Body Cavity Effusions and Washings

9

Xin Jing

Contents

Frequently Asked Questions

1. What is the main purpose of cytologic examination of body fluids? How to report cytologic findings?

The cytologic examination of effusions collected from pericardial, pleural, and peritoneal cavities or fluids obtained from peritoneal/pelvic washings is performed in order to determine the presence or absence of malignant cells. The examples of benign conditions causing effusions include congestive heart failure, hepatic cirrhosis, chronic renal failure, hypoalbuminemia, infection, trauma, etc. The malignant entities include carcinomas, mesothelioma, lymphomas, melanoma, soft tissue malignancy, etc. In general, "positive for malignant cells" and "no malignant cells identified" are reported when malignant cells are definitively present and absent, respectively. When metastatic malignant cells are identified, ancillary tests (i.e., immunostains, flow cytometry) may be applied for categorization of the malignancy (i.e., carcinoma, lymphoma, melanoma, etc.) and determination of primary site. When the cells show atypical features concerning for malignancy, however, the extent of atypia is quantitatively and/or qualitatively insufficient to be categorized as malignancy, a diagnosis of atypical cells or suspicious for malignant cells may be rendered. The definitive criteria for distinguishing the two less-definitive categories (atypical vs. suspicious) are lacking. As a result, both intraobserver variation and interobserver variation are commonly seen in real-life practice.

2. What is the minimum volume of effusion fluid required for an optimal cytologic evaluation?

There is no consensus regarding minimum fluid volume submitted for an optimal cytologic assessment. It is considered adequate regardless of the specimen volume if malignant cells are identified. A large-scale study of pleural fluids demonstrates that a minimum fluid volume of 75 mL is required to ensure a true benign diagnosis. Accordingly, fluid volumes of less than <75 mL increase the risk of a false-negative, indeterminate, or non-diagnostic result. The authors recommend a disclaimer to all benign specimens of <75 mL, suggesting that the low volume may have compromised specimen evaluation (Rooper et al. 2014) [1].

3. What cytologic preparations are commonly employed?

Effusion fluids are often processed using conventional cytopreparatory technique and/or liquid-based cytology. Various results have been reported in terms of cellularity, cell distribution, and cytomorphology in comparison of these two processing methods. With regard to the cytomorphology, some authors have observed that liquid-based cytology (i.e., ThinPrep preparation) demonstrated better nuclear chromatin morphology and significant shrinkage of cell size. It is noteworthy to mention that liquid-based cytology has cleaner background and less screening time. However, both methods offer compatible diagnostic sensitivity [2–4]. In addition, part of the fluids may be processed as cell block which may

X. Jing (✉)
Department of Pathology, University of Michigan-Michigan Medicine, Ann Arbor, MI, USA
e-mail: xinjing@med.umich.edu

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show histology pattern and be utilized for immunocytochemical staining if needed [5].

4. What are collagen balls? Do collagen balls have any clinical significance?

Collagen balls are tissue fragments with a smooth contour that are composed of mesothelial cells intermingling with or surrounding collagenous stroma. The mesothelial cells surrounding the stroma often become flat. They are found in women only and present in pelvic washing or peritoneal washing specimens. The collagen balls are probably originated from the surface of the ovaries and any structure covered by mesothelium. Collagen balls are non-specific findings and should not be mistaken as neoplasms [6] (Fig. 9.1a, b).

5. Does the presence of psammoma bodies indicate malignancy?

A psammoma body appears as a sphere with a laminated calcification and may present in pleural, peritoneal, and pericardial fluids involved by metastatic carcinomas of the thyroid, lung, ovary, and uterus. However, the presence of psammoma bodies does not necessarily imply a malignant condition and its presence may also be associated with benign conditions, i.e., ovarian cystadenoma or cystadenofibroma, papillary mesothelial hyperplasia, endosalpingiosis, and endometriosis, etc. [7] On Diff-Quik-stained smears, a psammoma body does not pick up stain and appears as colorless, refractile material. On Papanicolaou-stained conventional smears and liquid-based preparations, a psammoma body appears as a sphere with a laminated calcification (Fig. 9.2a, b).

6. What are the cytomorphological features of endometriosis and endosalpingiosis?

Müllerian epithelial cells may appear in pelvic washing specimens from women with benign conditions, i.e., endometriosis and endosalpingosis. The presence of endometrial epithelial cells and hemosiderin-laden histiocytes is suggestive of endometriosis. The epithelial cells along with stromal cells are arranged as honeycomb or syncytial sheets or tight clusters [8]. However, stromal cells and hemosiderin-laden histiocytes may be absent in some cases [9].

Typical features of endosalpingiosis include epithelial cells arranged as small clusters or branching tubular structures. Commonly, the epithelial cells have a cuboidal to columnar appearance with uniform nuclei, smooth nuclear membrane, and fine chromatin. Occasionally, the epithelial cells exhibit moderate cytologic and/or nuclear atypia which pose diagnostic challenges [9]. The presence of ciliated epithelial cells favors benign endosalpingiosis (Fig. 9.3).

7. How to distinguish reactive mesothelial cells from adenocarcinoma?

Adenocarcinomas account for the majority of malignant pleural, pericardial, and peritoneal effusions. Malignant pleural effusions are often caused by carcinoma of lung, followed by breast, ovarian, and gastrointestinal origin. Adenocarcinomas of lung and breast are also the first and second most common metastatic carcinoma in malignant

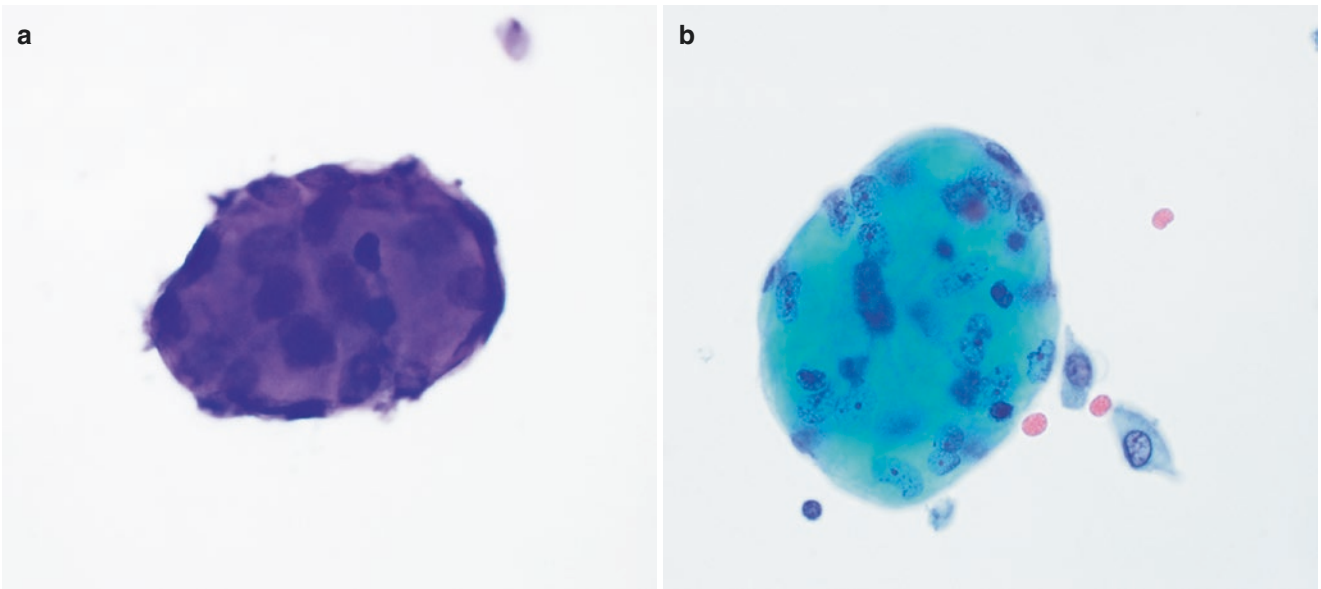

Fig. 9.1 Collagen balls. Mesothelial cells which are intermingling with or surrounding collagenous stroma. (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

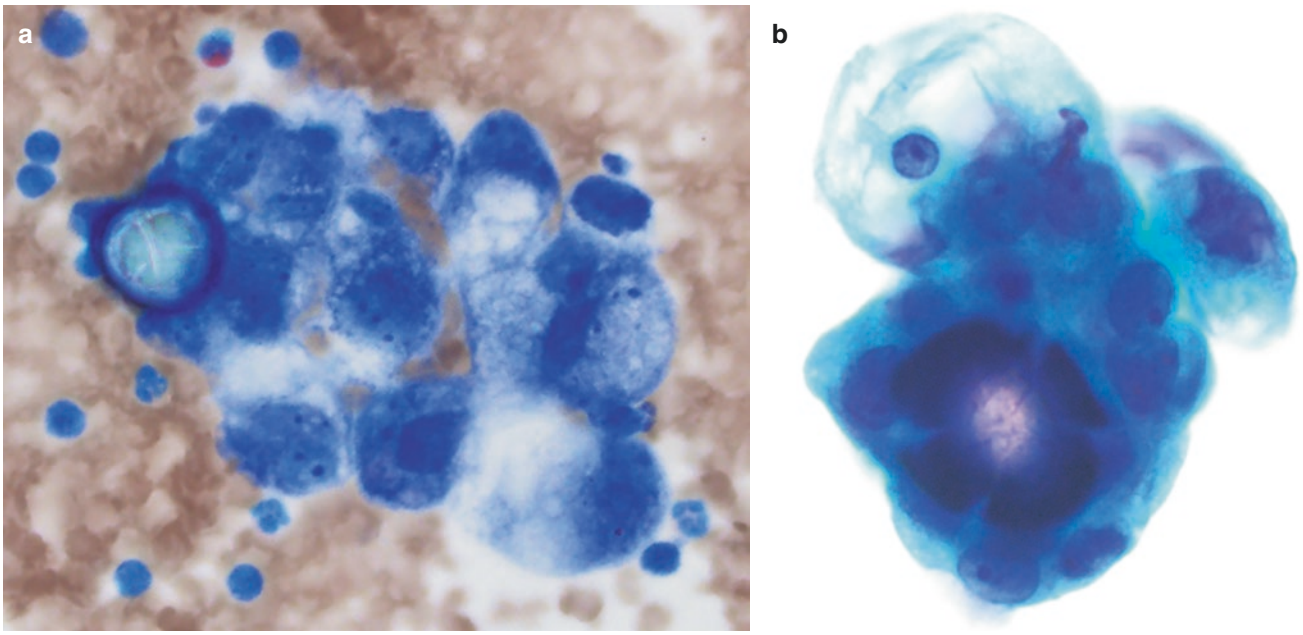

Fig. 9.2 Psammoma bodies. Acellular, calcified spheres within the clusters of epithelial cells, with colorless refractile (**a**, Diff-Quik stain) and laminated appearance (**b**, Papanicolaou stain)

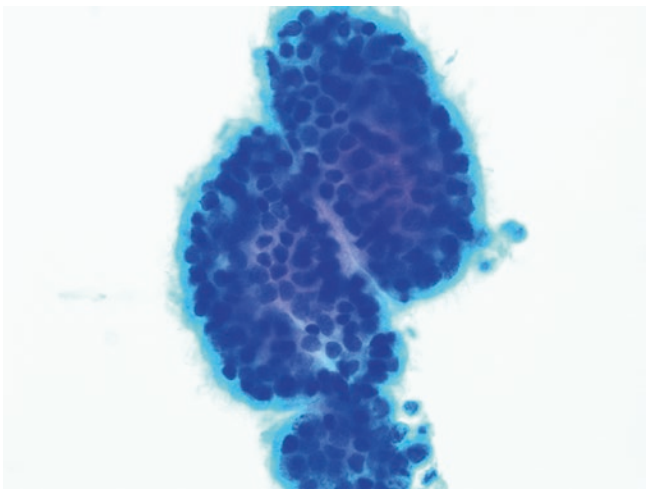

Fig. 9.3 Endosalpingiosis. Cohesive group of ciliated epithelial cells with moderate cytologic/nuclear atypia. Cilia and terminal bar are very prominent (Papanicolaou stain)

pericardial effusions [10]. Malignant peritoneal effusions are commonly associated with adenocarcinoma of ovary followed by gastrointestinal, pancreatic, or breast [11, 12].

Reactive mesothelial cells may show atypical features, including a notable amount of cell clusters, vacuolated cytoplasm, irregular nuclear membrane, and prominent nucleoli. Unlike adenocarcinoma, reactive mesothelial cells appear monotonous with fine chromatin and lack marked variation in cell/nuclear size and shape. When in doubt, workup with a panel of immunostains consisting of 2–3 mesothelial markers (i.e., WT-1, D2-40, calretinin, CK5/6, desmin) and 2–3 epithelial markers (i.e., MOC-31, EMA, B72.3, BerEp4) may help to distinguish reactive mesothelial cells from adenocarcinoma. Adenocarcinoma cells stain positive for epithelial cell markers while being negative for mesothelial markers. After adenocarcinoma is confirmed, immunostains with organ-associated markers may be added if the specimen is collected from the patient with adenocarcinoma of known origin. In case of unknown origin, more organ-associated markers should be attempted in hope of identifying the primary site of adenocarcinoma.

8. How to distinguish mesothelial cell hyperplasia from mesothelioma?

It is not uncommon that florid hyperplasia of mesothelial cells may present in effusions associated with benign conditions. Features favoring reactive hyperplasia are cellular specimens with one-cell population, monotonous cells arranged as single cells or loose clusters, with a subtle variation in size and shape of the cells/nuclei. Features favoring mesothelioma include hypercellularity, monotonous cells arranged as single cells, spheres with smooth borders, or tight/loose clusters with scalloped borders, numerous multinucleated giant mesothelial cells, a wide range of cell size and markedly enlarged cells/nuclei, as well as very prominent nucleoli. However, mesothelioma cells can also be deceptively bland and mimic benign mesothelial cells. Thus, distinguishing reactive mesothelial cells from mesothelioma based on morphologic features can be a challenge

and performing a panel of immunostains on the cell block preparation may be necessary in order to establish a definitive diagnosis. In this regard, the combination of positive expression for EMA with an enhanced membranous staining pattern and negative expression for desmin strongly favors mesothelioma; on the other hand, a combination of negative reaction with EMA (non-membranous staining) and positive reaction with desmin favors reactive mesothelial cells. Further, strong membranous positivity for GLUT-1 and/or strong nuclear staining for p53 favor mesothelioma. A Ki67 proliferative index showed no significant difference between reactive mesothelial hyperplasia and mesothelioma [13]. In addition, a greater expression for insulin-like growth factor-II mRNA-binding protein 3 (IMP3) has been seen in mesothelioma compared to reactive mesothelial cells [14].

9. What are the common immunostaining markers used for distinguishing epithelioid mesothelioma from carcinomas?

Very useful, positive mesothelioma markers include calretinin, CK5/6, WT-1, and D2-40. However, each of these markers has some limitations. In this regard, Calretinin and WT-1 has either limited or no value for distinguishing mesothelioma from serous or breast carcinomas; CK5/6 is not useful for distinguishing mesothelioma from serous, squamous, or breast carcinomas; D2-40 has little or no value for distinguishing mesothelioma from serous or squamous cell carcinomas. Very useful, positive carcinoma markers include MOC-31, BerEp4, BG-8, and CEA. Other markers are B72.3 and CD15. Among these markers, MOC-31, BerEp4, BG-8, CEA, and B72.3 have no values for discriminating mesothelioma from renal cell carcinoma. In addition, CEA and B72.3 are not useful while differentiating mesothelioma from serous and squamous cell carcinomas, respectively (Ordonez 2013) [15]. It is recommended to use a panel of immunostaining that consists of 2–3 mesothelial markers and 2–3 epithelial markers while making a distinction between mesothelioma and carcinomas.

10. What are the cytomorphological features favoring adenocarcinoma?

The presence of foreign (malignant) cell populations and background reactive mesothelial cells raises concerns for adenocarcinoma. The malignant cells may appear as single cells and/or present as various sizes of clusters which may show three-dimensional arrangement with smooth community outlines or papillary configuration with scalloped borders. Intercellular windows are lacking. The malignant epithelial cells contain granular to vacuolated cytoplasm, enlarged nuclei with high N/C ratio, moderate to severe nuclear atypia which is manifested by marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin, and prominent nucleoli.

11. What are the cytomorphological features and immunocytochemical profile of malignant effusions associated with metastatic breast carcinoma?

Effusion with metastatic ductal carcinoma of the breast shows various amounts of malignant cells. Some of the cells appear as single cells while others may be arranged as three-dimensional, loose clusters with irregular contours, or tight spheres with smooth borders resembling cannonballs. Nuclear pleomorphism, nuclear enlargement, high N/C ratio, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are evident. Metastatic lobular carcinoma usually presents as single, dispersed cells and a linear pattern may be seen. Vacuolated cytoplasm may be present and large vacuoles may push the nuclei to the side, resulting in an appearance of signet-ring cells. The cells have mild nuclear atypia, granular to coarse chromatin, and inconspicuous nucleoli. In a difficult case, immunostaining for gross cystic disease fluid protein-15 (GCDFP-15), mammaglobin, ER, PR, and GATA-3 may be useful to confirm the breast origin. Metastatic breast carcinoma may stain positive for these markers in various degree (Fig. 9.4a–e).

12. Does the presence of cannonballs in effusion fluids specifically indicate metastatic ductal adenocarcinoma of breast?

Metastatic breast carcinoma cells exfoliated into effusion fluids may show a cohesive, three-dimensional ball-like pattern with smooth community contours, resembling cannonballs. The malignant cells may appear relatively bland without marked nuclear atypia. Although the presence of cannonballs favors ductal adenocarcinoma of breast, it may be seen in metastatic carcinoma of primary sites other than breast, such as metastatic small cell carcinoma of lung [16] and well-differentiated neuroendocrine carcinoma of thymus [17].

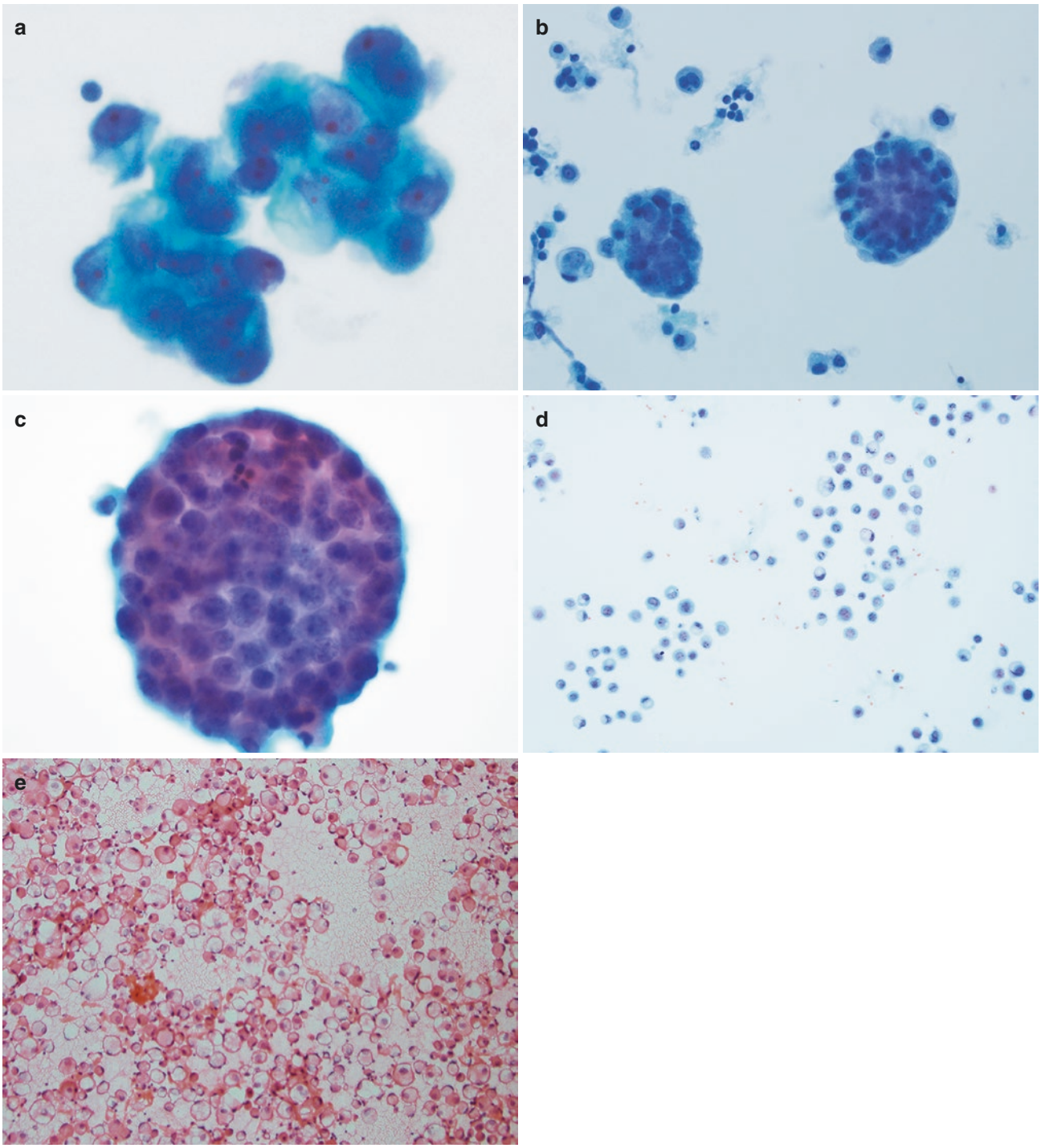

Fig. 9.4 Metastatic breast carcinoma. Ductal carcinoma cells are arranged as disorganized, three-dimensional clusters, cannonballs, and single cells (**a**, **b** and **c**, Papanicolaou stain). Lobular carcinoma shows single dispersed cells with eccentrically located nuclei. Some cells have an appearance of signet-ring cell (**d** and **e**, Papanicolaou and HE stain, respectively)

13. What are the cytomorphological features and immunocytochemical profile of malignant effusions associated with metastatic adenocarcinoma of lung?

Effusion with metastatic pulmonary adenocarcinoma contains malignant cells which appear as single cells or are arranged as three-dimensional clusters with nuclear overlapping/crowding. Delicate cytoplasm and cytoplasmic vacuoles, variation in nuclear size and shape, nuclear enlargement with high N/C ratio, fine to coarse chromatin, and prominent nucleoli are present. TTF-1 and napsin A are useful markers which show positive expression in metastatic adenocarcinoma of lung (Fig. 9.5a, b).

14. How to distinguish metastatic non-keratinizing squamous cell carcinoma from mesothelioma and adenocarcinoma?

Non-keratinizing squamous cell carcinoma shows nuclear pleomorphism, nuclear enlargement, high N/C ratio, coarse chromatin, and prominent nucleoli. Compared to adenocarcinoma, non-keratinizing squamous carcinoma cells have well-defined cell borders and dense cytoplasm with characteristic endo-ectoplasmic demarcation. The cells are arranged as single cells and/or flat groups. Three-dimensional clusters may be occasionally seen. When facing the challenge of distinguishing the cells of squamous cell carcinoma from benign or malignant mesothelial cells, immunostains for CK5/6, p40, and p63 may be performed on the cell block material. All three markers are expressed by squamous cell carcinoma, whereas mesothelial cells stain positive for CK5/6 while being negative for p40 and p63. To make a distinction from adenocarcinoma, applying a panel of immunostaining consisting of p63 and/or p40, TTF-1, and napsin A is helpful. Squamous cell carcinoma is diffusely and strongly positive for p40 and/or p63 while being negative for TTF-1 and napsin A (Fig. 9.6a–c).

15. What are the features of malignant effusions associated with metastatic small cell carcinoma of lung?

Effusion fluids involved by metastatic small cell carcinoma contain single cells and/or groups of cells with nuclear molding. The tumor cells show a scant amount of cytoplasm, high N/C ratio, salt and pepper chromatin, and inconspicuous nucleoli. However, features resembling non-small cell carcinoma may be present, including large cell clusters, coarse chromatin, and conspicuous nucleoli. To differentiate it from poorly differentiated non-small cell carcinoma and non-Hodgkin lymphoma, immunostains for pancytokeratin, CD45, and neuroendocrine markers including synaptophysin, chromogranin A, and CD56 should be performed. Small cell carcinoma shows positive dot-like cytoplasmic staining pattern for pancytokeratin and some if not all three endocrine markers while being negative for CD45 (Fig. 9.7a, b).

16. What are the cytomorphological features of malignant effusions associated with metastatic pancreatic adenocarcinoma?

Metastatic adenocarcinoma of pancreas often shows features that are seen in typical adenocarcinoma. The cells appear as

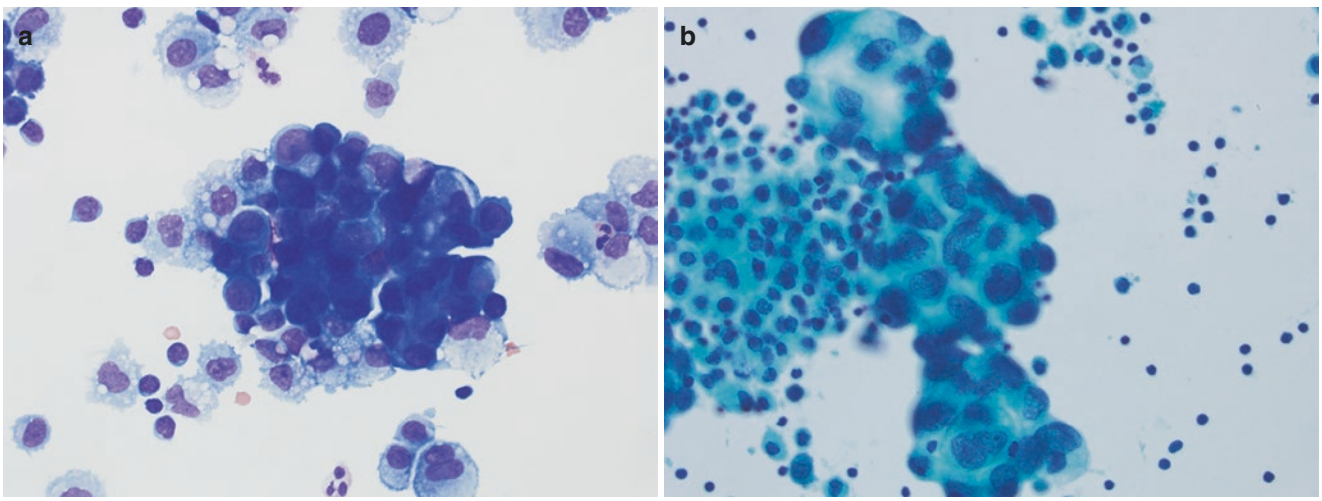

Fig. 9.5 Metastatic pulmonary adenocarcinoma. Carcinoma cells are arranged as single cells or three-dimensional clusters with nuclear overlapping. Delicate and vacuolated cytoplasm, nuclear pleomorphism, fine chromatin, and conspicuous nucleoli are present (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

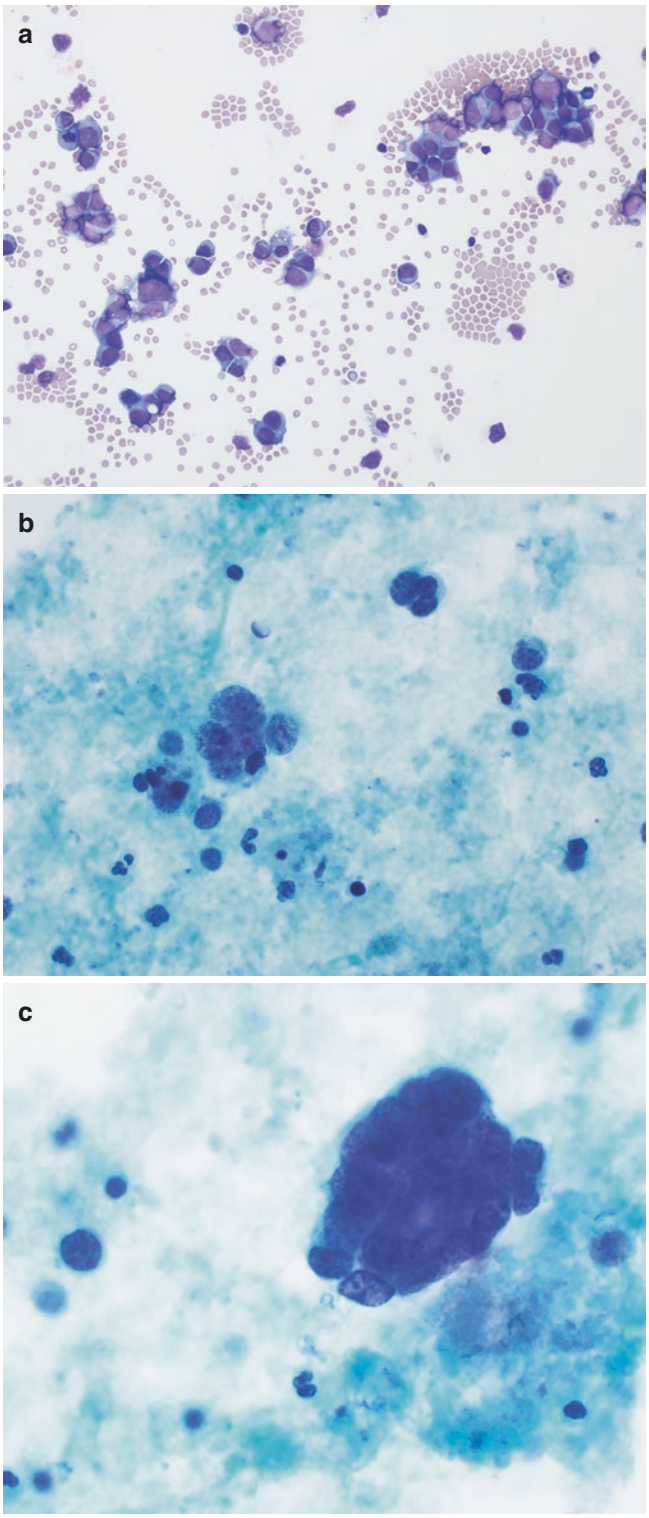

Fig. 9.6 Non-keratinizing squamous cell carcinoma. The cells are arranged as single cells and/or flat groups with well-defined cytoplasmic borders and dense cytoplasm (**a**, Diff-Quik stain). Occasionally, three-dimensional clusters may be seen (**b** and **c**, Papanicolaou stain)

single cells or three-dimensional disorganized clusters. Granular to vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin and prominent nucleoli are present. Positive immunohistochemical reaction for CK7, CK19, Mesothelin, napsin, placental S100 (S100P), and insulin-like growth factor-II mRNA-binding protein 3 (IMP3) in pancreatic ductal carcinoma has been reported [18]. However, clinical and imaging correlation is important to confirm pancreatic origin (Fig. 9.8a).

17. What are the cytomorphological features of malignant effusions associated with high-grade papillary serous carcinoma of the female genital tract?

Regardless of its origin (i.e., ovary, uterus, or fallopian tube), the carcinoma shows similar cytomorphological features. The cells are often arranged as papillary clusters with crowded nuclei and slit-like spaces. Some papillae may contain psammoma bodies. Vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are evident. Immunostains may be helpful for determining primary site. In this regard, positive staining for WT-1 is seen in a significant proportion of ovarian serous carcinomas compared to the serous carcinomas originating from the uterus and fallopian tube. Further, HER2/neu overexpression is seen exclusively in serous carcinomas of endometrial origin [19] (Fig. 9.9a–c).

18. What are the cytomorphological features of pseudomyxoma peritonei?

Typically, the effusion will contain predominantly thick mucin. The epithelial cells show various degrees of atypia depending on the differentiation of the original mucinous neoplasm. Epithelial cells with bland appearance or mild atypia are noted in low-grade mucinous neoplasm whereas markedly atypical epithelial cells are present in mucinous adenocarcinoma (Fig. 9.10).

19. What are the organ-associated immunostaining markers that are commonly used during the workup of primary site(s) of metastatic carcinomas?

TTF-1 and napsin A are useful for identification of adenocarcinoma of lung. Some clear cell and papillary renal cell carcinomas also express napsin A. Both PAX-8 and PAX 2 are expressed in renal cell carcinoma and serous carcinomas. PAX-8 is also positive in carcinomas that develop from thyroid follicular cells. CDX-2 is positive in adenocarcinoma of gastrointestinal or pancreatobiliary origin [15]. Over 50% of breast carcinomas express GCDFP-15 and/or mammaglobin. Further, GATA3 has been shown to be a sensitive marker for

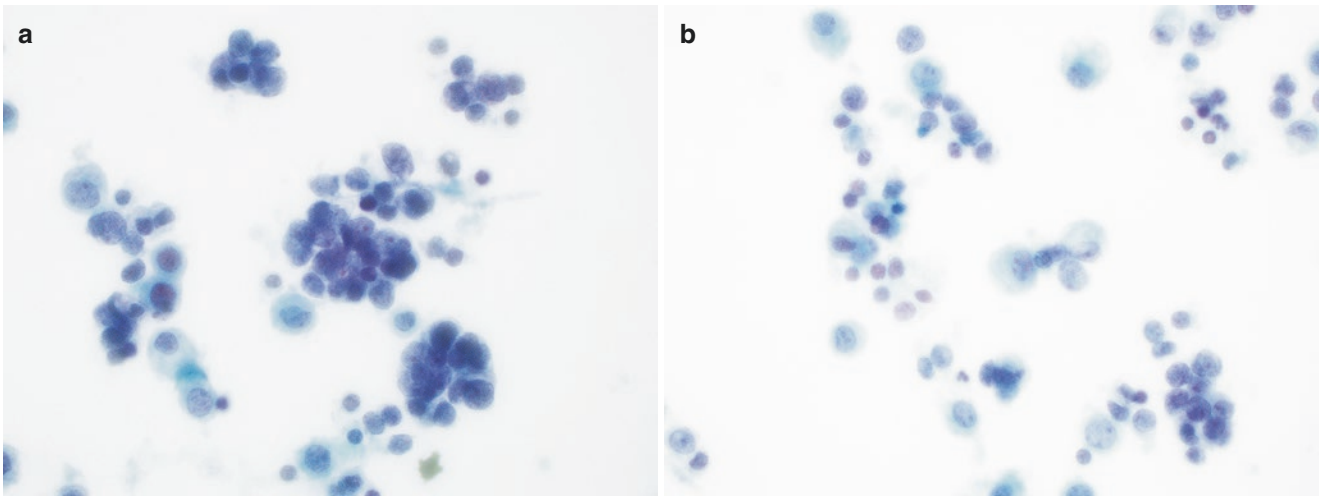

Fig. 9.7 Small cell carcinoma. Single cells and/or groups of cells with nuclear molding are present. The cells show scant amount of cytoplasm, high N/C ratio, salt and pepper chromatin, and inconspicuous nucleoli. The size of the tumor cell is usually one and a half to four times that of a lymphocyte (**a** and **b**, Papanicolaou stain)

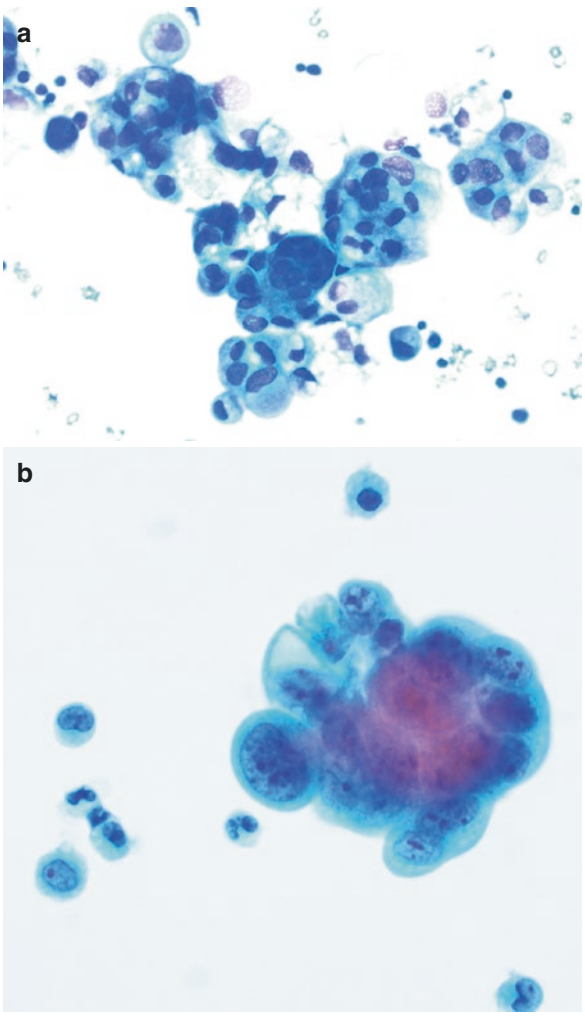

Fig. 9.8 Metastatic adenocarcinoma of pancreas. The cells are arranged as three-dimensional disorganized clusters. Granular to vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are present (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

metastatic breast carcinomas in effusions. However, it is not specific for breast origin and positive staining for GATA3 may be seen in carcinomas from other primary sites [20, 21]. It is noteworthy to mention that GATA3 may be positive in mesothelioma and over half (58%) of malignant mesotheliomas showed nuclear GATA3-positivity on histology specimens [22].

20. What are the features of metastatic papillary thyroid carcinoma?

There are a couple of case studies documenting cytomorphological features of effusion involved by metastatic thyroid carcinoma (Olson et al. 2013; Lew et al. 2015) [23, 24].

Accordingly, psammoma bodies were seen in both studies; one study observed classic features of conventional PTC, such as abundant papillae, nuclear enlargement and overlapping, intranuclear grooves, and pseudoinclusions. On the contrary, the malignant cells did not reveal the aforementioned classic features in the other study; instead, the metastatic papillary thyroid carcinoma cells showed a moderate amount of delicate and/or vacuolated cytoplasm, ovoid nuclei, and irregular nuclear contours. The lack of hallmark features of papillary thyroid carcinoma raises a great diagnostic challenge. In addition, these malignant cells may show positive staining for epithelial markers (i.e., MOC31, CEA, BerEp4), TTF-1, and napsin A. Without additional markers including thyroglobulin and/or PAX-8, misinterpretation of the findings as metastatic adenocarcinoma of lung origin may occur. Taken together, including PAX-8, thyroglobulin, and TTF-1 when performing immunostaining workup in an appropriate clinical context is crucial for establishing an accurate diagnosis (Fig. 9.11a, b).

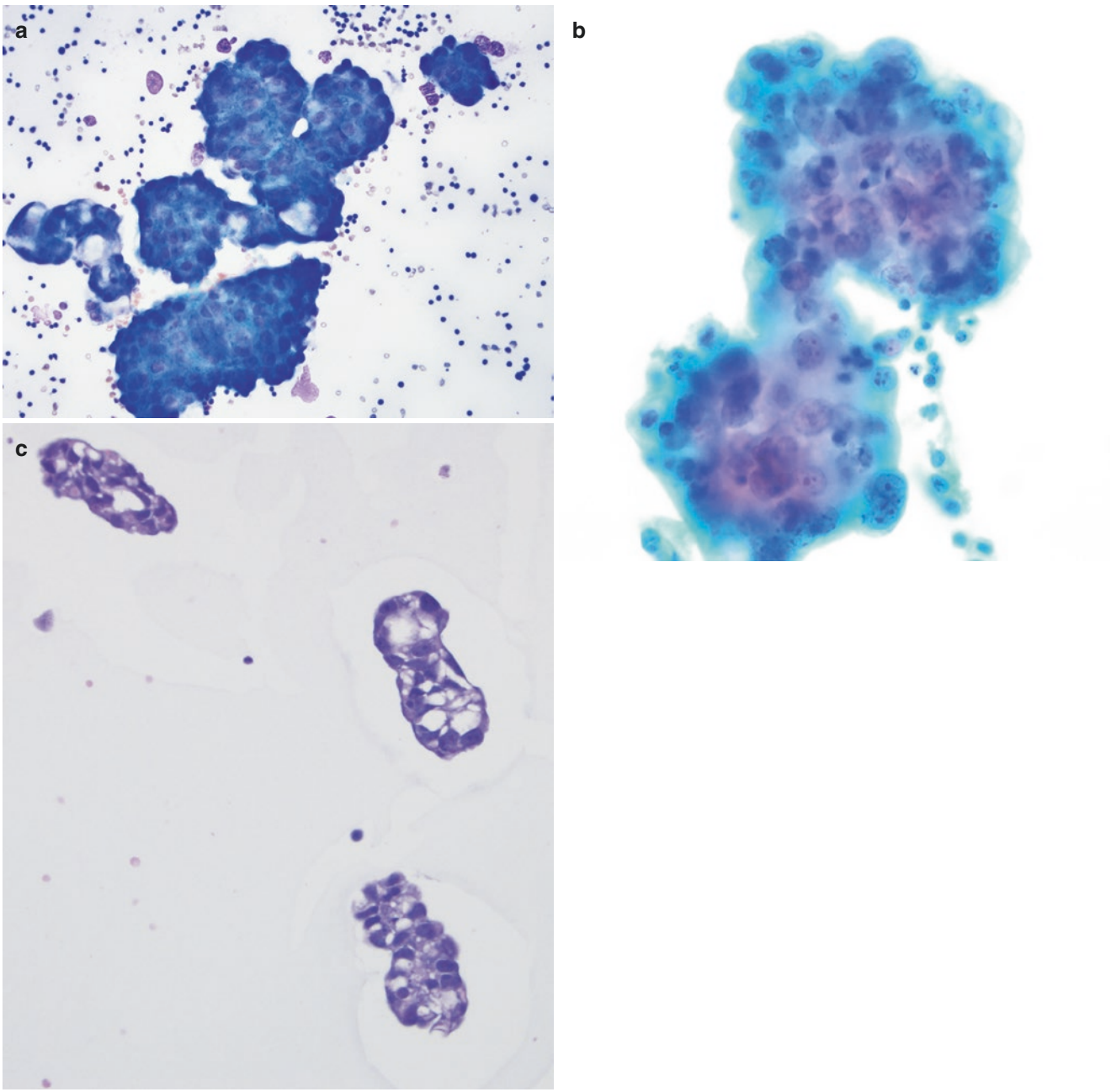

Fig. 9.9 Metastatic papillary serous carcinoma of endometrium. The carcinoma cells are arranged as complex, papillary clusters with crowded nuclei and slit-like spaces. Vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are present. (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively; **c**, H&E stain cell block)

21. What are the cytomorphological features of metastatic urothelial cell carcinoma?

Metastatic urothelial cell carcinoma in effusions is commonly poorly differentiated. The cells are arranged as single cells or clusters. Well-defined cell borders, dense cytoplasm, nuclear pleomorphism, nuclear enlargement with high N/C ratio, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are easily appreciated. Occasionally, vacuolated cytoplasm and cell-in-cell pattern may present. Metastatic urothelial cells may show positive for staining for GATA-3 and p63 (Fig. 9.12a–c).

22. What are the cytomorphological features of metastatic melanoma? What ancillary study can be used to make a definitive diagnosis?

Melanoma is notorious for its great variety of morphological features. The specimens have various amounts of malignant cells which are arranged as single cells, acini, and loose or three-dimensional clusters. The malignant cells appear epithelioid and pleomorphic with well-defined cytoplasmic borders. Larger neoplastic cells show various shaped nuclei, bi- or multinucleations, and cell-in-cell engulfment. The smaller neoplastic cells have a plasmacytoid appearance with round,

eccentrically located nuclei, coarse chromatin, and prominent nucleoli. Dark, coarse pigments may or may not be seen. Occasionally, intracytoplasmic vacuoles may present and some cells may show a signet-ring appearance, mimicking adenocarcinoma. A panel of immunostains consisting of melanoma markers (S-100, Mart-1/Melan-A, HMB-45, or SOX10) should be performed on the cell block material in order to establish a definitive diagnosis [25] (Fig. 9.13a, b).

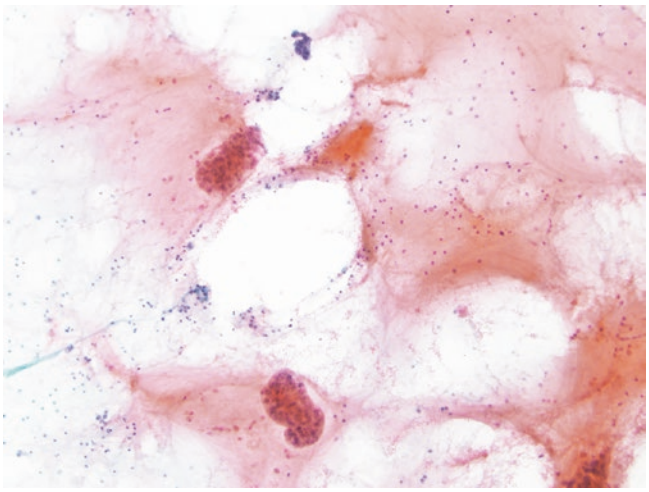

Fig. 9.10 Pseudomyxoma peritonei. Abundant thick mucin in the background. There are rare clusters of epithelial cells with mild atypia (Papanicolaou stain)

23. What role does flow cytometry or immunocytochemistry play in the diagnosis of non-Hodgkin's lymphomas involving effusion? How often is peritoneal involvement of lymphoma? pericardial?

Pleural effusion occurs in 20–30% of non-Hodgkin's lymphomas with a wide variation in rate of positive cytologic findings (22.2–94.1%) [26]. The involvement of peritoneal and pericardial cavities is less common. Flow cytometry analysis or immunocytochemistry as an adjunct to cytologic evaluation of effusions plays an important role in the diagnosis of non-Hodgkin's lymphomas, especially in situations of low to intermediate grade lymphoma involving the serous cavity and/or presence of a small proportion of malignant lymphoid cells. In these clinical scenarios, a definitive diagnosis of lymphomas is difficult to make based on cytomorphological finding alone as the atypical/suspicious cells may mimic reactive cells [27].

24. What are the cytomorphological features suggestive of multiple myeloma involving effusion?

The effusion contains single, dispersed cells, nuclear pleomorphism, bi- or multinucleation, eccentrically located nuclei, prominent nucleoli, and characteristic clock-face chromatin (Fig. 9.14).

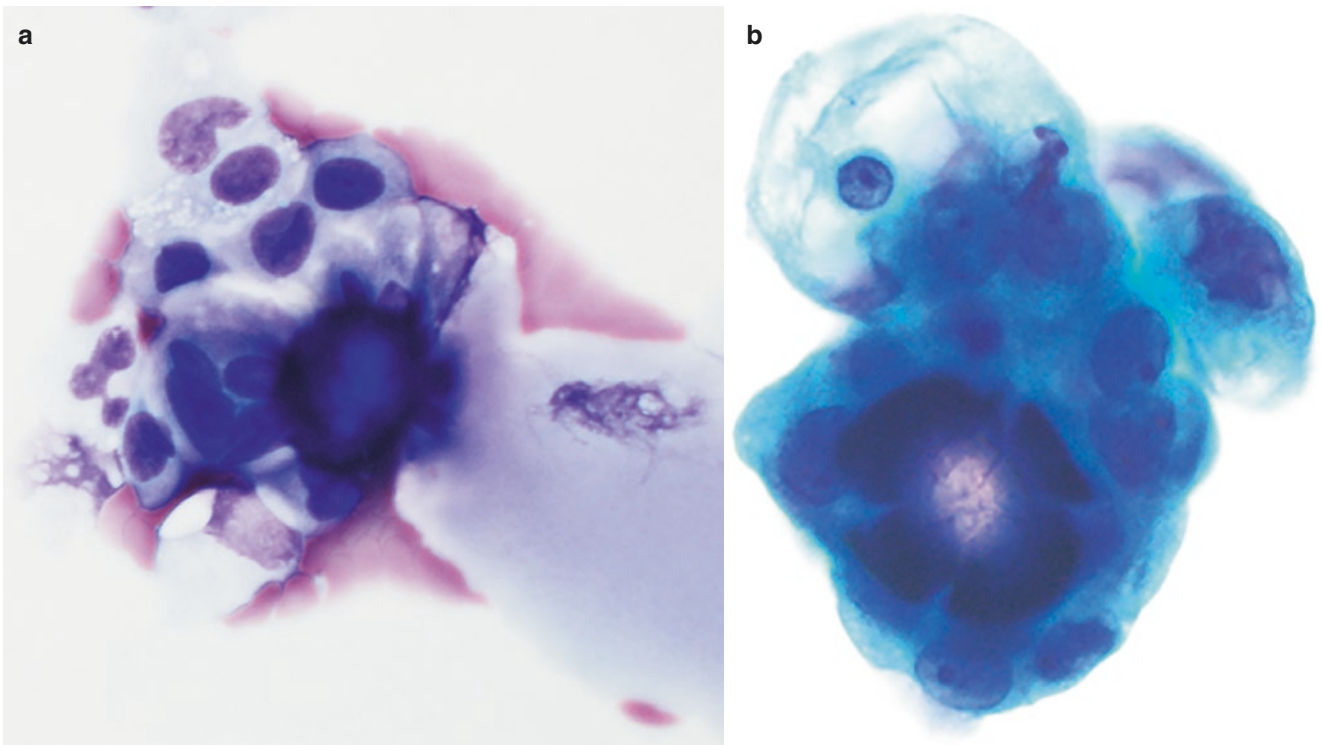

Fig. 9.11 Metastatic papillary thyroid carcinoma. Clusters of malignant cells with Psammoma bodies. Nuclear enlargement, irregular nuclear membrane, and intranuclear grooves are present. The presence of vacuolated cytoplasm mimics adenocarcinoma (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

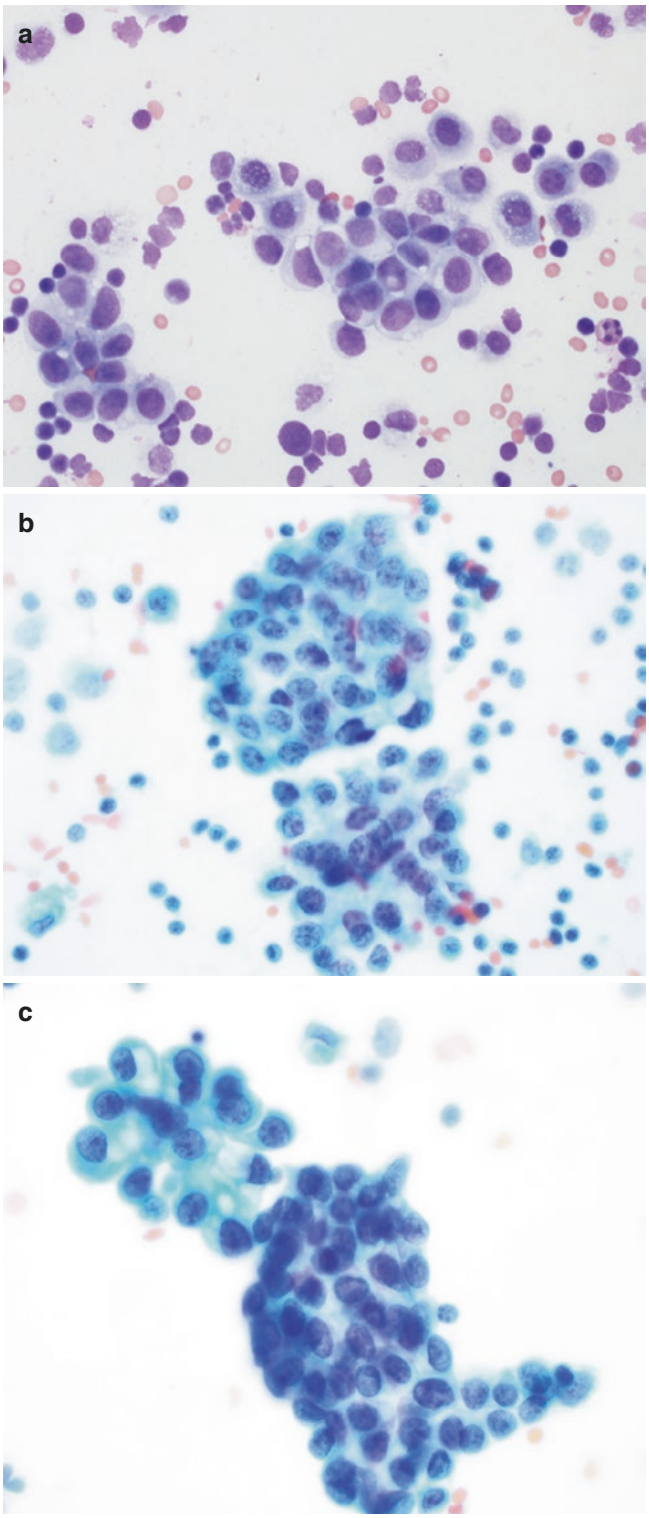

Fig. 9.12 Metastatic urothelial cell carcinoma. The cells are arranged as single cells or clusters. Well-defined cell borders, dense cytoplasm, nuclear pleomorphism, nuclear enlargement with high N/C ratio, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are easily appreciated. Vacuolated cytoplasm may present (**a**, Diff-Quik stain; **b** and **c**, Papanicolaou stain)

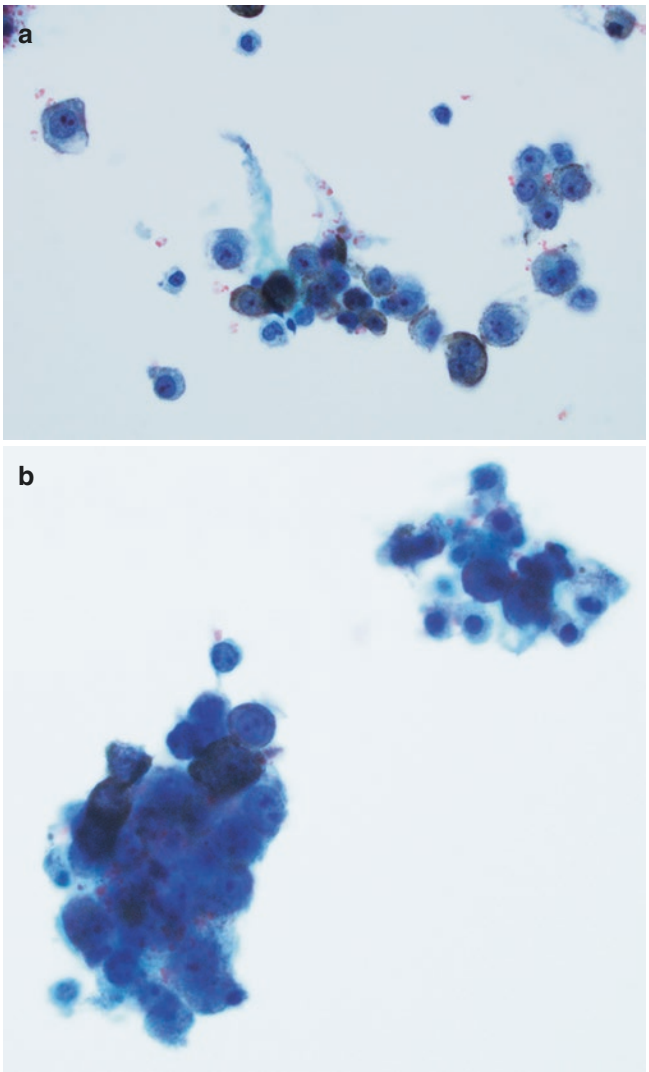

Fig. 9.13 Metastatic melanoma. Single and clusters of malignant cells have enlarged nuclei, coarse chromatin, and prominent nucleoli. Some cells contain dark, coarse pigments (**a** and **b**, Papanicolaou stain)

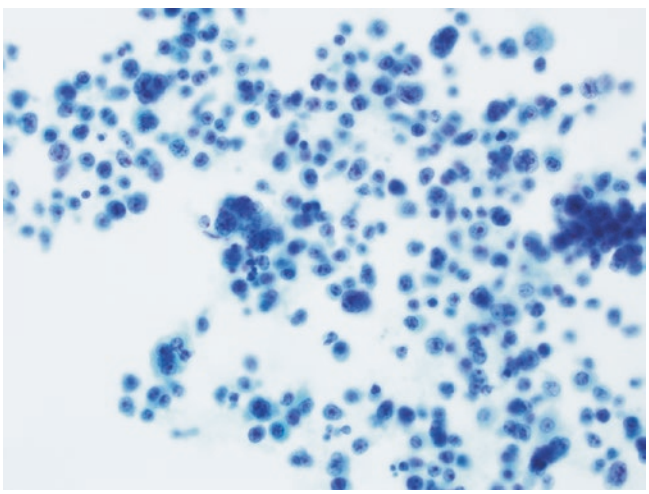

Fig. 9.14 Multiple myeloma. Single, dispersed cells, nuclear pleomorphism, bi- or multinucleation, eccentrically located nuclei, prominent nucleoli, and characteristic clock-face chromatin (Papanicolaou stain)

Case Presentation

Case 1

Clinical history:

- A 36-year-old female presents with left pleural effusion. She has a history of esophageal adenocarcinoma. Thoracentesis was performed and a total of 1000 mL of fluid is submitted for cytologic evaluation.

Cytomorphological findings:

- Both the Diff-Quik-stained conventional smear and Papanicolaou-stained ThinPrep® smear reveal scattered, single malignant-looking cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contour, coarse chromatin, and prominent nucleoli.

Differential diagnosis:

- Metastatic carcinoma
- Reactive mesothelial cells
- Mesothelioma

Immunostains performed on the cell block material:

- Positive for CK7, BerEp4, EMA, MOC-31, and CDX-2

Negative for TTF-1, GATA-3, and ER (Fig. 9.15a–e)

Final diagnosis:

- Metastatic adenocarcinoma, consistent with esophageal origin

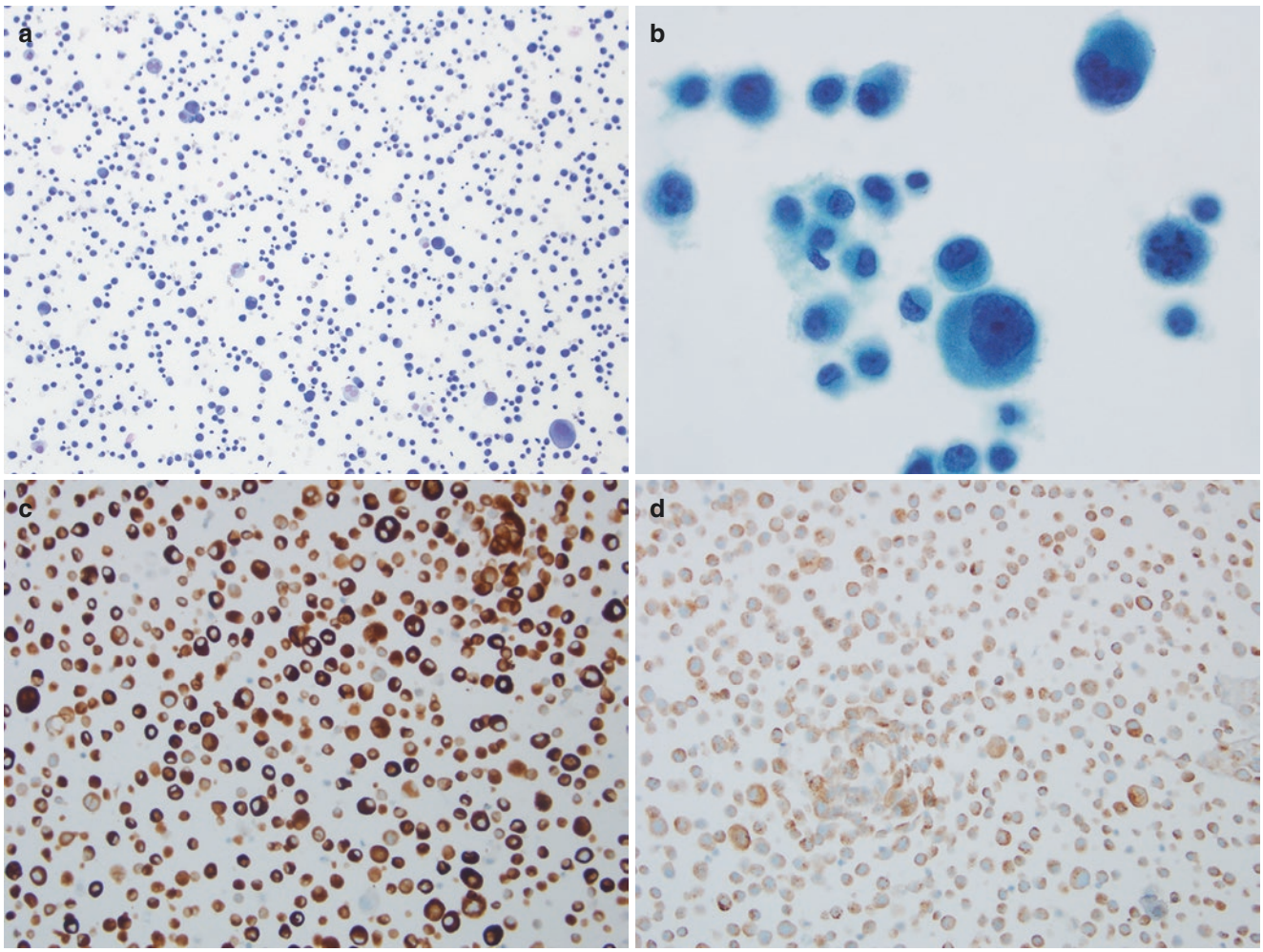

Fig. 9.15 Metastatic adenocarcinoma of esophagus. Single, dispersed malignant cells are present in both Diff-Quik- (**a**) and Papanicolaou-stained smears (**b**). The malignant cells contained in the cell block are positive for EMA (**c**), MOC-31 (**d**), and CDX-2 (**e**)

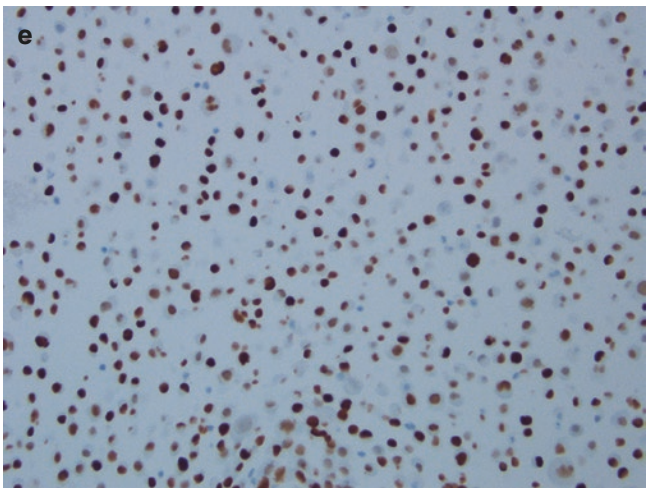

Fig. 9.15 (continued)

Case 2

Clinical history:

- A 76-year-old female presents with dyspnea. Imaging studies show right pleural effusion and thickening endometrium. Her past medical history is non-significant. Thoracentesis was performed and a total of 400 mL of fluid is submitted for cytologic evaluation.

Cytomorphological findings:

- The Papanicolaou-stained ThinPrep® smear reveals numerous single dispersed malignant-looking cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contour, coarse chromatin, and prominent nucleoli. Extremely large nuclei and multinucleation are seen.

Differential diagnosis:

- Metastatic carcinoma
- Mesothelioma
- Malignant neoplasm other than carcinoma

Immunostains performed on the cell block material:

- Positive for EMA, MOC-31, BerEp4, PAX-8, CK7, and CK20

Negative for WT-1, TTF-1, GATA-3, ER, and CDX-2 (Fig. 9.16a–d)

Final diagnosis:

- Metastatic adenocarcinoma, suggestive of Müllerian origin

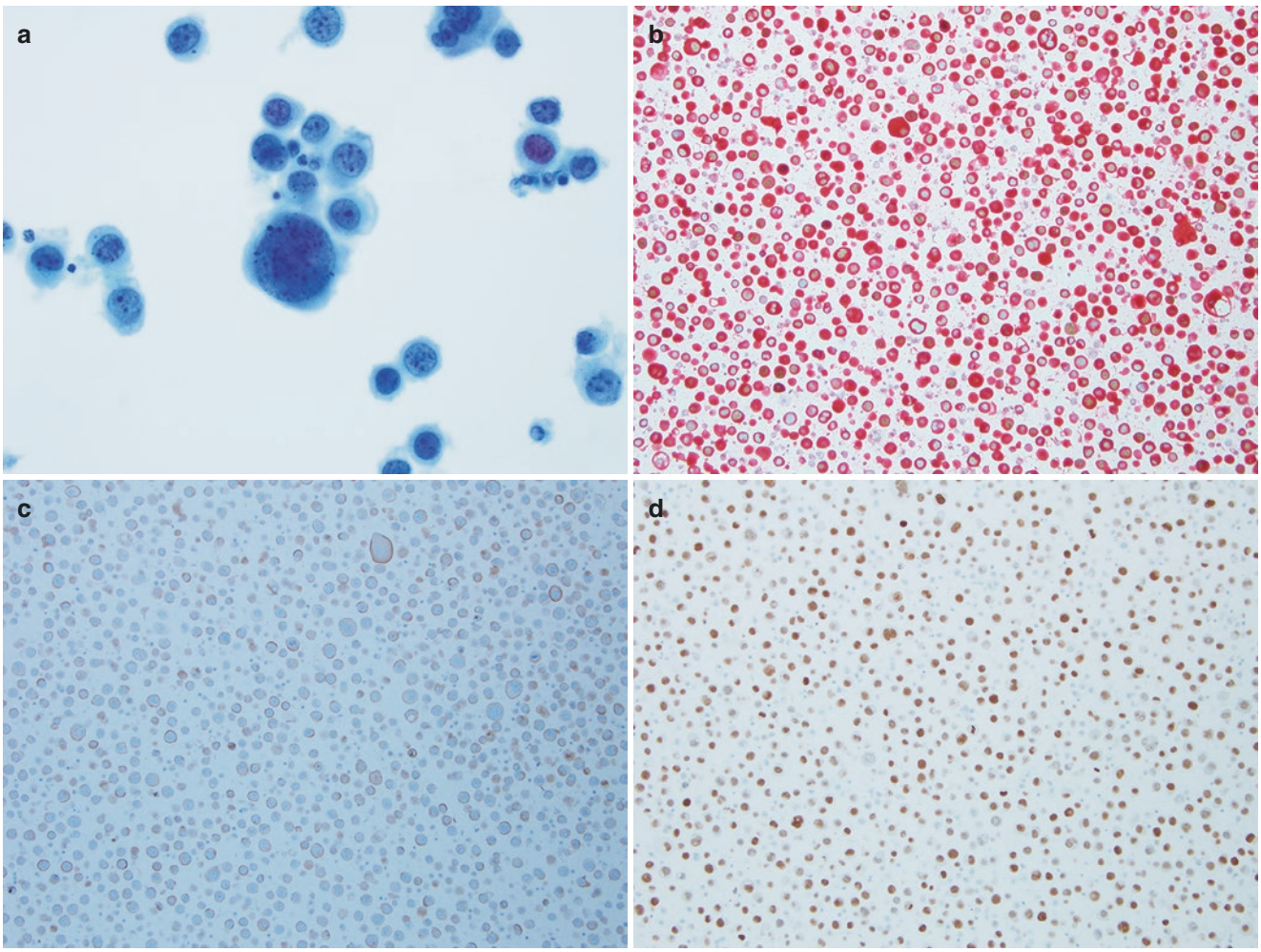

Fig. 9.16 Metastatic adenocarcinoma of Müllerian origin. The Papanicolaou-stained ThinPrep® smear reveals single dispersed malignant cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contours, coarse chromatin, and prominent nucleoli (**a**). The malignant cells are positive for EMA while being negative for WT-1 (**b**). The malignant cells are also positive for MOC-31 (**c**) and PAX-8 (**d**)

Case 3

Clinical history:

- An 87-year-old female presents with pleural effusion. Cytology smears and cell block prepared from the pleural fluid at the referring institution are received for consultation.

Cytomorphological findings:

- The provided smears show numerous malignant cells which are arranged as single cells or tight clusters with scalloped borders. The malignant cells show marked pleomorphism in nuclear size and shape, nuclear enlargement along with high N/C ratio, coarse chromatin, and prominent nucleoli. Cell-in-cell pattern and gigantic, multinucleated cells are easily seen.

Differential diagnosis:

- Metastatic carcinoma
- Mesothelioma
- Malignant neoplasm other than carcinoma

Immunostains performed on the cell block material:

- Positive for D2–40, WT-1, and EMA (enhanced membranous staining)
- Negative for desmin, B72.3, CEA, CD15, and PAX-8 (Fig. 9.17a–d).

Final diagnosis:

- Positive for malignant cells, favor mesothelioma

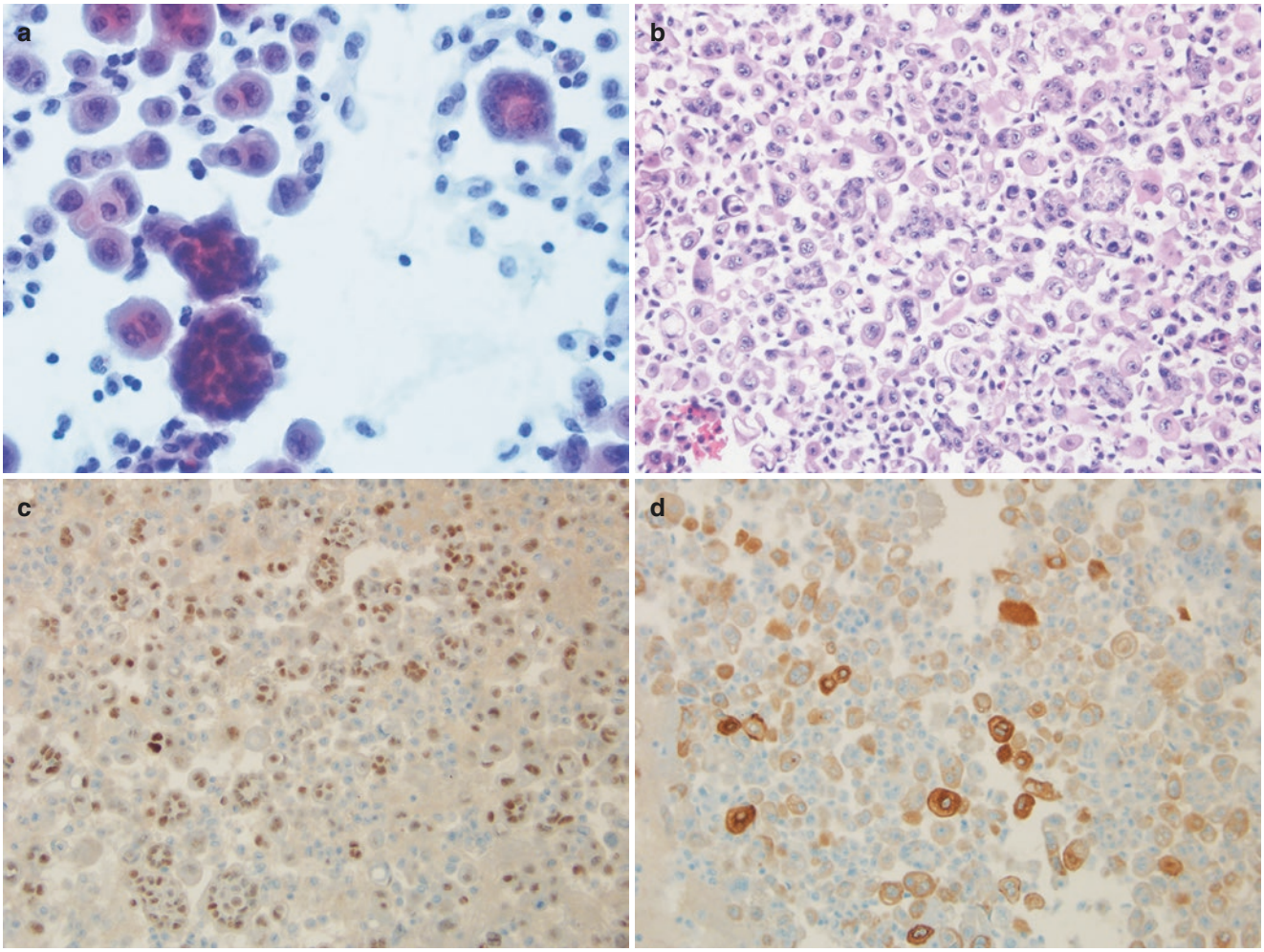

Fig. 9.17 Mesothelioma. Numerous malignant cells present in Papanicolaou-stained smear (**a**) and cell block (**b**). The cells are arranged as single cells or tight clusters with scalloped borders. Nuclear pleomorphism, gigantic multinucleated cells, cell-in-cell pattern, nuclear enlargement along with high N/C ratio, coarse chromatin, and prominent nucleoli are easily seen. The malignant cells stain positive for WT-1 (**c**, nuclear staining) and EMA (**d**, enhanced membranous staining)

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