Lung and Mediastinum

6

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Summary of Pearls and Pitfalls

- The concept of personalized medicine and the remarkable advances in lung cancer genetics and therapy in the past decade have changed lung pathology practice dramatically.
- The 2015 World Health Organization (WHO) classification of tumors of the lung specifies that immunohistochemistry is required for lung cancer diagnosis, not only for small biopsies and fine needle aspiration (FNA) specimens but also for certain resected specimens such as solid adenocarcinoma (ADC), nonkeratinizing squamous cell carcinoma, large cell carcinoma, neuroendocrine tumors, and sarcomatoid carcinomas.
- New criteria and terminology for the diagnosis of lung cancer based on small biopsies and cytology are proposed in the 2015 WHO classification and are summarized in Tables 6.1 and 6.2.
- The role of cytopathologists has expanded to not only making a specific diagnosis, including histopathological subtyping of tumors, but also to thoughtfully utilizing the limited material for necessary genetic studies to help personalize treatment strategies for advanced lung cancer patients.
- Thyroid transcription factor 1 (TTF1) and napsin A are accepted markers for ADC differentiation; p40 is reported to be the most specific and sensitive marker for squamous cell differentiation. A reasonable recommendation is that, when immunohistochemistry is deemed necessary, at least one antibody each for squamous and glandular differentiation, but no more than two antibodies, should be used for an initial workup (e.g., TTF1 and p40 or p63). Thus a limited panel of TTF1 and p40 (or p63) is suggested for subtyping the tumor to preserve tissue for molecular testing.
- Molecular testing for epithelial growth factor receptor (*EGFR*) mutation and anaplastic lymphoma kinase (*ALK*) rearrangement is recommended in tumors classified as ADC and in cases where an ADC component cannot be excluded.
- When evaluating a computed tomography (CT)-guided FNA of a lung lesion in the periphery, mesothelial cells are frequently seen on smears; reactive mesothelial cells may mimic carcinoma, and caution should be taken.
- Diagnosis of ADC with a lepidic growth pattern can be challenging in FNA specimens. Both quality (degree of atypia) and quantity (groups of atypical cells) need to be considered. In a typical case of ADC with a lepidic growth pattern, the smears tend to be very cellular, containing many groups of atypical epithelial cells. In contrast, in a reactive condition, such as pulmonary infarction, atypical reactive pneumocytes are usually less numerous.
- In mucinous ADC with a lepidic growth pattern, the neoplastic epithelial cells may mimic pulmonary macrophages. Features such as a mucinous background, cytoplasmic mucin/vacuoles, eccentrically located nuclei, and more abundant cytoplasm are helpful in reaching a correct diagnosis.
- Basaloid squamous cell carcinoma may mimic small cell carcinoma; therefore, cellblock preparation in conjunction with immunostains will be helpful.
- Infection such as aspergillosis may result in squamous cells with significant cytological atypia; therefore, infectious etiologies should be excluded before a diagnosis of well-differentiated squamous cell carcinoma is rendered.

F. Lin et al., *Handbook of Practical Fine Needle Aspiration and Small Tissue Biopsies*, DOI 10.1007/978-3-319-57386-1_6

- A lesion from the mediastinum should be considered if cytological features do not fit the description of a typical "lung lesion."
- Thymoma is rare in young patients and children. In lymphocyte-dominant thymoma, the main component of the aspirate may contain cortical thymocytes with expression of terminal deoxynucleotidyl transferase (TdT) and cluster of differentiation (CD)99; therefore, caution should be taken to avoid misdiagnosing it as a lymphoblastic lymphoma.
- Thymic neuroendocrine neoplasm may mimic an epithelial-dominant thymoma; therefore, immunohistochemistry should be performed in cases with equivocal features.

Lung

Tables 6.1 and 6.2 summarize new criteria and terminology for the diagnosis of lung cancer based on small biopsies and cytology from the 2015 WHO classification.

Normal Cytology

Upper Respiratory Tract

- Ciliated columnar cells, see Fig. 6.1.
- Squamous cells, see Fig. 6.2.

Lower Respiratory Tract

- Trachea and bronchi
  - Ciliated columnar cells
  - Goblet cells, see Fig. 6.3.
  - Basal/reserve cells, see Fig. 6.4.
- Terminal bronchioles
  - Non-ciliated cuboidal/columnar cells (Clara cells)
  - Alveoli
  - Type I and II pneumocytes
  - Alveolar macrophages, see Fig. 6.5.

Respiratory Infections

Viral Infections

Specific viral infections, such as herpes simplex (Fig. 6.6) and cytomegalovirus (Fig. 6.7), can cause significant cyto-

Table 6.1 Terminology and criteria for adenocarcinoma, squamous cell carcinoma, and non-small cell carcinoma, not otherwise specified, in small biopsies and cytology specimens compared with those for resection specimens

Small biopsy/cytology terminology	Morphology/immunohistochemistry	2015 WHO classification of resection specimens
ADC (describe patterns)	Morphologic ADC patterns clearly present	ADC predominant pattern: lepidic, acinar, papillary, solid, and micropapillary
ADC with lepidic pattern (if pure, add note: an invasive component cannot be excluded)		Minimally invasive ADC, ADC in situ, or an invasive ADC with a lepidic component
Invasive mucinous ADC (describe patterns; use term mucinous ADC with lepidic pattern if pure)		Invasive mucinous ADC
ADC with colloid features		Colloid ADC
ADC with fetal features		Fetal ADC
ADC with enteric features		Enteric ADC
NSCC, favor ADC	Morphologic ADC patterns not present but supported by special stains (i.e., TTF1+)	ADC (solid pattern may be just one component of the tumor)
SqCC	Morphologic squamous cell pattern clearly present	SqCC
NSCC, favor SqCC	Morphologic squamous cell patterns not present but supported by stain (i.e., p40+)	SqCC (solid pattern may be just one component of the tumor)
NSCC NOS	No clear ADC, squamous or neuroendocrine morphology or staining pattern	Large cell carcinoma

Adapted with permission of Elsevier from Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JH, Beasley MB, et al.; WHO Panel. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol. 2015;10(9):1243–60

ADC adenocarcinoma, *TTF1* thyroid transcription factor 1, *SqCC* squamous cell carcinoma, *NSCC* non-small cell carcinoma, *NOS* not otherwise specified, *WHO* World Health Organization

Table 6.2 Diagnostic terminology for small biopsy/cytology compared with that of the 2015 World Health Organization (WHO) in resection specimens with small cell carcinoma, LCNEC, adenosquamous carcinoma, and sarcomatoid carcinoma

Small biopsy/cytology terminology/criteria	2015 WHO classification of resections
Small cell carcinoma	Small cell carcinoma
NSCC with NE morphology and positive NE markers, possible LCNEC	LCNEC
NSCC with NE morphology. If negative NE markers comment: This is an NSCC where LCNEC is suspected, but stains fails to demonstrate NE differentiation	LCNEM
Morphologic squamous cell and ADC patterns present: NSCC, NOS. Comment that ADC and squamous components are present, and this could represent adenosquamous carcinoma	Adenosquamous carcinoma (if both components ≥10%)
No morphologic squamous cell or ADC patterns, but immunostains favor separate squamous and ADC components: NSCC, NOS. Specify the IHC results and the interpretation. Comment that this could represent adenosquamous carcinoma	ADC, SqCC, adenosquamous carcinoma, or large cell carcinoma with unclear IHC features
NSCC with spindle cell and/or giant cell carcinoma (mention if ADC or SqCC is present)	Pleomorphic, spindle cell, and/or giant cell carcinoma

Adapted with permission of Elsevier from Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JH, Beasley MB, et al.; WHO Panel. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol. 2015;10(9):1243–60

WHO World Health Organization, *LCNEC* large cell neuroendocrine carcinoma, *NSCC* non-small cell carcinoma, *NE* neuroendocrine, *LCNEM* large cell carcinoma with neuroendocrine morphology, *ADC* adenocarcinoma, *NOS* not otherwise specified, *SqCC* squamous cell carcinoma, *IHC* immunohistochemistry

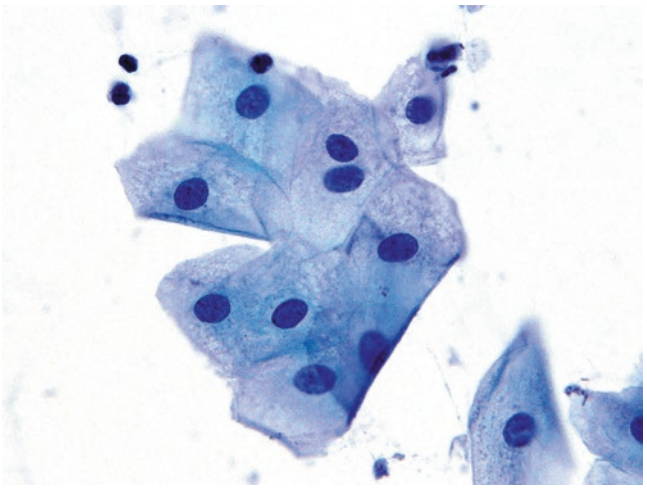

Fig. 6.2 Normal squamous cells, Pap stain

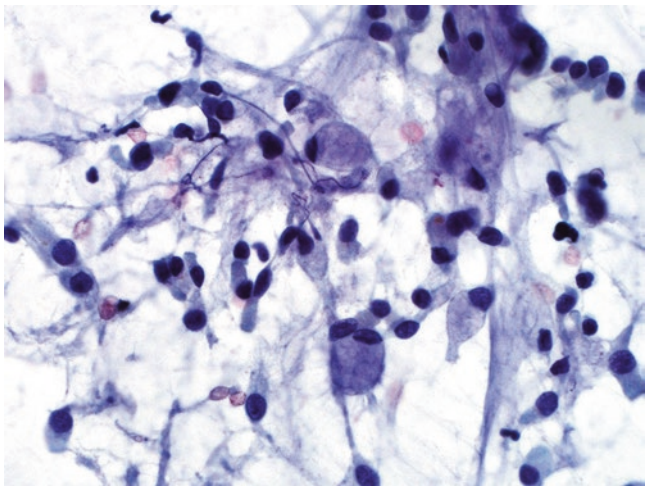

Fig. 6.3 Benign goblet cells, Pap stain

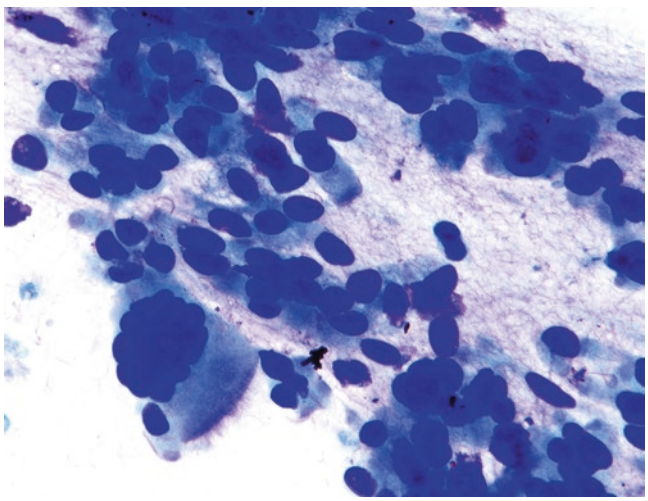

Fig. 6.1 Normal ciliated respiratory columnar cells, Diff-Quik stain

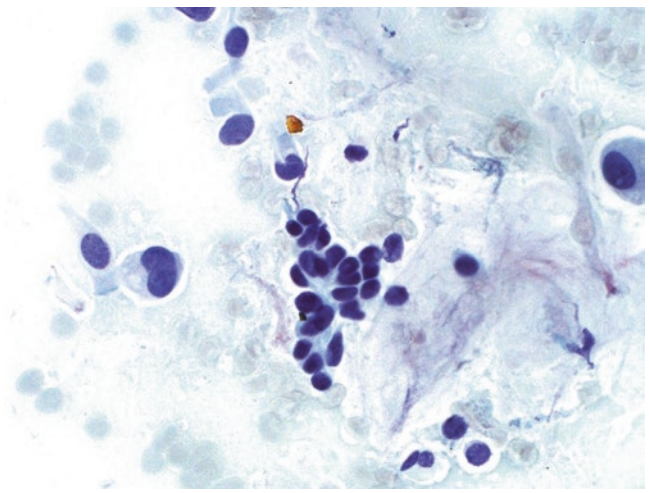

Fig. 6.4 Normal reserve cells, Pap stain

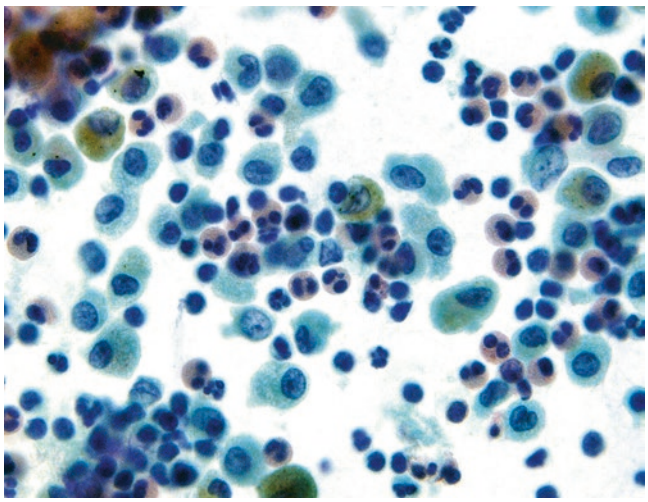

Fig. 6.5 Pulmonary macrophages, Pap stain

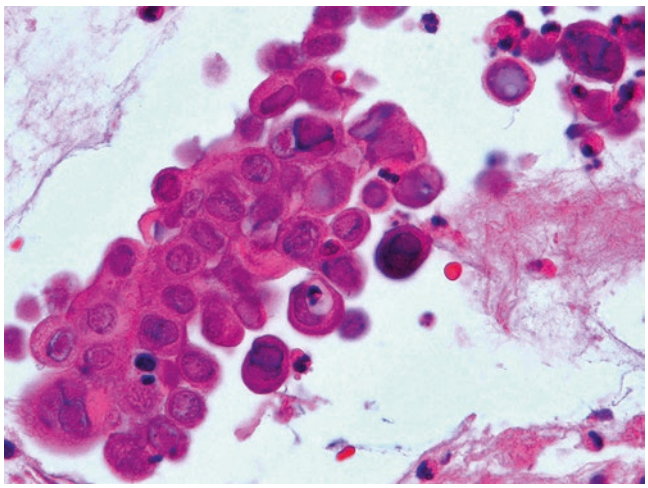

Fig. 6.6 Herpes simplex viral inclusions, H&E

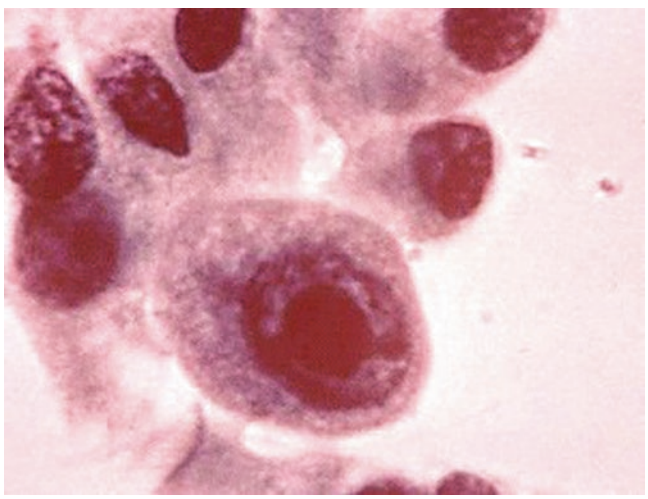

Fig. 6.7 Cytomegaloviral inclusion, Pap stain

logical changes that may mimic a malignant process. The diagnosis can be confirmed by immunoperoxidase studies.

Severe acute respiratory syndrome (SARS) caused by a novel coronavirus (SARS-CoV) became a worldwide outbreak in 2003, affecting more than 8000 patients, with a fatality rate of 9.2%. SARS-CoV belongs to a family of large, positive, single-stranded ribonucleic acid (RNA) viruses. The key pathologic finding is diffuse alveolar damage (DAD). Depending on different phases in the disease progression, the composition of inflammatory cells may vary; however, macrophages (including multinucleated forms) and lymphocytes usually predominate. Other pathologic findings, such as fibrosis, prominent vascular injury, hemophagocytosis, squamous metaplasia, apoptosis, and atypical pneumocytes, including multinucleated giant pneumocytes with irregularly distributed nuclei or pneumocytes with large atypical nuclei, prominent eosinophilic nucleoli, and granular amphophilic cytoplasm, were reported. Ancillary tests, such as in situ hybridization, immunohistochemistry, viral isolation, or reverse transcription polymerase chain reaction (RT-PCR), are necessary to confirm the diagnosis. Representative images are shown in Fig. 6.8a, b.

Fungal Infections

FNA is a useful means of diagnosing pulmonary fungal infection, which should be suspected whenever there is granulomatous inflammation. Silver or periodic acid-Schiff (PAS) stains are used on cellblock sections.

The common fungal infections include (1) *Candida* species; (2) *Aspergillus* species, Fig. 6.9; (3) phycomycosis (such as mucormycosis); (4) *Cryptococcus neoformans*, Fig. 6.10a–d; (5) *Histoplasma capsulatum*, Fig. 6.11a, b; (6) *Blastomyces dermatitidis*, Fig. 6.12a, b; (7) *Coccidioides immitis*, Fig. 6.13a, b; and (8) *Pneumocystis carinii*, Fig. 6.14a–c. The morphological features of these fungi are summarized in Table 6.3.

Strongyloidiasis

Pulmonary strongyloidiasis affects immunocompetent and, more commonly, immunosuppressed persons presenting with pneumonitis with hemoptysis. The etiological agent is the nematode. *Strongyloides stercoralis* in sputum is shown in Fig. 6.15.

Bacterial Infection

Infection by *Mycobacterium tuberculosis* is often a granulomatous inflammation containing clusters of epithelioid histiocytes, lymphocytes, and Langhans giant cells, with or without necrosis. In immunocompromised patients there may be abundant acid-fast organisms without obvious granulomatous inflammation.

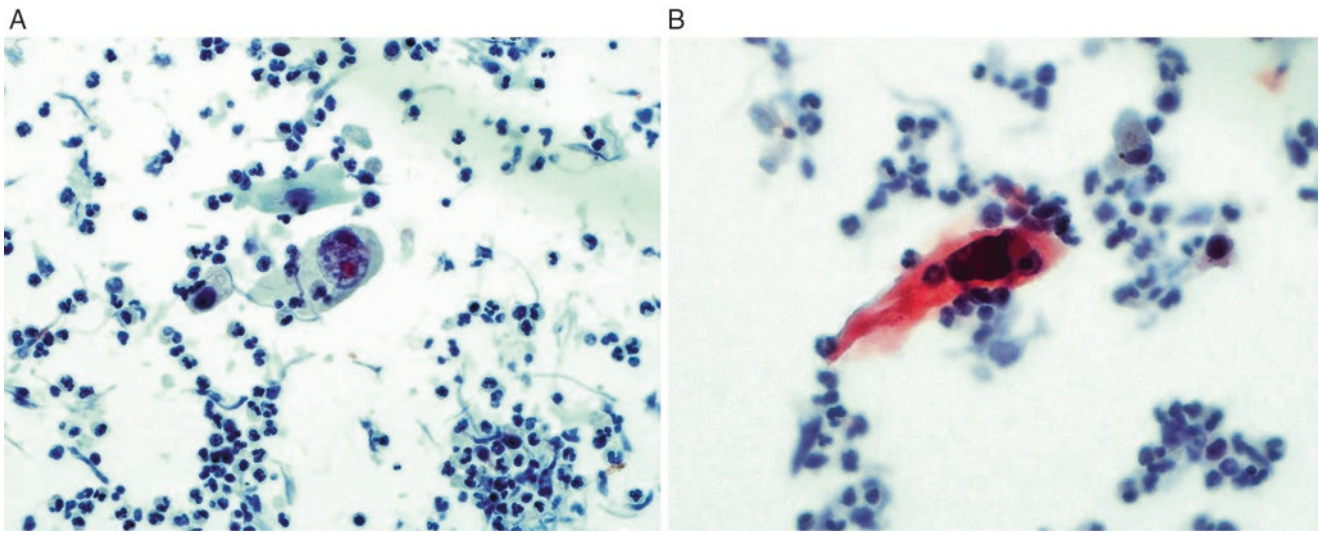

Fig. 6.8 Alveolar lavage from a patient with severe acute respiratory syndrome, with confirmed coronavirus infection. (**a**) Atypical pneumocytes with large nuclei, prominent eosinophilic nucleoli, and granular amphophilic cytoplasm in a background of marked acute inflammation, Pap stain; (**b**) typical metaplastic squamous cells with enlarged nuclei, hyperchromatic, smudged chromatin, Pap stain

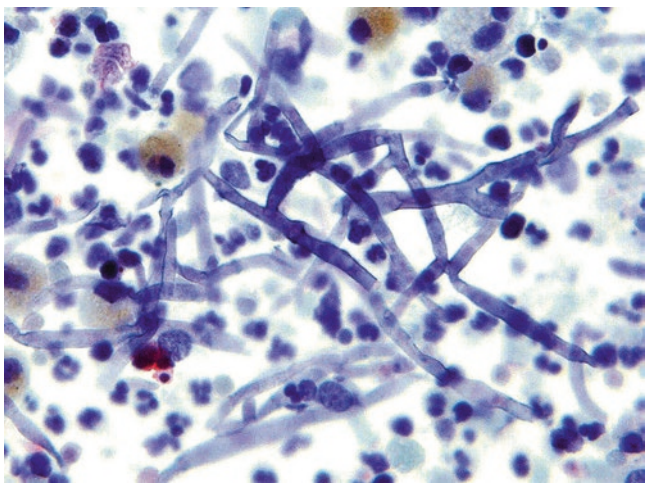

Fig. 6.9 *Aspergillus* species, alveolar lavage, Pap stain. The fungal hyphae exhibit septation, with dichotomous 45 degree angle branching. There are pulmonary macrophages and neutrophil-predominant mixed inflammatory cells in the background

Acquired immunodeficiency syndrome (AIDS) patients are especially susceptible to *Mycobacterium avium-intracellulare*, an acid-fast organism producing negative images on Romanowsky stain. Special stains on cellblock section are particularly helpful.

Nocardia, a weakly acid-fast filamentous organism, often infects immunocompromised patients, producing cavitary nodules on radiographs, which may mimic a neoplastic process.

Other Types of Granulomatous Inflammation

Sarcoidosis

Key Clinical Features

- Sarcoidosis is characterized by non-caseating granulomas in many organs, most commonly the lung.

Key Radiological Features

- Chest x-ray: bilateral hilar adenopathy is a classic finding; variable lung parenchyma changes, from normal, diffuse reticular, or ground glass opacities, nodular consolidation or cystic scarring.
- CT and positron emission tomography (PET) scan can also be used.

Cytological Features

- Aggregates of epithelioid histiocytes, with or without Schaumann and asteroid bodies
- Multinucleated giant cells and lymphocytes

Differential Diagnosis

An essential part of the diagnosis of sarcoidosis is the exclusion of alternative possibilities:

- Granulomas caused by infectious agents, such as mycobacterial infection and fungal infection
- Drug-induced, hypersensitivity pneumonitis, or foreign body granulomatosis

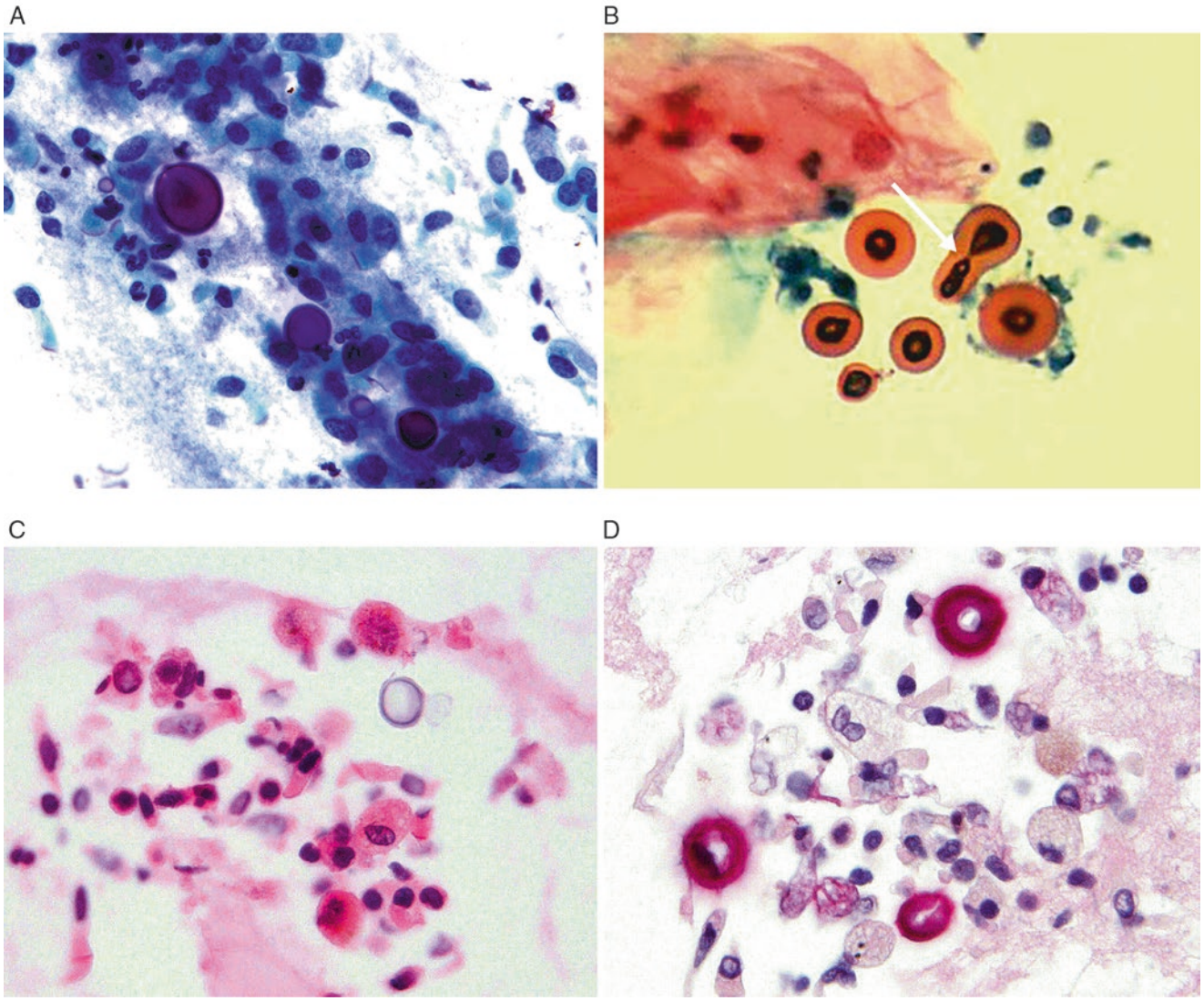

Fig. 6.10 *Cryptococcus neoformans*, bronchial brushing and alveolar lavage. (**a**) Round/oval, variable size yeast forms in background of respiratory epithelial cells, bronchial brushing, Pap stain; (**b**) alveolar lavage, showing slightly variable size, round/oval yeast forms with thick capsule and narrow-necked budding, H&E; (**c**) alveolar lavage, cellblock, H&E, showing round to oval yeast form; (**d**) alveolar lavage, the thick mucinous capsule showing bright red stain with mucicarmine, cellblock

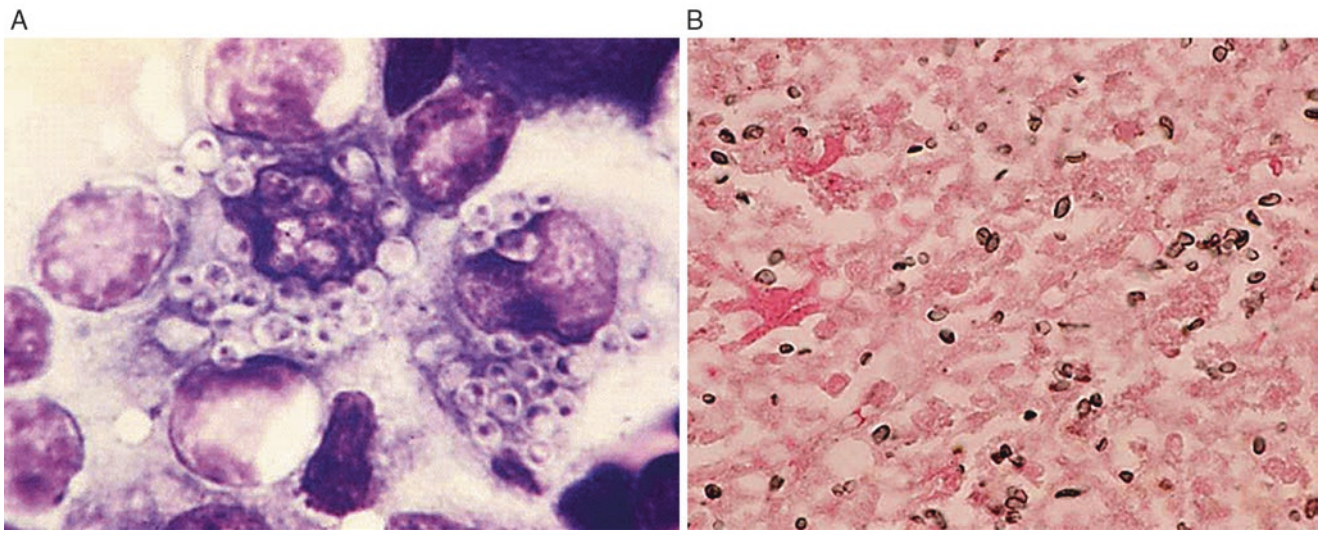

Fig. 6.11 *Histoplasma capsulatum*, lung lesion, fine needle aspiration. (**a**) Small, budding, intracellular yeast forms, 2–5 microns, Diff-Quick; (**b**) cellblock, Grocott's methenamine silver (GMS) stain, showing small yeast forms

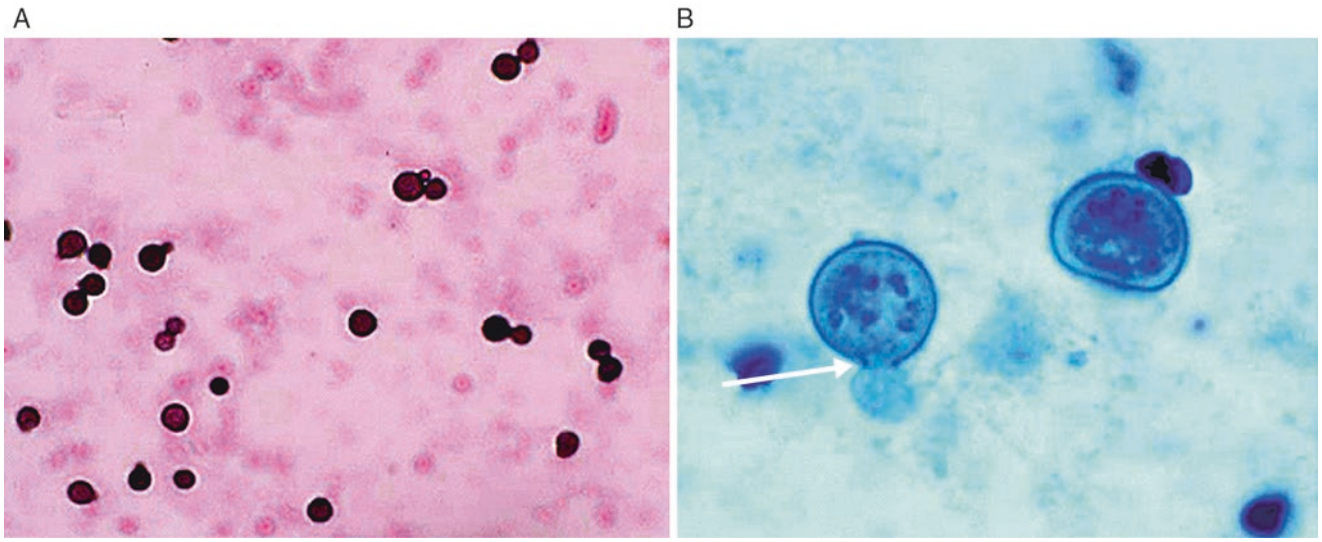

Fig. 6.12 *Blastomyces dermatitidis*, lung lesion, sputum. (**a**) Budding yeast form, broad base, cellblock, H&E; (**b**) sputum, broad-based budding yeast form, Pap stain

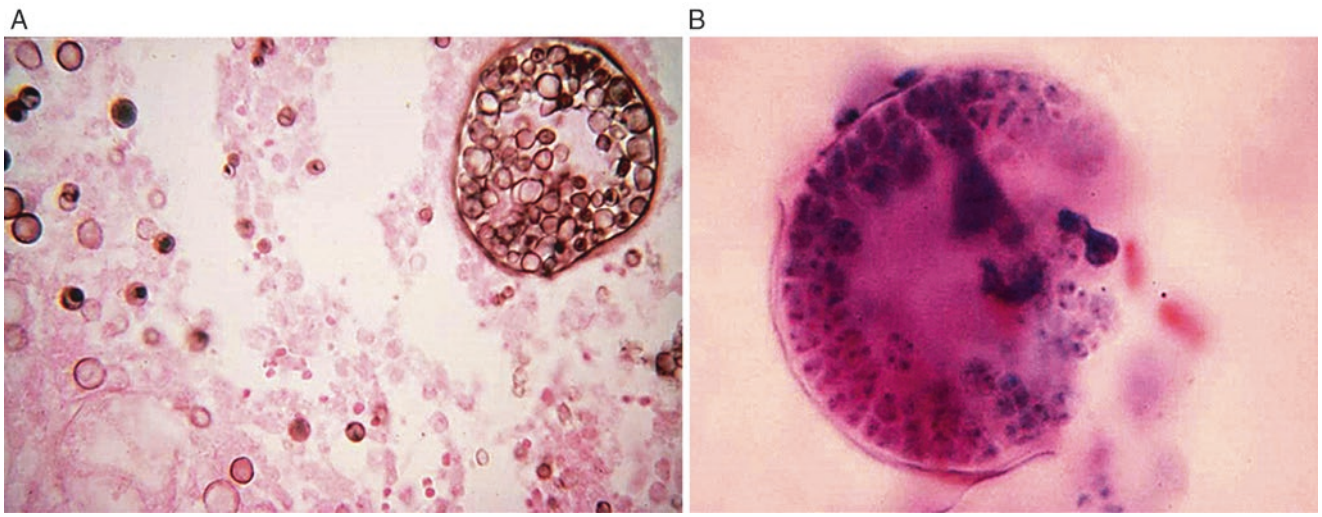

Fig. 6.13 *Coccidioides immitis*. (**a**) Cellblock, spherule form containing endospores, H&E; (**b**) spherule form containing endospores, Pap stain

- Pulmonary histiocytic disorders
- Granulomatous disorders associated with vascular inflammation such as Wegener granulomatosis and Churg-Strauss syndrome

Wegener Granulomatosis

Key Clinical Features

- Wegener granulomatosis is characterized by necrotizing vasculitis and may present as a lung mass with or without involvement of nasal passages and kidneys.

Key Radiological Features

- Multiple pulmonary nodules on imaging

Cytological Features

- Neutrophils, giant cells, necrotic collagen, and epithelioid histiocytes
- The findings are nonspecific; therefore, serologic studies are necessary.

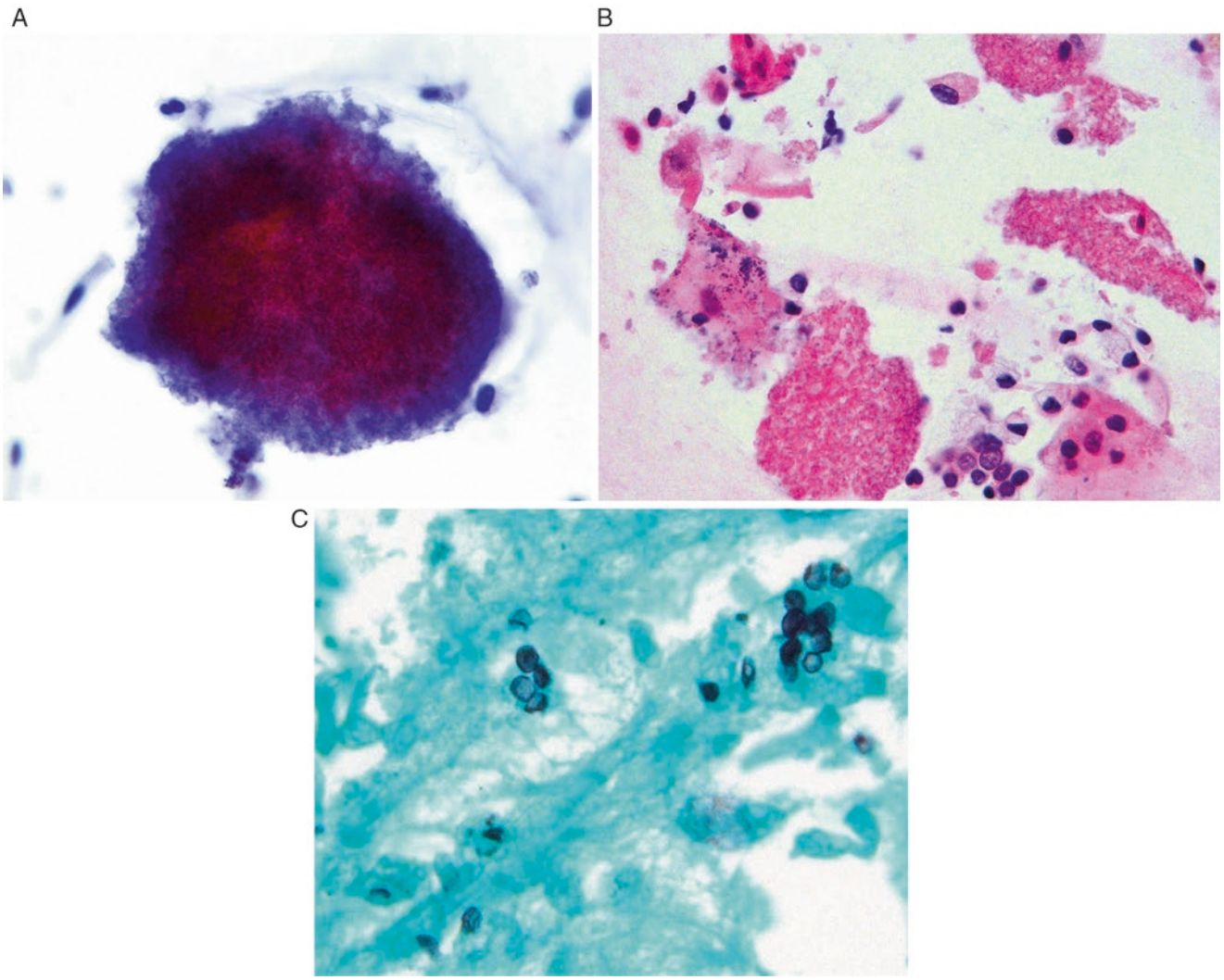

Fig. 6.14 *Pneumocystis carinii*, alveolar lavage. (**a**) Foamy proteinaceous spheres, Pap stain; (**b**) foamy proteinaceous spheres, cellblock, H&E; (**c**) GMS stain reveals small oval or cup-shaped yeast forms, cellblock

Table 6.3 Morphological features of fungi commonly seen in pulmonary cytological samples

Fungi	Forms	Hyphal branches	Average diameter (um)	Budding pattern
Candida spp.	Budding yeast Pseudohyphae	Not applicable	4	Constricted neck
Aspergillus spp.	Hyphae with 45-degree angle	45 degree	5–10	Not applicable
Mucormycosis	Hyphae with acute angle	90 degree	10–15	Not applicable
Histoplasma capsulatum	Budding yeast	Not applicable	2–4	Constricted neck
Cryptococcus neoformans	Yeast	Not applicable	5–8	Constricted neck
Blastomyces dermatitidis	Yeast	Not applicable	8–15	Broad-based
Coccidioides immitis	Endospores Spherules	Not applicable	Endospores 2–5 Spherules −100	Not applicable
[a]*Pneumocystis carinii*	Helmet-shaped cysts	Not applicable	6–8	Not applicable

[a]Genetic data suggest *Pneumocystis carinii* is more closely related to fungi
spp species (many)

Differential Diagnosis

- Granulomatous inflammations
- Metastatic diseases
- Non-Hodgkin lymphomas

Nodular Pulmonary Amyloidosis (Amyloid Tumor)

Key Clinical Features

- Amyloid deposits in the lung, forming discrete nodular masses

Key Radiological Features

- Discrete pulmonary nodules/masses (amyloidomas) on imaging

Cytological Features

- Irregular, waxy, amorphous, hypocellular material with a scalloped, occasionally cracked appearance, which is blue-green on Papanicolaou (Pap) stain and shows apple-green dichroism under polarized light after staining with Congo red

Differential Diagnosis

- Granulomatous inflammations
- Metastatic diseases
- Non-Hodgkin lymphomas

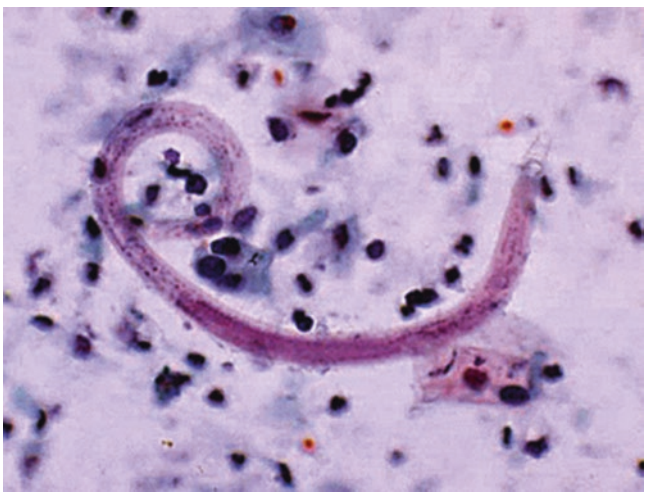

Fig. 6.15 Strongyloides stercoralis, sputum, Pap stain

Pulmonary Neoplasms

The 2015 *WHO Classification of Tumors of the Lung, Pleura, Thymus and Heart* has been published recently. There are numerous important changes in the classification of lung tumors, as summarized in Table 6.4.

Table 6.4 2015 World Health Organization (WHO) classification of lung tumors

Epithelial tumors	
Adenocarcinoma	Neuroendocrine tumors (continued)
Lepidic adenocarcinoma	Preinvasive lesion
Acinar adenocarcinoma	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
Papillary adenocarcinoma	Large cell carcinoma
Micropapillary adenocarcinoma	Adenosquamous carcinoma
Solid adenocarcinoma	Sarcomatoid carcinomas
Invasive mucinous adenocarcinoma	Pleomorphic carcinoma
Mixed invasive mucinous and nonmucinous adenocarcinoma	Spindle cell carcinoma
Colloid adenocarcinoma	Giant cell carcinoma
Fetal adenocarcinoma	Carcinosarcoma
Enteric adenocarcinoma	Pulmonary blastoma
Minimally invasive adenocarcinoma	Other and Unclassified carcinomas
Nonmucinous	Lymphoepithelioma-like carcinoma
Mucinous	Nuclear protein in testis (NUT) carcinoma
Preinvasive lesions	Salivary gland-like tumors
Atypical adenomatous hyperplasia	Mucoepidermoid carcinoma
Adenocarcinoma in situ	Adenoid cystic carcinoma
Nonmucinous	Epithelial-myoepithelial carcinoma
Mucinous	Pleomorphic adenoma
Squamous cell carcinoma	Papillomas
Keratinizing squamous cell carcinoma	Squamous cell papilloma
Nonkeratinizing squamous cell carcinoma	Exophitic
Basaloid squamous cell carcinoma	Inverted
Preinvasive lesion	Glandular papilloma
Squamous cell carcinoma in situ	Mixed squamous and glandular papilloma
Neuroendocrine tumors	Adenomas
Small cell carcinoma	Sclerosing pneumocytoma
Combined small cell carcinoma	Alveolar adenoma
Large cell neuroendocrine carcinoma	Papillary adenoma
Combined large cell neuroendocrine carcinoma	Mucinous cystadenoma
Carcinoid tumors	Mucous gland adenoma
Typical carcinoid tumor	
Atypical carcinoid tumor	

(continued)

Table 6.4 (continued)

Mesenchymal tumors	
Pulmonary hamartoma Chondroma PEComatous tumors Lymphangioleiomyomatosis PEComa, benign Clear cell tumor PEComa, malignant Congenital peribronchial myofibroblastic tumor Diffuse pulmonary lymphangiomatosis Inflammatory myofibroblastic tumor	Epithelioid hemangioendothelioma Pleuropulmonary blastoma Synovial sarcoma Pulmonary artery intimal sarcoma Pulmonary myxoid sarcoma with *EWSR1-CREB1* translocation Myoepithelial tumors Myoepithelioma Myoepithelial carcinoma
Lymphohistiocytic tumors	
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) Diffuse large cell lymphoma Lymphomatoid granulomatosis Intravascular large B-cell lymphoma Pulmonary Langerhans cell histiocytosis Erdheim-Chester disease	
Tumors of ectopic origin	
Germ cell tumors Teratoma, mature Teratoma, immature Intrapulmonary thymoma Melanoma Meningioma, not otherwise specified (NOS)	
Metastatic tumors	

Used with permission from Travis WD, Brambilla E, Burke AP, Marx A, Nicholson A. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon, France: IARC; 2015

Representative Benign Neoplasms of the Lung

Pulmonary Hamartoma

Key Clinical Features

- The most common benign pulmonary neoplasm
- Usually asymptomatic, incidental finding
- Common in males, especially elderly men

Key Radiological Features

- Chest x-ray: solitary or multiple discrete, round, golf ball-like lesions ("coin lesions"), usually peripherally located, about 10% endobronchial

Cytological Features

- A mixture of mesenchymal and epithelial elements
- Immature fibromyxoid matrix
- Mature cartilage with chondrocytes in lacunae
- Benign glandular cells
- Adipocytes
- Representative images are shown in Fig. 6.16a, b.

Differential Diagnosis

- Epithelial malignancy

Histology

- Round to multilobulated, well-circumscribed tumor nodules composed of mesenchymal tissues, including chondroid or chondromyxoid tissue, fat, connective tissue, smooth muscle, and bone in various proportions, intermixed with clefts of respiratory epithelial cells
- Chondromyxoid tissue usually predominates.
- Representative images are shown in Fig. 6.16c, d.

Immunohistochemistry

- Immunohistochemistry is usually not necessary for diagnosis.
- Pulmonary hamartomas have a high frequency of translocation t(3;12)(q27–28;q14–15) leading to a gene fusion of the high mobility group protein gene AT-hook 2 (*HMGA2)* and the lipoma preferred partner (*LPP)* gene.

Inflammatory Myofibroblastic Tumor

Key Clinical Features

- Most often young patients, under the age of 40
- Male = female
- The most common endobronchial mesenchymal lesion in childhood

Key Radiological Features

- Chest x-ray: a peripheral, discrete, solitary nodule. If endobronchial location, post-obstructive pneumonia and atelectasis may be evident.

Cytological Features

- Spindle cells
- Storiform pattern
- Polymorphous inflammatory cells
- Minimal to no necrosis

Differential Diagnosis

- Sarcoma
- Mesothelioma
- Solitary fibrous tumor

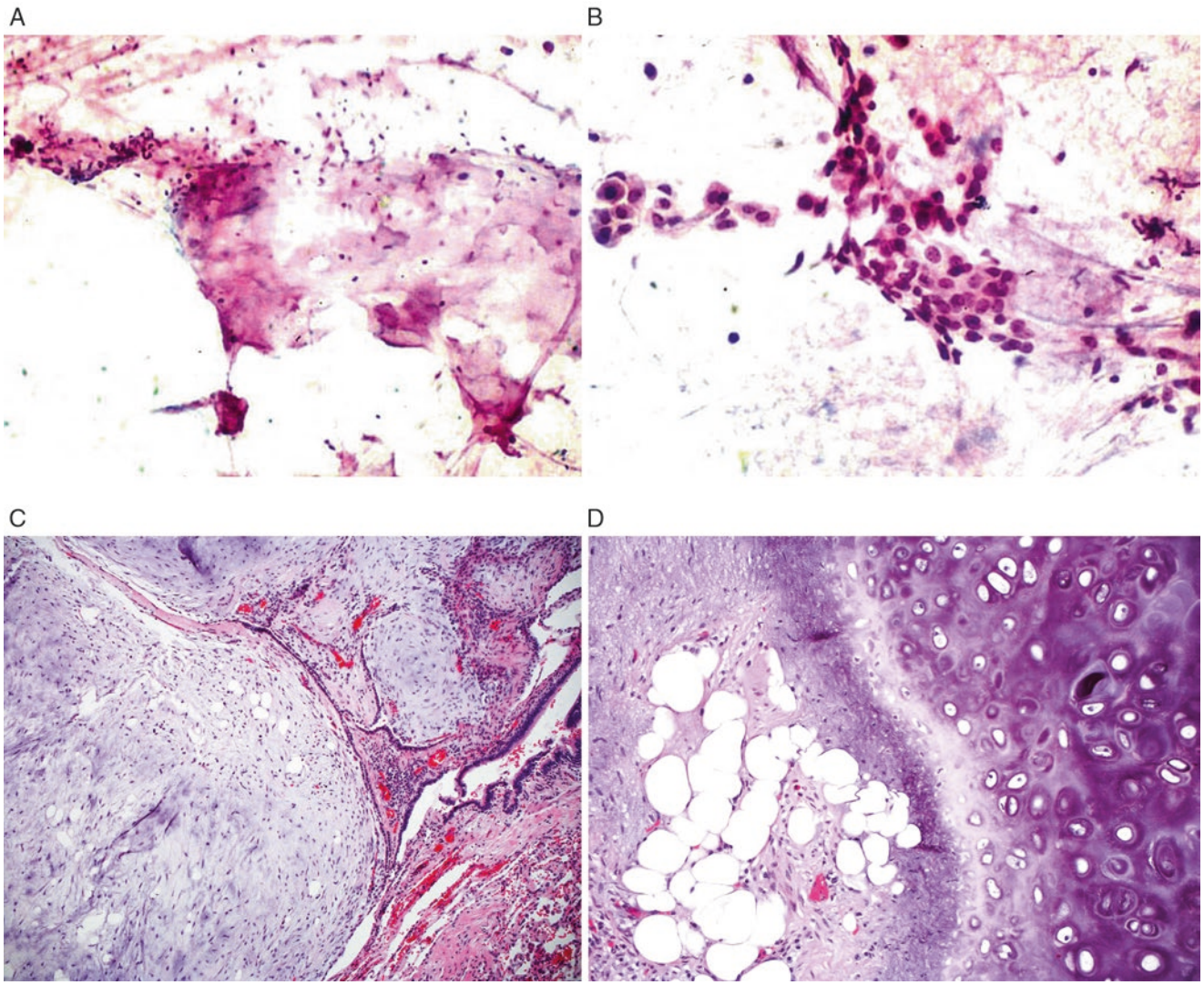

Fig. 6.16 Pulmonary hamartoma. (**a**) Sparsely cellular chondromyxoid matrix material containing scattered, benign, spindled, and stellate mesenchymal cells, Pap stain; (**b**) clusters of epithelial cells embedded in stromal, Pap stain; (**c**, **d**) histopathology, well-circumscribed, lobulated nodules of mesenchymal tissue including chondroid or chondromyxoid tissue, fat, and connective tissue intermixed with clefts of respiratory epithelial cells, H&E

Histology

- Composed of a mixture of spindle cells and obscuring inflammatory cell infiltrates, including lymphocytes, plasma cells, and histiocytes.
- Touton giant cells are often seen.
- The spindle cells contain oval nuclei with a fine chromatin pattern, inconspicuous nucleoli, and abundant bipolar lightly eosinophilic cytoplasm arranged in fascicles or a storiform architecture.
- Representative images are illustrated in Fig. 6.17a, b.

Immunohistochemistry

- Positive for vimentin, smooth muscle actin (SMA), and rarely to desmin
- Negative for myogenin, myoglobin, CD117, and S100 protein
- Focal cytokeratin (CK) positivity was reported in 1/3 of cases, likely due to alveolar entrapment.
- Anaplastic lymphoma kinase 1 (ALK1) expression was noted in 40% of cases.
- p53 is negative; however, positivity is associated with recurrence and malignant transformation.
- Representative images are illustrated in Fig. 6.17c, d.

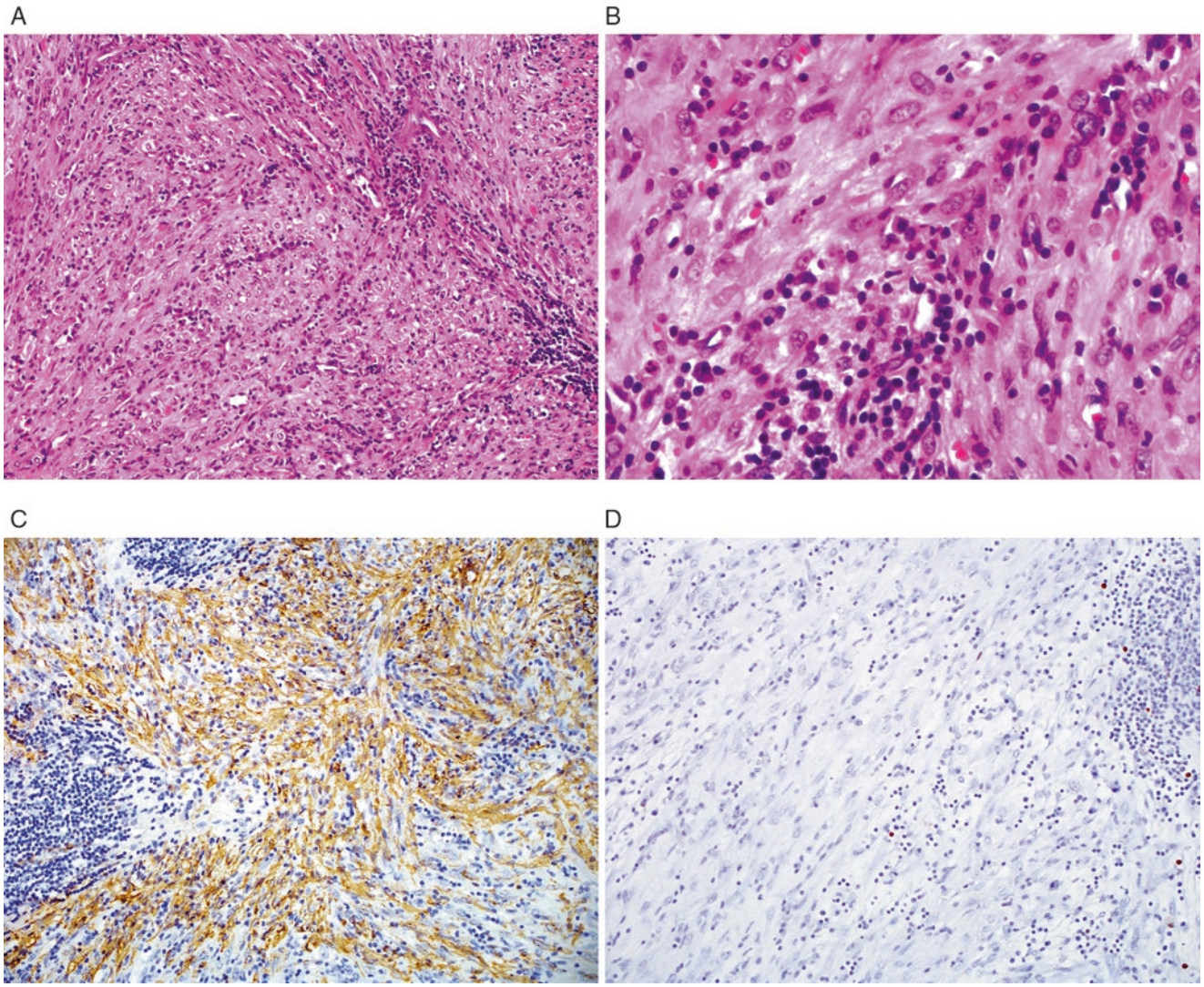

Fig. 6.17 Inflammatory myofibroblastic tumor. (**a**, **b**) Histopathology, a mixture of spindle cells and obscuring inflammatory cell infiltrates including lymphocytes, plasma cells, and histiocytes, H&E; (**c**) positive for SMA, IHC; (**d**) negative for S100, IHC

Representative Malignant Neoplasms of the Lung

Squamous Cell Carcinoma

Squamous cell carcinomas are malignant tumors that either morphologically show squamous cell differentiation (keratinization and/or intercellular bridges) or are morphologically undifferentiated non-small cell carcinomas but show squamous cell differentiation immunohistochemically. The 2015 WHO classifications of tumors of the lung reclassified squamous cell carcinomas into keratinizing, nonkeratinizing, and basaloid subtypes. The nonkeratinizing tumors require immunohistochemical proof of squamous differentiation.

Key Clinical Features

- About 20% of all pulmonary malignancies
- Clinical presentation is similar to other non-small cell carcinomas.

Key Radiological Features

- Usually a central mass with cavitation and post-obstructive pneumonia

Cytological Features

Keratinizing squamous cell carcinoma:

- Abundant dyshesive cells with dense cytoplasm, may be orangeophilic
- Polygonal, rounded, or elongated cells
- Tadpole or fiber-like cells
- Pleomorphic, pyknotic nuclei with obscured nucleoli and chromatin detail
- Anucleated cells and twisted keratin strands (Herxheimer spirals)
- Representative images are shown in Fig. 6.18a–d.

Nonkeratinizing or basaloid squamous cell carcinoma:

- Cohesive groups of cells with larger nuclei and coarsely granular chromatin
- Cyanophilic cytoplasm on Pap stain
- Rare or no keratinization
- Representative images are shown in Fig. 6.19a–f.

Differential Diagnosis

- Squamous metaplasia
- Cavitary infection
- Pemphigus vulgaris

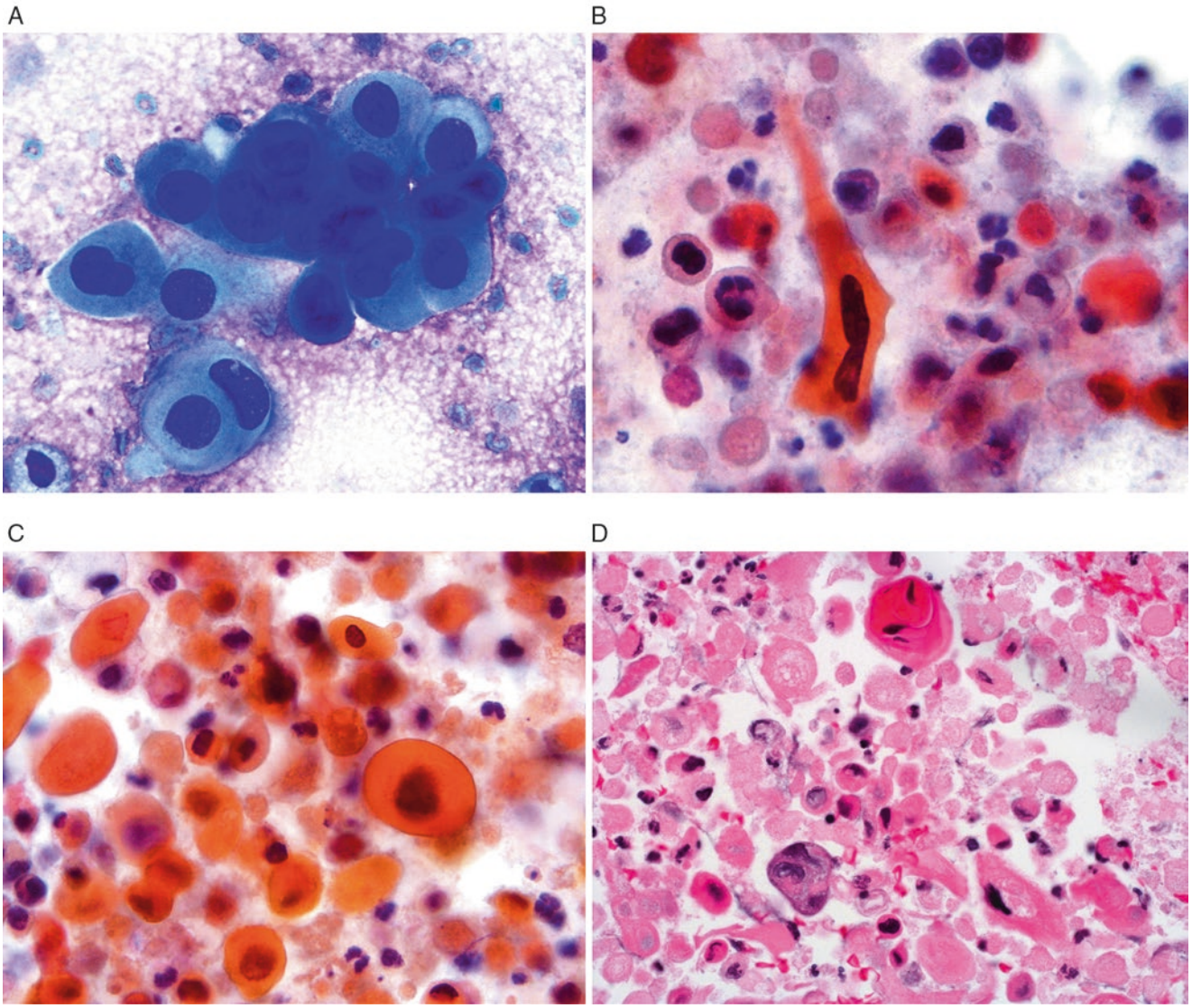

Fig. 6.18 Keratinizing squamous cell carcinoma, cytology. (**a**) Polygonal to round cells with hyperchromatic nuclei and hard cytoplasm, Diff-Quik; (**b**, **c**) polygonal or tadpole cells with pyknotic nuclei and dense, orangeophilic cytoplasm, Pap stain; (**d**) keratin pearls and debris, cell block, H&E

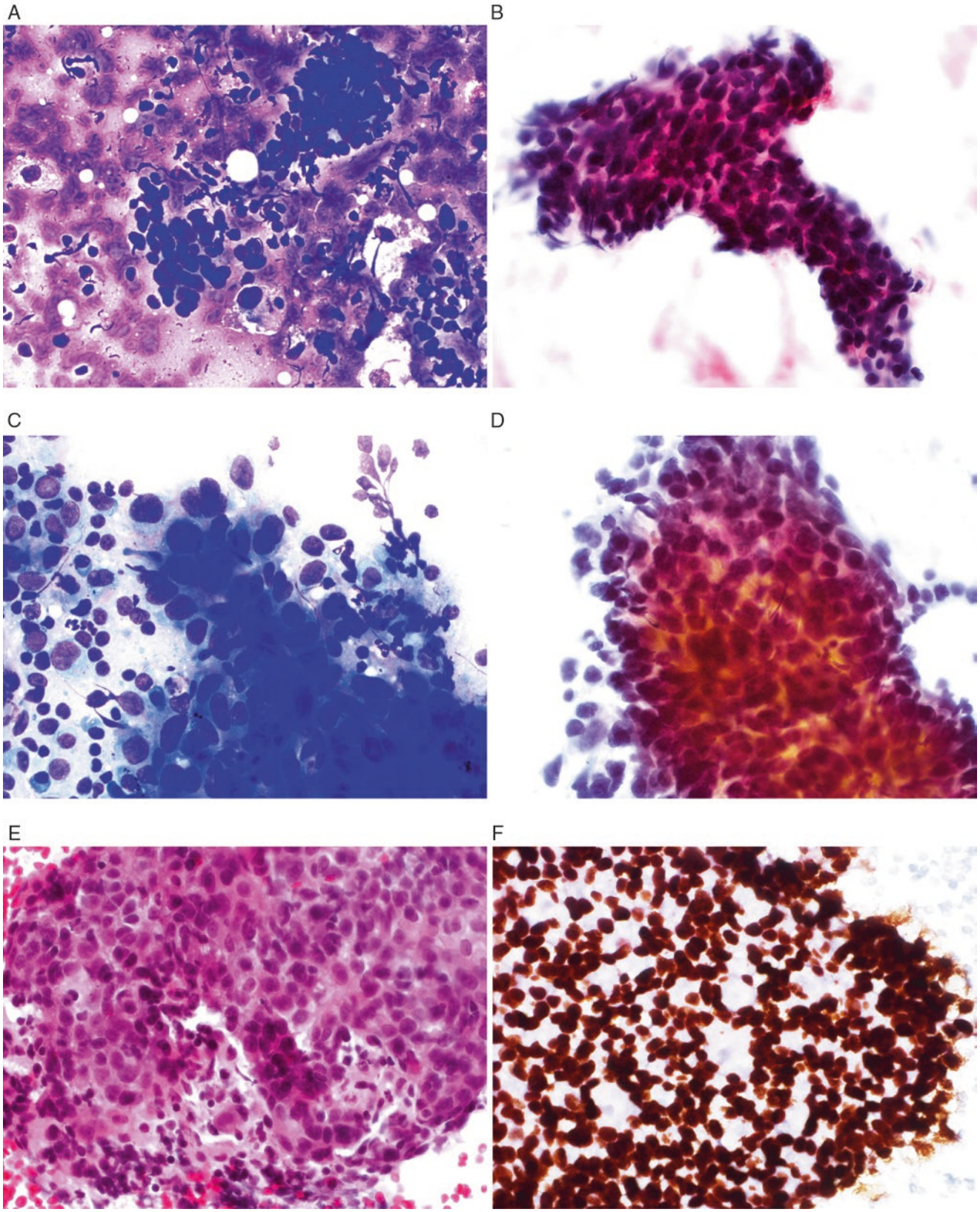

Fig. 6.19 Nonkeratinizing, basaloid squamous cell carcinoma, cytology. (**a**) Cohesive groups of atypical cells with hyperchromatic nuclei and coarse, granular chromatin texture, basaloid, Diff-Quik; (**b**) similar, basaloid, Pap stain; (**c**) cohesive cluster of atypical epithelial cells, no keratinization identified, Diff-Quik; (**d**) similar to C, Pap stain; (**e**) tumor cell cluster in cellblock, H&E; F. p40-decorated tumor nuclei, IHC

- Radiation/chemotherapy effect
- Other non-small cell carcinoma
- Small cell carcinoma
- Upper airway cancer contamination

Histology

- Three subtypes of squamous cell carcinoma in the 2015 WHO classification of tumors of the lung: keratinizing, nonkeratinizing, and basaloid subtypes:
  1. Keratinizing squamous cell carcinomas exhibit recognizable keratinization, keratin pearls, and/or intercellular bridges, as illustrated in Fig. 6.20a, b.
  2. Nonkeratinizing squamous cell carcinomas are without recognizable keratinization, keratin pearls, or intercellular bridges, as illustrated in Fig. 6.20c.
  3. Basaloid squamous cell carcinomas are tumors with a basaloid component in greater than 50% of the tumor, regardless of the presence of any keratinization, as illustrated in Fig. 6.20d.

Immunohistochemistry

- Immunohistochemistry is required for the diagnosis of nonkeratinizing squamous cell carcinomas.
- Squamous cell carcinomas express p40, p63, and CK5/6.
- TTF1 is usually negative in keratinizing squamous cell carcinomas, may be weakly focally positive in nonkeratinizing squamous cell carcinomas.
- Representative images are illustrated in Fig. 6.21a–d.
- Squamous cell carcinomas of lung are characterized by complex genomic alterations, frequently involving the following pathways:

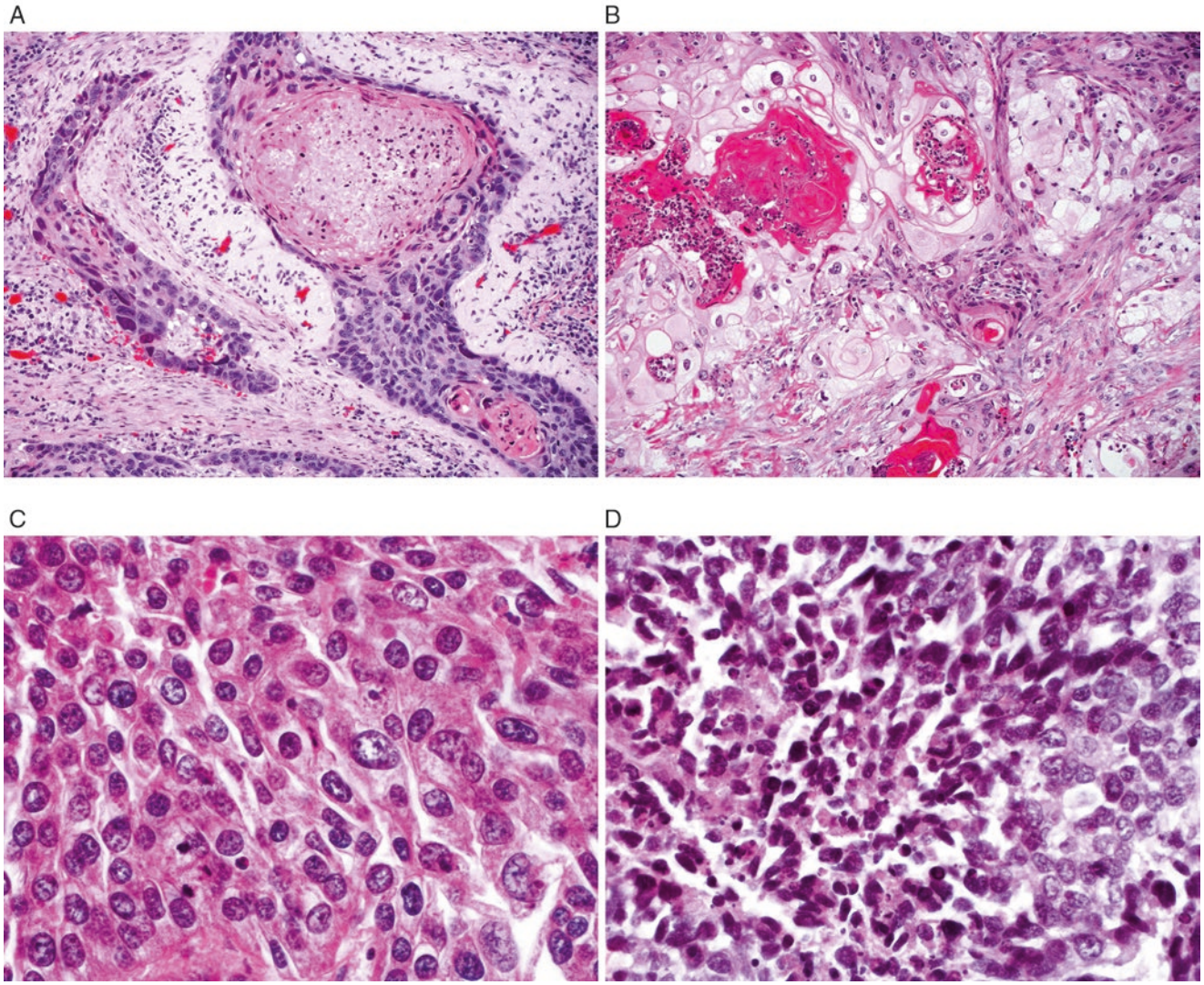

Fig. 6.20 Squamous cell carcinoma, histopathology. (**a**, **b**) Keratinizing squamous cell carcinoma, H&E; (**c**) nonkeratinizing squamous cell carcinoma, H&E; (**d**) basaloid squamous cell carcinoma exhibits solid islands of larger, hyperchromatic tumor cells with peripheral palisading, H&E

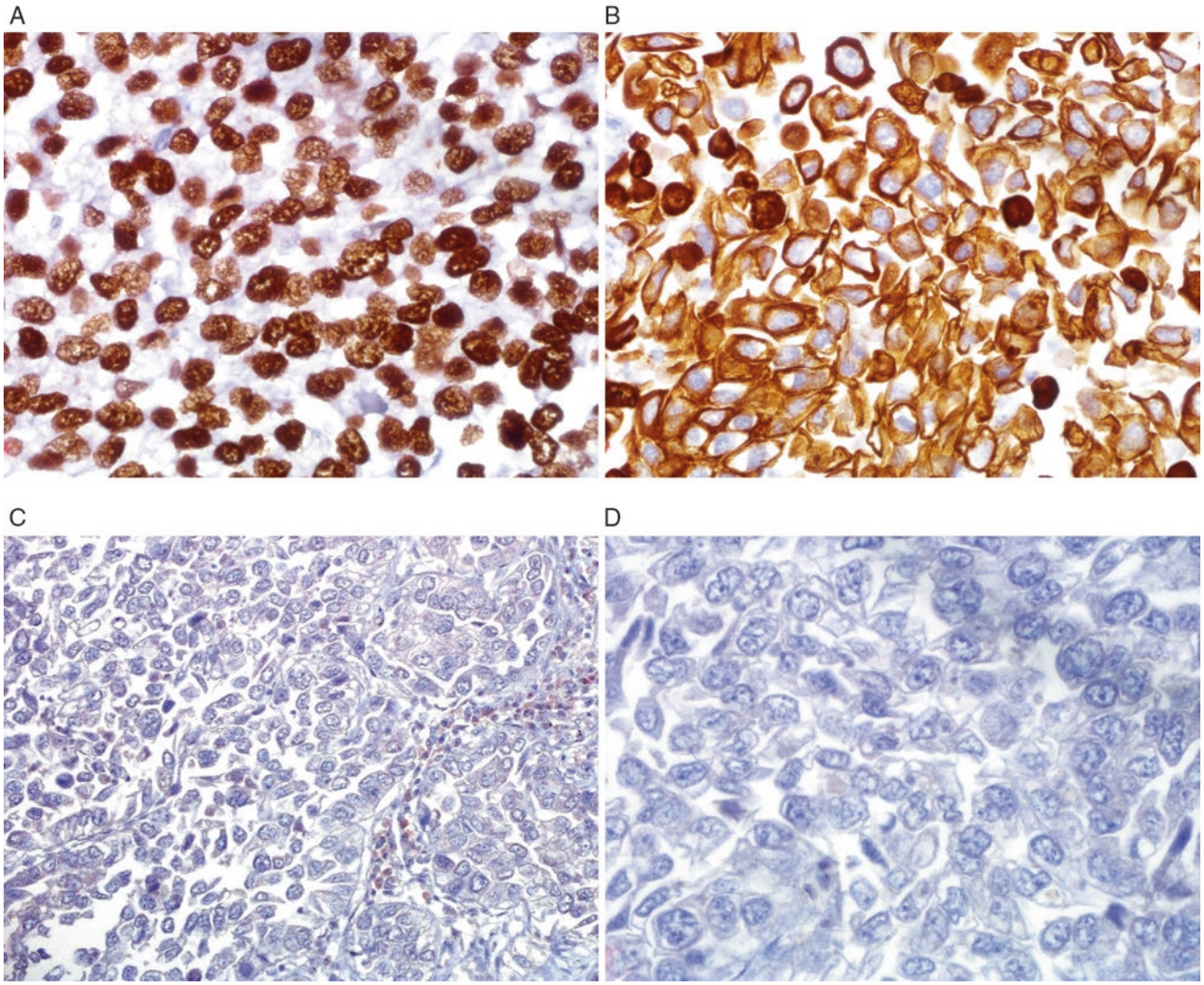

Fig. 6.21 Immunophenotype of squamous cell carcinoma. (**a**) Positive nuclear staining for p40, IHC; (**b**) positive CK5/6 staining, IHC; (**c**) negative TTF1 staining, IHC; (**d**) negative CK7 staining, IHC

1. Cyclin-dependent kinase inhibitor 2A/retinoblastoma 1/nuclear factor, erythroid 2 like 2/Kelch-like ECH-associated protein 1/cullin 3 (CDKN2A/RB1, NFE2L2/KEAP1/CUL3)
2. Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT)
3. Sex-determining region Y box 2/tumor protein 63/Notch (Drosophila) homolog 1(*SOX2/TP63/NOTCH1)*

Gene copy number alterations involving chromosomes 3q (*SOX2, TP63*), 7p (*EGFR*), and 8p (fibroblast growth factor receptor 1 [*FGFR1]*) are characteristic. Almost all tumors display somatic mutation of tumor protein 53 (*TP53).* Deletion of chromosome 9p (*CDKN2A*) was observed in 72% of cases.

Adenocarcinoma (ADC)

Key Clinical Features

- The most common primary pulmonary malignancy, accounting for about 40% of cases.
- Mortality and incidence rates have generally been highest in high-income countries but are now declining, especially in younger males and females.
- Has been more common in men than in women, but has begun to converge.

Key Radiological Features

- Usually a solitary peripheral nodule/mass

Cytological Features

- Cohesive, three-dimensional groups.
- Eccentric, irregular nuclei with granular chromatin, and prominent nucleoli.
- Transparent, foamy cytoplasm, may have secretory vacuoles.
- Invasive mucinous ADC: high cellularity, sheets and three-dimensional groups of relatively uniform cells, nuclear enlargement and irregular contour, and nucleoli. May have pseudoinclusions, psammoma bodies, and nuclear grooves/clearing.
- Poorly differentiated ADC: cohesive groups of overtly malignant cells with nuclear pleomorphism, nuclear membrane irregularity, and coarse chromatin pattern; cytoplasmic mucin is inconspicuous.
- Representative images are shown in Fig. 6.22a–h.

Differential Diagnosis

- Metastatic ADC
- Reactive/reparative changes
- Goblet cell hyperplasia
- Granulomatous inflammation
- Vegetable cells

Histology

- In 2011, the new International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) classification of lung ADC proposed significant changes to the 2004 WHO classification for resected tumors:
  1. Discontinuing the terms bronchioloalveolar carcinoma (BAC) and mixed subtype ADC
  2. Adding adenocarcinoma in situ (AIS) as a preinvasive lesion to join atypical adenomatous hyperplasia
  3. Adding minimally invasive adenocarcinoma (MIA)
  4. Classifying invasive ADCs according to the predominant subtype after comprehensive histologic subtyping in 5% increments
  5. Using of "lepidic" to describe a noninvasive component (previously BAC) as part of an invasive ADC
  6. Replacing mucinous BAC with invasive mucinous ADC excluding tumors that meet the criteria for AIS or MIA
  7. Discontinuing the subtypes of clear cell and signet ring ADC
  8. Discontinuing the term mucinous cystadenocarcinoma and including these under the category of colloid ADC
- In the 2015 WHO classification, large cell carcinomas with expression of TTF1 and/or napsin A are reclassified as solid ADCs even if mucin is absent. The solid ADC must be distinguished from squamous cell carcinomas and large cell carcinomas by the identification of at least two high-power fields with five or more cells showing intracytoplasmic mucin and/or the expression of TTF1 and/or napsin A.
- The diagnostic criteria for AIS:
  1. Solitary, small tumor ≤3 cm.
  2. Purely lepidic growth pattern.
  3. No invasion: stromal, vascular, or pleural.
  4. No pattern of invasive ADC: acinar, papillary, micropapillary, solid, colloid, enteric, fetal, or invasive mucinous ADC.
  5. No spread through air spaces.
  6. Mainly nonmucinous cell type, rare mucinous.
  7. No or inconspicuous nuclear atypia.
  8. Septal widening with sclerosis/elastosis is common.
- The diagnostic criteria for MIA:
  1. Solitary, small tumor ≤3 cm.
  2. Predominantly lepidic growth pattern.
  3. ≤0.5 cm invasive component in greatest dimension in any one focus.
  4. The invasive component to be measured includes any histologic subtype other than a lepidic pattern; tumor cells infiltrating myofibroblastic stroma.
  5. MIA diagnosis is excluded if the tumor invades lymphatics, blood vessels, air spaces, or pleura, contains tumor necrosis, and spreads through air spaces.
  6. The cell type is mostly nonmucinous, but rarely may be mucinous.
- The 2015 WHO classification of tumors of lung classifies ADC as lepidic, acinar, papillary, micropapillary, or solid according to the predominant pattern after a comprehensive histologic subtyping to identify all of the different histologic patterns in 5% increments.
- Other variants of ADCs include invasive mucinous ADC, colloid ADC, fetal ADC, and enteric ADC.
- Representative images are shown in Fig. 6.23a–d.

Immunohistochemistry

- Positive for TTF1 and napsin A: commonly used markers for pneumocytes, with comparable sensitivity, about 75%; much lower TTF1 expression in solid ADCs and mucinous ADCs
- Positive for CK7
- Negative for squamous cell markers; however, p63 positivity was reported in up to 30% of lung ADCs.

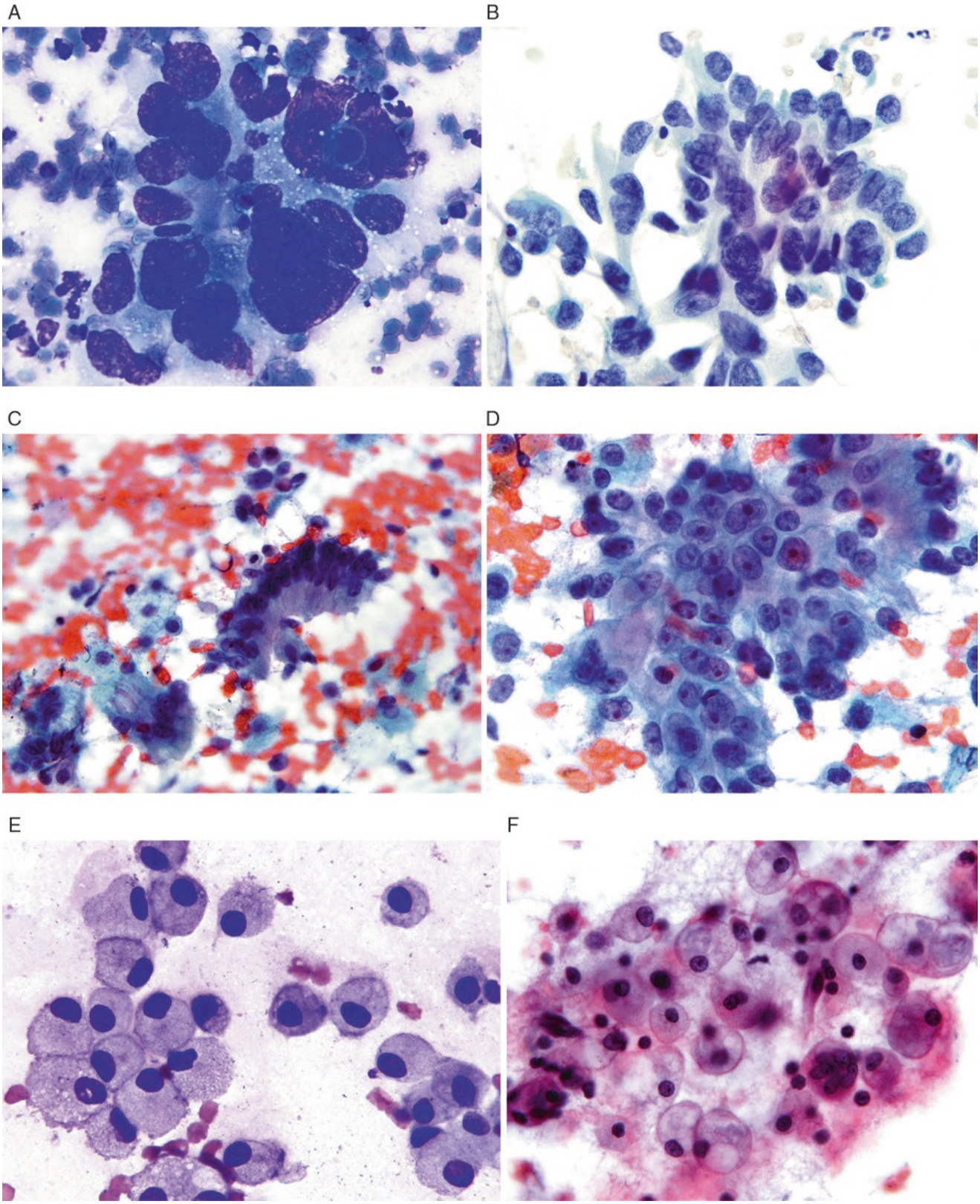

Fig. 6.22 Adenocarcinoma, cytology. (**a**) Cohesive groups of tumor cells with nuclear pleomorphism, nucleoli, and abundant foamy cytoplasm, forming acinar or ductal structure, Diff-Quik; (**b**) the same, Pap stain; (**c**, **d**) invasive mucinous adenocarcinoma, basally located nuclei with no atypia, Pap stain; (**e**) mucinous tumor cells mimic macrophages in background of mucin, Diff-Quik; Pap stain; (**f**) mucinous tumor cells mimic macrophages in background of mucin, Pap stain; (**g**) poorly differentiated adenocarcinoma showing pleomorphic tumor cells with no mucinous features identified, Diff-Quik; (**h**) poorly differentiated adenocarcinoma, Pap stain

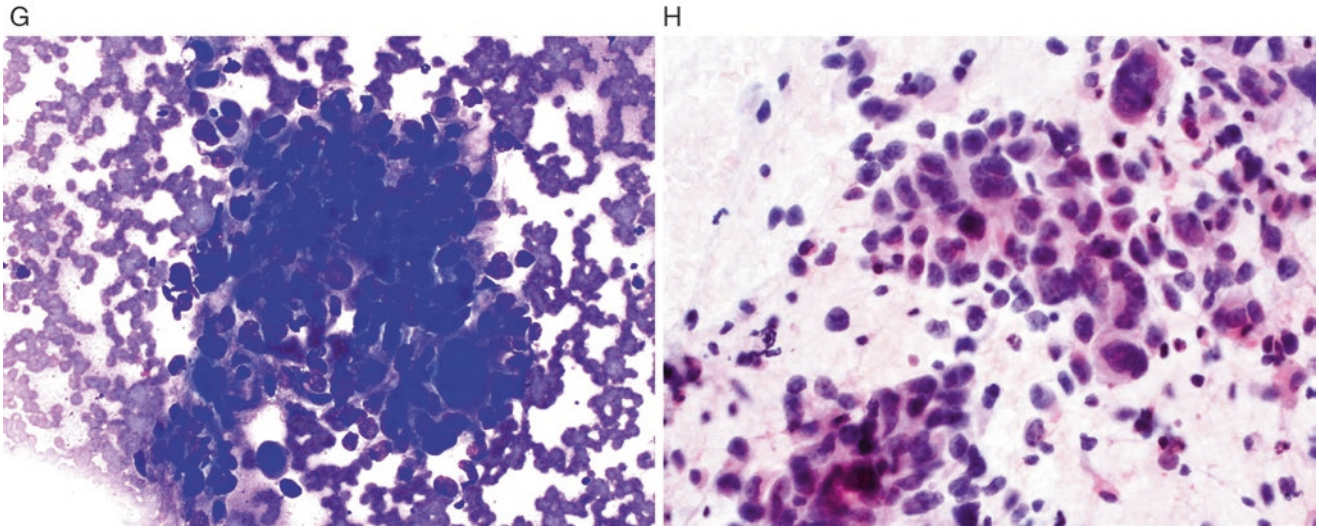

Fig. 6.22 (continued)

- Representative images are shown in Fig. 6.23e–h.
- EGFR, Kirsten rat sarcoma viral oncogene (KRAS), and ALK mutations are specific for lung ADCs. The ADCs with *EGFR* or *ALK* alteration are usually located in the periphery; in contrast, the ADCs with *KRAS* mutation are frequently located in hilar region. The prevalence for *KRAS* and *EGFR* mutations is 10–30%; the transforming fusion gene echinoderm microtubule-associated protein-like 4 (*EML4)-ALK* is found in 5% of lung ADCs. The characteristics of patients with *EGFR* mutation are Asians, never smokers, with nonmucinous tumors; for *ALK* mutation, patients are younger age, male gender, and never or light smokers; for *KRAS* mutation patients are non-Asians, smokers, with invasive mucinous ADCs usually negative for TTF1, and positive for mucin (MUC) 2,5,6 immunophenotypes.

Neuroendocrine Tumors

This group of tumor represents a morphologic and biologic spectrum of tumors that is classified by the 2015 WHO classification of tumors of the lung into four types: preinvasive lesion (including diffuse idiopathic pulmonary neuroendocrine cell hyperplasia), carcinoid tumors (including typical and atypical carcinoid tumors), large cell neuroendocrine carcinoma (LCNEC), including combined LCNEC, and small cell carcinoma (including combined small cell carcinoma).

Carcinoid Tumor

Carcinoid tumors are neuroendocrine epithelial malignancies, including typical carcinoids, defined as those with <2 mitoses/per 2 mm^2, lacking necrosis, ≥0.5 cm in size, and atypical carcinoids, which are those with 2–10 mitoses/per 2 mm^2 and/or foci of necrosis.

Key Clinical Features

- Account for <1% of all lung cancers.
- Majority (70–90%) are typical carcinoids.
- More common in female, white, and <60 years old.
- Clinical syndromes are uncommon, including carcinoid syndrome, Cushing syndrome, and acromegaly.

Key Radiographic Findings

- Chest x-ray: usually a centrally located, lobulated mass with a prominent endobronchial component; one third of tumors in periphery.
- CT using intravenous contrast medium shows considerable enhancement; calcification, especially in centrally located tumors; atelectasis; bronchiectasis; and hyperlucency for tumors with bronchial involvement.

Cytological Features

- Loosely cohesive groups and single uniform cells with granular nuclei and ample eosinophilic cytoplasm and naked nuclei.
- Round, columnar, or plasmacytoid cells, forming acinar or rosette-like structures.
- Branching capillaries.
- Clean background.

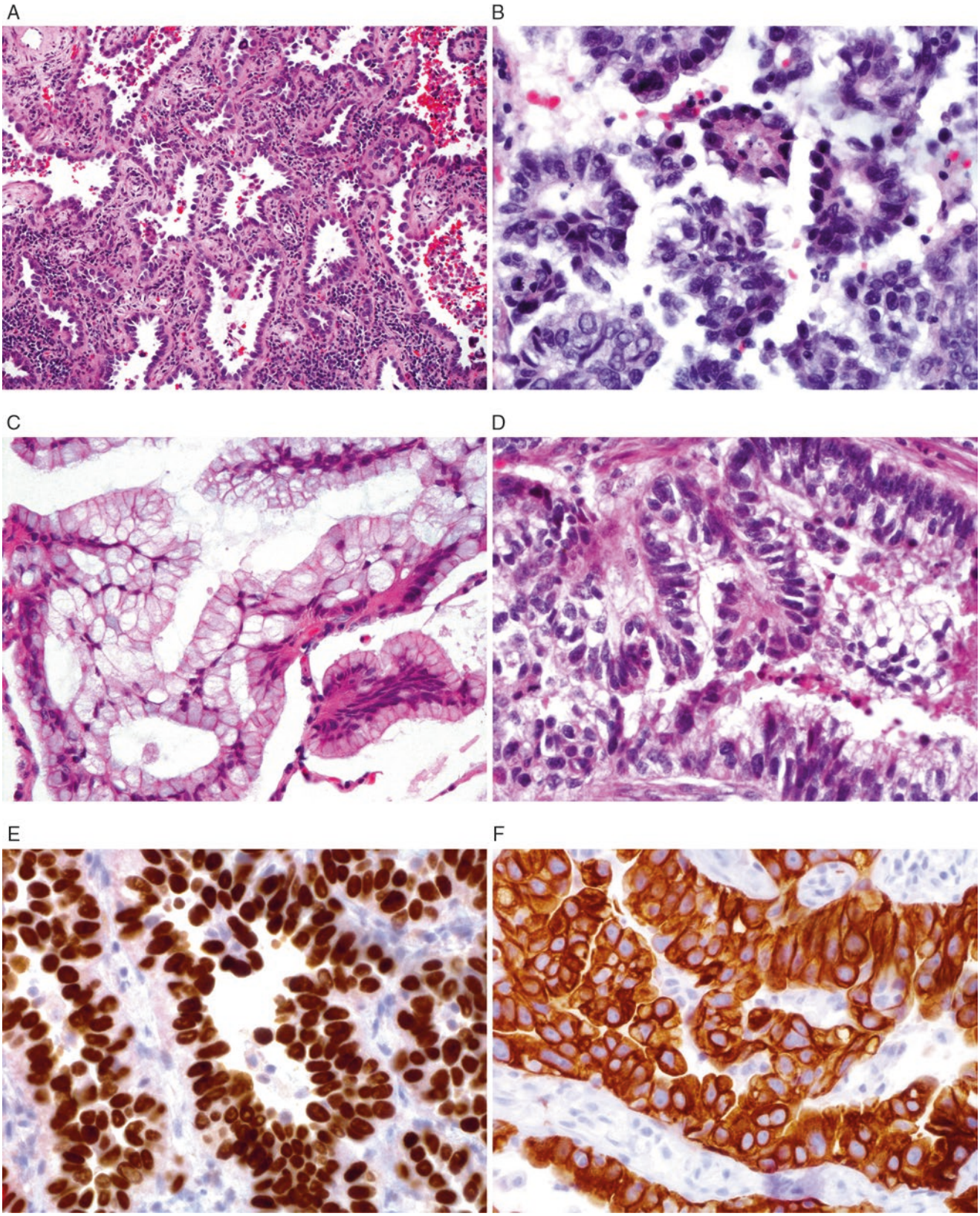

Fig. 6.23 Adenocarcinoma, histopathology and immunophenotype. (**a**) Lepidic growth pattern, H&E; (**b**) micropapillary pattern, H&E; (**c**) mucinous, H&E; (**d**) fetal adenocarcinoma, H&E; (**e**) nuclear staining for TTF1, IHC; (**f**) positive for CK7, IHC; (**g**) positive for napsin-A, IHC; (**h**) negative for p40, IHC

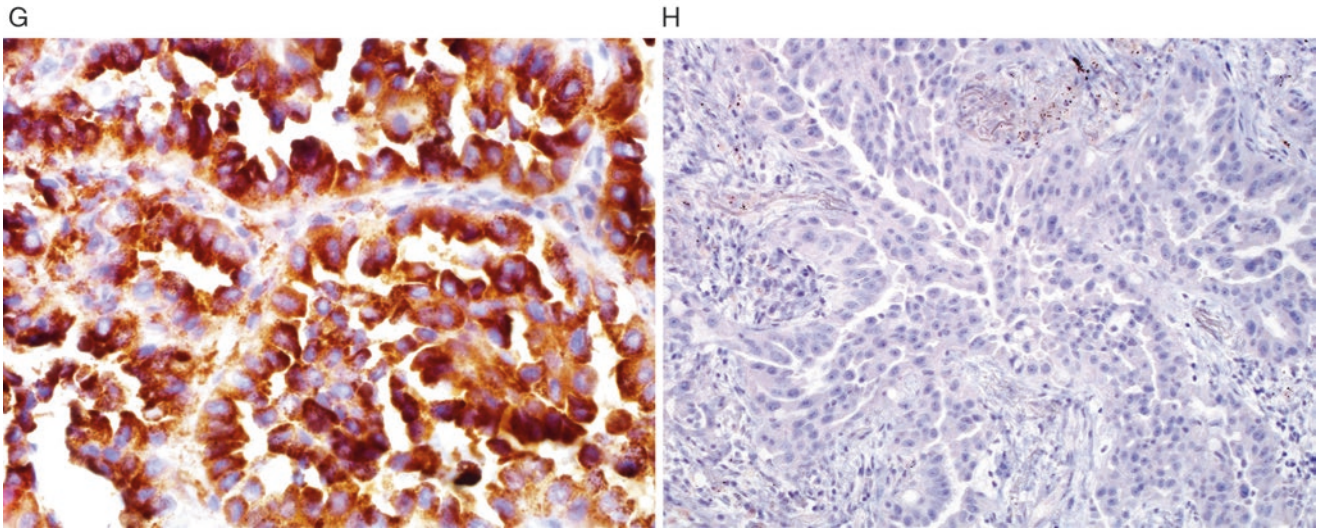

Fig. 6.23 (continued)

- May show pleomorphism and prominent nucleoli in atypical carcinoids.
- Mitoses uncommon, no necrosis in typical carcinoids; necrosis and mitosis seen in atypical carcinoids.
- Representative images are shown in Fig. 6.24a, b.

Differential Diagnosis

- Small cell carcinoma
- Lymphoma
- Benign bronchial epithelial cells
- Mesenchymal tumor (spindle cell carcinoid)
- ADC

Histology

- Usually uniform polygonal or spindled cells arranged in organoid or trabecular patterns.
- Other growth patterns also seen: rosette formation, papillary, pseudoglandular, or follicular patterns.
- Significant pleomorphism and prominent nucleoli may be seen in typical carcinoids.
- The differential features for atypical carcinoids are the presence of 2–10 mitoses per 2 mm^2 and/or necrosis. These changes may be focal.
- Representative images are shown in Fig. 6.24c–f.

Immunohistochemistry

- Immunohistochemistry is recommended for the diagnosis of neuroendocrine tumors, not only in small biopsy or cytology cases but also in resected specimens.
- The typical phenotype expresses neuroendocrine markers (CD56, synaptophysin, chromogranin); most are positive for panCK and negative for high molecular weight cytokeratins (HMWCKs) and TTF1. However, TTF-1 expression was reported in 43–53% of cases.
- The proliferative index (ki-67) is valuable in distinguishing carcinoids from high-grade neuroendocrine carcinomas, especially in small biopsies or cytology specimens with significant crush artifact. Small cell carcinomas have high proliferative index (>50%) in contrast to <10–20% in carcinoids.
- Ki-67 is not recommended for the distinction of typical from atypical carcinoids due to the lack of cutoff value.
- Representative images are shown in Fig. 6.25a–d.

Large Cell Neuroendocrine Carcinoma (LCNEC)

Key Clinical Features

- Heavy smokers in >90% of cases.
- Paraneoplastic syndrome is uncommon.

Key Radiological Findings

- Usually a periphery mass with irregular margin, with or without intratumoral calcification
- The tumors are usually large, showing central inhomogeneous enhancement on CT with contrast.
- Cavitation uncommon

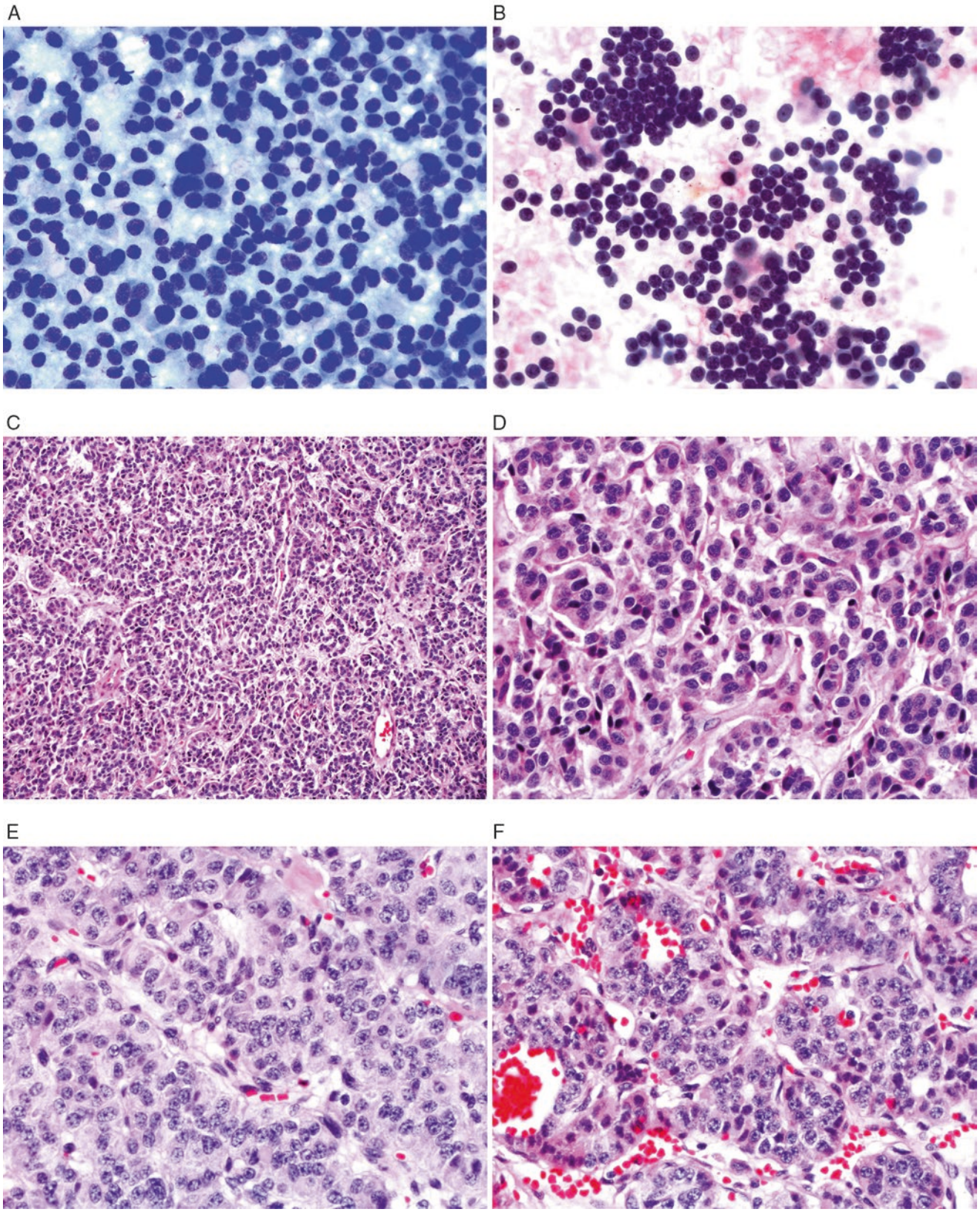

Fig. 6.24 Carcinoid tumors, cytology. (**a**) Loosely cohesive groups and single uniform round to oval cells with granular nuclei and ample eosinophilic cytoplasm, Diff-Quik stain; (**b**) same, Pap stain; (**c**, **d**) typical carcinoid, composed of uniform polygonal and spindled cells arranged in organoid and trabecular patterns, H&E; (**e**, **f**) atypical carcinoid, the cells are more atypical with focal prominent nucleoli, rare necrosis, and 2–10 mitoses/per 2 mm^2

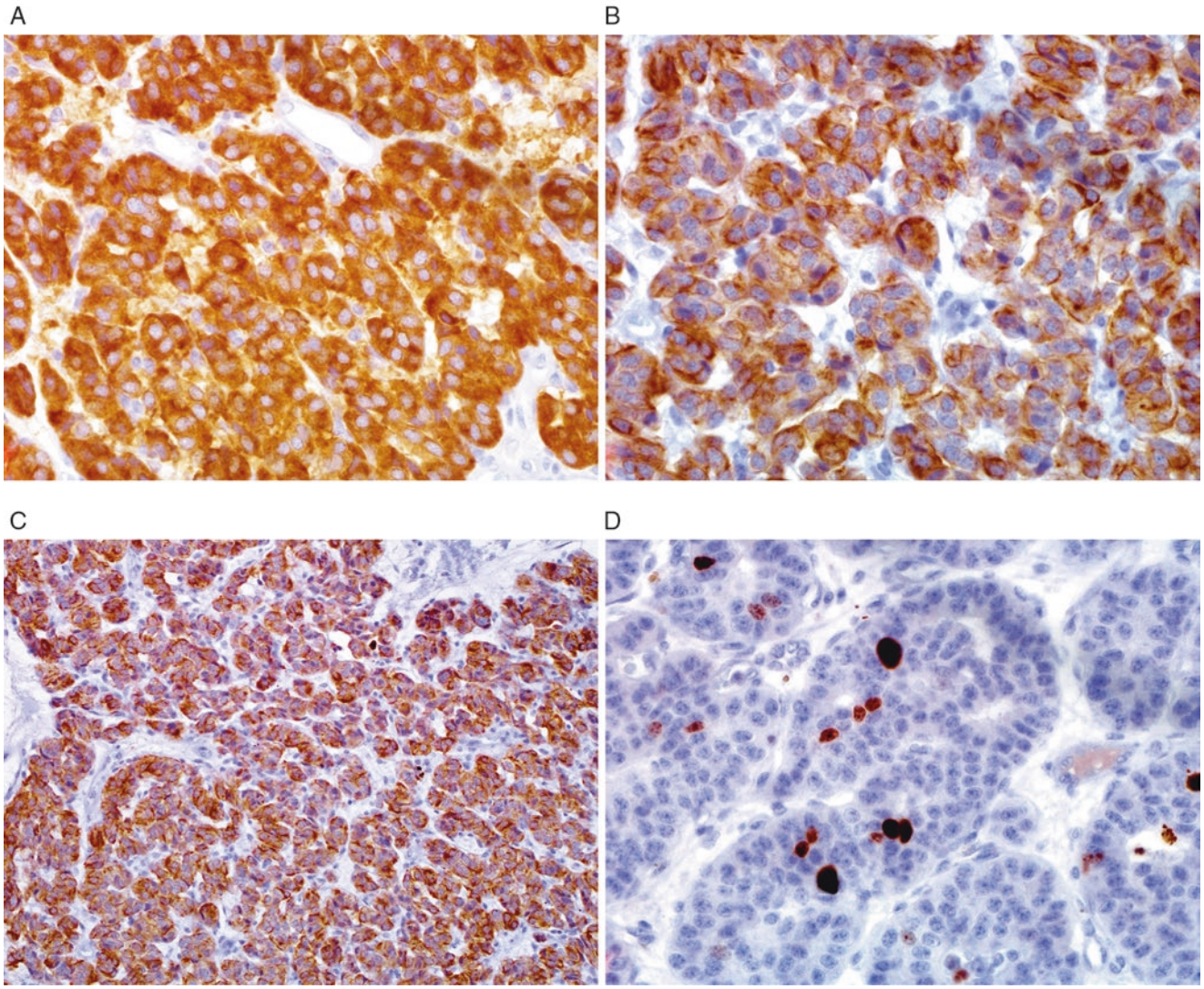

Fig. 6.25 Carcinoids, immunohistophenotype. (**a**) Positive for synaptophysin, IHC; (**b**) positive for chromogranin, IHC; (**c**) positive for AE1/3, IHC; (**d**) very low MIB-1, IHC

Cytological Features

- Overlapping with other neuroendocrine tumors and ADCs
- Loosely cohesive or single, monotonous tumor cells with a hyperchromatic nuclear chromatin pattern but easily appreciated nucleoli, nuclear membrane irregularity, and preserved moderate to abundant, delicate cytoplasm
- Necrosis or apoptotic debris may be seen.
- Representative images are shown in Fig. 6.26a, b.

Differential Diagnosis

- Other neuroendocrine tumors, including small cell carcinoma
- Other non-small cell carcinomas, such as ADC, basaloid squamous cell carcinoma, and large cell carcinoma
- Melanoma
- Metastasis

Histology

- Usually arranged in typical neuroendocrine tumor growth patterns, such as organoid, trabecular, rosette-like, peripheral palisading, or solid patterns.
- The tumor cells are often larger, with nucleoli (often prominent) and moderate to abundant cytoplasm.
- Mitotic figures >10/per 2 mm^2 (with an average of 75) of viable tumor; tumors with <30 mitoses/per 2 mm^2 are rare.

- The proliferative index (ki-67) is usually in the range of 40–80%.
- Necrosis is common.
- Representative images are shown in Fig. 6.26c, d.

Immunohistochemistry

- Immunohistochemical markers such as synaptophysin, chromogranin, and CD56 are required to confirm neuroendocrine differentiation of the tumor.
- CD56 was reported in 92–100% of cases, chromogranin in 80–85%, and synaptophysin in 50–60%.
- About 50% of cases are positive for TTF1.
- PanCK, low molecular weight cytokeratin (LMWCK), and CK7 are expressed in either dot-like or diffuse cytoplasmic patterns.
- CD117 positivity was reported in >70% of cases.

Small Cell Carcinoma

Key Clinical Features

- Thirteen percent of all newly diagnosed lung cancers are small cell carcinomas.
- Virtually all are heavy smokers, male predominant.

Key Radiological Findings

- Usually a centrally located lobulated mass, with occasional endobrochial involvement; 5% peripherally located
- A large hilar mass with bulky mediastinal lymph nodes is characteristic; invasion of hilar vessels and vena cava is common; cavitation is rare.

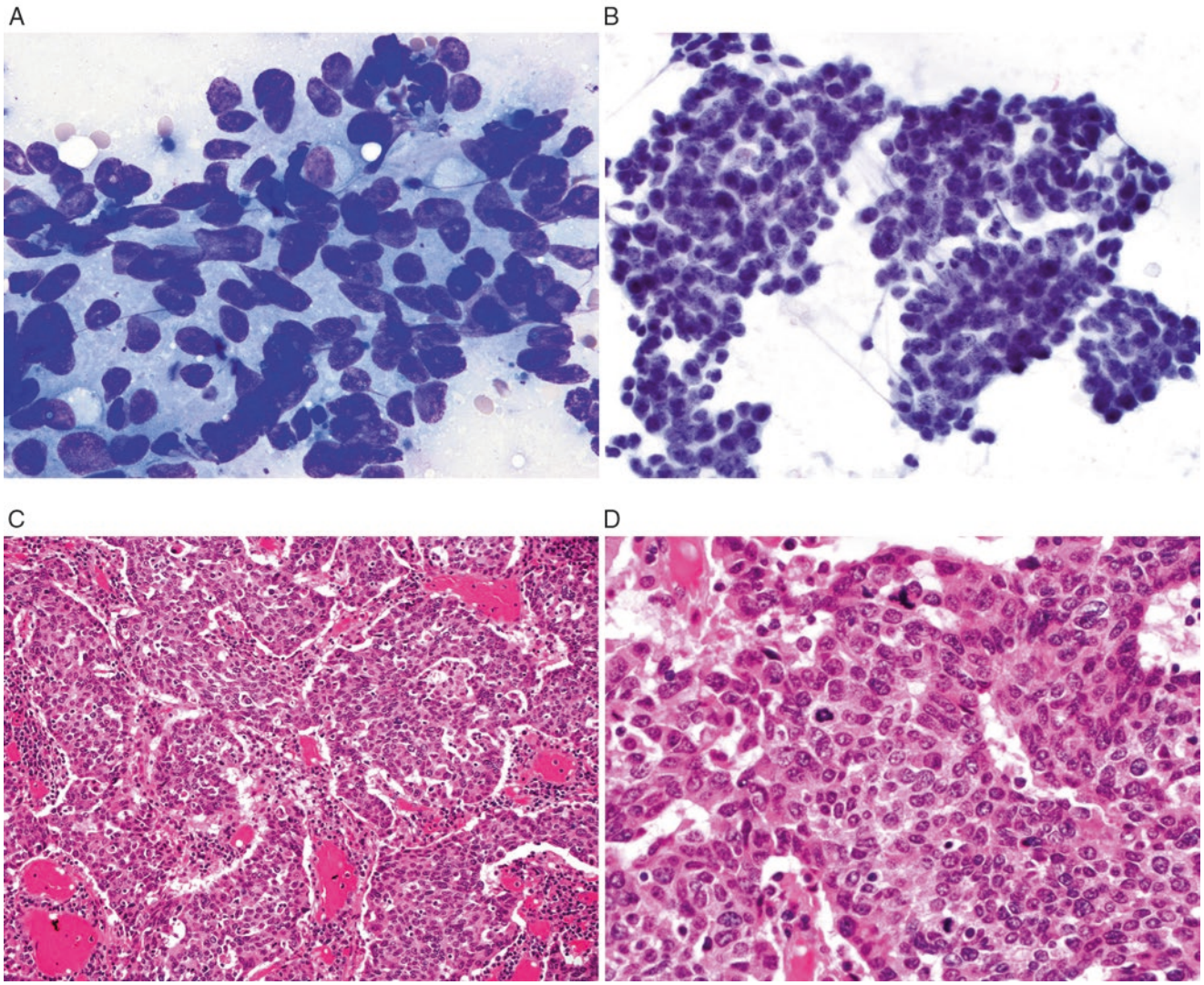

Fig. 6.26 Large cell neuroendocrine carcinoma. (**a**, **b**) Loosely cohesive cluster of mildly pleomorphic tumor cells with hyperchromatic nuclear chromatin pattern but easily appreciated nucleoli, nuclear membrane irregularity, and preserved moderate to abundant, delicate cytoplasm, Diff-Quik (**a**) and Pap (**b**) stains; (**c**) large tumor cells with prominent nucleoli and moderate to abundant cytoplasm, arranged in irregular nests, H&E; (**d**) mitotic figures are easily identified, H&E

Cytological Features

- Small cells with hyperchromatic nuclei, powdery chromatin texture, indistinct nucleoli, nuclear molding, and scant cytoplasm
- Marked mitosis and single cell necrosis
- Nuclear debris and crush artifact in the background
- Representative images are shown in Fig. 6.27a–d.

Differential Diagnosis

- Reserve cell hyperplasia
- Carcinoids
- Small blue cell tumors, such as lymphoma, Ewing sarcoma, and rhabdomyosarcoma
- Non-small cell carcinoma
- Pulmonary blastoma
- Merkel cell carcinoma

Histology

- The tumor usually presents in a sheetlike pattern.
- The tumor cells are small, containing round, oval, or spindled nuclei, with a fine granular chromatin pattern, inconspicuous nucleoli, and scant cytoplasm, without defined cell borders.
- Nuclear molding is frequent.
- High mitotic rate is seen, at least 10 mitoses/per 2 mm^2, with an average of 60 mitoses/per 2 mm^2. The proliferative index (ki-67) is >50%, with an average of >80%.
- May show extensive necrosis
- Representative image is shown in Fig. 6.27e.

Immunohistochemistry

- Immunohistochemical studies may be required to confirm neuroendocrine and epithelial differentiation of the tumor.
- CK AE1/3, CAM5.2, and MNF116 are positive in nearly 100% of cases, with either dot-like, paranuclear, or diffuse cytoplasmic staining patterns.
- Neuroendocrine markers (CD56, synaptophysin, and chromogranin) are reactive in the majority of cases; CD56 and synaptophysin are usually diffuse and strong, but chromogranin is often focal and weak.
- <10% of small cell carcinomas may lack or have only very focal expression of neuroendocrine markers.
- TTF1 is positive in up to 90–95% of cases.
- CD117 positivity was reported in 60% of cases.
- Representative images are shown in Fig. 6.27f–j.

The features of differential diagnosis for neuroendocrine tumors are summarized in Table 6.5.

Large Cell Carcinoma

Key Clinical Features

- About 2.3% of all pulmonary malignancies.
- Usually in the sixth decade, male predominant.
- Most patients are smokers.

Key Radiographic Features

- Chest x-ray: usually a peripheral mass, rarely may be cavitated

Cytological Features

- Single cells or loose clusters
- Vesicular, pleomorphic nuclei with irregular nuclear membrane, multiple nucleoli and high nuclear-to-cytoplasmic ratio
- Ill-defined, feathery cytoplasm

Differential Diagnosis

- Poorly differentiated non-small cell carcinoma
- Melanoma
- Metastatic carcinoma
- Radiation reaction
- Large cell non-Hodgkin lymphoma
- Anaplastic lymphoma
- Sarcoma

Histology

- Large cell carcinoma can only be diagnosed in a resected specimen, after ruling out the presence of squamous cell carcinoma, ADC, and small cell carcinoma morphologically, immunohistochemical evidence of squamous or ADC differentiation, and mucin stain.
- For small biopsy or cytology cases, large cell carcinomas can only be diagnosed as non-small cell lung carcinoma, not otherwise specified (NSCLC, NOS).
- Composed of sheets or nests of polygonal cells with vesicular nuclei, prominent nucleoli, and moderate amounts of cytoplasm.

Immunohistochemistry

- Immunohistochemical evaluation for TTF1, p40, and, if indicated, neuroendocrine markers (synaptophysin, chromogranin, and CD56) to exclude the presence of ADC, squamous cell carcinoma, and high-grade neuroendocrine carcinomas (small and large cell).

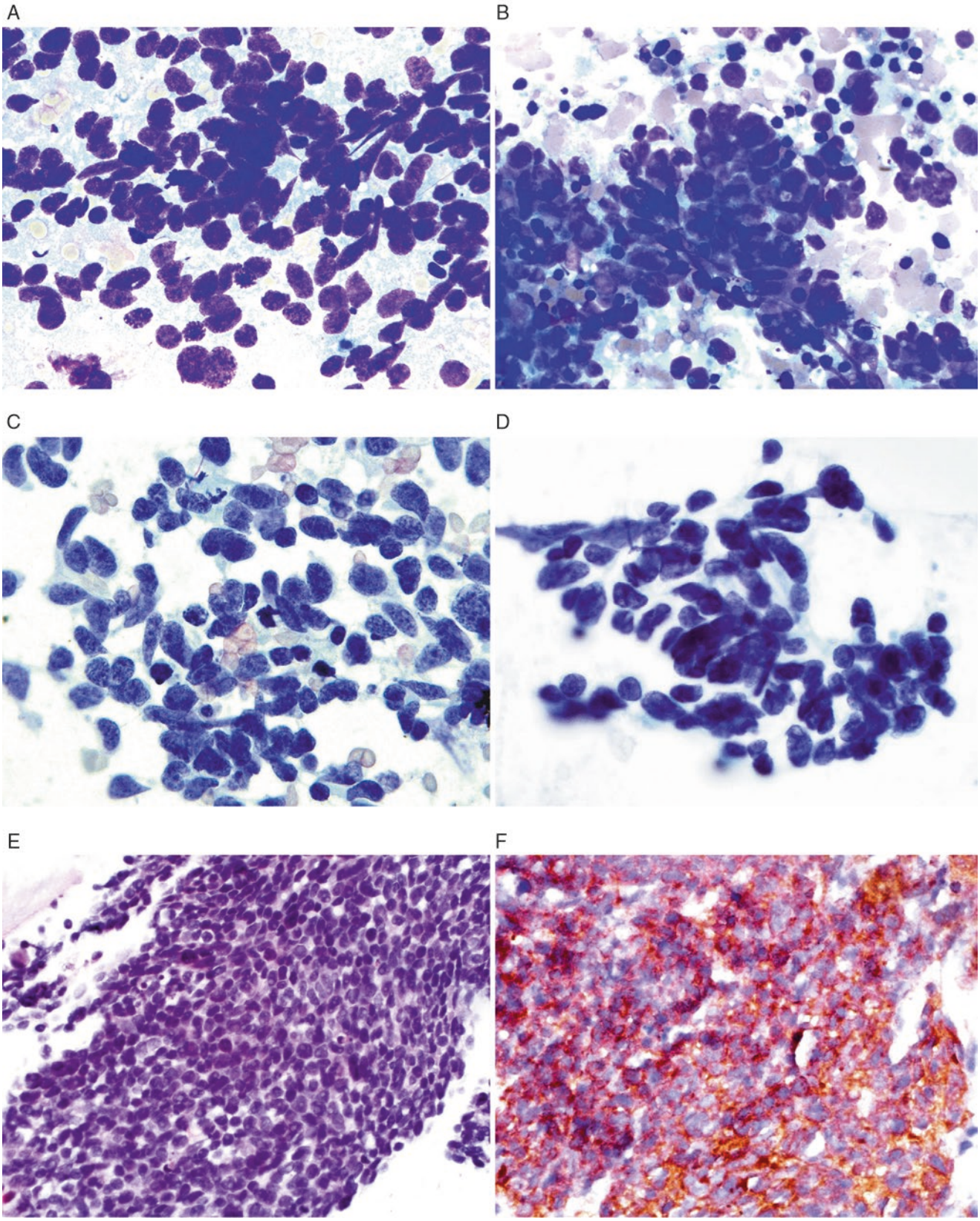

Fig. 6.27 Small cell carcinoma, cytology with immunophenotype. (**a**, **b**) Small uniform tumor cells with nuclear hyperchromasia, molding, powdery chromatin pattern, very scant cytoplasm, and abundant apoptotic cells, Diff-Quik; (**c**, **d**) lack of nucleoli, frequent mitoses, in clean background, Pap stain; (**e**) cellblock preparation shows thin cores of tumor tissue comprised of small, round to oval to spindled tumor nuclei with fine, granular chromatin pattern, inconspicuous nucleoli, and scant cytoplasm without defined cell borders, as well as abundant mitoses, H&E; (**f**) tumor cells are decorated by synaptophysin, IHC; (**g**) focal weak positivity with chromogranin, IHC; (**h**) diffuse nuclear staining for TTF-1, IHC; (**i**) positive CK7 staining, in a granular and dot-like pattern, IHC; (**j**) proliferative index (MIB-1) is estimated at 95%, IHC

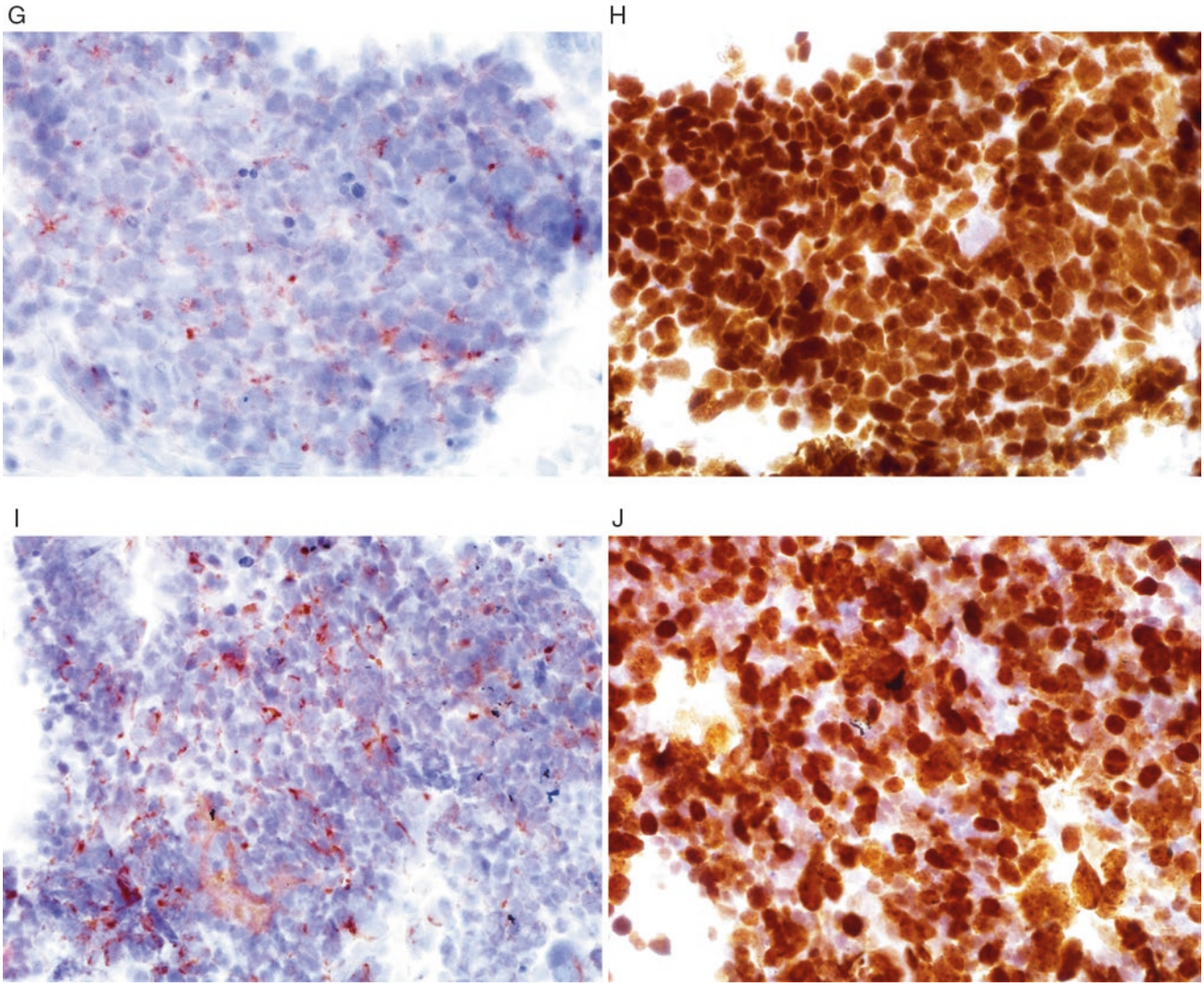

Fig. 6.27 (continued)

Table 6.5 Differential diagnosis of neuroendocrine tumors

	Typical carcinoid	Atypical carcinoid	LCNEC	SCNC
Clinical features				
Age	6th decade	6th decade	7th decade	7th decade
Gender	Female	Female	Male	Male
Smoker	No	Variable	Yes	Yes
Diagnostic criteria				
Mitosis per 2 mm^2	0–1	2–10	> 10 (Med 70)	> 10 (Med 80)
Necrosis	No	Focal if any	Yes	Yes
Ki-67 index	Up to 5%	Up to 20%	40–80%	50–100%
IHC				
TTF1	Mostly –	Mostly –	+ in 50%	+ in 90–95%
SYN	+	+	+ in 80–90%	+ in 80–90%
CHR	+	+	+ in 80–90%	+ in 80–90%, F
CD56	+	+	+ in 80–90%	+ in 80–90%

LCNEC large cell neuroendocrine carcinoma, *SCNC* small cell neuroendocrine carcinoma, *Med* median, *IHC* immunohistochemistry, *TTF1* thyroid transcription factor 1, *SYN* synaptophysin, *CHR* chromogranin, *CD56* cluster of differentiation 56, *F* focal

- Only cases with negative phenotypes for those markers or cases with unclear patterns are classified as large cell carcinomas.
- Three subtypes of large cell carcinomas are based on immunophenotype:
  1. Large cell carcinoma with null immunohistochemical features: mucin −; cytokeratin +; TTF1 −; p63/p40/CK5/6 −
  2. Large cell carcinoma with unclear immunohistochemical features: mucin −; cytokeratin +; TTF1 −; p63/p40/CK5/6: any one of them showing focal +
  3. Large cell carcinoma with no additional stains: no immunohistochemical or mucin staining available
- Genomic data are limited to marker-null large cell carcinomas. The mutations found are those showing association with ADCs, such as *KRAS* and occasional *EGFR*. *TP53* mutations, *CDKN2A* deletions, and *MYC* as well as cyclin E1 (*CCNE1)* amplifications are also reported, with a similar frequency to that of other histological types. Therefore, molecular testing is recommended for large cell carcinomas.

Sarcomatoid Carcinoma

Sarcomatoid carcinoma is a general term that includes pleomorphic carcinoma, carcinosarcoma, and pulmonary blastoma, which are individual entities in the 2015 WHO classification. This group of tumors accounts for 2–3% of all cancer cases in a surgical series and <1% of all lung cancers. Definitive diagnosis of this group of tumors is very difficult or impossible in small biopsies and cytology specimens.

The characteristic features of individual tumors in this group are summarized in Table 6.6.

Representative images are illustrated in Fig. 6.28a–h.

Nuclear Protein in Testis (NUT) Carcinoma

This is a new entity in the 2015 WHO classification of tumors of lung. The group of carcinomas associated with

Table 6.6 Features of sarcomatoid carcinomas

	Pleomorphic, spindle cell, and giant cell CA	Carcinosarcoma	Pulmonary blastoma
Clinical features	Often smokers	Often heavy smokers Men > women, 7–8 times Median age of 65 years	Often smokers No sex predominance Common in the fifth decade
Radiographic features	A large peripheral mass, favoring the upper lobes; CT: low central attenuation, with frequent invading to adjacent pleura.	Usually centrally located mass with necrosis	Usually solitary, large, peripherally located mass
Cytologic features	1. Both malignant epithelial (ADC or SqCC) and mesenchymal-like elements 2. Discohesive, round to oval tumor giant cells with single or multiple large, irregular, lobulated nuclei, and abundant eosinophilic cytoplasm 3. Collagen or myxoid stroma, mitosis, necrosis, lymphocytes, and neutrophils	1. Both malignant epithelial and mesenchymal elements 2. The epithelial component is often SqCC with marked keratinization 3. The mesenchymal component can be heterologous differentiation	1. Biphasic tumor with fetal ADC (LG) and primitive mesenchymal stroma 2. Fetal ADC: uniform, small columnar cells with small nuclei, inconspicuous nucleoli and clear vacuolated cytoplasm 3. Stroma: loose aggregate or single dispersed small homogeneous cells with oval to elongated nuclei without nucleoli and high N/C ratio; may be embedded in myxoid tissue fragment 4. Reticulated, bubbly background
Histology	1. Pleomorphic CA: a mixture of giant and/or spindle cell elements (at least 10%) and epithelial elements, commonly ADC (31–72%), followed by undifferentiated NSCC (up to 43%) and SqCC (12–26%) 2. Spindle cell CA: composed of almost entirely malignant spindle cells in fascicular or storiform patterns 3. Giant cell CA: composed almost entirely pleomorphic giant tumor cells	1. Feature intimately admixed NSCC and sarcoma 2. The NSCLC component is often SqCC, followed by ADC, adenosquamous CA, and large cell CA 3. The sarcomatous components include, in descending order of frequency, rhabdomyosarcoma, chondrosarcoma, and osteosarcoma; often in combination	1. Areas of epithelial and mesenchymal differentiation in various proportions 2. Epithelial element: essentially LG fetal ADC, composed of branching tubules or glandular structures lined by pseudostratified columnar cells with bland morphology and clear to weakly eosinophilic cytoplasm 3. Mesenchymal element: tightly packed primitive cells with oval or elongated nuclei and high N/C ratio in myxoid or fibrous background; heterologous elements may be seen

(continued)

Table 6.6 (continued)

	Pleomorphic, spindle cell, and giant cell CA	Carcinosarcoma	Pulmonary blastoma
Immunohistochemistry	1. Positive for vimentin and fascin 2. Epithelial elements: positive for corresponding differentiation markers 3. Keratin expression is not required in giant or spindle cell elements	1. Helpful to highlight the epithelial and sarcomatous differentiation 2. The NSCLC component express markers of the conventional count parts 3. The sarcomatous component expresses corresponding linage markers	1. Epithelial element: positive for CK7, CK AE1/3, CEA, EMA, and TTF1; focally positive for CHR, SYN, and vimentin 2. Mesenchymal element: positive for vimentin, MSA, focal to CK AE1/3; heterologous elements: positive for linage specific markers 3. N/C accumulation of β-catenin: both epithelial and mesenchymal elements
Molecular Genetics	*KRAS* mutation in up to 38% of cases; *EGFR* mutation in up to 25% of cases; *TP53* mutation in spindle cell and pleomorphic CAs	Frequent for *TP53* mutation Less frequent for *KRAS* mutation Very uncommon for *EGFR* mutation	Frequent missense mutations in exon 3 of *CTNNB1* *TP53* mutation and both p53 and MDM2 protein accumulation occasionally detected

CA carcinoma, *CT* computed tomography, *ADC* adenocarcinoma, *SqCC* squamous cell carcinoma, *LG* low-grade, *N/C* nuclear to cytoplasmic ratio, *NSCC* non-small cell carcinoma, *NSCLC* non-small cell lung carcinoma, *CK* cytokeratin, *CEA* carcinoembryonic antigen, *EMA* epithelial membrane antigen, *TTF1* thyroid transcription factor 1, *CHR* chromogranin, *SYN* synaptophysin, *MSA* muscle-specific actin, *KRAS* Kirsten rat sarcoma oncogene, *EGFR* epithelial growth factor receptor, *TP53* tumor protein 53, *CTNNB1* catenin beta 1, *MDM2* mouse double minute 2 homolog

chromosomal rearrangement in the *NUT* gene is designated as NUT carcinoma.

Key Clinical Features

- Affects people of all ages, although, it was originally reported in children and younger adults
- Male equals female
- Fewer than 100 cases reported
- Usually presents at an advanced stage, with pleural effusion, chest pain, weight loss, and respiratory symptoms

Key Radiological Features

- Chest x-ray: extremely rapid-growing tumor, with complete opacification of the thorax within 2–8 weeks
- CT: a hypoattenuating, heterogeneously enhancing, often extensively necrotic mass with poorly defined, infiltrative borders. High fluorodeoxyglucose (FDG) uptake is characteristic.

Cytologic Findings

- Usually cellular smears
- Discohesive clusters and single of small to intermediate size, monomorphic cells with irregular nuclear membrane, granular to coarse chromatin pattern, and discrete nucleoli
- Mitoses, necrotic debris, and crush artifact are common.
- Representative image is shown in Fig. 6.29a.

Differential Diagnosis

- Poorly differentiated malignant neoplasms
- Basaloid squamous cell carcinoma
- Small cell carcinoma
- Ewing sarcoma
- Germ cell tumor
- Acute leukemia

Histology

- NUT carcinoma, also known as NUT midline carcinoma
- Usually composed of sheets and nests of small- to intermediate-size undifferentiated cells with a monomorphic appearance
- The tumor cells are primitive-appearing, with irregular nuclear membrane, distinct nucleoli, granular to coarse chromatin, and pale eosinophilic to basophilic cytoplasm.
- Neutrophil infiltration is a prominent feature.
- Foci of abrupt keratinization are characteristic.
- Representative image is shown in Fig. 6.29b.

Immunohistochemistry

- Speckled nuclear positivity in more than 50% of tumor cells with NUT antibody is a constant finding and is diagnostic.
- Broad-spectrum cytokeratins are positive in majority of cases.
- Other epithelial markers, such as epithelial membrane antigen (EMA), epithelial cell adhesion molecule (BerEP4), and carcinoembryonic (CEA) antigen showed variable results.
- Most of cases are positive for p63/p40 and CD34.
- Occasional reactivity to synaptophysin, chromogranin, and even TTF1 was observed.

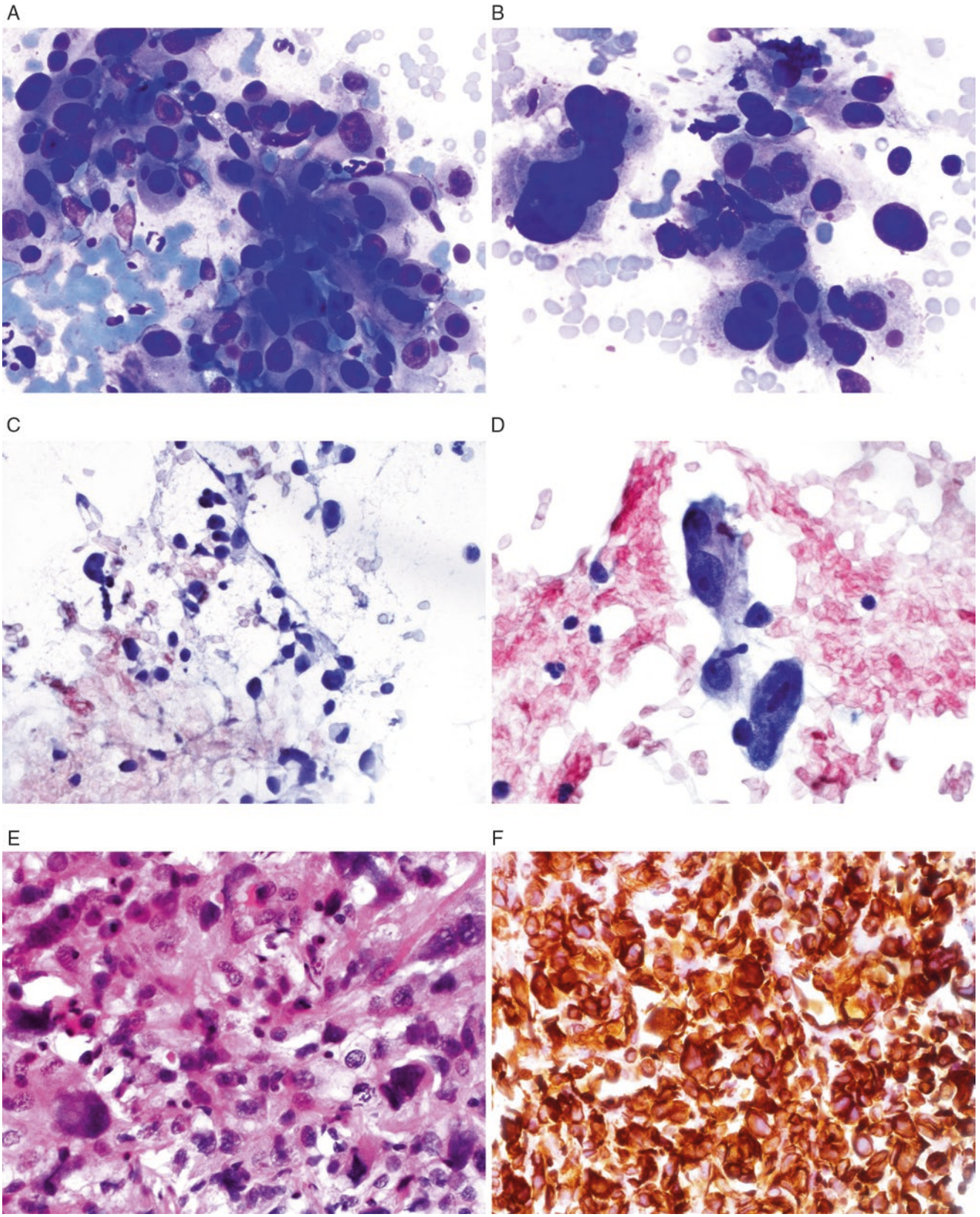

Fig. 6.28 Pleomorphic, spindle cell and giant cell carcinoma. (**a**, **b**) Discohesive, round to oval tumor giant cells with multiple large, irregular, lobulated nuclei and abundant eosinophilic cytoplasm, rare squamoid cells, and neutrophils are present, Diff-Quik; (**c**, **d**) spindled and giant tumor cells, Pap stain; (**e**) focal squamous differentiation is seen; spindled and giant tumor cells with abundant mitoses and necrosis (not shown), H&E; (**f**) vimentin positive, IHC; (**g**) AE1/3 positive, IHC; (**h**) focal CK5/6 positive, IHC

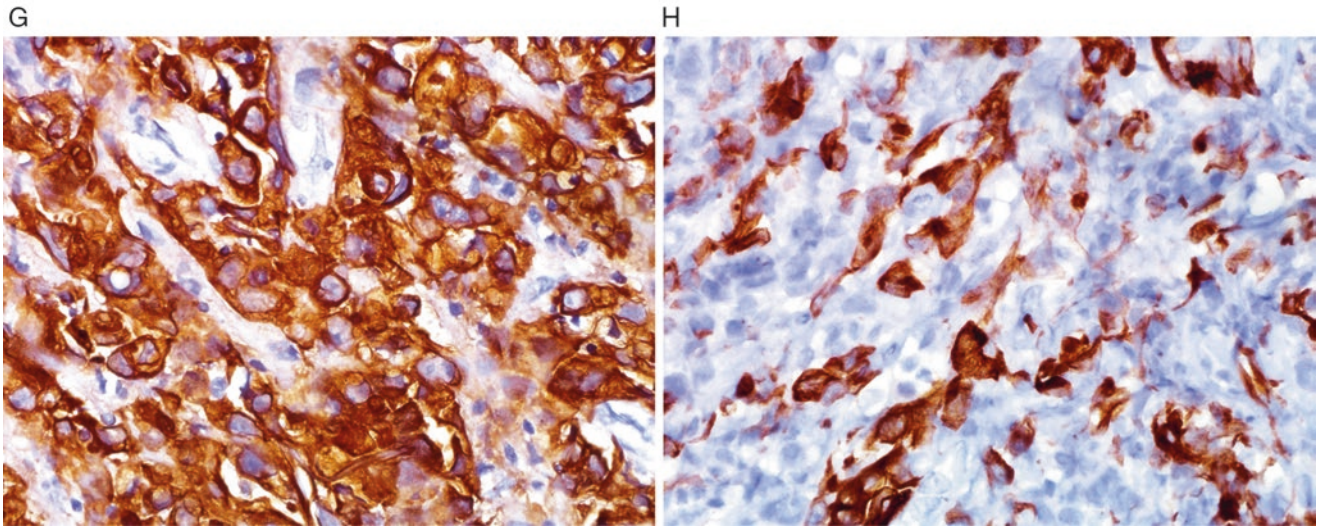

Fig. 6.28 (continued)

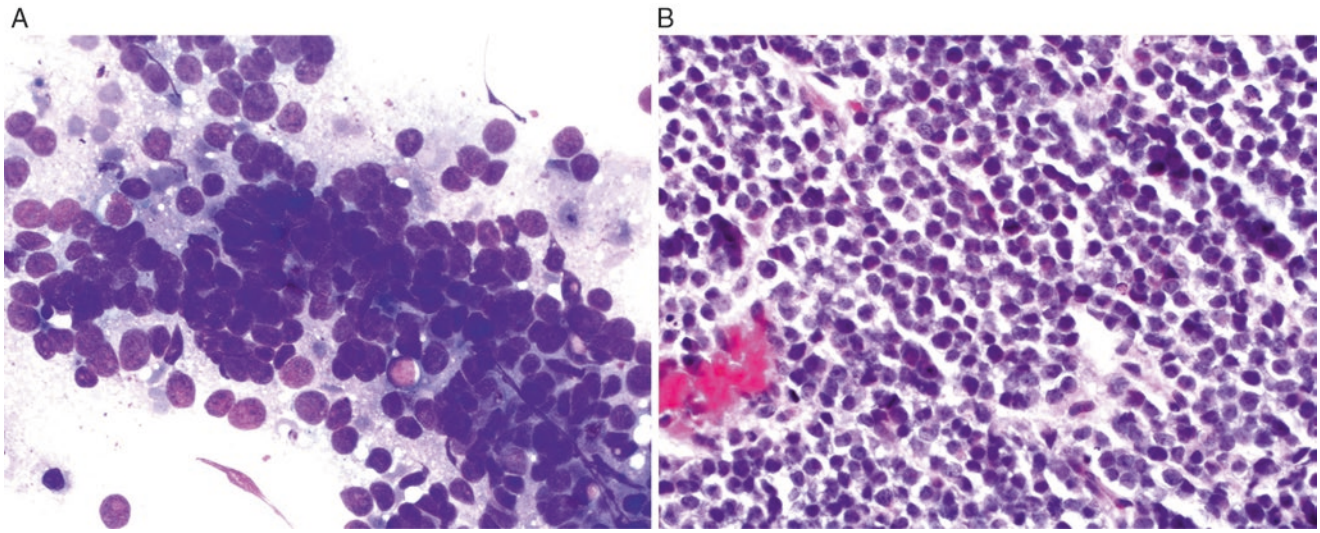

Fig. 6.29 NUT carcinoma. (**a**) Highly cellular smears contain discohesive clusters and single of small to intermediate sized, monomorphic cells, Diff-Quik; (**b**) sheets of small- to intermediate-sized, primitive-appearing cells with distinct nucleoli, granular to coarse chromatin, and pale eosinophilic to basophilic cytoplasm, H&E

- NUT carcinomas are defined by the presence of NUT gene rearrangement, which is a chromosomal translocation between the *NUT* gene (*NUTM1*) on chromosome 15q14 and other genes: Bromodomain containing *4* (*BRD4*) on chromosome 19p13.1 (70%), *BRD3* on chromosome 9q34.2 (6%), or an unknown partner gene (24%).

Metastasis to the Lung (See Chap. 7 on the Liver and Other Chapters)

The most common metastatic tumors are carcinomas, especially from the gastrointestinal tract, gynecological tract, breast, urothelial, head and neck, prostate, and other sites, followed by sarcomas, melanomas, and germ cell tumors. Sex and age distribution depend on tumor types, such as colorectal cancers in elderly patients of both sexes, breast cancer and melanoma in younger adults, and germ cell tumors and sarcomas in young adults or children. Metastases can be single or multiple, usually involving the lung parenchyma and the pleura. However, bilateral, multiple, peripherally located round, variable-size nodules (hematogenous metastases), or diffuse thickening of the interstitium (lymphangitic carcinomatosis) are typical.

Cytological features, histological findings, and immunoprofile are the same as the primary tumors. The differential immunophenotypes for the most common metastatic carcinomas are summarized in Table 6.7.

Table 6.7 Typical immunophenotypes of the common metastatic carcinomas

Antibody	Upper GI	Lower GI	Breast	GYN	Thyroid	Prostate	Urinary Bladder
CK7	+	−	+	+	+	−	+
CK20	+/−	+	−	−/+	−	−	+/−
TTF1	−	−	−	−	+	−	−
PAX8	−	−	−	+	+	−	−
GATA3	−	−	+	−	−	−	+
ER	−	−	+/−	+	−	−	−
SATB2	−	+	−	−	−	−	−
NKX3.1	−	−	−	−	−	+	−
Thyroglobulin	−	−	−	−	+	−	−
p63	−	−	−	−	−	−	+/−
CK5/6	−	−	−/+, F	−	−	−	+

− positive in <5% of cells, + positive in >70% of cells, +/− positive in 50–70% of cells, −/+ positive in <50% of cells
GI gastrointestinal tract, *GYN* gynecologic, *CK* cytokeratin, *TTF1* thyroid transcription factor-1, *PAX8* paired box gene 8, *GATA3* GATA binding protein 3, *ER* estrogen receptor, *SATB2* special AT-rich sequence-binding protein 2, *NKX3.1* NK3 homeobox 1, *F* focal

Table 6.8 2015 World Health Organization (WHO) classification of tumors of the pleura

Mesothelial tumors	*Mesenchymal tumors*
Diffuse malignant mesothelioma	Epithelioid hemangioendothelioma
Epithelioid mesothelioma	Angiosarcoma
Sarcomatoid mesothelioma	Synovial sarcoma
Desmoplastic mesothelioma	Solitary fibrous tumor
Biphasic mesothelioma	Malignant solitary fibrous tumor
Localized malignant mesothelioma	Desmoid-type fibromatosis
Epithelioid mesothelioma	Calcifying fibrous tumor
Sarcomatoid mesothelioma	Desmoplastic round cell tumor
Biphasic mesothelioma	
Well-differentiated papillary mesothelioma	
Adenomatoid tumor	
Lymphoproliferative disorders	
Primary effusion lymphoma	
Diffuse large B-cell lymphoma associated with chronic inflammation	

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Pleura

The WHO classifications of tumors of the pleura, 2015, are summarized in Table 6.8.

Mesothelioma

Key Clinical Features

- Usually occurs in older adults, ≥60 years
- More often in men
- A strong association with asbestos exposure
- Pleural effusion is very common.

Key Radiological Features

- Variety of presentations, classically exhibiting a diffuse circumferential ring of nodular pleura associated with ipsilateral effusion
- Less commonly presents with pleural effusion without obvious pleural nodularity
- Pleural plaques, especially associated with calcifications, are suggestive of asbestos exposure.

Cytological Features

- Hypercellular specimen with mixed epithelial and spindle cells.
- Can be epithelial or spindle cell dominate.
- Resemble ordinary mesothelial cells.
- Tumor giant cells, granulomas, and psammoma can be seen.
- Representative images are shown in Fig. 6.30a–c.

Differential Diagnosis

- Pulmonary ADC (Table 6.9)
- Metastatic carcinoma
- Reactive mesothelial cells (Table 6.10)

Histology

Epithelioid Mesotheliomas

- Account for 60–80% of malignant mesotheliomas
- Variety of histological patterns. Most common patterns: solid, tubulopapillary, and trabecular; less common patterns: micropapillary, adenomatoid (microcystic), clear cell, transitional, deciduoid, and small cell

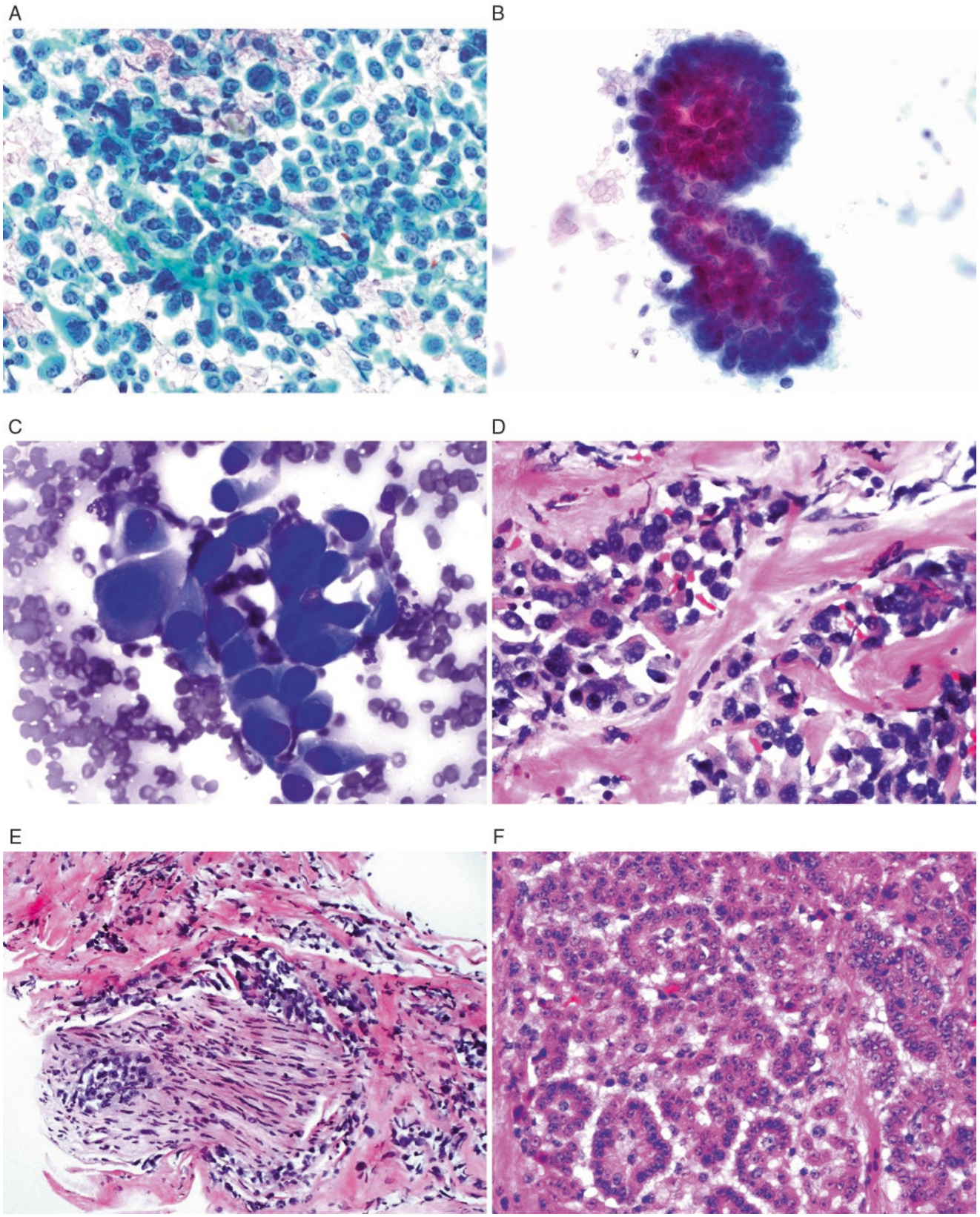

Fig. 6.30 Mesothelioma cytology and histopathology. (**a**) Cellular smears contain single and loosely clusters of plump and spindled cells, Pap stain; (**b**) epithelioid cells in papillary configuration, Diff-Quik; (**c**) atypical epithelioid cells, Diff-Quik; (**d**) histopathology, mesothelioma in nested pattern infiltrating dense fibrous tissue, H&E; (**e**) histopathology, perineural invasion, H&E; (**f**) histopathology, mesothelioma with papillary pattern, H&E

Table 6.9 Distinction of lung adenocarcinoma from mesothelioma

Markers	Mesothelioma	Adenocarcinoma
Calretinin	+	–
Cytokeratin 5/6	+	–
WT1	+	–
D2–40	+	–
MOC-31	–	+
CEA	–	+
TTF1	–	+
LeuM1	–	+
BerEP4	–	+
B72.3	–	+

"+" positive in majority of cases, "-" negative in majority of cases
Note: Two positive markers and two negative markers are usually needed to make a diagnosis of mesothelioma: (1) Two positive markers: calretinin and cytokeratin 5/6 (or Wilms tumor 1[WT1]); (2) two negative markers: CEA and MOC-31 (or B72.3)
WT1 Wilms tumor 1, *D2–40* podoplanin, *MOC-31* epithelial-related antigen, *CEA* carcinoembryonic antigen, *TTF1* thyroid transcription factor 1, *LeuM1* cluster of differentiation (CD)15, *BerEP4* epithelial cell adhesion molecule, *B72.3* tumor-associated glycopotein (TAG)72

Table 6.10 Benign mesothelial cells vs. mesothelioma

Markers	Benign mesothelial cells	Mesothelioma
GLUT1	–/+	+
CD146	–	+
IMP3	–/+	+
E-Cadherin	–	+
EMA	+/–	+
p53	–/+	+
Desmin	+	–/+
p16	+/–	–/+

– positive in <5% of cells, + positive in >70% of cells, +/– positive in 50–70% of cells, –/+ positive in <50% of cells
GLUT1 glucose transporter 1, *CD146* cluster of differentiation 146, *IMP3* insulin-like growth factor II messenger RNA binding protein-3, *EMA* epithelial membrane antigen

- The tumor cells are usually bland, with eosinophilic cytoplasm and a bland vesicular chromatin pattern. Occasional anaplastic forms may be observed.
- The fibrous stroma can vary from scant to prominent, showing varying degree of cellularity from hyalinized acellular to highly cellular stroma. Myxoid changes may be seen in 5–10% of cases, with nests of bland-looking, vacuolated epithelioid cells floating in the matrix.
- Representative images are shown in Fig. 6.30d–f.

Sarcomatoid Mesotheliomas

- Characterized by a proliferation of spindle cells arranged in fascicles or with a haphazard pattern and involves the adipose tissue of the parietal pleura or the adjacent lung parenchyma.
- The tumor cells show variety of morphology, from plump to thin, elongated cells with scant cytoplasm.
- The degree of nuclear atypia, mitotic activity, and necrosis vary from minimal to moderate to marked.

Desmoplastic Mesotheliomas

- Characterized by areas of atypical spindle cells arranged in a so-called patternless pattern within a dense, hyalinized, fibrous stroma constituting at least 50% of the tumor
- Invasion of adipose tissue is the most reliable criterion to distinguish desmoplastic mesothelioma from organizing pleuritis.

Biphasic Mesotheliomas

- Show any combination of the patterns described above constituting at least 10% of the tumor

Immunohistochemistry

- Positive for CK AE1/3, calretinin, CK5/6, WT1, and D2–40.
- Negative for BerEP4, MOC-31, B72.3, and CEA.
- See Tables 6.8 and 6.9 for differential diagnosis.
- Multiple chromosomal alterations, more common with chromosomal losses, especially on chromosomal arms 1p, 3p, 4q, 6q, 9p, 13q, 14q, and 22q; alterations in several tumor suppressor genes, including neurofibromin 2 (NF2), CDKN2A (p16INK4a), CDKN2B (p15INK4b), and BRCA1-associated protein 1 (BAP1).
- Representative images are shown in Fig. 6.31a–f.

Mesenchymal Tumors

Solitary Fibrous Tumor

Key Clinical Features

- Most common in sixth to seventh decades
- No sex predisposition
- Accounting for <5% of primary pleural tumors
- Unknown etiology
- Slow-growing, relatively benign tumors, up to 10% malignant
- Usually asymptomatic, incidental finding

Key Radiological Features

- Usually solitary, well-circumscribed mass arising from visceral pleura
- Can be multiple and distributed throughout the pleural cavity

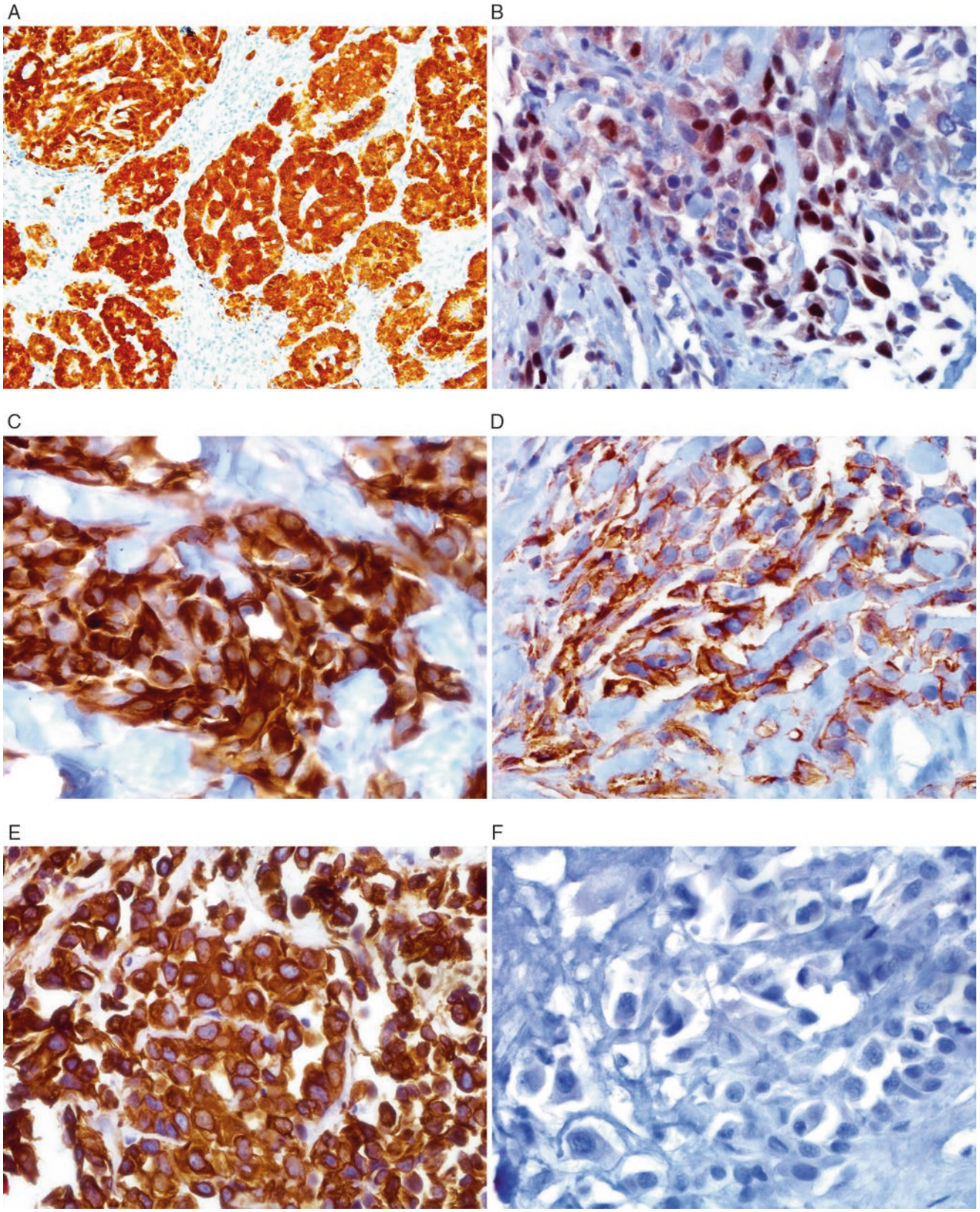

Fig. 6.31 Mesothelioma, immunophenotype. (**a**) Calretinin decorates tumor cells, IHC; (**b**) positive for WT-1, IHC; (**c**) CK5/6 positive, IHC; (**d**) positive for GLUT1, IHC; (**e**) positive for AE1/3, IHC; (**f**) MOC31 negative, IHC

Cytological Features

- Cellular smear with bland-appearing, small, oval to spindled cells
- No mitosis or necrosis
- Representative images are shown in Fig. 6.32a, b.

Differential Diagnosis

- Neuroendocrine tumors
- Synovial sarcoma
- Sarcomatoid mesothelioma
- Nerve sheath tumor
- Type A thymoma

Histology

- Uniform fibroblastic spindle cells with tapering nuclei, scant, pale, indistinct cytoplasm, variable cellularity, and patternless architecture.
- Focal storiform or fascicular growth pattern may be seen.
- Prominent stromal and perivascular hyalinization.
- Branching hemangiopericytoma-like vessels of varying size and number.
- Usually <3 mitoses/per 2 mm^2; malignant: > 4 mitoses/per 2 mm^2, rarely showing dedifferentiation.
- Representative images are shown in Fig. 6.32c, d.

Immunohistochemistry

- Positive for signal transducer and activator of transcription 6 (STAT6): > 95% of cases, specific.
- Positive for CD34, B-cell CLL/lymphoma 2 (Bcl2), and CD99: nonspecific.
- Occasionally may be positive for SMA, epithelial membrane antigen (EMA), keratin, S100, or desmin.
- Representative images are shown in Fig. 6.32e, f.
- The tumor harbors characteristic gene fusion NGFI-A binding protein 2 (*NAB2)-STAT6*.

Mediastinum

Overview

The mediastinum can be divided into four hypothetical compartments: superior, anterior, middle, and posterior. The distribution of organs and tumors of the mediastinum is summarized in Table 6.11.

In adults, the distribution of tumors is as follows: 46% thymic epithelial tumors, 23% lymphomas, 16% endocrine tumors, and 15% germ cell tumors. In children, the frequency of tumors is 47% neurogenic neoplasms, 19% germ cell tumors, 12% lymphomas, 10% thymic epithelial tumors, 6% cysts, and 6% mesenchymal tumors. The 2015 WHO classification of tumors of the thymus is summarized in Table 6.12.

Selected entities will be discussed in the section below.

Cysts

Key Clinical Features

- Bronchogenic cyst –any part of the mediastinum
- Enteric cyst – posterior mediastinum
- Thymic cyst – anterior mediastinum
- Pericardial cyst –middle mediastinum

Cytological Features

- Hypocellular smear
- Dependent upon the type of the cyst

Epithelial Tumors

Thymoma

Key Clinical Features

- Rare malignancy overall, but the most common mediastinal tumors in adults
- Median age of 50 years, rare in children
- May present as a mass lesion or an incidental finding
- May be associated with myasthenia gravis, pure red cell aplasia, hypogammaglobulinemia (Good syndrome), and/or other autoimmune disorders

Key Radiological Features

- Usually a mass lesion, well-circumscribed or invasive borders, depending on the subtypes of thymoma

Cytological Features

- Usually moderate to hypercellular smear
- Two populations of relatively bland neoplastic epithelial cells and lymphocytes
- The proportion of epithelial cells and lymphocytes is dependent upon subtype.
- In epithelial subtype, epithelial cells are dominant; in lymphocyte-predominant subtype, small mature lymphocytes are dominant; and in spindle cell type, spindle epithelial cells are predominant.
- Neoplastic epithelial cells tend to be cohesive and have a delicate nuclear membrane, fine nuclear chromatin, and small nucleoli.
- Few or no mitoses are present.
- Representative images are shown in Fig. 6.33a–f.

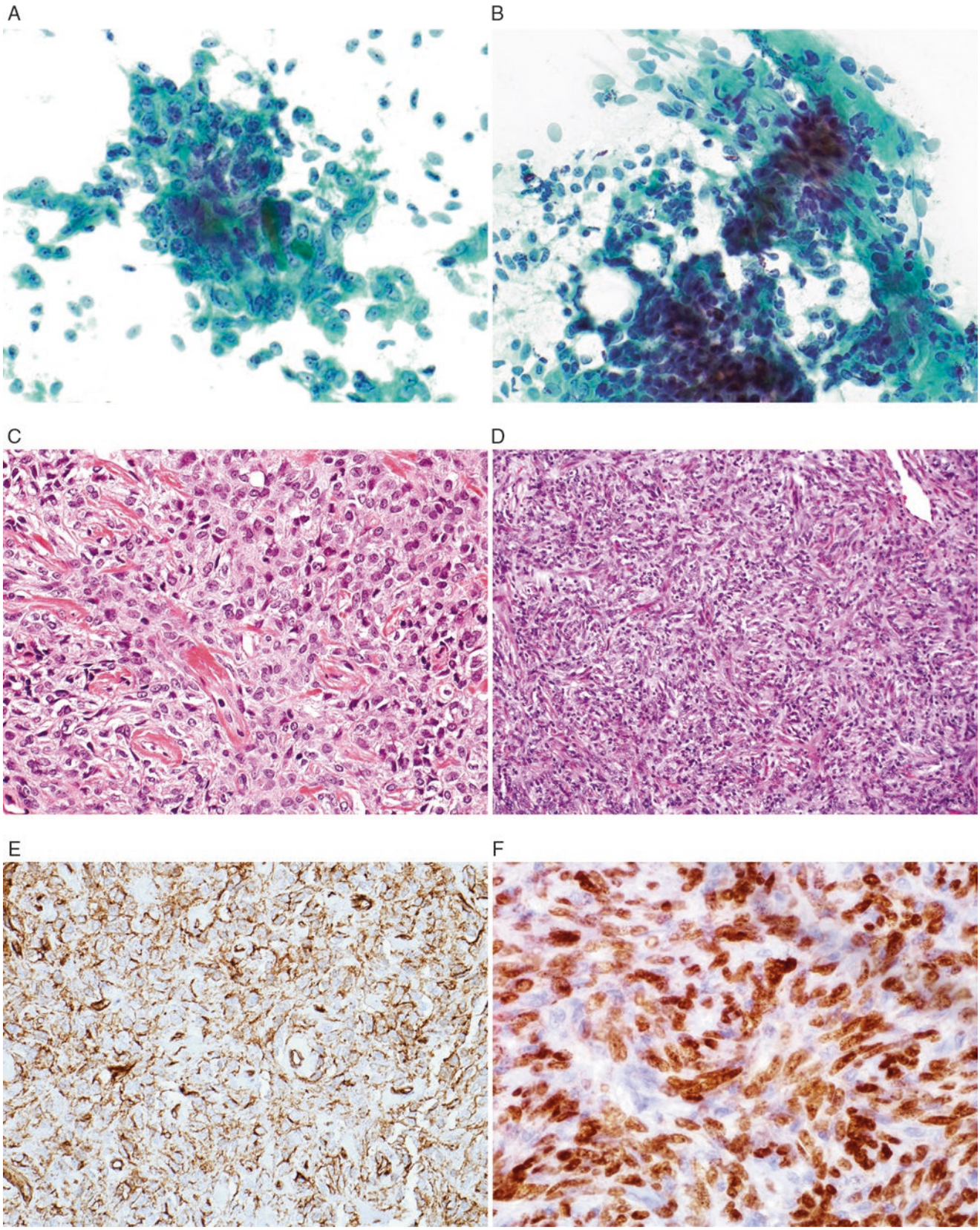

Fig. 6.32 Solitary fibrous tumor. (**a**, **b**) Cellular smear with bland-appearing, small, oval to spindled cells, Pap stain; (**c**, **d**) surgical resection tissue showing patternless spindle cells with significant hyalinized stroma, H&E; (**e**) diffuse CD34 positivity, IHC; (**f**) positive for STAT6, IHC

Table 6.11 Distribution of organs and tumors of the mediastinum

Superior					
Thymus (thymoma, cyst, carcinoid, lymphoma) *Lymph node* (metastases, lymphoma, inflammation) *Thyroid* (goiter, neoplasm) *Parathyroid* (adenoma, cyst) *Other* (cysts, aneurysm)					
Anterior	*Thymus* (thymoma, cyst, carcinoid, lymphoma) *Germ cell tumors* *Lymph node* (metastases, lymphoma, inflammation) *Thyroid* (goiter, neoplasm) *Parathyroid* (adenoma, cyst) *Paraganglia tumors* *Other* (angioma, lipoma, other mesenchymal tumors	Middle	*Cysts* (pericardial, bronchogenic) *Lymph node* (metastases, lymphoma, inflammation) *Other* (atrial myxoma, mediastinal fibrosis)	Posterior	*Neurogenic* (nerve sheath and sympathetic, paraganglioma) *Lymph node* (metastases, lymphoma, inflammation) *Cyst* (gastroenteric) *Other* (esophageal lesions, abscess, aneurysm, meningocele)

Table 6.12 2015 World Health Organization (WHO) classification of tumors of the thymus

Epithelial tumors	
Thymoma Type A thymoma, including atypical variant Type AB thymoma Type B1 thymoma Type B2 thymoma Type B3 thymoma Micronodular thymoma with lymphoid stroma Metaplastic thymoma Other rare thymoma Microscopic thymoma Sclerosing thymoma Lipofibroadenoma Thymic carcinoma Squamous cell carcinoma Basaloid carcinoma Mucoepidermoid carcinoma Lymphoepithelioma-like carcinoma Clear cell carcinoma Sarcomatoid carcinoma Adenocarcinomas Papillary adenocarcinoma Thymic carcinoma with adenoid cystic carcinoma-like features Mucinous adenocarcinoma Adenocarcinoma, not otherwise specified (NOS)	Thymic carcinoma (continued) NUT carcinoma Undifferentiated carcinoma Other rare thymic carcinomas Adenosquamous carcinoma Hepatoid carcinoma Thymic carcinoma, NOS Thymic neuroendocrine tumors Carcinoid tumors Typical carcinoid Atypical carcinoid Large cell neuroendocrine carcinoma Combined large cell neuroendocrine carcinoma Small cell carcinoma Combined small cell carcinoma Combined thymic carcinomas
Germ cell tumors of the mediastinum	
Seminoma Embryonal carcinoma Yolk sac tumor Choriocarcinoma Teratoma Teratoma, mature Teratoma, immature	Mixed germ cell tumors Germ cell tumors with somatic-type solid malignancy Germ cell tumors with associated hematological malignancy
Lymphomas of the mediastinum	
Primary mediastinal large B-cell lymphoma Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) Other mature B-cell lymphomas T lymphoblastic leukemia/lymphoma	Anaplastic large cell lymphoma (ALCL) and other rare mature T- and natural killer (NK)-cell lymphomas ALCL, ALK-positive (ALK+) ALCL, ALK-negative (ALK-) Hodgkin lymphoma B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell and classical Hodgkin lymphoma
Histiocytic and dendritic cell neoplasms of the mediastinum	
Langerhans cell lesions Thymic Langerhans cell histiocytosis Langerhans cell sarcoma Histiocytic sarcoma	Follicular dendritic cell sarcoma Interdigitating dendritic cell sarcoma Fibroblastic reticular cell tumor Indeterminate dendritic cell tumor

(continued)

Table 6.12 (continued)

Myeloid sarcoma and extramedullary acute myeloid leukemia	
Soft tissue tumors of the mediastinum	
Thymolipoma	Vascular neoplasms
Lipoma	Lymphangioma
Liposarcoma	Hemangioma
Well-differentiated	Epithelioid hemangioendothelioma
Dedifferentiated	Angiosarcoma
Myxoid	Neurogenic tumors
Pleomorphic	Tumors of peripheral nerves
Solitary fibrous tumor	Ganglioneuroma
Malignant	Ganglioneuroblastoma
Synovial sarcoma	Neuroblastoma
Synovial sarcoma, NOS	
Synovial sarcoma, spindle cell	
Synovial sarcoma, epithelioid cell	
Synovial sarcoma, biphasic	
Ectopic tumors of the thymus	
Ectopic thyroid tumors	
Ectopic parathyroid tumors	
Other rare ectopic tumors	

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Differential Diagnosis

- Thymic carcinoma
- Thymic hyperplasia
- T-cell lymphoblastic lymphoma
- Neuroendocrine neoplasm

Histology

- Type A thymoma:
  1. Complete or incomplete fibrous capsule may display coarse lobulation with thick fibrous bands.
  2. Microcystic pattern is most common, more prominent in subcapsular areas.
  3. Other patterns: rosettes (with or without a central lumen), glandular or glomeruloid structures, Masson's hemangioma-like papillary projections in cystic spaces, meningioma-like whorls, fascicular growth, and storiform growth.
  4. Hassall corpuscles are absent.
  5. The tumor cells are spindled and/or oval-shaped with bland nuclei, finely dispersed powdery chromatin, and inconspicuous nucleoli.
  6. Low mitotic activity, usually <4 mitoses/per 2 mm^2.
  7. No or very few immature lymphocytes.
  8. When hypercellularity, increased mitotic counts, and focal necrosis are present, designate as atypical type A thymoma variant.
  9. Representative images are shown in Fig. 6.34a, b.
- Type AB thymoma:
  1. Well-demarcated and encapsulated tumor showing a lobulated growth pattern.
  2. Highly variable mixture of a lymphocyte-poor type A component and a more lymphocyte-rich type B-like component including a prominent infiltrate (often in dense clusters) of immature, TdT+ T cells. Infiltrate of TdT+ lymphocytes in >10% of the tumor area is a feature used to distinguish from type A thymoma.
  3. The tumor cells are small, oval to plump, spindled, or polygonal shaped, containing round to oval pale-staining nuclei showing dispersed chromatin and inconspicuous nucleoli.
  4. Medullary islands are very rare; Hassall corpuscles are absent.
  5. Summary of three distinctive features of type AB thymoma: (a) an admixed spindle cell-predominant, lymphocyte-poor component and lymphocyte-rich component; (b) bland, spindly, oval, and focally polygonal thymic epithelial cells; and (c) a focal or diffuse abundance of immature T cells.
- Type B1 thymoma:
  1. Thymus-like architecture with predominance of cortical areas.
  2. Either absence of lobulation or lobulated with larger lobules traversed by hypocellular, collagenous septa.
  3. The neoplastic epithelial cells are individually embedded in densely packed nonneoplastic immature lymphocytes; the epithelial cells have poorly defined, pale

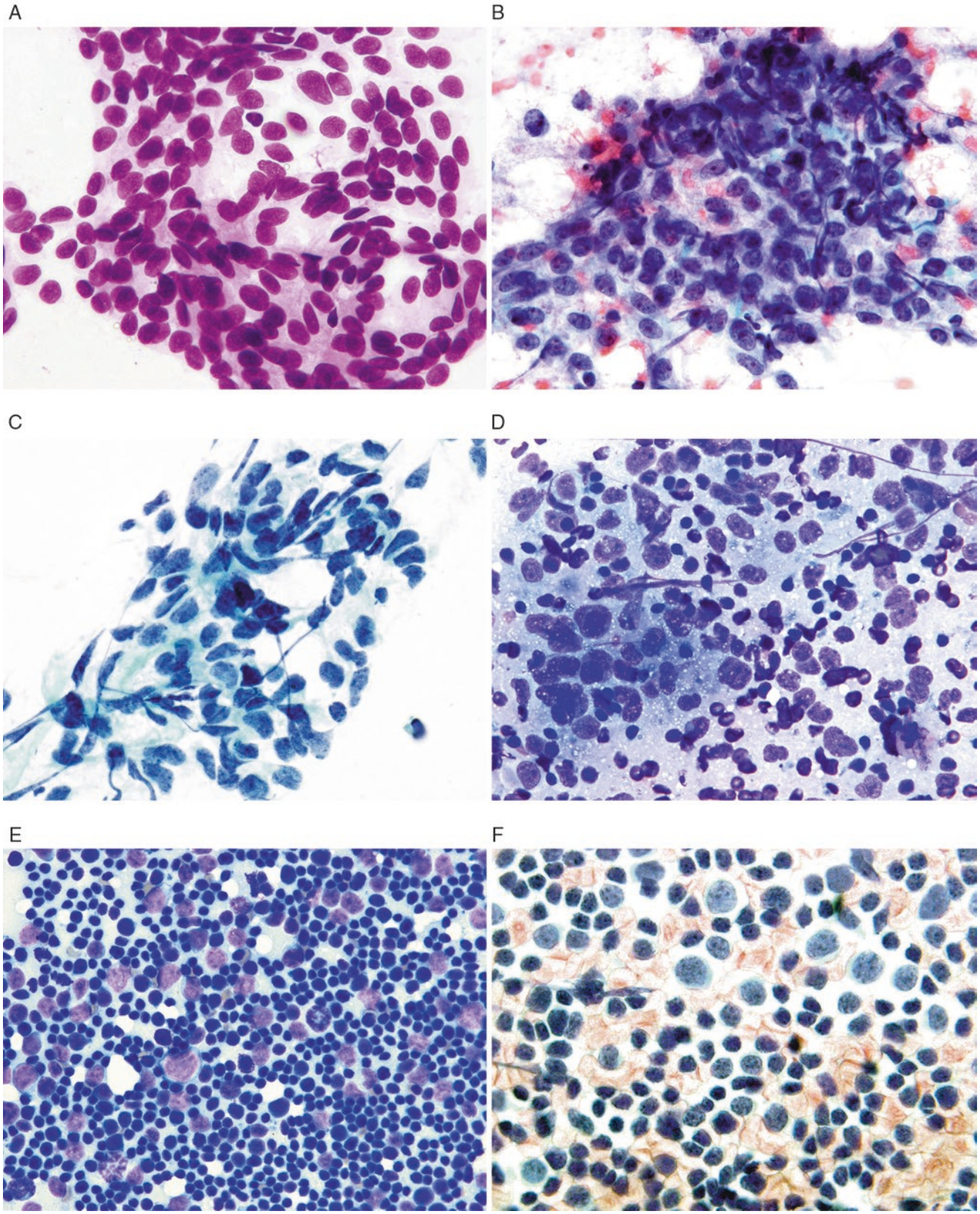

Fig. 6.33 Thymoma, cytology. (**a**, **b**) Epithelial prominence, showing round to slightly oval nuclei with vesicular chromatin and small but prominent nucleoli, Diff-Quik (**a**) and Pap (**b**) stains; (**c**) spindle cell predominant, bland-appearing, Pap stains; (**d**) mixed epithelial and lymphocytes, Diff-Quik; (**e**, **f**) lymphocyte predominant, Diff-Quick (E) and Pap (F) stains

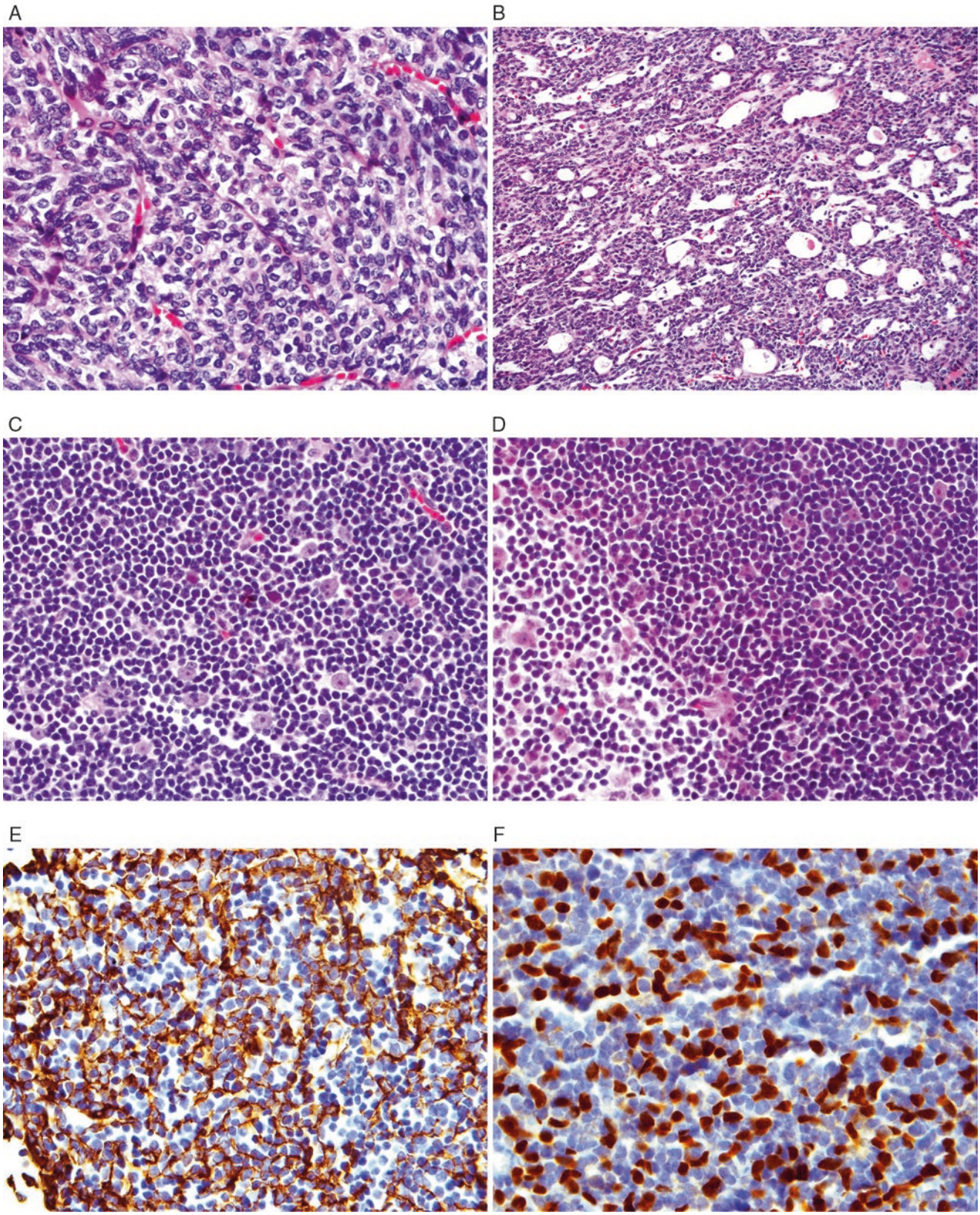

Fig. 6.34 Thymoma, histopathology. (**a**) Spindle cell predominant, type A, H&E; (**b**) type A, microcystic growth pattern, H&E; (**c**) lymphocyte-predominant, individual embedded epithelial tumor cells, type B1, H&E; (**d**) type B1, pale-stained medullary island left lower corner), H&E; (**e**) cytokeratin AE1/3 decorates tumor epithelial cells, IHC; (**f**) p63 positive in epithelial tumor cells, IHC; (**g**) TdT-positive mature T lymphocytes, IHC; (**h**) positive for CD5, mature T lymphocytes, IHC

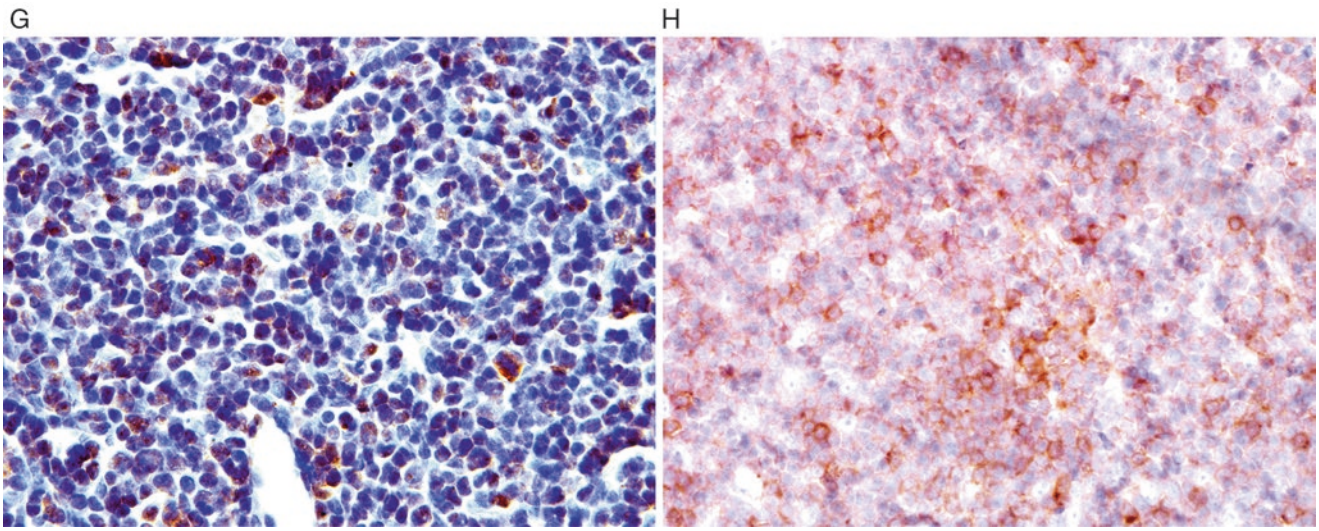

Fig. 6.34 (continued)

eosinophilic cytoplasm, and relatively uniform, oval to slightly irregular round nuclei, showing pale chromatin, distinct nuclear membranes, and small central nucleoli.
4. Medullary islands are always present and may contain Hassall corpuscles and myoid cells; perivascular spaces are often seen.
5. Summary of two distinctive features of type B1 thymoma: (a) close resemblance to the normal, non-involuted thymic cortex; (b) the consistent presence of medullary islands.
6. Representative images are shown in Fig. 6.34c, d.

- Type B2 thymoma:
  1. Encapsulated tumor, composed of lymphocyte- predominant, irregular size and shape tumor lobules surrounded by delicate fibrous septa, giving a lobular architecture and blue appearance on hematoxylin and eosin (H&E) stain.
  2. Interspersed among the lymphoid cells are single or poorly defined clusters of epithelial cells (≥ 3 cells).
  3. The epithelial cells have round or slightly oval nuclei with vesicular chromatin and small but prominent nucleoli; rare focal anaplasia may be seen.
  4. Perivascular spaces composed of a central venule surrounded by a clear space containing proteinaceous fluid or lymphocytes.
  5. Hassall corpuscles are rare; medullary islands are uncommon.
  6. Summary of distinctive features of type B2 thymoma: (a) polygonal (non-spindle shaped) neoplastic thymic epithelial cells in clusters; (b) high content of intermingled immature T cells.
- Type B3 thymoma:
  1. Tumor lobules separated by fibrous septa, pushing borders at the invasion front, prominent perivascular spaces with epithelial palisading, rare Hassall corpuscles.
  2. Tumor cells are polygonal with eosinophilic or clear cytoplasm and slightly/moderately atypical, round to elongated, grooved, or raisinoid nuclei.
  3. Summary of distinctive features of type B3 thymoma: (a) predominance of polygonal epithelial cells forming solid sheets, resulting in a pink appearance on H&E; (b) paucity of admixed nonneoplastic immature T cells.

Immunohistochemistry

- Epithelial cells: usually positive for AE1/3, CK7, CK19, p63, PAX8, forkhead box N1 (FOXN1), CD57, and CD205; negative for EMA, CD117, and CD5.
- Small lymphocytes (thymocytes) are mature T-cells, but positive for CD3, CD5, TdT, CD99, with a high MIB1 (Ki-67) proliferative index.
- Representative images are shown in Fig. 6.34e–h.

Thymic Carcinomas

Thymic carcinomas include of a variety of histological types of tumors, accounting for approximately 22% of all thymic epithelial neoplasms. The squamous cell carcinomas are the most common type, accounting for approximately 70% of all thymic carcinoma cases. Lymphoepithelioma-like carcinomas are rare, accounting for 6–32% of all thymic carcinomas; sarcomatoid carcinomas account for 5–10%; and basaloid carcinomas account for <5%. Other types, such as mucoepidermoid carcinomas, clear cell carcinomas, ADCs,

Table 6.13 Features of thymic carcinomas of the most common types

	Squamous cell carcinoma	Lymphoepithelioma-like carcinoma	Sarcomatoid carcinoma
Clinical features	1. 70% of all cases 2. Most common in sixth decade 3. Men slightly more than women 4. <5% associated with myasthenia gravis	1. 6–32% of all cases 2. Median age: 41; bimodal peaks at 14 and 48 years 3. Male/female: 2:1 4. Association with EBV in ~50% of cases, especially in children and young adults	1. 5–10% of all cases 2. In late adulthood, fourth–eighth decades, mean 47 years 3. Unknown etiology
Radiographic features	Usually an anterior mediastinal mass, often with poorly defined, irregular lobular margins, or with necrotic/cystic changes, which on CT appear as low-attenuation regions, on MRI with greater tumor heterogeneity	1. a large mass in the anterior mediastinum with necrosis (low-attenuation on CT) 2. Often invading surrounding tissues: pleura, lung, diaphragm, and pericardium	1. Usually a large, lobulated mass in the anterior mediastinum with necrosis (low-attenuation on CT) 2. Often invading surrounding tissues: pleura, lung, diaphragm, and pericardium; with encroachment on the major vessels
Cytologic features	Single or clusters of large atypical cells with enlarged nuclei showing coarse chromatin and macronucleoli and a moderate amount of cytoplasm (Fig. 6.35)	1. Malignant epithelial cells: clusters of large, relatively uniform cells with vesicular nuclei, one or more distinct nucleoli, indistinct cell borders; polygonal squamoid cells in some 2. Many lymphocytes and plasma cells	1. Type A thymoma component: spindled epithelial cells with bland nuclei, powdery chromatin, and inconspicuous nucleoli 2. Malignant spindle cells: coarse chromatin, prominent nucleoli, and frequent mitoses; may show heterologous sarcomatous fragments
Histology	1. Infiltrative, usually smooth contoured sheets, islands, and cords of large polygonal cells accompanied by broad zones of desmoplastic to sclerohyaline stroma 2. Polygonal tumor cells: large vesicular or hyperchromatic nuclei, distinct nucleoli, eosinophilic cytoplasm, and vague to obvious intercellular bridges; may be keratinized with keratin whorls mimic Hassall corpuscles 3. Foci of coagulative necrosis are common	1. Anastomosing sheets, nests, and cords of CA cells accompanied by dense lymphocytes and plasma cells 2. CA cells: large vesicular nuclei, one or more distinct nucleoli, and indistinct cell borders; the nuclei are relatively uniform, crowded, overlapping 3. Focal squamous differentiation 4. Coagulative necrosis 5. Many lymphocytes and plasma cells; may see germinal center, eosinophils, and granulomas	1. Usually a mixture of conventional type A thymoma and areas of malignant spindle cells 2. Gradual or abrupt transition between the two 3. Malignant cells: coarse chromatin. Prominent nucleoli, frequent mitoses 4. May see small areas of lymphoepithelioma-like CA or prominent squamous differentiation 5. May have heterologous mesenchymal elements
IHC	1. Positive for CKs, p63 (83%), PAX8 (~75%); CD5, CD117, GLUT1, and MUC1 (~80%); FOXN1 (68–76%), CD205 (10–59%) 2. Focal expression of NE markers: 64% cases 3. Lack TdT+ lymphocytes	1. for panCKs, p63, CD117, and CD5 (variable) 2. Negative for CK7 and CK20 3. Admixed CD3+/TdT- T cells and B cells; the plasma cells are polytypic for Ig.	1. Type A thymoma component: same as conventional 2. Malignant spindle cells: positive for CK, EMA; may or may not be CD5-positive 3. The heterologous sarcomatous elements express markers for their differentiation
Molecular Genetics	1. Chromosomes loss (16q, 6, 3p, and 17p); gain (1q, 17q, and 18) 2. Mutations of *KIT* (11%), *TP53* (~20%) 4. Copy number gain of *Bcl2*, loss of *p16 (CDKN2A)* 5. Missense mutation of *GTF2I* gene: <10%	1. EBV is almost always positive in children and young adults, uncommon positive in adults >30 years 2. The most reliable test: in situ hybridization for EBV-encoded small RNA	Information limited

EBV Epstein-Barr virus, *CT* computed tomography, *MRI* magnetic resonance imaging, *CA* carcinoma, *IHC* immunohistochemistry, *CK(s)* cytokeratin(s), *CD* cluster of differentiation, *GLUT1* glucose transporter 1, *MUC1* mucin 1, *FOXN1* forkhead box N1, *NE* neuroendocrine, *TdT* terminal deoxynucleotidyl transferase, *Ig* immunoglobulin, *EMA* epithelial membrane antigen, *KIT* a type III transmembrane receptor tyrosine kinase, *TP53* tumor protein 53, *Bcl2* B-cell CLL/lymphoma 2, *CDKN2A* cyclin-dependent kinase inhibitor 2A, *GTF2I* general transcription factor IIi, *RNA* ribonucleic acid

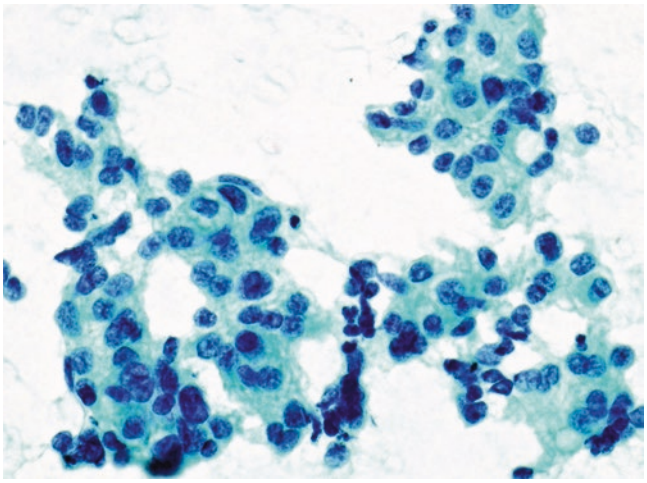

Fig. 6.35 Thymic carcinoma, showing single and clusters of large atypical cells with enlarged nuclei, coarse chromatin, macronucleoli, and moderate amount of cytoplasm, Pap stain

NUT carcinomas, and undifferentiated carcinomas, are very few. The characteristic features of the three most common types are summarized in Table 6.13.

Thymic Neuroendocrine Tumors

Thymic neuroendocrine tumors are categorized into two major groups: (1) low-grade typical carcinoids and intermediate-grade atypical carcinoids, which always show characteristic morphological and immunohistochemical neuroendocrine features, and (2) high-grade LCNECs and small cell carcinomas, which may lack some neuroendocrine features.

Key Clinical Features

- No established association with smoking.
- More aggressive clinical course: propensity for recurrence, lymph node or distant metastases, and tumor-associated death.
- Atypical carcinoids account for the majority of the tumors.
- Only carcinoids reported in setting of multiple endocrine neoplasia type 1 (MEN1).
- Average age at presentation: 49 years.
- Carcinoids show a strong male predominance.

Key Radiological Features

- Typically a lobulated, heterogeneous mass in the anterior mediastinum

Cytological Features

- Same as pulmonary neuroendocrine tumors; please refer that section in this chapter.
- Representative images are shown in Fig. 6.36a–i.

Differential Diagnosis

- Pulmonary neuroendocrine tumors: careful clinical and radiological correlation is the primary way to distinguish pulmonary from thymic neuroendocrine tumors.
- Thymoma, especially type A, to distinguish from carcinoids.
- Thymic carcinoma usually shows focal and/or weak expression of neuroendocrine markers.

Histology

The same as pulmonary neuroendocrine tumors, respectively.

Immunohistochemistry

Same as the pulmonary neuroendocrine tumors, respectively.

Germ Cell Tumors of the Mediastinum

Key Clinical Features

- Accounts for 3–4% of all germ cell tumors; up to 16% of all mediastinal tumors
- In prepubertal patients (mean <8 years): teratomas (58%), yolk sac tumors +/− teratoma (42%)
- In postpubertal female patients: teratoma (93%), seminoma (dysgerminoma) (4%), embryonal carcinoma (2%), and others (<1%)
- In postpubertal male patients (mean 25–29 years): teratoma (35%), seminoma (32%), mixed (16%), yolk sac tumor (10%), embryonal carcinoma (4%), and choriocarcinoma (3%)
- In patients with Klinefelter syndrome: mediastinal non-seminomatous germ cell tumors particularly common but not seminomas or testicular germ cell tumors
- Depending on tumor type, may have increased serum levels of the β-subunit of human chorionic gonadotropin (βhCG), α-fetoprotein (AFP), and lactate dehydrogenase

Key Radiological Features

- Anterior mediastinal mass: uncalcified and homogeneous for seminomas, heterogeneous with central attenuation, and a frond-like periphery for non-seminomatous germ cell tumors.
- Multilocular cystic lesions can accompany any type of mediastinal germ cell tumor (particularly seminomas), also others, such as thymomas, thymic carcinomas, Hodgkin or non-Hodgkin lymphomas, and metastases.

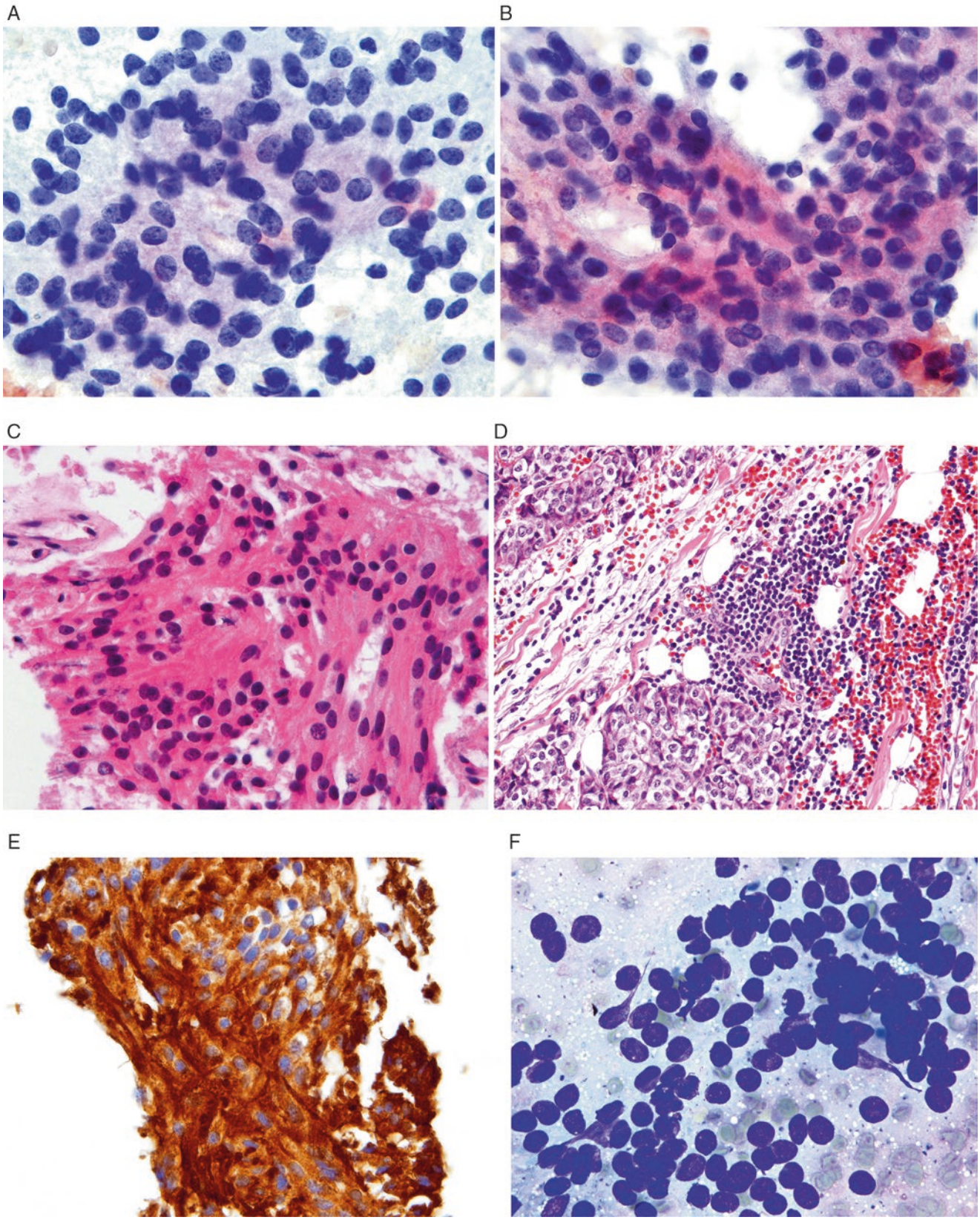

Fig. 6.36 Thymic neuroendocrine tumors. (**a**, **b**) Typical carcinoid, showing loosely cohesive and single of uniform cells with powdery chromatin pattern and discernible nucleoli, Pap stain; (**c**) atypical carcinoid, cellblock material, showing tumor cells forming rosette-like structure, H&E; (**d**) atypical carcinoid, tissue section showing nests of tumor cells, H&E; (**e**) atypical carcinoid, diffuse staining for chromogranin, IHC; (**f**, **g**) high-grade neuroendocrine carcinoma showing discohesive cells with moderate nuclear pleomorphism, prominent nucleoli and frequent mitoses, most compatible with large cell type, Diff-Quik (**f**) and Pap (**g**) stains; (**h**, **i**) high-grade neuroendocrine carcinoma showing discohesive small cells with nuclear molding, inconspicuous nucleoli and frequent mitoses, most compatible with small cell type, Diff-Quik (**h**) and Pap (**i**) stains

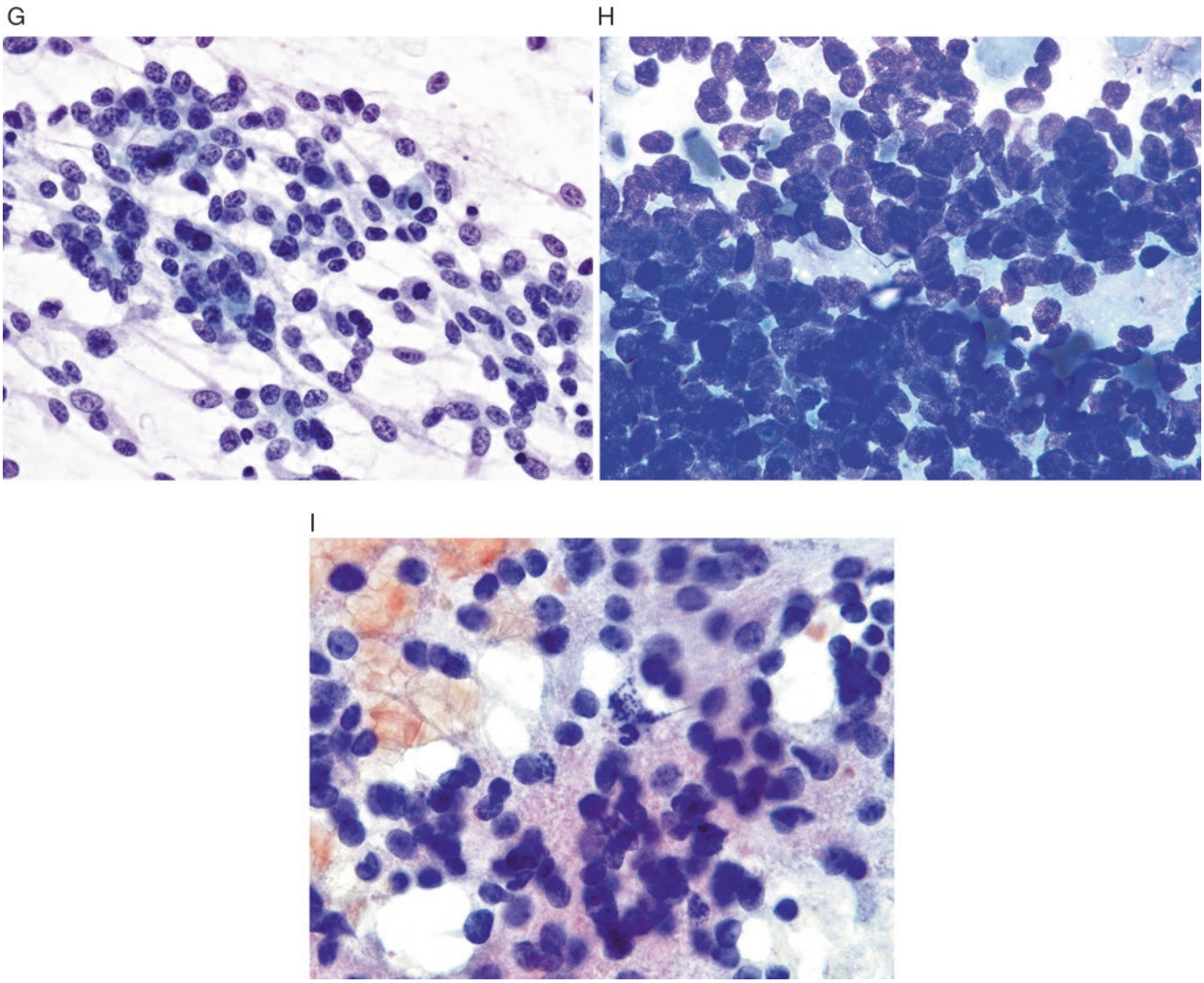

Fig. 6.36 (continued)

Cytological Features

- Seminomas:
  1. Highly cellular smears composed of small loose syncytium-like clusters or isolated large tumor cells with centrally located round nuclei containing one or more prominent, irregular nucleoli, and clear to granular cytoplasm.
  2. Mitoses present.
  3. Admixed with polymorphous lymphocytes, occasional epithelioid histiocytes.
  4. A characteristic tigroid background.
  5. Representative images are shown in Fig. 6.37a–h.
- Embryonal carcinomas:
  1. Highly cellular smears
  2. Three-dimensional clusters or sheets of large, pleomorphic tumor cells with high nuclear-to-cytoplasmic ratio, irregular nuclear contours, coarse chromatin, and several nucleoli
  3. Indistinct pale cytoplasm
  4. Bizarre-shaped cells, mitoses, and necrosis frequent
- Yolk sac tumors:
  1. Clusters of medium to large pleomorphic tumor cells with irregular nuclear contours, vesicular nuclei, prominent nucleoli, and eosinophilic or vacuolated cytoplasm.
  2. Some tumor cells contain intracytoplasmic dense hyaline globules.
  3. Granular debris and mucoid or metachromatic material in background.
  4. No lymphocytes or epithelioid histiocytes.
- Choriocarcinomas:
  1. Cellular smears
  2. Malignant cytotrophoblasts: mononucleated, medium-size cells with vacuolated, basophilic cytoplasm, and eccentric nuclei
  3. Malignant syncytiotrophoblasts: very large tumor cells with one or several nuclei, distinct nucleoli, and eosinophilic cytoplasm

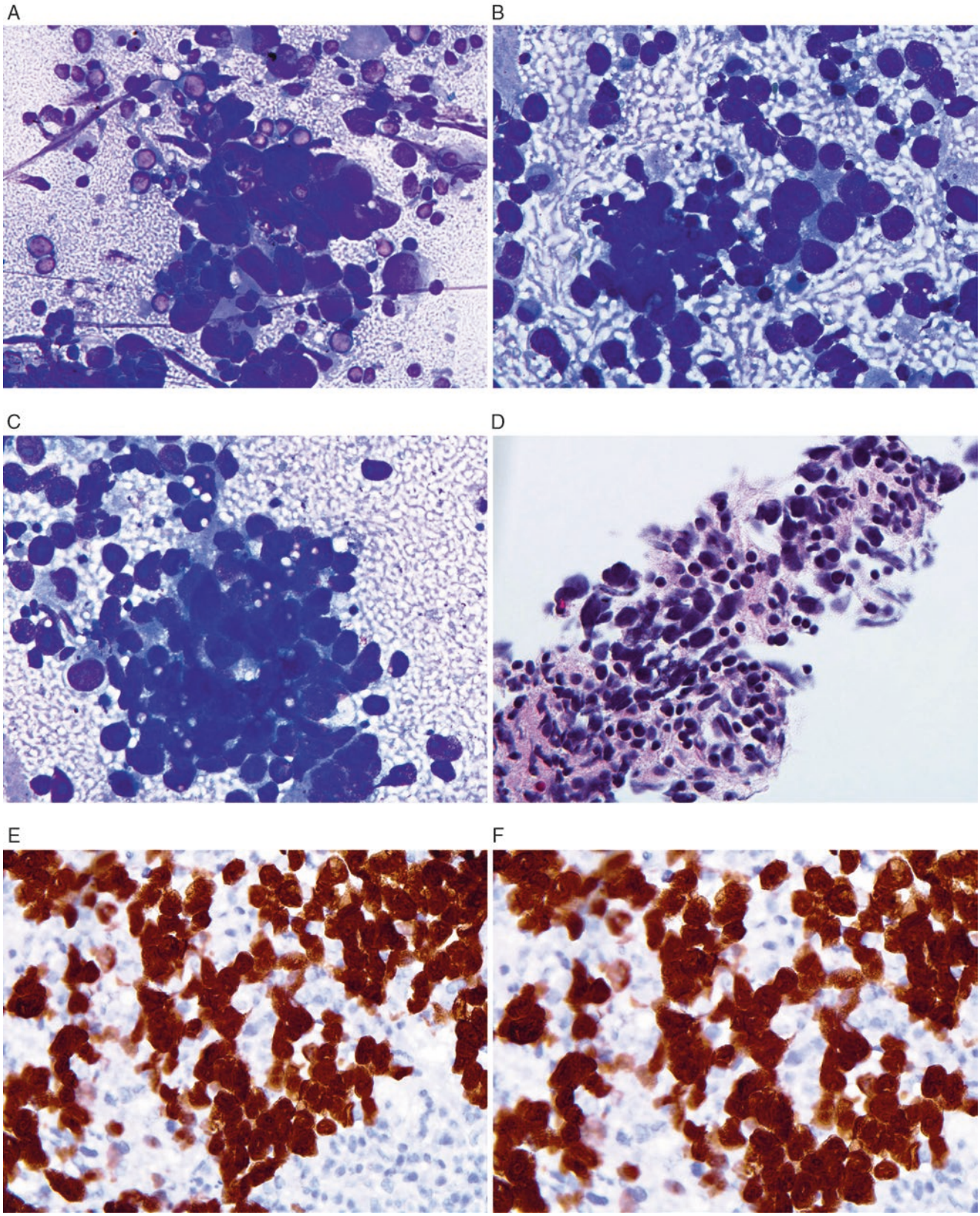

Fig. 6.37 Seminoma, mediastinum. (**a**–**c**) The smears reveal small loose syncytium-like clusters and isolated large tumor cells with centrally located round nuclei containing one or more prominent, irregular nucleoli, and clear to granular cytoplasm, with admixed polymorphous lymphocytes, in a characteristic tigroid background, Diff-Quik; (**d**) cell block preparation reveals thin core of tumor tissue showing small clusters of atypical cells with prominent nucleoli admixed with lymphocytes, H&E; (**e**) positive for SALL4, IHC; (**f**) positive for OCT4, IHC; (**g**) positive for CD117, IHC; (**h**) negative for CD30, IHC

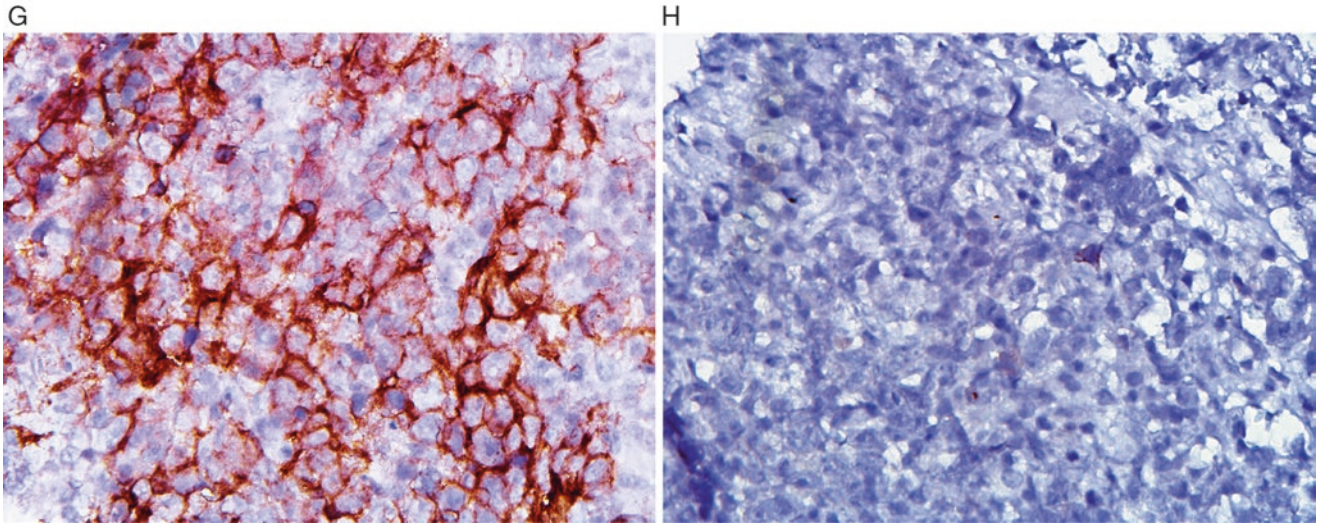

Fig. 6.37 (continued)

4. Atypical mitoses
5. Significant hemorrhage and necrosis in the background

- Teratoma:
  1. Mature teratoma: cytology diagnosis is difficult; may be paucicellular with few anucleated squamous cells and macrophages in a proteinaceous background; ciliated bronchial cells, smooth muscle, and cartilage are present; a mucoid background with bland-looking signet ring cell-like mucus cells may be seen.
  2. Immature teratoma: may be cellular, composed of aggregates or individual small, round, hyperchromatic cells with high nuclear-to-cytoplasmic ratio, and inconspicuous nucleoli; rare rosettes with neuropils, rhabdomyoblasts, immature cartilage, and blastema-like stromal cells may be identified.

Differential Diagnosis

- Metastatic gonadal germ cell tumors
- Metastatic carcinomas
- Small round blue cell tumors
- Sarcomas

Histology

- Seminoma:
1. Sheets, cords, or irregular lobules of monotonous, round to polygonal tumor cells with central nuclei, macronucleoli, and abundant clear to lightly eosinophilic cytoplasm.
2. Delicate fibrous septa, with infiltrates of lymphocytes, may form germinal centers.
3. Granulomatous reaction and fibrosis.
4. Syncytiotrophoblastic cells may be scattered throughout.

- Embryonal carcinoma:
1. Sheets, tubules, and papillary structures of large polygonal or columnar cells with round to oval, vesicular, hyperchromatic nuclei containing one or multiple nucleoli and a moderate amount of cytoplasm.
2. Numerous mitoses, apoptotic bodies, and coagulated necrosis are common.
3. Scattered single or small clusters of syncytiotrophoblasts in one third of cases.

- Yolk sac tumor:
1. Various patterns: reticular-microcystic, spindle cell, solid, macrocystic, papillary, forming Schiller-Duval bodies, polyvesicular vitelline, glandular-alveolar, and hepatoid patterns.
2. The tumor cells may be flat or columnar, with intracellular and extracellular hyaline globules, as well as basement membrane-like material deposition between the cells.

- Choriocarcinoma:
1. Two cell components, syncytiotrophoblasts and cytotrophoblasts, arranged in solid nests or sheets, occasionally in a villus-like arrangement.
2. The syncytiotrophoblasts are large, vacuolated, multinucleated giant cells with dark eosinophilic cytoplasm.
3. The cytotrophoblasts are uniform, medium-size, polygonal cells with abundant clear cytoplasm and distinct cytoplasmic borders.
4. Hemorrhage and necrosis are frequently observed.

- Teratoma:
1. Mature teratoma: solid or multicystic; cysts may be filled with clear, keratinous, gelatinous, or mucinous material; cartilage, spicules of bone, patches of pigmented tissue, or brain tissue may be discernible; an admixture of ectoderm, endoderm, and mesoderm is seen, assembled in either a disorganized or organized pattern.

Table 6.14 Immunophenotypic features of mediastinal germ cell tumors

Tumors/Antibody	CK	OCT3/4	SALL4	CD117	CD30	AFP	βhCG	Gly-3
Seminoma	−/+	+	+	+	−	−	−[e]	−
Embryonal CA	+	+	+	−/+	+	−/+	−[e]	−/+
Yolk Sac Tumor	+	−	+	+/−	−	+	−	+
Choriocarcinoma	+	−	+[b]	−	−	−	+	+/−[f]
Teratoma	+[a]	−	−/+[c]	−/+	−	−/+[d]	−	+/−

CK cytokeratin, *OCT3/4* octamer-binding transcription factor 3/4, *SALL4* Sal-like protein 4, *CD* cluster of differentiation, *AFP* α-fetoprotein, *βhCG* B subunit of human chorionic gonadotropin, *Gly-3* glypican 3, *CA* carcinoma
[a]Epithelial component
[b]Mononuclear trophoblast
[c]Focal staining in enteric glands, primitive neuroepithelium, and blastema-like stroma of immature teratoma
[d]AFP can be positive in fetal gut, liver, or neuroepithelium in immature teratoma
[e]Positive in scattered syncytiotrophoblasts
[f]Positive in syncytiotrophoblasts

2. Immature teratoma: the immature teratomatous elements are mostly cellular spindle mesenchymal components, but immature neural and epithelial elements can be seen; frequent mitoses; embryonic rhabdomyoblastic tissue, blastomatous tissue resembling embryonic kidney or lung, primitive neuroectodermal tumor (PNET), and other epithelial or mesenchymal malignant transformations can be seen.

Immunohistochemistry
The immunophenotypes of mediastinal germ cell tumors are summarized in Table 6.14.

Lymphomas of the Mediastinum

Primary Mediastinal Large B-Cell Lymphoma

Key Clinical Features

- An aggressive large B-cell lymphoma, of putative thymic B-cell origin
- Accounting for 2–3% of all non-Hodgkin lymphomas
- Predominantly in young adults, third to fourth decades
- Female predominance
- No known risk factors

Key Radiographic Features

- Anterior-superior mediastinal mass, often bulky, invading adjacent structures
- The mass typically shows a regular contour.

Cytological Features

- Atypical medial to large-size, monomorphic lymphoid cells with irregular, round to oval nuclei, small nucleoli and clear to eosinophilic cytoplasm
- Background of lymphoglandular bodies and abundant necrosis

Differential Diagnosis

- Diffuse large B-cell lymphoma
- Hodgkin lymphoma

Histology

- Diffuse, sheets, or clusters of medium to large, monomorphic tumor cells with irregular, round to oval nuclei, small nucleoli, and clear or eosinophilic cytoplasm; some cases show cells with pleomorphic nuclei and abundant amphophilic cytoplasm resembling Hodgkin lymphoma or non-lymphoid tumors.
- Admixed, especially at the periphery, are reactive lymphocytes, macrophages, and granulocytes.
- Distinct fibrosis to form irregular collagen bands dividing tumor into variable size compartments.

Immunohistochemistry

- Express CD19, CD20, CD22, and CD79a.
- Express PAX5, B-cell Oct-binding protein 1 (BOB1), OCT2, and PU.1.
- Lack expression of immunoglobulin; CD10 is often negative.
- CD30 expression in >80% of cases, but usually weak and heterogeneous.
- Positive for interferon regulatory factor 4/multiple myeloma oncogene 1 (IRF4/MUM1) (75%), CD23 (70%), and variable to Bcl2 and Bcl6.
- Positive for myelin and lymphocyte (MAL) antigen, CD54, CD95, tumor necrosis factor receptor 1 (TRAF1), p63, and nuclear REL.

Other Types of Lymphomas

Refer to Chap. 4 on lymph nodes.

Case Presentations

Case Scenario 1: Reactive Metaplastic Changes in Viral Infection

Learning Objectives

1. To describe the cytological features of this entity
2. To become aware of its cytologic atypia, a potential mimic of malignancy
3. To become familiar with the utility of lab tests in the diagnosis of this entity

Case

A 14-year-old female with a medical history of Rett syndrome presented with respiratory illness, fatigue, and pneumonia for 2–3 months. Respiratory secretion for culture and RT-PCR and bronchial washing for cytology and differential cell count were received.

The bronchial washing specimen revealed scattered atypical squamoid cells with large nuclei, high nuclear-to-cytoplasmic ratio, and hyperchromatic, smudged chromatin with a suggestion of nuclear inclusions in background of intense acute inflammation, reactive respiratory epithelial cells, and pulmonary macrophages. The cellblock material showed similar findings. Immunohistochemically, the scattered atypical cells were decorated by CK7, but nonreactive to p40; the profile suggested that those cells are pneumocytes. Representative images are shown in Fig. 6.38a–d.

SARS, a new human emergent infectious disease mainly involving the lower respiratory tract, became a worldwide outbreak in 2003, affecting more than 8000 patients, with a fatality rate of 9.2%. A novel coronavirus (SARS-CoV), a member of a family of large, positive, single-stranded RNA viruses, was identified as the etiological agent for this disease. The key pulmonary pathology is that of DAD, featured by pronounced pulmonary edema and hyaline membrane formation. There are interstitial thickening, fibrosis, and intra-alveolar exudates with granulation tissue formation, as well as sparsely inflammatory cell infiltrates, including macrophages (often multinucleated forms) and lymphocytes. Depending on different phases in the disease progression, the degree of fibrosis and the composition of inflammatory cells may vary. Other pathologic findings, such as prominent vascular injury, hemophagocytosis, squamous metaplasia, apoptosis, and atypical pneumocytes, including multinucleated giant pneumocytes with irregularly distributed nuclei or pneumocytes with large atypical nuclei, prominent eosinophilic nucleoli, and granular amphophilic cytoplasm, were reported. However, distinct viral inclusions were not apparent. Ancillary tests, such as in situ hybridization, immunohistochemistry, viral isolation, or RT-PCR are necessary to confirm the viral infection.

For the index case, serology testing and culture of the respiratory secretion were positive for coronavirus OC43. Electron microscopy (EM) revealed viral-like particles in pneumocytes, but not in macrophages or other cell types of lung. The findings confirmed the viral infection.

The atypical pneumocytes, metaplastic squamous cells, and apoptosis may mimic malignancy. Caution should be exercised in the interpretation of specimens from patients with a history of SARS and coronavirus infection. The awareness of clinical history and the limited amount of atypical cells should alert the pathologists to avoid overdiagnosis in this clinical setting.

Case Scenario 2: Metastatic Melanoma

Learning Objectives

1. To describe the cytological features of this entity
2. To become aware of the common differential diagnoses of this tumor
3. To become familiar with the potential utility of the immunohistochemical markers in the diagnosis of this tumor

Case

An 81-year-old gentleman with no documented past history of malignancy presented with dizziness and difficulty of breathing. CT imaging of the chest revealed multiple lung nodules and lymphadenopathy. In addition, a possible brain lesion was noted on imaging. An ultrasound-guided FNA of the hilar lymph node was performed. The smears were highly cellular, composed of clusters, aggregated cell groups, and abundant single spindled to plump cells with hyperchromatic nuclei without prominent nucleoli. Some rosette-like structures were seen, as well as scattered pigment-laden macrophages. The pigments were dusky and brown granules on Pap stain and blue-black on Diff-Quik stain. Abundant necrosis was noted. Representative images of the FNA smears and cellblock material were shown in Fig. 6.39a–d.

The cytological features raise a differential diagnosis that includes neuroendocrine tumor, spindle cell tumors, including sarcomatoid carcinoma and metastatic melanoma of the spindle cell type. A limited panel of immunoassays was performed, revealing tumor cells with strong and diffuse nuclear staining for

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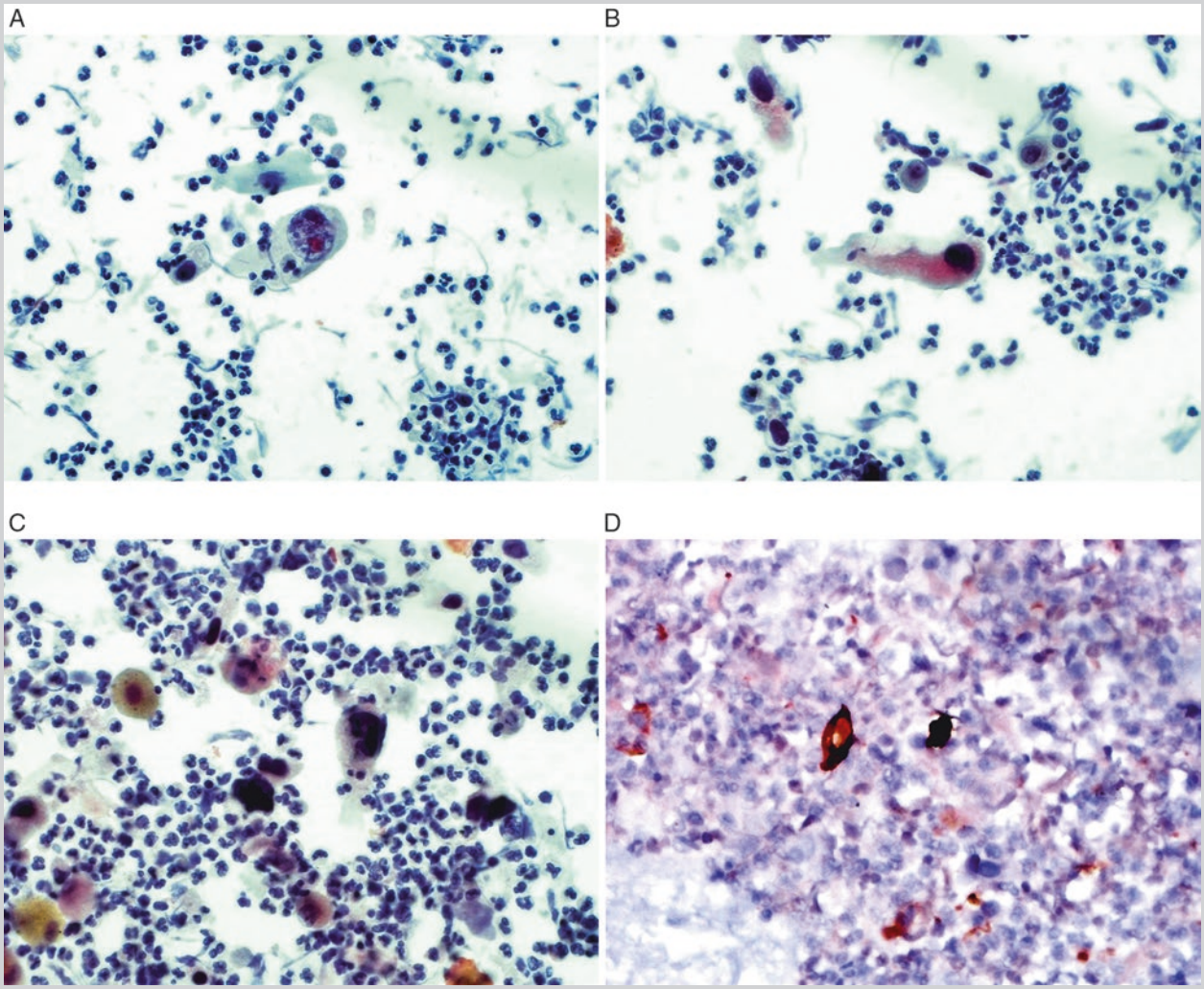

Fig. 6.38 Bronchial washing, in patient with coronavirus infection. (**a**) Atypical pneumocytes with large atypical nuclei, prominent eosinophilic nucleoli, and granular amphophilic cytoplasm, Pap stain; (**b**, **c**) scattered atypical metaplastic squamous cells with smudged chromatin, Pap stain; (**d**) CK7 highlights the atypical pneumocytes, IHC

sex determining region Y box 10 (SOX10) and cytoplasmic staining for vimentin and human melanoma black 45 (HMB45), focally for melanoma antigen recognized by T cells (MART1) and S100 (both nuclear and cytoplasmic), while CK AE1/3 and neuroendocrine markers (synaptophysin, chromogranin and CD56) were nonreactive. Representative images are illustrated in Fig. 6.39e–h. The overall findings are those of a metastatic spindle cell melanoma.

The diagnosis of metastatic spindle cell melanoma can be challenging on FNA specimens. The spindled tumor cells can display a wide range of morphologies, from deceptively bland-appearing reactive fibroblast-like cells to highly pleomorphic, high-grade sarcomatous spindle cells. The diagnostic cytological features of conventional melanoma, such as a dispersed single cell pattern, eccentric nuclei, cytoplasmic melanin pigments, intranuclear pseudoinclusions, prominent macronucleoli, and bi- or multinucleations, are often subtle or even lacking. For the index case, the presence of melanin pigments is a valuable clue to raise the suspicion for melanoma which, in conjunction with the immunophenotype, justified the diagnosis of spindle cell melanoma. The presence of cytoplasmic melanin

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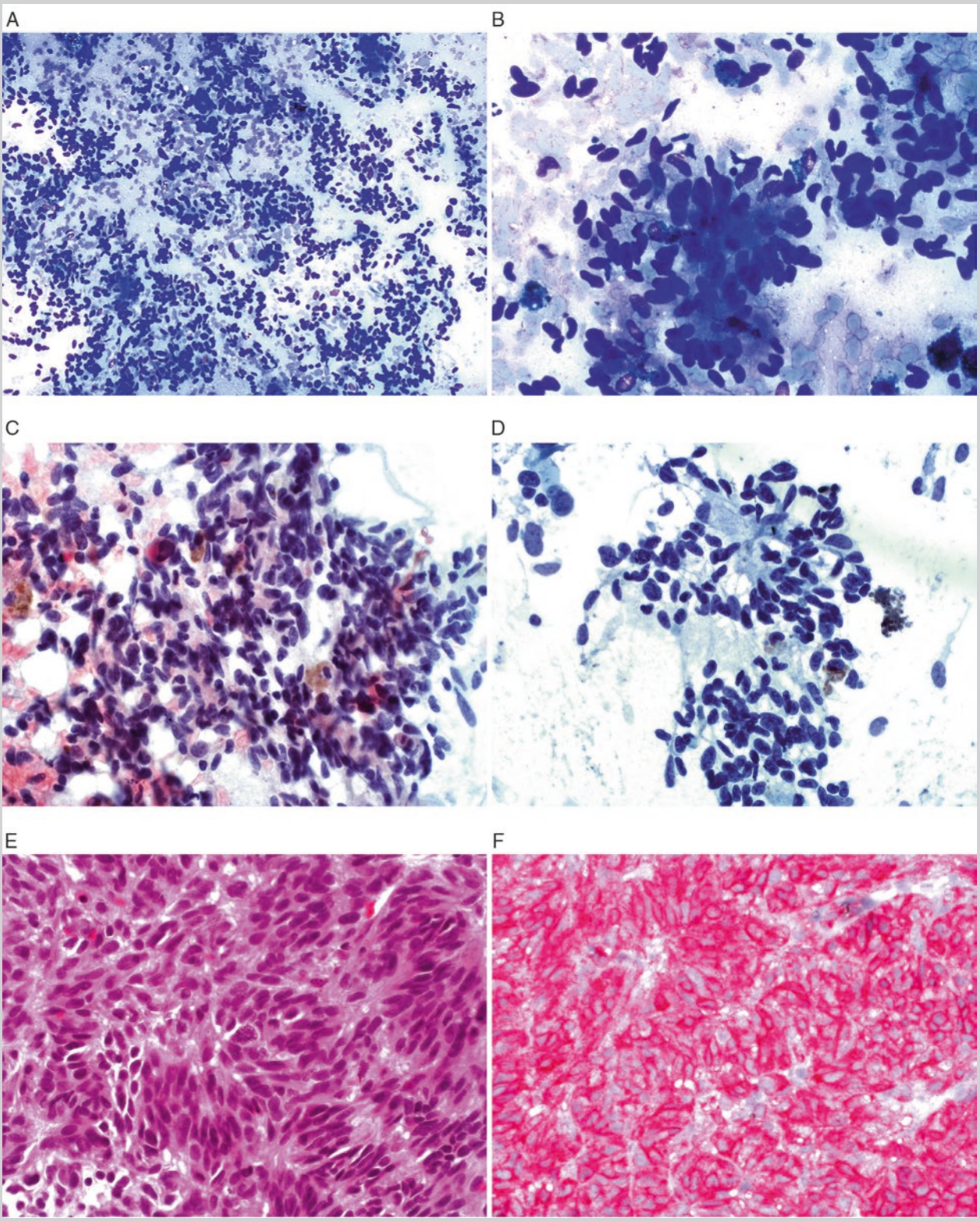

Fig. 6.39 Metastatic spindle cell melanoma. (**a**) Highly cellular smears, comprised of clusters and abundant singly spindle to plump cells with hyperchromatic nuclei without discernible nucleoli, Diff-Quik; (**b**) some rosette-like structures are seen, as well as scattered pigment laden macrophages; the pigments are dusky, blue-black, Diff-Quik; (**c**, **d**) same as **b**, the pigments are brown, granular, Pap stain; (**e**) cellblock preparation reveals spindled cells with perivascular palisading; scattered pigment-laden macrophages are seen, but not shown, H&E; (**f**) diffuse positive for HMB-45, IHC; (**g**) strong, diffuse SOX10 nuclear staining, IHC; (**h**) no CK AE1/3 expression, IHC

(continued)

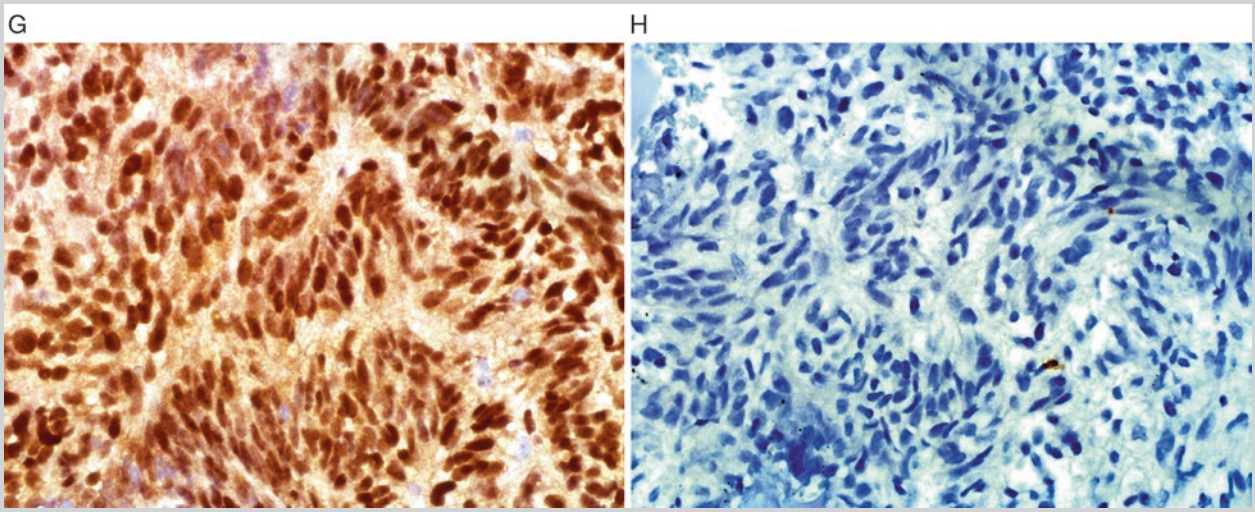

Fig. 6.39 (continued)

pigments in FNA samples varies, reported in the range of 30–80%. However, these pigments can occasionally be identified in melanotic schwannomas and melanotic malignant peripheral nerve sheath tumors (MPNSTs).

Immunohistochemistry plays a critical role in the diagnosis of spindle cell melanoma, especially in the clinical scenario of metastasis. Metastatic spindle cell melanomas tend to lose expression of some melanoma markers. Piao and colleagues reported that spindle cell melanomas express S100, HMB45, and MART1 in 67%, 50%, and 18% of cases, respectively. Although the sensitivity of S100 for melanoma in general is very high (~93–100%), its specificity is low. The expression of S100 can be seen in a variety of tumors. HMB45 is expressed in melanocytic tumors, also in clear cell sarcomas, perivascular epithelial cell tumors (PEComas), melanocytic schwannomas, meningeal melanocytomas, some ovarian steroid tumors, and renal cell carcinomas with the t(6;11)(p29;q12) translocation. The sensitivity of HMB45 for melanoma in general is in the range of 70–90%, but only 0–30% for desmoplastic melanomas. MART1 has a sensitivity of ~85–97% for primary and ~57–92% for metastatic melanomas, but only 0–33% for desmoplastic melanomas; its specificity for melanoma is 95–100%. MART1 expression is also seen in PEComas and clear cell sarcomas.

SOX10 is a transcription factor that is essential for the survival of neural crest-derived cells and for the maintenance of the multipotency of neural crest cells. The cells derived from neural crest multipotential cells include neurons and glial cells in the peripheral nervous system, melanocytes of the skin, C cells of the thyroid, catecholaminergic cells of the adrenal gland, and cartilage and bone of the face. Studies have shown that, among tumors, SOX10 is commonly expressed in melanomas, including desmoplastic melanomas, tumors with Schwann cell differentiation, myoepithelial cell tumors of the soft tissue and some salivary gland neoplasms, particularly those with myoepithelial differentiation, and acinic cell carcinomas. When compared with other melanocytic-associated markers, SOX10 is highly sensitive and specific and can assist in the differential diagnosis of melanomas. Even in desmoplastic melanomas, SOX10 expression was reported in 100% of cases. Studies of SOX10 expression in tumors of the nervous system reported that SOX10 is commonly expressed in schwannomas (100%), neurofibromas (98–100%), and, less frequently, MPNSTs (~50–55%).

The differential diagnosis of this case is summarized in Table 6.15.

Table 6.15 Immnohistochemical differential diagnosis of spindle cell melanoma

	MSM	NET	SC	MPNST
SOX10	+	–	–	–/+
S100	+	–	–	–/+
CK AE1/3	–	+	+	–
SYN	–	+	–	–
CHR	–	+	–	–
HMB45	–/+	–	–	–
MART1	–/+	–	–	–

MSM malignant spindle cell melanoma, *NET* neuroendocrine tumor, *SC* sarcomatoid carcinoma, *MPNST* malignant peripheral nerve sheath tumor, *SOX10* sex determining region Y box 10, *CK* cytokeratin, *SYN* synaptophysin, *CHR* chromogranin, *HMB45* human melanoma black 45, *MART1* melanoma antigen recognized by T cells

(continued)

Case Scenario 3: Metastatic Carcinoma

Learning Objectives

1. To describe the cytological features of this entity
2. To become aware of the subtle differences in cytology and to raise the question of a metastasis
3. To become familiar with the potential utility of immunohistochemical markers in the diagnosis of this tumor

Case

A 72-year-old female with a past medical history of noninvasive low-grade papillary urothelial carcinoma a year ago presented with coughing and chest pain for month. CT imaging of the chest revealed multiple lung nodules and lymphadenopathy. An ultrasound-guided FNA and biopsy of the hilar lymph node were performed. The smears were highly cellular, composed of loosely cohesive clusters and single spindle to plump cells with hyperchromatic nuclei, coarse chromatin, occasional discernible to prominent nucleoli, and abundant delicate cytoplasm without defined borders. Some of the cells appeared spindled, columnar or racket-shaped, with eccentrically located nuclei and cytoplasmic tails, also called cercariform cells. Abundant necrosis was noted in the background. Representative images of the FNA smears and biopsy are shown in Fig. 6.40a–f.

The cytological features raise a differential diagnosis that includes a non-small cell carcinoma of lung, especially squamous cell carcinoma, a sarcomatoid carcinoma, and metastatic carcinoma, especially metastatic urothelial carcinoma. A panel of immunoassays was performed, revealing that the tumor cells were positive for CK7, CK20 (focal), CK5/6, uroplakin II, p40, and placental S100 (S100P) while negative for TTF1, napsin A, synaptophysin, and chromogranin. Representative images are illustrated in Fig. 6.40g–j. The final diagnosis is metastatic carcinoma with urothelial differentiation

There are limited reports in literature regarding the cytology of metastatic urothelial carcinomas. The following features were reported: (1) loosely cohesive cells occurring singly and in syncytial clusters; (2) large hyperchromatic nuclei with irregularly distributed granular chromatin and abundant granular or fibrillar cytoplasm; (3) distinct cell borders; (4) multilayered papillary fragments; (5) cells with eccentric nuclei, multiple nucleoli, and intracytoplasmic vacuoles; and (6) cercariform cells, “bipolar” cells, and spindled-shaped cells. Among those, multilayered papillary fragments and cercariform cells are the most helpful features in the distinction of urothelial carcinoma from others. The cercariform cells, first described in 1993 by Johnson and Kini and further defined in 1995 by Powers and Elbadawi, are fusiform, pyramidal, or racket-like cells with eccentric nuclei that form non-tapering, flattened, bulbous, or fishtail-like cytoplasmic extensions in varying lengths. The presence of a small vacuole in the bulbous tail was also a helpful criterion. These cells, which are encountered in 57–100% of metastatic urothelial carcinomas, are interpreted in favor of urothelial carcinoma, particularly when they are observed in large numbers. However, they are not specific and must be considered alongside other clinical and morphological characteristics and immunohistochemical phenotypes. The cytological features of metastatic urothelial carcinomas are very difficult to differentiate from mesenchymal tumors and squamous cell carcinomas. Immunohistochemical analyses play a crucial role.

Urothelial cell carcinomas express both CK7 and CK20. In addition, they express HMWCKs (CK5/6, CK903), p63, GATA binding protein 3 (GATA3), uroplakin II, and S100P. GATA3 is a zinc finger transcription factor with a diverse range of biologic roles. GATA3 is a newer generation of urothelial specific marker. GATA3 expression was reported in approximately 80% of urothelial carcinomas, over 90% of breast carcinomas, 100% of parathyroid gland tissue or tumors, salivary gland tumors, especially the salivary ductal carcinomas (~90%), and transitional proliferations of the gynecological tract. In addition, 0–12% of pulmonary squamous cell carcinomas and approximately 10% of pancreatic carcinomas were also reported to be positive for GATA3, although weakly and focally in the majority of positive cases.

The immunohistochemical differential diagnosis of metastatic urothelial carcinomas is summarized in Table 6.16.

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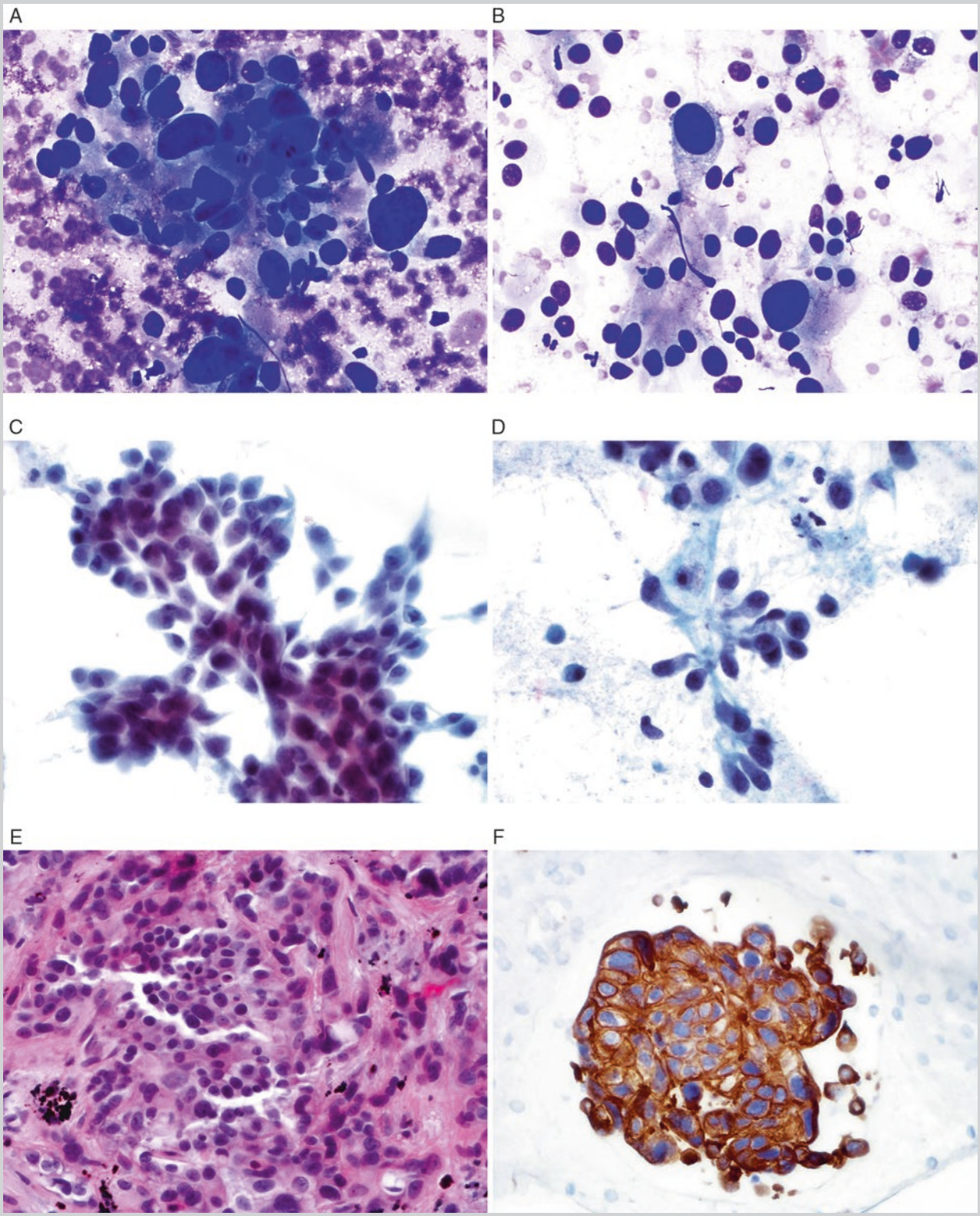

Fig. 6.40 Metastatic urothelial carcinoma, hilar lymph node, fine needle aspiration with biopsy. (**a**) Loosely cohesive atypical cells with pleomorphic and hyperchromatic nuclei, Diff-Quik; (**b**) the tumor cells show eccentrically placed, oval-shaped nuclei, and abundant cytoplasm, Diff-Quik; (**c**) multilayered papillary fragments, Pap stain; (**d**) many racket-shaped cells, also called cercariform cells showing eccentrically located nuclei with cytoplasmic tails, Pap stain; (**e**) biopsy, H&E; (**f**) positive for CK7, IHC; (**g**) positive for CK20, IHC; (**h**) positive for GATA3, IHC; (**i**) positive for uroplakin II, IHC; (**j**) positive for S100P, IHC

(continued)

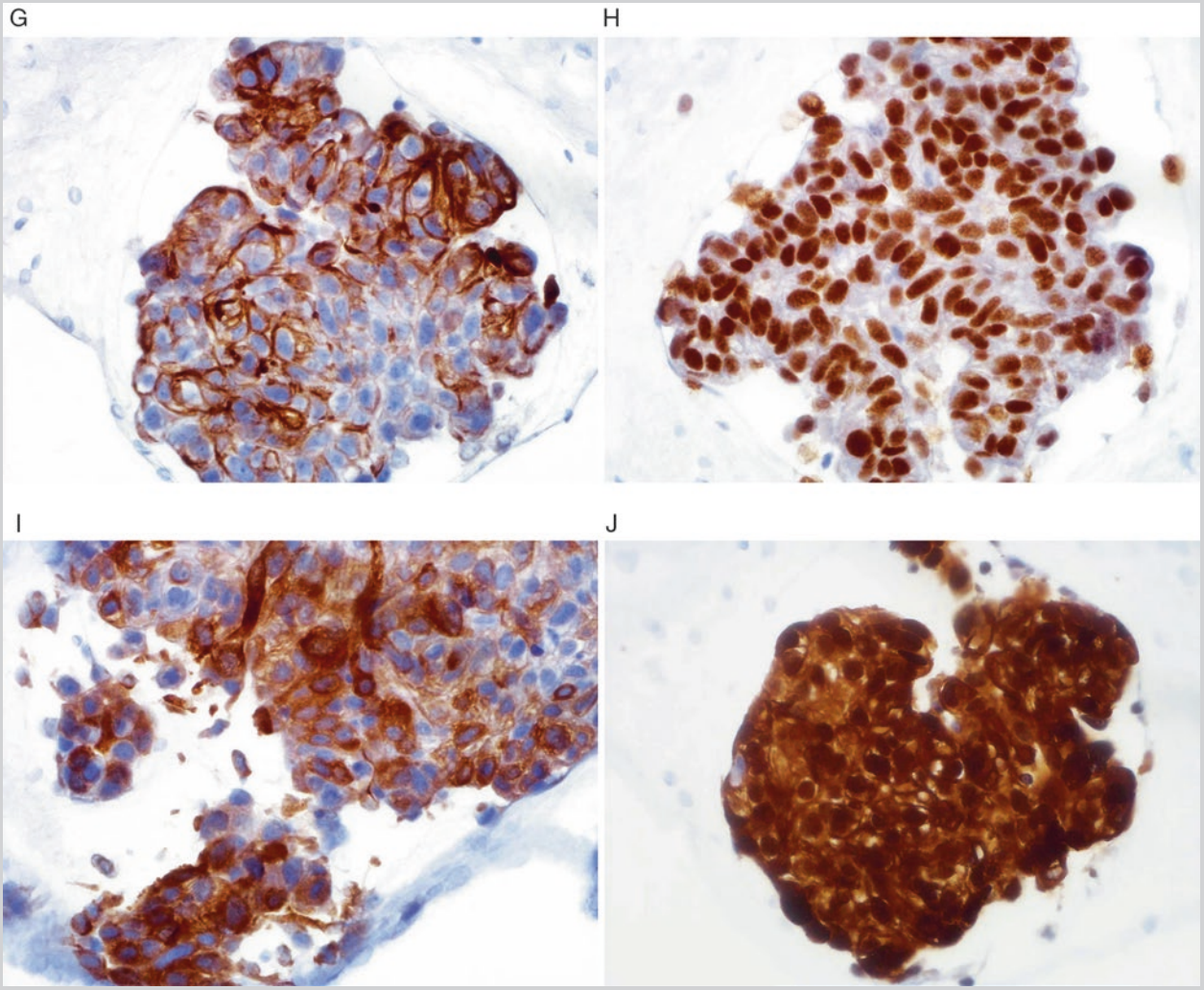

Fig. 6.40 (continued)

Table 6.16 Immunohistochemical differential diagnosis of metastatic urothelial carcinoma

	Met UCA	SqCC	Sarcomatoid CA
CK7	+	−	−/+
CK20	+/−	−	−
CK5/6	+	+	+/−
TTF1	−	−	−/+
GATA3	+	−/+[a]	−
Uroplakin II	+	−	−
p63	+/−	+	−/+
S100P	+	−	−

Met UCA metastatic urothelial carcinoma, *SqCC* squamous cell carcinoma, *CA* carcinoma, *CK* cytokeratin, *TTF1* thyroid transcription factor 1, *GATA3* GATA binding protein 3, *S100P* placental S100

[a]GATA3 expression in squamous cell carcinoma of lung was reported in 0–12% of cases; when positive, it is usually weak and focal.

Abbreviations List

Abbreviation	Full Text
ADC	Adenocarcinoma
AFP	Alpha-fetoprotein (α-fetoprotein)
AIDS	Acquired immunodeficiency syndrome
AIS	Adenocarcinoma in situ
AKT	Protein kinase B
ALCL	Anaplastic large cell lymphoma
ALK	Anaplastic lymphoma kinase
ATS	American Thoracic Society
B72.3	Tumor-associated glycoprotein (TAG)72
BAC	Bronchioloalveolar carcinoma
BAP1	BRCA1 associated protein 1
Bcl (Bcl2, Bcl6)	B-cell CLL/lymphoma 2, 6
BerEP4	Epithelial cell adhesion molecule
BOB1	B-cell Oct-binding protein 1
BRD	Bromodomain containing (BRD3, BRD4)
CA	Carcinoma
CCNE1	Cyclin E1
CD	Cluster of differentiation
CDKN2	Cyclin-dependent kinase inhibitor 2 (CDKN2A, CDKN2B)
CEA	Carcinoembryonic antigen
CHR	Chromogranin (table only)
CK	Cytokeratin
CREB1	CAMP responsive element binding protein
CT	Computed tomography
CTNNB1	Catenin beta 1
CUL3	Cullen 3
DAD	Diffuse alveolar damage
EBV	Epstein-Barr virus
EGFR	Epithelial growth factor receptor
EM	Electron microscopy
EMA	Epithelial membrane antigen
EML4	Echinoderm microtubule-associated protein-like 4
ERS	European Respiratory Society
EWSR1	Ewing sarcoma break point region 1
F	Focal (table)
FDG	fluorodeoxyglucose
FGFR1	Fibroblast growth factor receptor 1
FNA	Fine needle aspiration
FOXN1	Forkhead box N1
GATA3	GATA binding protein 3
GI	Gastrointestinal
GLUT1	Glucose transporter 1
Gly3	Glypican 3
GMS	Grocott's methenamine silver
GTF2I	General transcription factor IIi
GYN	Gynecologic
H&E	Hematoxylin and eosin
HMB45	Human melanoma black 45
HMGA2	High-mobility group AT-hook 2
HMWCK	High molecular weight cytokeratin
IASCL	International Association for the Study of Lung Cancer
Ig	Immunoglobulin
IHC	Immunohistochemistry (only in table)
IMP3	Insulin-like growth factor II messenger RNA protein
IRF4	Interferon regulatory factor 4
KEAP1	Kelch-like ECH-associated protein 1
KIT	
KRAS	Kirsten rat sarcoma viral oncogene
LCNEC	Large cell neuroendocrine carcinoma
LCNEM	Large cell carcinoma with neuroendocrine morphology (in table only)
LeuM1	Cluster of differentiation (CD)15
LG	Low grade (table only)
LMWCK	Low molecular weight cytokeratin
LPP	Lipoma preferred partner
MAL	Myelin and lymphocyte
MALT	Mucosa-associated lymphoid tissue
MART1	Melanoma antigen recognized by T cells
MDM2	Mouse double minute 2 homolog
Med	Median (table)
MEN1	Multiple endocrine neoplasia type 1
Met UCA	Metastatic urothelial carcinoma
MIA	Minimally invasive adenocarcinoma
MIB1	Mindbomb E3 ubiquitin protein ligase 1
MPNST	Malignant peripheral nerve sheath tumor
MRI	Magnetic resonance imaging
MSA	Muscle-specific actin
MSM	Malignant spindle cell melanoma
MUC	Mucin (e.g., MUC1)
MUM1	Multiple myeloma oncogene 1
N/C	Nuclear-to-cytoplasmic ratio
NATB2	NGFI-A binding protein 2
NE	Neuroendocrine
NET	Neuroendocrine tumor
NF2	Neurofibromin 2
NFE2L2	Nuclear factor, erythroid 2 like 2
NK	Natural killer

Abbreviation	Full Text
NKX3.1	NK3 homeobox 1
NOS	Not otherwise specified
NOTCH1	Notch (Drosophila) homolog 1
NSCC	Non-small cell carcinoma (in tables only)
NSCLC	Non-small cell lung carcinoma
NUT	Nuclear protein in testis
OCT3/4	Octamer-binding transcription factor 3/4
OT2	
Pap	Papanicolaou
PAS	Periodic acid-Schiff
PAX	Paired box gene (PAX5, PAX8)
PEComa	Perivascular epithelioid cell tumor
PEComatous	Perivascular epithelioid cell (PEC) omatous tumors
PET	Positron emission tomography
PNET	Primitive neuroectodermal tumor
PU.1	
RB1	Retinoblastoma 1
REL	
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
S100P	Placental S100
SALL4	Sal-like protein 4
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome caused by coronavirus (SARS coronavirus)
SATB2	Special AT-rich sequence-binding protein 2
SC	Sarcomatoid
SCNC	Small cell neuroendocrine carcinoma
SMA	Smooth muscle actin
SOX	Sex-determining region Y box (SOX1, SOX10, etc.)
spp.	Species (many)
SqCC	Squamous cell carcinoma (in tables only)
STAT6	Signal transducer and activator of transcription 6
SYN	Synaptophysin
TdT	Terminal deoxynucleotidyl transferase
TP	Tumor protein (TP53, TP63)
TRAF	Tumor necrosis factor receptor
TTF1	Thyroid transcription factor 1
WHO	World Health Organization
WT1	Wilms tumor 1
βhCG	B subunit of human chorionic gonadotropin

Suggested Reading

Cytology/Fine Needle Aspiration

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