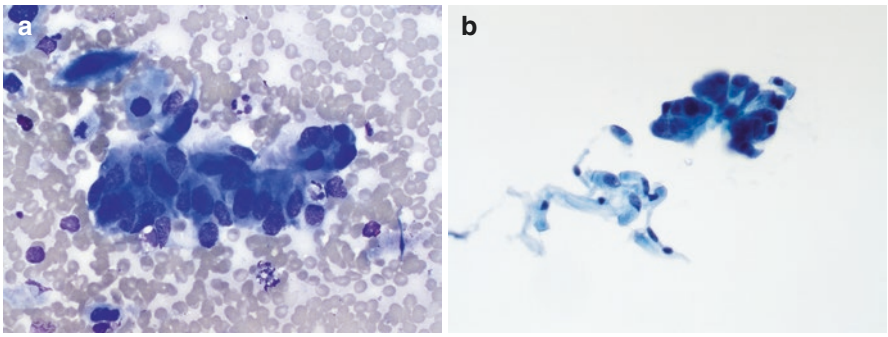

Fig. 11.3 Single and clusters of bronchial epithelial cells with cilia ((**a**) Diff-Quik stain; (**b**) Papanicolaou stain)

Goblet Cells

The mucus producing goblet cells are less predominant than ciliated columnar cells. They are wider than ciliated cells, have a basally placed nucleus with distended supranuclear cytoplasm containing mucinous vacuoles (Fig. 11.4). Goblet cell hyperplasia is seen in asthma and other chronic irritation conditions [6].

Basal or Reserve Cells

The basal or reserve cells form a layer of cells attached to the basement membrane, which are capable of regenerating and proliferating in response to inflammation or injury. These cells are small and have scant cytoplasm (Fig. 11.5). Immunohisto-

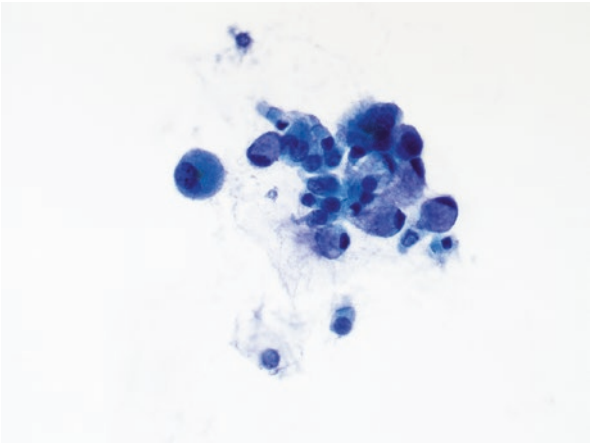

Fig. 11.4 Goblet cells admixed with bronchial epithelial cells (Papanicolaou stain)

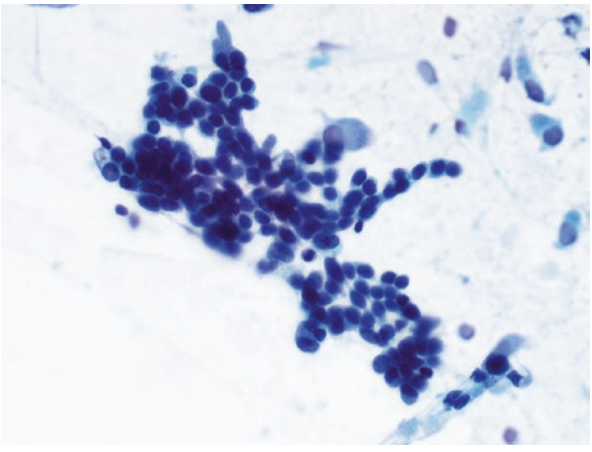

Fig. 11.5 Bronchial reserve/basal cells with a few ciliated bronchial cells (Papanicolaou stain)

chemically, they usually stain positive for p40, which therefore can be used as an internal control when working up subclassification of non-small cell carcinoma.

Squamous Cells

The exfoliated superficial squamous cells predominate the saliva and mostly originate from the superficial and intermediate cells of squamous epithelium. Squamous cells with relatively large but uniform nuclei may be present singly or more often in clusters hinting towards an underlying inflammatory disorder. Squamous cells in the lower respiratory tract likely represent reactive/metaplastic changes. During an inflammatory process, squamous cells may show significant cytologic atypia.

Macrophages

Alveolar macrophage in a cytology sample confirms the origin of the material from pulmonary alveoli. They are the predominant cell type in BAL specimen. They are spherical to oval cells, with amphophilic vacuolated cytoplasm containing variable amounts of brown-black dust particles (dust cells), and round-oval, or kidney-shaped nuclei with fine chromatin (Fig. 11.6). Binucleation can also be seen, also large multinucleated macrophages are not rare. Macrophages may contain other material such as hemosiderin pigments and lipid droplets in case of pulmonary hemorrhage and aspiration pneumonia.

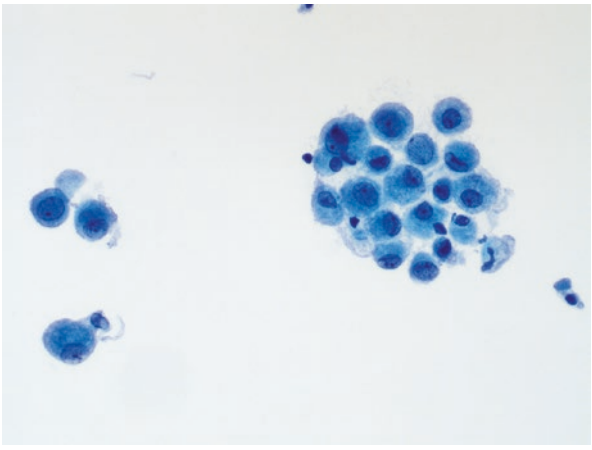

Fig. 11.6 Alveolar macrophages (Papanicolaou stain)

Mesothelial Cells

Mesothelial cells line the serosal surface of the lung. They are not infrequently seen as the contaminant in the percutaneous transthoracic needle biopsy of the peripheral lung lesions. These cells form flat clusters of uniform cells separated by slits or windows.

Non-cellular material (Table 11.2)

Curschmann's Spiral

Casts of inspissated mucus taking the shape of small bronchiolar lumen. It has a coiled appearance with a dark center and translucent periphery (Fig. 11.7). These spirals can be conveniently seen in chronic inflammatory conditions due to excess mucin production.

Inspissated Mucus

Small, dark staining structureless blob, as small as a naked nucleus and can be mistaken for a malignant cell.

Table 11.2 Cytomorphology and diagnostic implications of non-cellular material

Acellular material	Morphology	Differential diagnosis	Diagnostic clue
Curschmann's spiral	Spiral-shaped inspissated mucus cast of bronchioles	Parasite	Non-refractile, stains positive for mucin
Inspissated mucus	Small, dark stained blob-like size of nucleus	Malignant cell	No internal structures
Amyloid	Homogenous-amorphous material which shows apple-green birefringence post Congo red stain	Pseudoamyloid	Pseudoamyloid is fibrillar and does not show birefringence
Ferruginous body	Asbestos fiber coated with iron and protein	Non-ferruginous body	Ferruginous bodies are transparent, segmented bamboo shaped. Non-ferruginous bodies have opaque fibrous core, and may be curved or branched

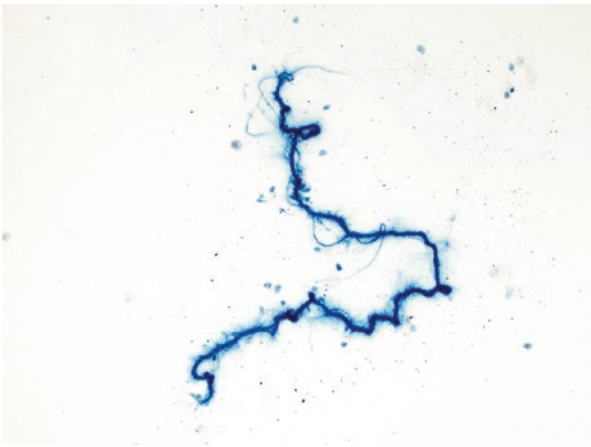

Fig. 11.7 Curschmann's spiral (Papanicolaou stain)

Corpora Amylacea

These are proteinaceous spherical translucent structures with concentric lamination. They are often seen in patients with a history of pulmonary edema, infarction, and chronic bronchitis.

Amyloid and Pseudoamyloid

Amyloid is a homogenous, waxy amorphous material which shows apple-green birefringence under polarized light following Congo red staining. Amyloid deposition in the lung could be diffuse or localized [7]. Localized amyloid deposition, amyloidoma, in the lung parenchyma can present a mass lesion by imaging studies. Pseudoamyloid is fibrillar eosinophilic material with no birefringence which is associated with an underlying light chain deposition disease.

Ferruginous Bodies

Sputum or bronchial samples of patients exposed to asbestos may contain beaded or segmented bamboo-shaped asbestos fibers with knobbed or bulbous ends (Fig. 11.8). These are coated with protein and iron, which lend a golden-yellow hue to the ferruginous body. The presence of asbestos bodies does not necessarily imply asbestos-related disease, moreover, other mineral fibers may have similar appearance.

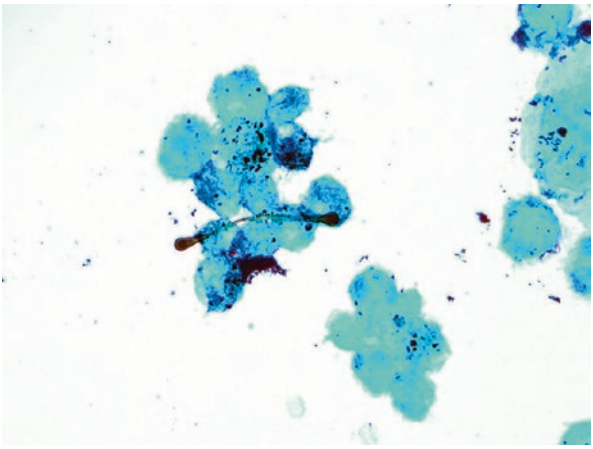

Fig. 11.8 Asbestos body with a few degenerated cells (Papanicolaou stain)

Reactive Cellular changes (Table 11.3)

Reactive Bronchial Cells

As a response to insult, bronchial cells undergo reactive changes including cytomegaly, nuclear enlargement, prominent nucleoli, and multinucleation. Such reactive changes can be observed even after minor trauma like repeated bronchoscopies or bouts of severe coughing, and in inflammatory processes such as bronchitis, bacterial or viral pneumonia, or tuberculosis.

Ciliocytophthoria

Ciliocytophthoria represents destruction of ciliated bronchial cells mostly due to an underlying viral pneumonia [8, 9]. The distal ciliated portion of the cells is pinched off, resulting in anucleated ciliated tufts, and nucleated cytoplasmic remnants. Nuclear degeneration is a common finding.

Creola Bodies

The chronic inflammatory conditions especially bronchiectasis, triggers papillary hyperplasia of the respiratory epithelium which sheds spherical or ovoid papillary clusters of bronchial cells [10]. The surface of this cluster consists of ciliated columnar cells while the core is composed of uniform small basal cells. It is important to

Table 11.3 Reactive cellular changes, differential diagnosis, and diagnostic pitfalls

	Cytomorphology	Differential Diagnosis	Diagnostic Clue
Reactive bronchial cells	Bi- or multinucleation, cytomegaly	Viral infection Adenocarcinoma	Smooth nuclear contours
Creola body	Detached portion of papillary hyperplasia of bronchial epithelium	Adenocarcinoma	Presence of cilia and goblet cells preserved nucleus to cytoplasm ratio
Basal cell hyperplasia	Clusters of small basal cells with hyperchromatic nuclei and scant cytoplasm	Small cell carcinoma	One of the edges is straight, tight clusters, with no nuclear molding
Squamous metaplasia	Clusters of cuboidal or polygonal cells with moderate eosinophilic cytoplasm	Squamous cell carcinoma	Smooth nuclear contour, preserved N:C ratio Some may show a straight edge or terminal plate
Bronchial metaplasia	Columnar epithelial cell accompanying alveolar macrophage in BAL specimen		
Reactive pneumocytes	Cuboidal cells with hyperchromatic nuclei and prominent nucleoli	Adenocarcinoma, atypical adenomatoid hyperplasia	Positive TTF-1 and Napsin A immunostaining

differentiate them from well-differentiated adenocarcinoma by paying attention to the presence of cilia, admixed goblet cells, and relatively maintained nuclear cytoplasmic ratio.

Basal Cell Hyperplasia

The small basal cells situated next to the basement membrane in the respiratory epithelium may undergo an abnormal multiplication to tide over unfavorable environment [11]. Normally, these cells are firmly adhered to the basement membrane hence rarely seen in the sputum. However, they can be seen in specimens obtained through instrumentation causing forceful detachment of the cells. The clusters of small, hyperchromatic cells are tightly packed, and may have a straight edge (Fig. 11.9). It can mimic small cell carcinoma; hence, keen observation is required [12].

Squamous Metaplasia

Respiratory epithelium of the trachea or bronchus can be replaced by squamous epithelium in response to inflammation, infection, or repeat instrumentation. Squamous metaplasia of the bronchial epithelium without atypia must not be considered as a precancerous lesion; however, atypical squamous metaplasia is a potential precursor of bronchogenic squamous cell carcinoma.

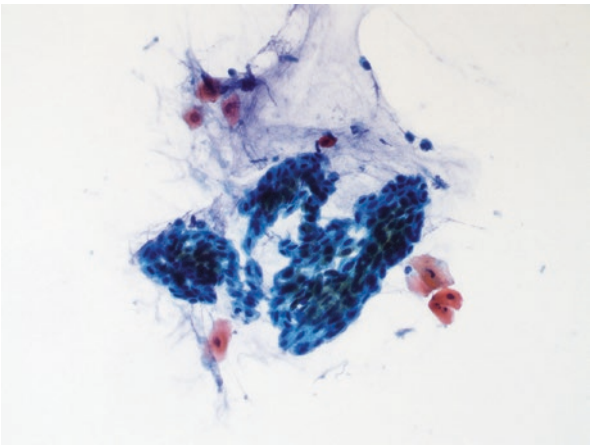

Fig. 11.9 Clusters of predominant reserve/basal cells (Papanicolaou stain)

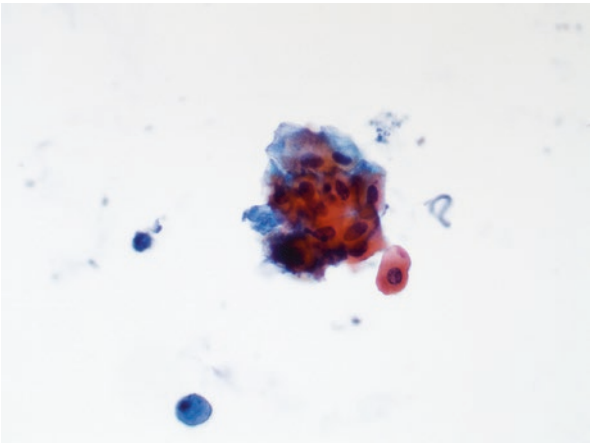

Fig. 11.10 Cluster of metaplastic squamous cells (Papanicolaou stain)

In sputum sample, it is difficult to differentiate the normal squamous cells from metaplastic. In bronchial washings, aspirates, and brush specimens, the metaplastic squamous cells can be well identified, when contamination from upper respiratory tract is excluded. Cytologically, the metaplastic squamous cells are small, adhered to each other in clusters or sheets (Fig. 11.10). They have round to oval nuclei and eosinophilic cytoplasm resembling parabasal cells.

Bronchial Metaplasia of Alveolar Epithelium

The pulmonary alveoli are lined by small cuboidal pneumocytes. In a variety of chronic inflammatory/obstructive processes, the pneumocytes can be replaced by one or more layers of columnar epithelial cells that are in continuity with the surrounding bronchioles. This phenomenon is commonly seen in areas adjacent to

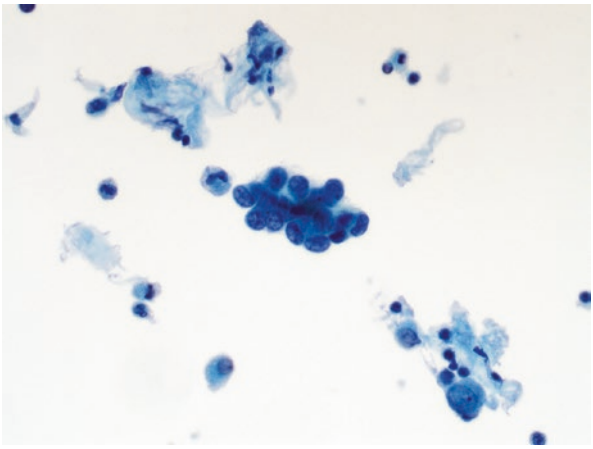

Fig. 11.11 Cluster of reactive pneumocytes with a few macrophages and lymphocytes (Papanicolaou stain)

scars or fibrosis and associated with cystic honeycomb lung changes. In setting of radiological findings suggestive of honeycomb lung changes, the presence of significant numbers of columnar epithelial cells accompanying alveolar macrophages in BAL specimens may suggest alveolar bronchial metaplasia.

Reactive Pneumocytes

Type II pneumocytes are highly reactive cells that respond to various pathologic processes. Reactive pneumocytes present individually or in sheets or rosette-like clusters with fine textured cyanophilic cytoplasm, occasional cytoplasmic vacuoles, and enlarged hyperchromatic nuclei with smooth nuclear contours and single or multiple prominent nucleoli (Fig. 11.11) [12]. The pulmonary diseases that are associated with reactive changes includes chronic pneumonia, viral pneumonitis, pulmonary fibrosis, infarcts, and chemo or radiation therapy.

Bacterial infections (Fig. 11.10)

Tuberculosis

It is caused by acid-fast mycobacterium tuberculosis, characterized by formation of caseating granulomas composed of epithelioid histiocytes, giant cells, and lymphocytes (Fig. 11.12). Epithelioid histiocytes have elongated nuclei and eosinophilic cytoplasm with poorly defined cell borders. Giant cells have multiple nuclei, which may be arranged with a peripheral wreath pattern, known as Langhans' cells [13]. Presence of Langhans' giant cell in cytology material is a non-specific finding and should not be considered as a hallmark feature for tuberculosis diagnosis.

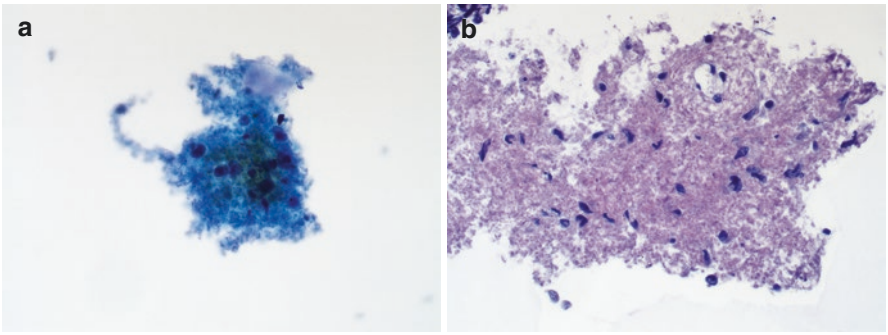

Fig. 11.12 Loose clusters of necrotic debris and scattered histiocytes ((**a**) Papanicolaou stain; (**b**) Hematoxylin-eosin stain)

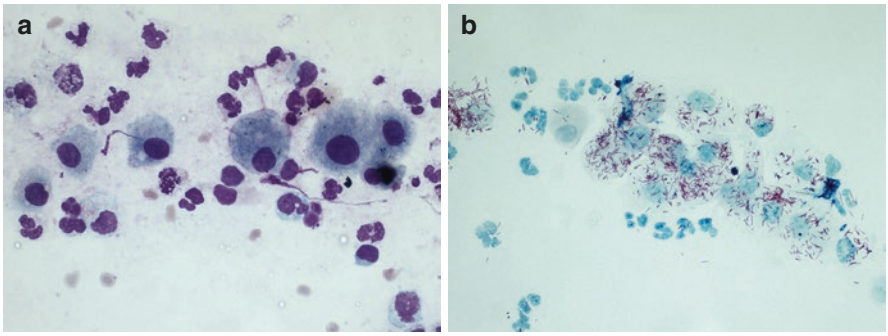

Fig. 11.13 Macrophages with intracytoplasmic negative images ((**a**) Diff-Quik stain) and acid-fast bacilli; ((**b**) Acid-fast bacilli stain)

Granuloma is rarely seen in exfoliative cytology specimens. Direct sampling of the lesions via FNA usually yields granular necrotic debris and a mixture of inflammatory cells. The presence of granuloma-like cluster of spindly epithelioid histiocytes with necrosis is not specific but should raise the possibility of tuberculosis. Mycobacterial organisms (*mycobacteria tuberculosis*) are sparse and difficult to identify even with special stains. Special attention should be given in high-risk patients with AIDS, in whom disease is severe and highly contagious. In such patients, the granulomas are poorly formed, and likely to contain a greater number of atypical mycobacteria (*mycobacterium avium-intracellulare*) (Fig. 11.13).

Actinomycosis

Suppurative infections caused by Gram-positive filamentous bacteria which are mostly saprophytic but can be pathogenic in immune compromised patients. They can commonly present as contaminants in the sputum, with no clinical significance.

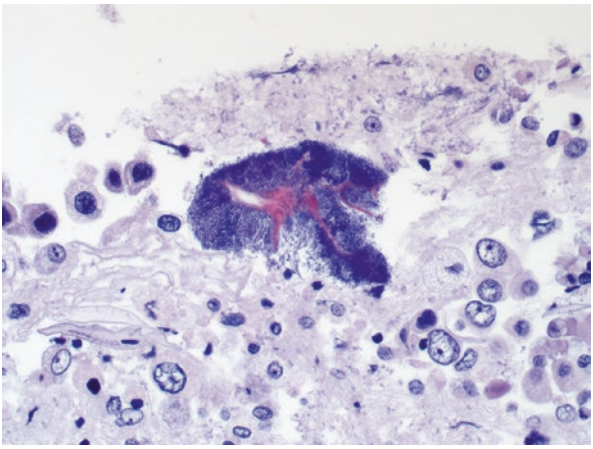

Fig. 11.14 Actinomycin colony with scattered macrophages and lymphocytes (Hematoxylin-eosin stain)

Pulmonary lesions sampled through bronchial brush or bronchial aspirate, usually represent secondary or complicating infections of already damaged or inflamed lung tissue. Actinomyces may produce lung abscesses, which can grow into pleura and chest wall resulting in empyema and fistulous tracts. They present as dense colonies made of hematoxylin-stain tangled filaments that radiate outwards and are more eosinophilic at the periphery (Fig. 11.14), known as sulfur granules [14].

Nocardiosis

Nocardiosis is aerobic branching filamentous bacteria that is Gram positive and resembles actinomyces but does not usually form colonies [15, 16]. Nocardia stains positive with Fite stain.

Viral Infections (Table 11.4)

Herpes Simplex Virus

Herpes infection is more often seen in immunosuppressed patients, which may progress to herpetic pneumonia [17]. Cytology specimen reveals multinucleated cells with moderately enlarged basophilic nuclei of ground glass appearance, or nuclei with margination of chromatin and molding. There are no cytoplasmic inclusions.

Table 11.4 Cytopathic effects of viral infections

Virus	Cytoplasmic Inclusions	Nuclear Inclusions	Giant Cells
Herpes simplex	No	Yes, ground-glass and eosinophilic	Yes
Cytomegalovirus	Yes, eosinophilic, or basophilic with halo	Yes, eosinophilic, or basophilic with large halo	Occasional
Adenovirus	No	Yes, multiple basophilic	No
Respiratory syncytial virus	Multiple basophilic halos	No	Always
Measles	Multiple small eosinophilic	Rare	Always

Cytomegalovirus (CMV)

CMV infection is mostly seen in immunocompromised patients. On cytology, CMV is characterized by significantly enlarged nuclei with large basophilic intranuclear inclusions surrounded by a clear halo. Multiple tiny basophilic cytoplasmic inclusions are sometimes seen. Virus can affect bronchiolar or alveolar epithelial cells, macrophages, and endothelial cells. In equivocal cases, immunocytochemistry, in situ hybridization, or molecular testing can help confirm the diagnosis.

Adenovirus

The cytopathic effects of this virus infection are characterized by enlarged nuclei containing multiple eosinophilic intranuclear inclusions with halos, which can merge into a single basophilic inclusion presenting as a smudge nucleus. The infected bronchial epithelial cells retain their cilia; however, ciliocytophthoria can be seen [18].

Respiratory Syncytial Virus

The infection predominantly occurs in infants or children with immunodeficiency but can also be seen in immunosuppressed adults. The classic finding is the presence of large syncytial groups of cells which contain multiple basophilic inclusions within the degenerated cytoplasm. Multinucleated giant cells with similar cytoplasmic inclusions.

Measles Virus

Measles is a common infection of childhood, usually in a transient nature, but can be complicated in immunocompromised patients. The hallmark feature is the formation of multinucleated giant cells (Warthin-Finkeldey cells) which can have up to

100 nuclei and contain spherical eosinophilic intracytoplasmic and intranuclear inclusions [19]. Also seen are syncytial epithelial giant cells which are formed by coalescence of type II pneumocytes and contain similar inclusions [20].

Viral Mimickers

Small eosinophilic intracytoplasmic inclusions can be seen in desquamated bronchial cells like the inclusions seen in ciliocytophthoria. These inclusions are aggregates of intermediate filaments and represent an underlying degenerative process.

Pulmonary Mycoses (Table 11.5)

Cryptococcus neoformans *(Hominis)*

Commonly seen in immunocompromised patients with leukemia, AIDS, etc. with meningitis being the typical presentation. However, lung is believed to be the site of entry for the fungus, hence early detection helps in preventing an accelerated disseminated form.

Table 11.5 Morphologic characteristics of pulmonary fungi

Fungal organism	Cytomorphologic features	Differential diagnosis
Cryptococcus neoformans	Transparent capsule, narrow based budding	Bronchogenic carcinoma
Blastomyces dermatitidis	Broad based budding, granuloma	Tuberculosis
Coccidioides immitis	Large thick wall spherules with multiple endospores	
Paracoccidioides brasiliensis	Large spore surrounded by buds (Ship's wheel)	
Histoplasma capsulatum	Tiny dot like structures with halo in macrophage	Tuberculosis
Sporothrix schenckii	Multiple, eosinophilic intracytoplasmic yeast in macrophages, non-staining cell wall	Candida albicans Histoplasma capsulatum
Aspergillus	Septate hyphae branching at acute angle. Oxalate crystals sign of *Niger* species	Mucor Tuberculosis
Mucor	Aseptate broad hyphae branching at obtuse angle	Aspergillus
Candida	Pseudohyphae, narrow based budding yeast	Trichophyton
Pneumocystis jirovecii	Uniform spherical or cup-shaped cysts with internal dots	Candida yeast

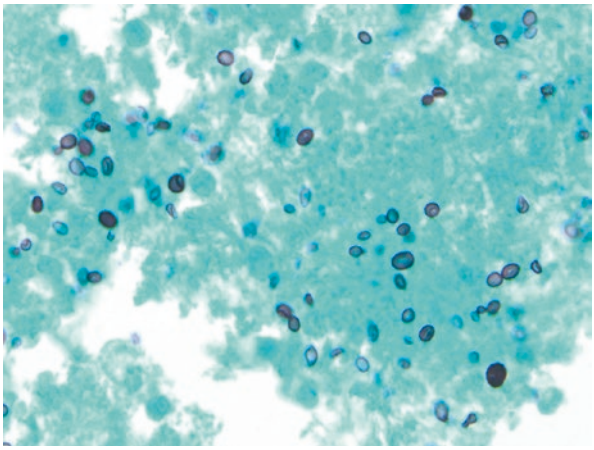

Fig. 11.15 Cryptococcus—pleomorphic organisms with uneven narrow-based budding (Grocott's methenamine silver stain)

The cytology shows spherical, pleomorphic, yeast-form organisms, measuring 6 to 20 mm, and have characteristic uneven narrow-based budding (Fig. 11.15). The organisms have thick sharply demarcated capsule and stain positive with mucicarmine, periodic acid-Schiff (PAS), and Gomori methenamine silver stains [21].

Blastomyces Dermatitidis

Pulmonary blastomycosis manifests as granulomatous lesions which can mimic malignancy. In cytology, the yeast forms of the organisms are spherical, relatively uniform, with broad based budding [22]. The organisms are about 20 mm in size, equal or larger to cryptococcus. *Blastomyces* has a refractile, thick wall but no mucoid capsule, stained by methenamine silver.

Coccidioidomycosis Immitis

The pulmonary form of the disease may show cavitary forms, which can be misdiagnosed as tuberculosis. The cytology shows large spherules with endospores [23], the spherules measure up to 100 mm and the endospores about 20 mm. The spherules can have a crushed appearance (Fig. 11.16).

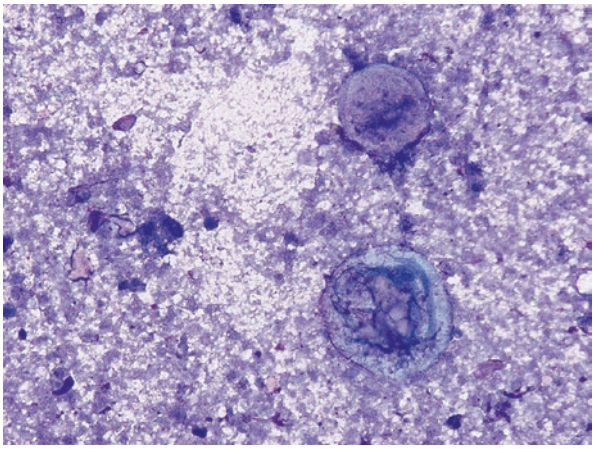

Fig. 11.16 Coccidioidomycosis—large, crushed spherules (Diff-Quik stain)

Paracoccidioides Brasiliensis

Paracoccidioidomycosis is characterized by large spores surrounded by multiple peripheral buds (ship's wheel), stained by methenamine silver [24]. The cytology specimens are usually hemorrhagic or purulent with epithelioid and multinucleated giant cells.

Histoplasma Capsulatum

The pulmonary form can mimic tuberculosis and cause sclerosing mediastinitis. The fungal organism fills the cytoplasm of macrophages with tiny dot-like structures with clear halos. The organisms are small with narrow-based budding and measure 2 to 4 mm. They are best seen by silver staining.

Sporothrix Schenckii

Sporotrichosis, caused by thermally dimorphic fungi *Sporothrix schenckii* complex, has become an emerging infection in recent years although pulmonary infection with *S. schenckii* still remains relatively uncommon [25]. The organisms are multiple, small, ovoid, eosinophilic intracytoplasmic yeasts seen within macrophages. Due to the non-staining of the organism wall, a thin halo is seen around. Elongated, budding cigar-shaped forms are also seen which helps to differentiate from histoplasma. This intracellular fungal species should not be confused with extracellular candida.

Aspergillus Species

Aspergillus can present as diffuse pulmonary infection or solitary aspergilloma and mainly infects the immunosuppressed patients [26]. The hyphae branch at 45 (acute angle), helping to differentiate them from *Mucor* species. Rarely calcium oxalate crystals can be seen, suggestive of *Aspergillus niger* species. The cavity wall of aspergilloma can undergo atypical squamous metaplasia, reactive basal epithelial cell hyperplasia, and marked inflammation.

Mucor Species

The family of fungus has strong predisposition towards angioinvasion and causing vascular thrombosis and infarcts. The morphology is characteristic, showing broad, ribbon-like and wavy, aseptate hyphae branching at obtuse angle [27].

Candida Species

Candida presents as pseudohyphae, rarely true hyphae, with narrow neck "teardrop"-shaped budding yeast are documented (Fig. 11.17). It is mostly seen in debilitated patients where it can even become invasive and disseminate. Trichophyton, a common contaminant of oral cavity should be differentiated from candida.

Pneumocystis Jirovecii

Pneumocystis jirovecii pneumonia (PJP) occurs primarily in immunocompromised patients, presenting as diffuse lung lesion on imaging studies. Rapid accurate diagnosis, often achieved by examination of BAL specimens, is crucial for patient

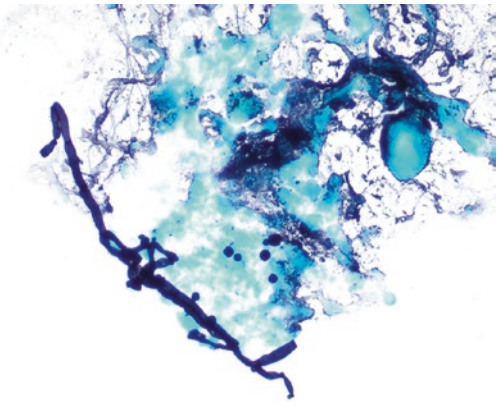

Fig. 11.17 Candida—mixed pseudohyphae and yeast (Grocott's methenamine silver stain)

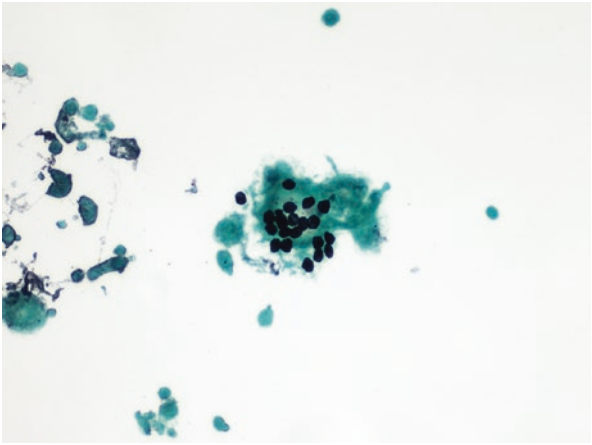

Fig. 11.18 Alveolar cast with pneumocystis jirovecii—uniform spherical organisms (Grocott's methenamine silver stain)

management. Typical findings of BAL specimens include foamy alveolar casts full of microorganisms. The organisms are best revealed by GMS stain, showing relatively uniform 4–6 μm, spherical or cup-shaped cyst structures with 1–2 internal dots (Fig. 11.18).

Other Lung disorders

Lipid Pneumonia

Exogenous lipid pneumonia results from aspiration of oily substance into the lungs. The radiologic appearance can be alarming as localized infiltrate or mass can mimic an underlying carcinoma [28]. The oil gets phagocytized by macrophages which can trigger a granulomatous response. Cytology can yield characteristic features of large macrophages with large cytoplasmic vacuoles or abundant bubbly vacuolated cytoplasm (Fig. 11.19). It is worth differentiating these lipid-rich macrophages from mucus producing cells, the latter tend to have atypia and commonly a single cytoplasmic vacuole.

Endogenous lipid pneumonia represents an underlying destructive process with release of tissue lipid that get phagocytized by macrophages. This is suggestive of involvement of lung by carcinoma, organizing pneumonia, necrotizing granulomatous inflammation, or other chronic inflammation. On cytology, small, finely vacuolated macrophages are seen as opposed to large cytoplasmic vacuoles seen in exogenous pneumonia [29]. Gaucher's disease and side effects of amiodarone should also be considered, while diagnosing lipid pneumonia.

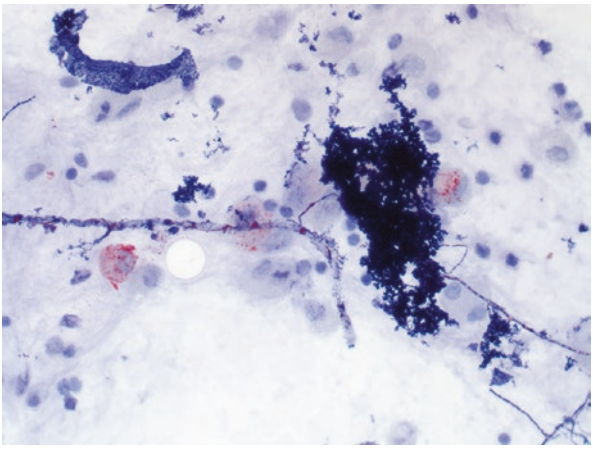

Fig. 11.19 A few macrophages with intracytoplasmic lipid droplets (Oil-Red-O stain)

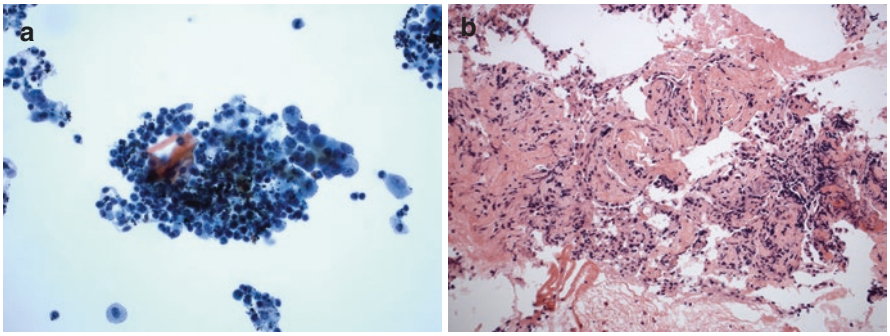

Fig. 11.20 Mixed neutrophils, lymphocytes, and macrophages in BAL specimen ((**a**). Papanicolaou stain) with current lung biopsy showing inflamed fibrotic lung parenchyma; ((**b**) Hematoxylin-eosin stain)

Interstitial Pneumonia

It is an umbrella term for disorders of unknown etiology, which have in common inflammation, progressive fibrosis, and synchronous dilatation of bronchioles forming pseudo-glandular spaces. On cytology marked bronchiolar atypia and pneumocyte hyperplasia can be seen, mimicking adenocarcinoma. The aspirate commonly shows papillary clusters of bronchiolar and alveolar cells with prominent nucleoli [30]. BAL of patients with interstitial pneumonia show an increase in macrophages, lymphocytes, and other inflammatory cells (Fig. 11.20).

Sarcoidosis

This granulomatous disease differs from tuberculosis that does not show caseous necrosis. A characteristic, though not specific feature is laminated crystalline inclusions (Schaumann's bodies) in multinucleated giant cells. Intracytoplasmic asteroid bodies may also be present. A constellation of findings should increase the suspicion of sarcoidosis: tight clusters of epithelioid histiocytes with lymphocytes (Fig. 11.21), alveolar macrophages, and multinucleated giant cells in a relatively clean background. The lymphocytosis in BAL specimen shows an increased ratio of CD4/CD8 with predominance of activated T-cells. In cases with concern for sarcoidosis, a transbronchial FNA of mediastinal lymph node probably has a higher diagnostic yield than percutaneous aspiration; however, final diagnosis requires clinical correlation [31].

Wegener's Granulomatosis

Vasculitis of small and medium size vessels and necrotizing granulomatous inflammation involving upper respiratory tract and lung, where it can mimic cavitating tuberculosis. The diagnosis can be suggested, not confirmed by FNA of lung yielding amorphous or filamentous necrotic tissue, and an inflammatory cellular infiltrate [30, 32]. It is however difficult to diagnose this disease on cytology specimens. The diagnosis is often rendered by a supplementary biopsy and positive anti-neutrophilic cytoplasmic antigen test (cANCA) and ELISA for proteinase 3.

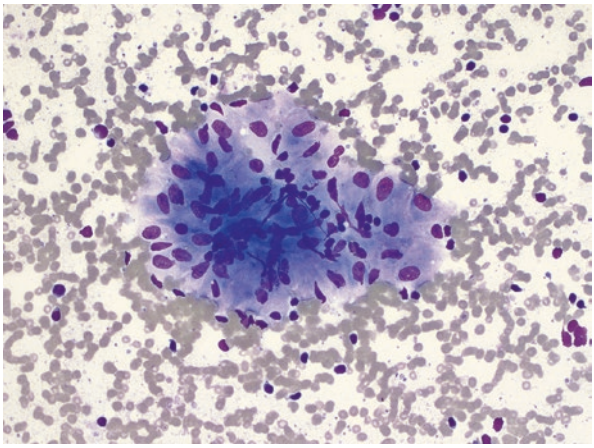

Fig. 11.21 A tight cluster of epithelioid histiocytes and scattered lymphocytes (Diff-Quik stain)

Langerhans Cell Histiocytosis

Pulmonary Langerhans cell histiocytosis is a proliferative, usually clonal, disorder of Langerhans cells, with associated interstitial changes in the lung tissue [33]. The diagnosis may be suspected when a BAL specimen shows more than 4–5% of CD1a positive large mononuclear cells, often with increase eosinophils and Charcot-Leyden crystals [34, 35]. Occasionally, transbronchial or percutaneous aspirate can show CD1a and S-100 positive Langerhans' cells with round to oval, cleaved, or convoluted nuclear contour, fine chromatin, and long cytoplasmic processes.

Rheumatoid Granuloma

Classic manifestation of pulmonary involvement by rheumatoid arthritis is seen in pleural effusion cytology, however, limited cytologic findings can be seen in sputum and bronchoalveolar specimens. Necrobiotic nodules, presented as mass-like lesions, may be subjected to FNA, showing multinucleated giant cells in a necrotic background [36]. The background necrosis on cytology specimen is generally considered to be the representation of the necrotic center of the granuloma.

Alveolar Proteinosis

Acquired autoimmune disorder caused by antibodies targeting surface receptor for granulocyte-macrophage colony stimulating factors, which is expressed on alveolar macrophages [37]. The phospholipid-rich proteinaceous material filling the alveoli is due to the defective clearance by macrophages. BAL specimens demonstrate globules of amorphous or fibrillar PAS-positive casts which is suggestive of the disease in the correct clinical settings [35, 38].

Therapy-Related Changes

Systemic or local therapies such as chemotherapy and radiation can cause significant cellular changes in squamous epithelium, respiratory epithelium, and pneumocytes of the respiratory system. The cellular changes are characterized by cytomegaly, cytoplasmic vacuolization, nuclear enlargement, prominent nucleoli, and occasionally multinucleation (Fig. 11.22). These changes, sometimes quite atypical, may overlap with cytomorphologic features of malignant tumors, raising a concern. It

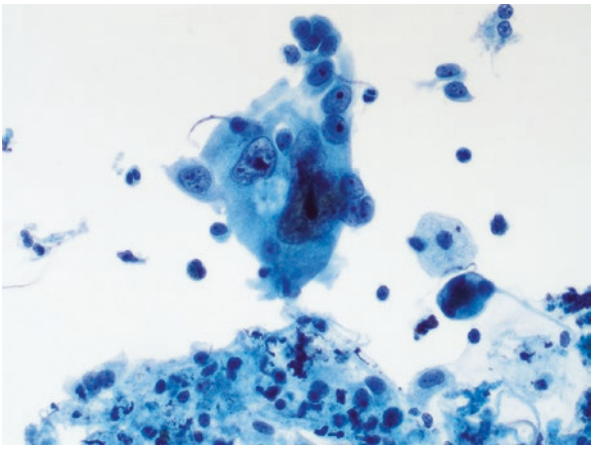

Fig. 11.22 Reactive bronchial cells with enlarged nuclei, multinucleation, and prominent nucleoli (Papanicolaou stain)

should be extremely cautious to interpret cytological atypia in the specimens from patients who receive therapies. The presence of cytoplasmic vacuoles with low nuclear-to-cytoplasmic ratios favors therapy-related atypia over an underlying malignancy.

Acknowledgments None.

Conflict of Interest None.

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