HHS Public Access

Author manuscript

J Clin Transl Pathol. Author manuscript; available in PMC 2024 October 04.

Published in final edited form as:

J Clin Transl Pathol. 2023 ; 3(4): 160–177. doi:10.14218/jctp.2023.00025.

The International System for Reporting Serous Fluid Cytopathology—An Updated Review

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Abstract

The International System (TIS) for reporting serous fluid cytopathology was published in December 2020 as a joint project by the International Academy of Cytology and the American Society of Cytopathology. The purpose was to standardize the diagnostic criteria and nomenclature used in reporting serous fluid samples, thereby improving the reproducibility of reports and improving communication between pathologists and clinicians. TIS defines a five-tier system consisting of nondiagnostic, negative for malignancy, atypia of uncertain significance, suspicious for malignancy and malignant categories. This review provides an updated summary of the reporting system, risk of malignancy, potential diagnostic pitfalls, and a practical diagnostic approach to serous fluid specimens.

Keywords

Serous fluid; International reporting system; Cytopathology; Risk of malignancy

Introduction

Serous effusion is defined as an excessive accumulation of fluid in a body cavity, which includes the pleural, pericardial, and peritoneal spaces.[1] Cytological evaluation of serous fluid is useful for detecting underlying etiologies, such as malignancy, and evaluating tumor stage, as well as providing information for treatment customization.[2,3] To standardize the diagnostic criteria and nomenclature used in fluid cytology reporting, The International System (TIS) for reporting serous fluid cytopathology was proposed by the International Academy of Cytology and the American Society of Cytopathology.[4,5] The purposes of such a standardized reporting system are to improve the reproducibility of cytopathology reports,

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Author contributions

Manuscript writing (MHW) and critical revision (MHW, AC, GC).

Conflict of interest

GPC is the Editor-in-Chief of *Journal of Clinical and Translational Pathology*. The authors have no other conflicts of interest to declare.

improve the communication between pathologists and clinicians, and provide guidance for patient management. TIS defines a five-tier category system consisting of nondiagnostic (ND), negative for malignancy (NFM), atypia of uncertain significance (AUS), suspicious for malignancy (SFM), and malignant (MAL) categories. The diagnostic criteria for each category are summarized in Table 1.[6]

ND

Definition—A specimen is considered ND if it provides no diagnostic information in the appropriate clinical context, as in the case of acellular, highly degenerated, or hemorrhagic samples.[6] The sample is deemed unsatisfactory for evaluation owing to insufficient cellularity with no cells or rare benign-appearing cells (fewer than 10 cells), such as lymphocytes, macrophages, or red blood cells. This diagnostic category should only be used after an adequate and representative amount of fluid has been processed and examined.[6]

Adequacy assessment—The adequacy of a specimen depends on several factors, including the volume and cellularity of the sample. Furthermore, degenerated cells, generalized hemolysis, and poorly preserved cells should be considered ND instead of interpreted as AUS. A minimum volume of at least 75–100 mL is recommended for optimal results in detecting malignancy.[7] However, smaller volumes should not be rejected as they may contain adequate cellularity and cells of interest. A comment about cellularity should be stated for specimens with low cellularity. Mesothelial cells are not required for specimen adequacy.[8] Specimens with no mesothelial cells can still be considered satisfactory for evaluation as long as the specimens are compatible with clinical settings. Situations such as tuberculosis and chylous effusion can result in lymphocyteonly specimens. Likewise, a sample taken from an acute infection with a neutrophil-predominant effusion can be considered adequate even without mesothelial cells. Blood-only specimens are mostly considered ND; however, it can be categorized as NFM in an appropriate clinical setting, such as tamponade, which can contribute to a blood-only pericardial fluid.[8] On the other hand, specimens with adequate volume and cellularity can still be considered ND if the specimens demonstrate extensive degenerative changes.

Risk of malignancy (ROM) and clinical management—The reported ROM for this category has varied from 0 to 100%, with a mean ROM of 17 ± 8.9% as reported by a recent meta-analysis.[6,9] An effusion with a ND result should be re-aspirated if clinically indicated.[10]

NFM

Definition—The specimen with a benign diagnosis shows no evidence of malignancy in the appropriate clinical setting.[6] A serous effusion specimen obtained from peritoneal, pleural, or pericardial cavities may be considered NFM when the specimen is composed of only benign or reactive cellular components, without MAL tumor cells or cells concerning for malignancy.[6]

Diagnostic considerations and pitfalls—By definition, these samples are adequate for evaluation although the specimens may be paucicellular. However, the scant material may

not be adequately representative of the underlying lesion and therefore could potentially result in a failure to detect an existing malignancy. In our practice, if there is no suspicion for malignancy based on cytomorphologic analysis, we usually do not perform immunohistochemistry. However, if the patients have a history of malignancy or are clinically suspicious for malignancy, we do recommend performing immunohistochemistry with a panel of two epithelial and two mesothelial markers. NFM will be rendered if epithelial markers are negative. If positivity of epithelial markers is detected, additional immunostains are performed to elucidate the origin of the tumor. In cases with low cellularity, an indeterminate diagnosis including AUS or SFM is likely to be reported with a recommendation for repeat sampling for a definite diagnosis.

ROM and clinical management—The recent meta-analysis has reported that the ROM of the NFM category ranges from 0–82% with a mean ROM of 21%.[6,9] Overall, serous effusion cytology has excellent diagnostic performance with a sensitivity of 73.1%, specificity of 99.9%, a positive likelihood ratio of 2112.2, and a negative likelihood ratio of 0.27.[9] Patients with a NFM diagnosis are generally followed-up clinically.[10]

AUS

Definition—The AUS diagnostic category is reserved for effusion specimens that lack quantitative or qualitative cytological features to be confidently diagnosed as either benign or MAL and exhibit sufficiently clear morphologic features to exclude the possibility of classifying them as ND.[6] Atypical cells may include atypical cells of undetermined origin, atypical mesothelial cells, or atypical lymphoid cells, with cytological features indefinite for a diagnosis of SFM.[6]

Diagnostic pitfalls—AUS should be a diagnosis of exclusion and its rate kept as low as possible in order to increase clinicians' confidence in cytologic reports. The atypical cells in this category cannot be confidently ruled out as MAL, but while they show morphological features overlapping with reactive changes, they are more likely to be only reactive changes. One of the common reasons for this uncertainty is that there is not sufficient material for further work-up.[11] Another common reason is the focal positivity of epithelial markers in atypical cells.

According to TIS, atypical cells include atypical epithelial cells, atypical mesothelial cells, and atypical lymphoid cells.

ROM and clinical management—The meta-analysis study has reported an estimated ROM of AUS of 66 ± 10.6%.[6,9] The management of AUS interpretation needs clinical correlation. If the patient does not have a history of malignancy or if fluid does not reaccumulate, in most cases follow-up is appropriate. Repeat sampling for further evaluation is recommended if the patients have a history of malignancy or fluid reaccumulates.[10]

SFM

Definition—The SFM category is defined as one in which the evidence falls short of confirming malignancy based on cytomorphology and results of any ancillary tests performed.[6]

Diagnostic considerations and pitfalls—Compared with AUS, cases with a SFM diagnosis are more likely to be MAL.[12] Abnormal cells in an SFM diagnosis have notable cytological atypia and are morphologically suspicious for malignancy. These cells are classified as SFM rather than MAL for the following reasons: (1) There is no cell block for further work-up; (2) Immunohistochemistry may be noncontributory due to the cells of interest not appearing on the levels used for immunostaining; (3) There is focal positivity of epithelial markers, but the nonspecific staining of the epithelial cells in histiocytes and mesothelial cells cannot be ruled out; or (4) Suspicious cells are sparse and admixed with mesothelial cells and histiocytes, and although these cells might be highlighted by epithelial markers, they are rarely identified in hematoxylin and eosin sections.

ROM and clinical management—The meta-analysis study has reported the ROM of SFM as 82 ± 4.8%.[6,9] SFM has a high ROM, which supports more aggressive management for patients diagnosed with SFM. In the appropriate clinical setting, cases with SFM may be managed similarly to MAL cases.[12–14]

MAL

Definition—Cases categorized as MAL are defined as effusion specimens that present cells showing cytomorphological features, either alone or in combination with ancillary studies, diagnostic of malignancy.[6] This category includes primary malignancies and secondary malignancies. Malignant mesothelioma is a primary malignancy originating from mesothelial cells, accounting for 1–6% of malignant effusion cases.[15] A majority of malignant effusions are metastatic carcinoma to the pleura, most commonly from the lung and breast. Other secondary malignancies include melanoma, sarcoma, and lymphoma.[15,16]

Diagnostic considerations and pitfalls

Mesothelial cells vs. metastatic carcinoma: Mesothelial cells, histiocytes, and small lymphocytes are normal elements in fluid specimens (Fig. 1). In daily practice, differentiating reactive mesothelial cells from malignant components is commonly encountered. To distinguish metastatic carcinoma from reactive mesothelial cells, the first step is to assess if there are a second population of cells, i.e. the alien epithelial component, in the effusion specimen. Metastatic carcinoma can form tightly packed clusters with a smooth border or are individually distributed. The challenge is that reactive mesothelial cells with cytologic atypia can form clusters even with a papillary architecture, morphologically mimicking epithelial clusters. Additionally, dispersed individual carcinoma cells may mimic reactive mesothelial cells, subsequently producing a picture of a single population of cells (Fig. 2).

For these reasons, an immunopanel of at least two epithelial markers and two mesothelial markers are recommended to differentiate metastatic epithelial cells from mesothelial

cells in effusion specimens. Mesothelial markers include calretinin, WT-1, D2–40, CK5/6, HBME-1, and thrombomodulin.[17–19] Epithelial markers include claudin-4, MOC31, Ber-Ep4, BG-8, B72.3, CD15 (LeuM1) and CEA.[17,19,20] Focal reactivity of epithelial markers, such as MOC31 and Ber-Ep4 can be found in reactive mesothelial cells as well as in up to 35% of mesotheliomas (Fig. 3).[21] Recent studies show that claudin-4 can be used as a single marker in effusion as it is highly sensitive for metastatic carcinoma and completely negative in mesothelial cell origin (Fig. 3).[21–24] A panel of claudin-4 and Ber-EP4 yields the highest combined sensitivity and specificity.[22] HEG1 also holds good potential as a marker for mesothelial differentiation because it is reported to be highly sensitive for mesothelial cells but negative for carcinoma cells.[25]

Tumor origin of secondary malignancy in effusions

Once the malignant component is identified, the next step is to identify the tumor origin of the metastatic carcinoma. Depending on the patient's sex, age, and malignancy history, organ-specific markers can be included in the diagnostic panel. If the patient has a documented history of malignancy, the cytomorphology of the tumor cells is typical of the particular tumor type, direct application of organ-specific markers would be a reasonable approach. Otherwise, an approach to initially narrow down possible tumor origins by examining the expression patterns of cytokeratin, CK7, and CK20 followed by organ-specific markers might be considered. The algorithm is summarized in Figure 4. Organ-specific markers are summarized below.

Lung

TTF1 and napsin A: TTF1 and napsin A are particularly useful for primary lung adenocarcinoma (Figs. 2 and 5). It should be brought to attention that histiocytes can be reactive to napsin A and may be misinterpreted as individual epithelial cells. To address this, duplex TTF1/napsin A shows nuclear TTF1 and cytoplasmic napsin A staining in different colors, which may be helpful in distinguishing histiocytes from epithelial cells, especially when tumor cells are scattered in fluid specimens.

Breast: GATA3, mammaglobin, and GCDFP-15 are the traditional markers for tumors of breast origin. GATA3 is probably the most used marker to confirm metastatic breast carcinoma (Fig. 6). However, GATA3 expression has also been noted in many other organs and pathogeneses, including urothelial cancer, a subset of lung cancer, and gynecologic cancer.[26–28] Therefore, a panel of mammaglobin and GCDFP-15 may be needed for distinguishing breast cancer from urothelial cancer. SOX10 and TRPS1 are newer markers which can help detect triple-negative breast cancer. Recently, TRPS1 has been reported to be a highly sensitive and specific marker for breast carcinoma, especially in triple-negative breast cancer.[29–31] TRPS1 could also be used to distinguish metastatic breast cancer from urothelial cancer as this marker is negative in tumors of urothelial origin.[29,31] It also should be noted that triple-negative breast carcinoma may show positivity for calretinin.[20]

Gastrointestinal tract: CDX2, SATB2, and CK20 can help distinguish gastrointestinal adenocarcinoma (Fig. 7). Mesothelial cells show focal positivity for CK20. Interpretation of CK20 staining needs to be combined with mesothelial markers and cytomorphology in

hematoxylin and eosinstained sections. CK7 and pan CK, however, have a limited role in effusion as these markers are diffusely positive in mesothelial cells and expressed at least in selective cases of gastrointestinal tumor.

Gynecologic origin: PAX-8, ER, WT1 can suggest a gynecologic primary site. In high-grade serous carcinoma, p53 shows aberrant expression, including overexpression (strong nuclear staining in at least 75% of tumor cells), null pattern (loss of staining), and cytoplasmic pattern (Fig. 8).

SOX10, HMB45, and melanin A are useful markers for confirmation of metastatic melanoma in effusion fluids (Fig. 9). INSM1, synaptophysin, chromogranin, and CD56 are the commonly used neuroendocrine markers (Fig. 10).

Prostatic adenocarcinoma: NKX3.1, PSA, and PSAP are shown in Figure 11. Compared with PSA and PSAP, NKX3.1 may show a higher sensitivity in identifying metastatic prostate cancer, especially those of poorly differentiated carcinoma.[32]

Thyroid: TTF1, PAX8, and thyroglobulin are common markers for tumors of thyroid origin. It should be noted that PAX8 might be the only marker, though, to be positive in anaplastic thyroid carcinoma.

Kidney: PAX8 is a pan-marker of tumors of renal origin, while CAIX is likely expressed in clear cell renal cell carcinoma.

Urothelial tract: GATA3 is the most commonly used marker for urothelial carcinoma. Uroplakins, p63, p40, and 34BE12 are the other markers likely expressed in tumors of urothelial origin.

Reactive mesothelial proliferation vs. malignant mesothelioma

Distinguishing malignant mesothelioma from florid reactive mesothelial cells can be difficult. The cytological diagnosis of malignant mesothelioma by effusion cytology might be controversial in clinical practice, and the diagnostic value of cytology may be questionable.[33,34] However, in recent years, molecular and biomarker testing that can distinguish neoplastic mesothelial cells from reactive/benign mesothelial cells has enhanced diagnostic accuracy of mesothelioma in effusions. It is currently becoming recognized that mesothelioma diagnosis in cytology specimens is possible and with high specificity and positive prediction value when an adequate sample is provided and ancillary tests are applied.[35–38]

Guidelines for the diagnosis of mesothelioma were proposed by the International Mesothelioma Interest Group 2015 Supplement and followed by the International Guidelines for Reporting Serous Fluids (Table 2).[6,39,40] Morphology, high cellularity, presence of complex papillary structures or tissue fragments, variation in cell size and shape, and marked cytologic atypia are useful features for distinguishing and identifying mesothelioma (Table 3) (Fig. 12). Nevertheless, the role of immunohistochemistry in distinguishing mesothelioma from reactive mesothelial cells is still limited. Benign mesothelial cells show strong

cytoplasmic staining for desmin and are negative for epithelial membrane antigen (EMA), while mesothelioma cells are negative for desmin but show membranous band-like staining with EMA.[20,41–44] However, due to background benign mesothelium, interpretation is not always confidently conclusive. Therefore, desmin and EMA tests are not recommended for diagnostic work-up in the determination of inclusion or exclusion of mesothelioma.

Homozygous deletion of CDKN2A (9p21) has been frequently detected in mesothelioma by fluorescence *in situ* hybridization testing.[45] In addition, homozygous co-deletion of the MTAP gene occurs in 80–90% of tumors with CDKN2A deletion.[45] MTAP expression can be detected by immunohistochemical methods, thus it can potentially be used as a surrogative marker for CDKN2A deletion.[25,46] MTAP shows both nuclear and cytoplasmic staining in normal mesothelial cells, while only the loss of cytoplasmic expression, which occurs in up to 65% of mesothelioma cases,[35] is considered abnormal.

BAP1 is a tumor suppressor gene product. Loss of BAP1 expression is found in up to 65% of mesotheliomas and is excluded in reactive mesothelial cells.[47] A combined immunoprofile of claudin-4, HEG1, MTAP and BAP1 is recommended for differentiating metastatic carcinoma and malignant mesothelioma on cytology and in biopsy specimens with HEG1 and claudin 4 to validate the cell origins and BAP1 and MTAP to confirm mesothelial malignancy.[21,25,48]

Atypical lymphoid proliferation

Differential diagnosis of lymphocyte-rich serous effusions includes reactive lymphocytes and involvement by a lymphoproliferative disorder. Lymphomatous effusion accounts for 3.6–10% of effusion malignancies, including diffuse large B cell lymphoma, lymphoblastic lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma, follicular lymphoma, Burkitt's lymphoma, anaplastic large cell lymphoma, peripheral T cell lymphoma, and Hodgkin's lymphoma.[49–56] The majority of lymphoproliferative involvement are of B cell origin,[52,53] and T-lymphoblastic lymphoma is perhaps the most common lymphoma in pediatric patients (36.9%).[49] Burkitt's lymphoma is more frequently seen in peritoneal fluid compared to pleural and pericardial fluids, and involvement of effusion by Hodgkin's lymphoma is extremely rare.[49,57]

High-grade non-Hodgkin's B cell lymphoma and T cell-origin lymphoma usually contain large atypical cells with evident morphologic atypia (Fig. 13); however, low-grade B cell non-Hodgkin's B cell lymphoma contains small to intermediate-sized lymphocytes, which are challenging to separate from reactive lymphocytes (Fig. 14). Monomorphic morphology is a valuable indicator to perform further work-up. If a cell block is available, immunohistochemical study with a B cell marker (CD20) and a T cell marker (CD3) is commonly used as an initial step to separate reactive lymphocytes from a possible lymphoproliferative disorder. Predominantly CD3-positive T cells admixed with sporadic CD20-positive B cells are characteristic of a reactive process. If a lymphoma is suspected, additional various antibodies might be needed to subtype the specific cell lineage and detect aberrant expression of markers. A panel of immunomarkers are determined based on morphologic differential diagnoses, including leukocyte common antigen (CD45), B cell

markers (CD20 and PAX5), T cell markers (CD3), and other markers such as CD5, CD10, CD23, BCL-2, BCL-6, cyclinD1, CD15, CD30, ALK-1, CD138 (Tables 4 and 5).[49]

Flow cytometry is essential for distinguishing lymphoma from reactive lymphocytes and for specifying lymphoma subtypes in fluid specimens.[50,58] It should be noted that neoplastic cells in large cell lymphoma are particularly fragile and can lose cell surface markers, which may result in noncontributory determination by flow cytometry analysis.[53] Another pitfall of flow cytometry is that it is not sensitive in detecting T cell-origin and Hodgkin's lymphoma.

In addition, a valuable technique is molecular testing for rearranged immunoglobulin or T cell receptor genes by PCR to assess the presence of clonal lymphoproliferation.[59] Detection of immunoglobulin heavy chain by fluorescence *in situ* hybridization can also be helpful.[60]

It is noteworthy that lymphoma is rarely manifested initially in effusion; rather, it is more commonly encountered as a secondary involvement, indicative of an advanced stage.[49,50] However, primary effusion lymphoma is a rare type of large B cell lymphoma that occurs primarily as pleural/pericardial/peritoneal effusion without lymph node involvement. This entity is strongly associated with HHV8 and HIV, and is usually Epstein–Barr virus-positive. The neoplastic cells are positive for CD45, CD30, EMA, HHV8, and Epstein–Barr virus EBER, but negative for CD20, CD5, CD22, CD23, and CD43.[61]

In conclusion, the combined application of cytological features, immunocytochemistry, flow cytometry, PCR, and fluorescence *in situ* hybridization can yield a correct diagnosis and classification of most lymphomatous cases.[60]

ROM and clinical management—The ROM of the MAL category is 99 ± 0.1% reported from the meta-analysis study.[6,9] The predicted positive value is almost 100%. Clinically, diagnosis is sufficient to start treatment of these patients with an advanced clinical stage of malignancy (stage IV).

Incorporation of molecular data

Molecular testing, in conjunction with cytomorphology and immunohistochemical studies, can assist in confirming the diagnosis of malignant mesothelioma, identifying a metastatic neoplasm, and determining the origin of a metastasis.[62] Paraffin-fixed cell blocks are usually prepared from effusion specimens and are essential for ancillary testing including immunohistochemistry, PCR, fluorescence *in situ* hybridization, and next-generation sequencing molecular testing, for both diagnostic and therapeutic purposes.[62] In addition, effusion supernatants, even after centrifugation, can still provide reliable material for analyzing DNA, RNA, microRNA, and proteins expressed by tumor cells. Both cells and supernatants can yield valuable molecular information that can assist in the diagnosis.[63,64]

Conclusions

Since the introduction of TIS, it has been applied to pleural, peritoneal, and pericardial fluids in several studies.[8,13,65–69] However, the ROM proposed in the TIS system was determined from a meta-analysis of historic data predating TIS.[9] We have summarized

recent studies that reclassified effusion cases using the TIS system and have provided an updated perspective on the distribution and ROM for each category in different body cavities. The distribution of each category is summarized in Table 6.[8,9,11–14,56,65,67–77] Reported ROMs for all categories are summarized in Table 7.[9,12–14,56,65,67–77] In summary, the distribution rates of ND, NFM, AUS, SFM and MAL range from 0–11%, 36–81%, 0.2–6%, 0.9–6.3% and 12.3–56.2%, respectively. The ROMs of ND, NFM, AUS, SFM, and MAL range from 0–100%, 1.8–51.6%, 0–88%, 49–93%, and 98–100%, respectively.

Overall, TIS defines clear diagnostic criteria for five categories: ND, NFM, AUS, SFM, and MAL. This five-tier system supports a standardized reporting structure for verifying malignancy, offering high specificity, positive predictive value, and modest to high sensitivity.[68,69] Increasingly available data provide evidence that the standardized categorization system effectively stratifies malignancy risks, facilitating the triage of clinical management.[78] However, despite the defined diagnostic criteria, the AUS category remains a diagnostically gray zone in effusion cytology. The definition of ND is defined, but its practical threshold varies, contributing to the wide variability in the ROM for the ND category.[79] Further validation studies should be conducted to contribute more data on the ROM in each category of specific body cavities, providing more evidence for clinical management and further refining the classification. Regardless, pelvic and peritoneal washing specimens still pose diagnostic dilemmas because of the presence of Müllerian borderline tumors. Addressing this issue may require a subdivision of the TIS, a topic we did not cover in this review but one that requires further investigation.[79]

Funding

NIH research grant P30CA016359 supported this study.

Abbreviations:

AUS	atypia of uncertain significance
BAP1	BRCA1-associated protein
CDKN2A	cyclin-dependent kinase inhibitor 2A
EMA	epithelial membrane antigen
MAL	malignant
MIS	Mesothelioma in situ
MTAP	methylthioadenosine phosphorylase
ND	nondiagnostic
NFM	negative for malignancy
ROM	risk of malignancy
SFM	suspicious for malignancy

TIS	The International System

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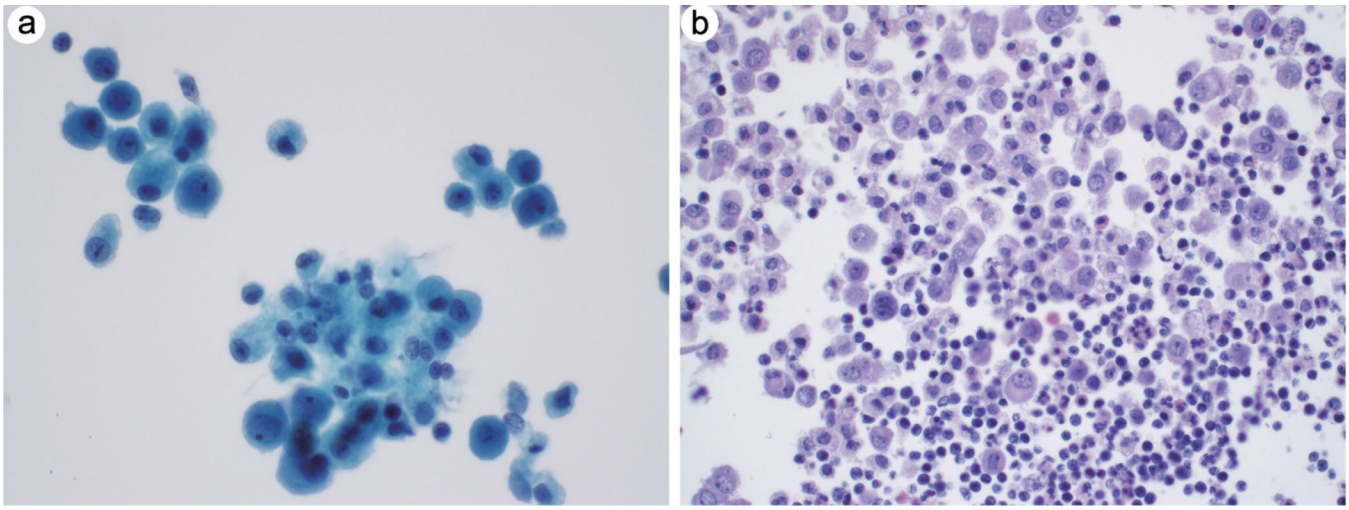

Fig. 1. Reactive mesothelial cells, histiocytes, and small lymphocytes.
(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 400×.

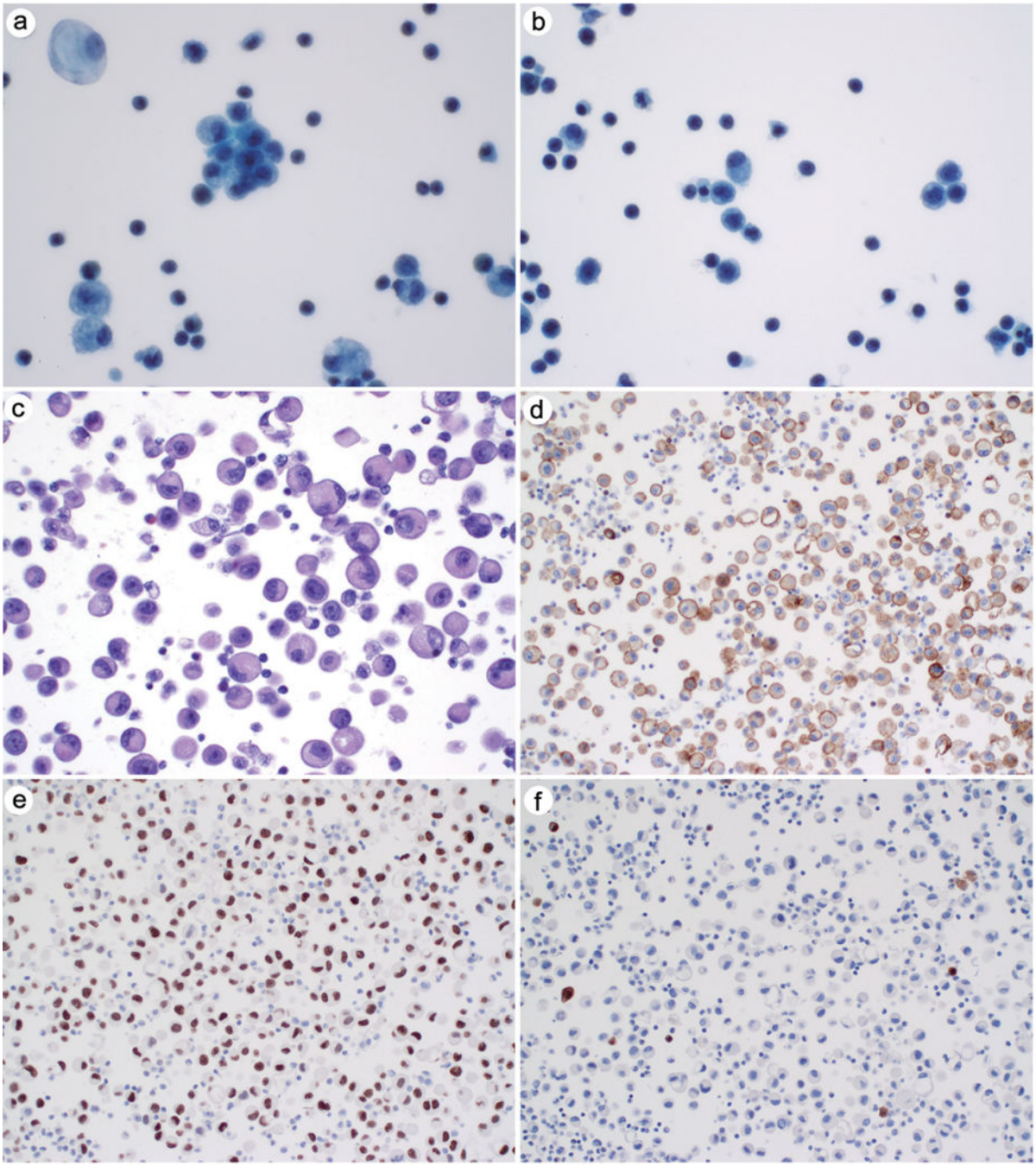

Fig. 2. Metastatic lung adenocarcinoma in pleural fluid.

Tumor cells show individual distribution or form loosely cohesive clusters, mimicking mesothelial cells or histiocytes. (a–b) ThinPrep, 600×. (c) Hematoxylin and eosin stain, 400×. (d–f) Tumor cells are positive for Ber-Ep4 (d) and TTF1 (e) but negative for calretinin (f); all, 200×.

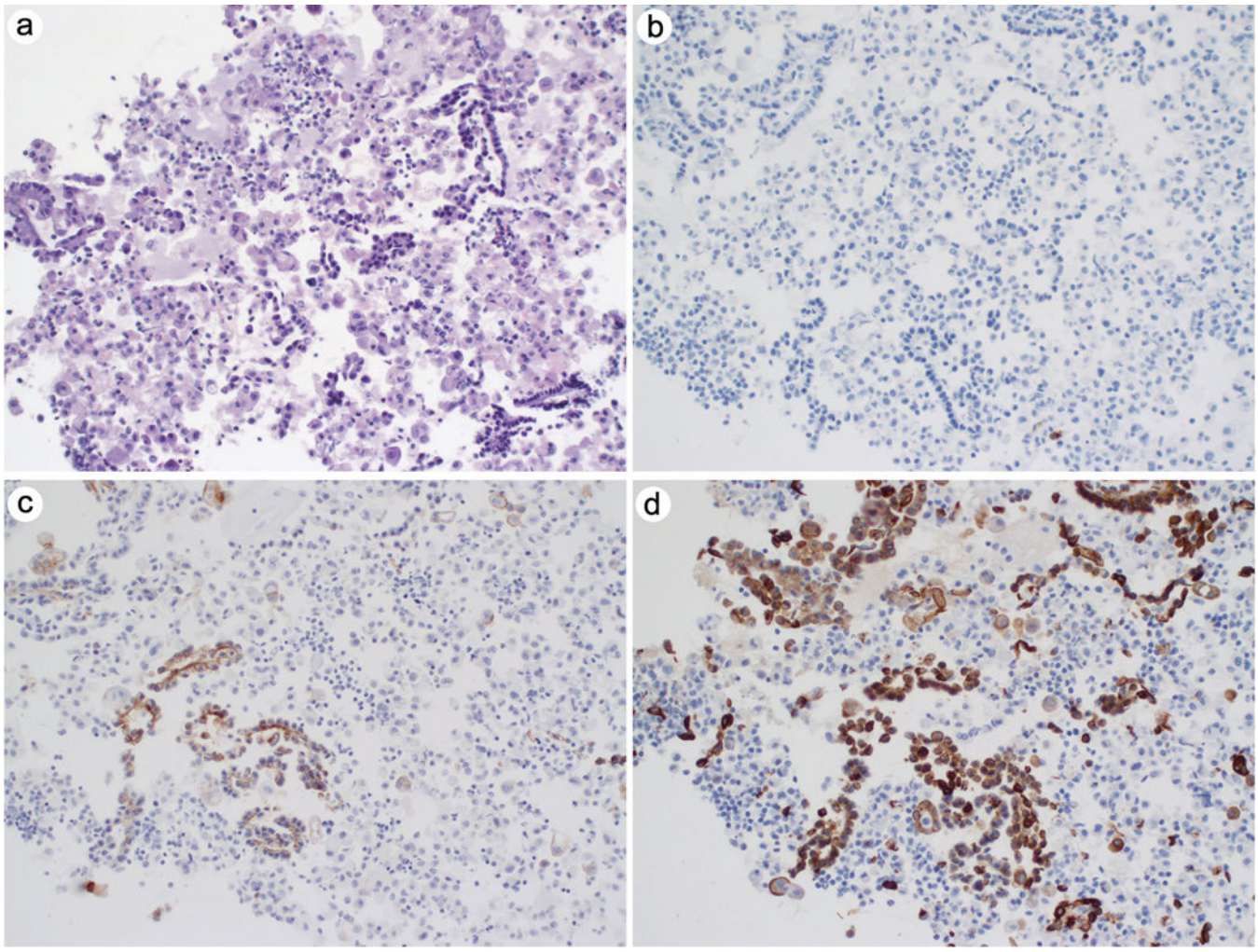

Fig. 3. Cells with BerEp4 positivity may be reactive mesothelial cells.

(a) Mesothelial cells in pericardial fluid show reactive changes, 200×. (b) Claudin-4 is negative in mesothelial cells, 200×. (c) Ber-Ep4 shows positivity in these reactive mesothelial cells, overlapping with CK5/6 staining, 200×. (CK5/6 highlights reactive mesothelial cells, 200×.

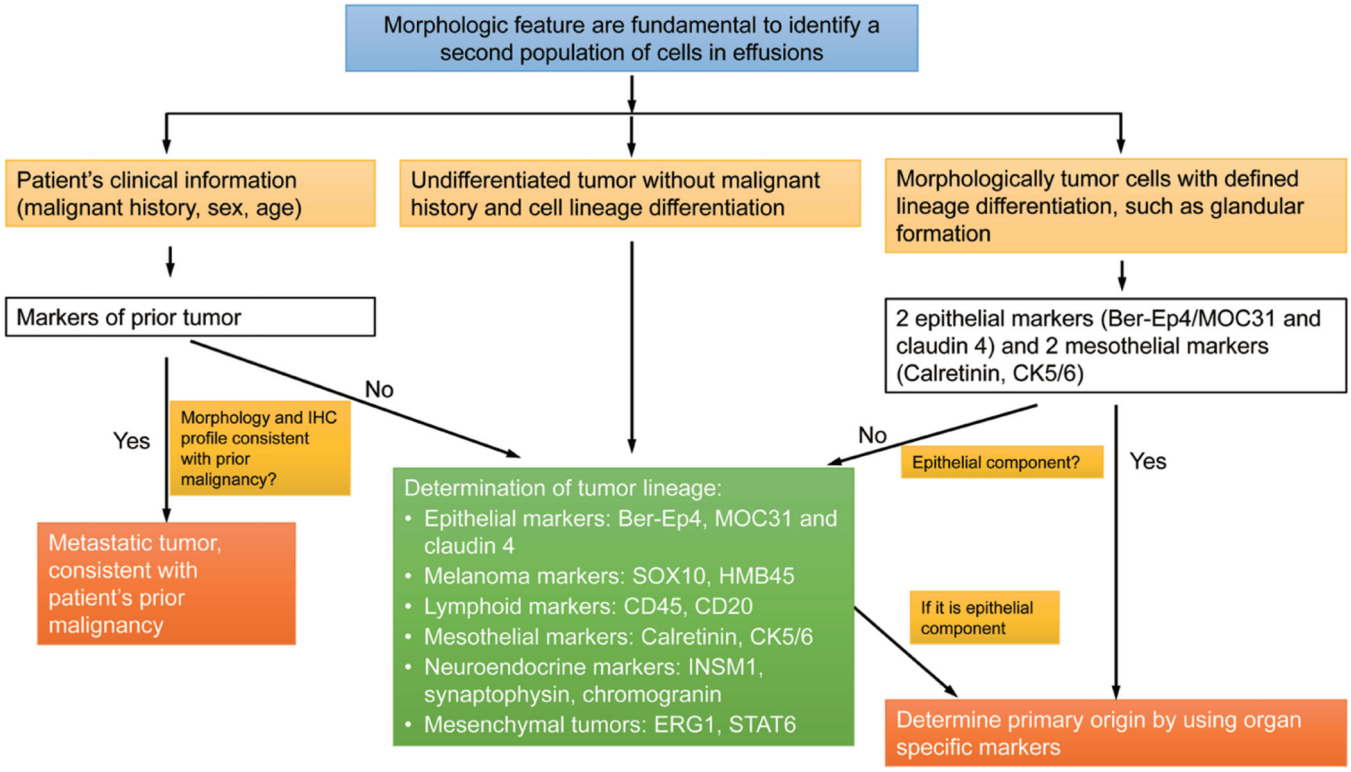

Fig. 4. Algorithm to identify the tumor origin in effusions.

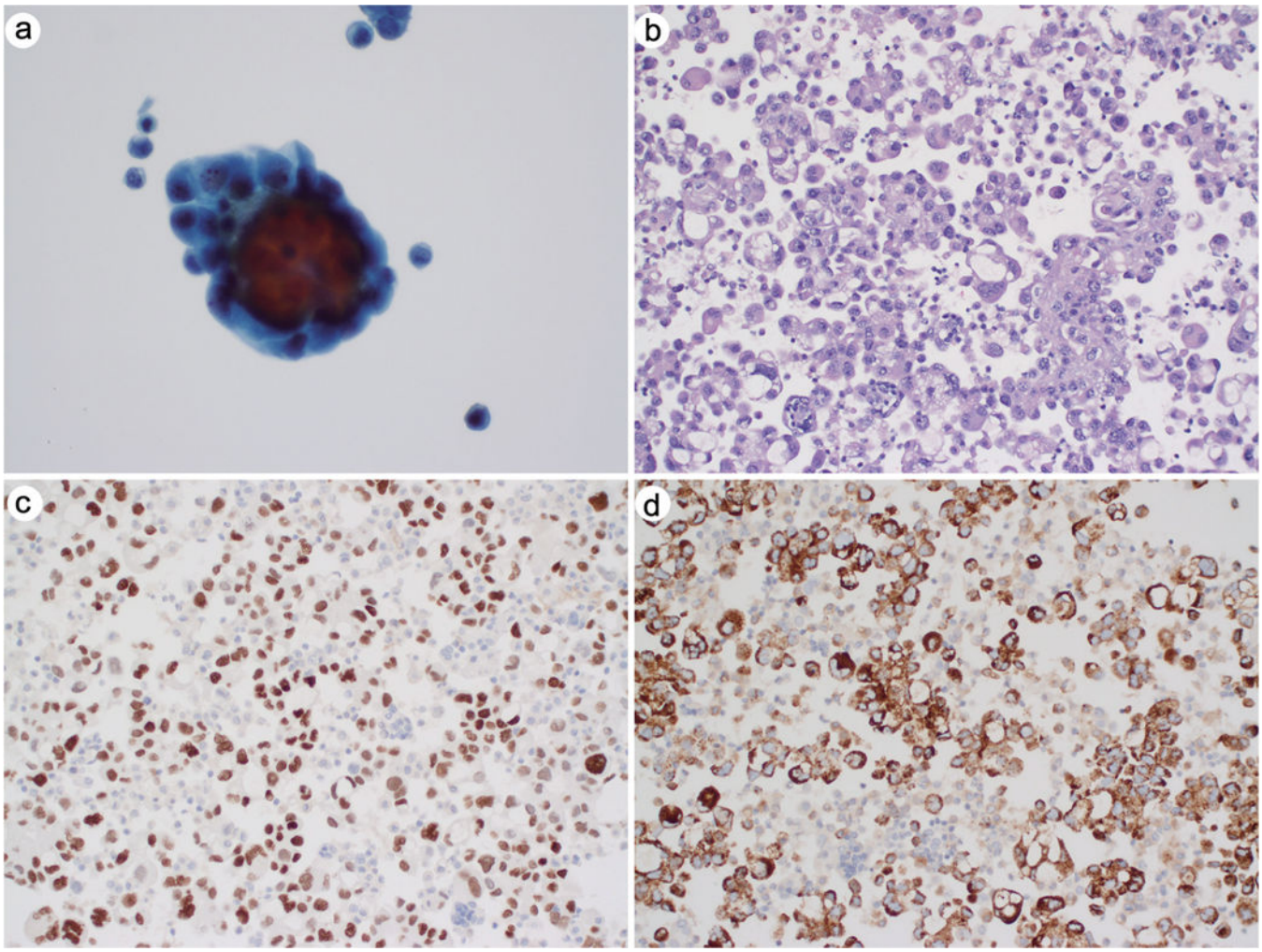

Fig. 5. Metastatic adenocarcinoma with lung primary.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 200×. (c–d) Tumor cells show positivity for TTF1 (c) and napsin A (d); all, 200×.

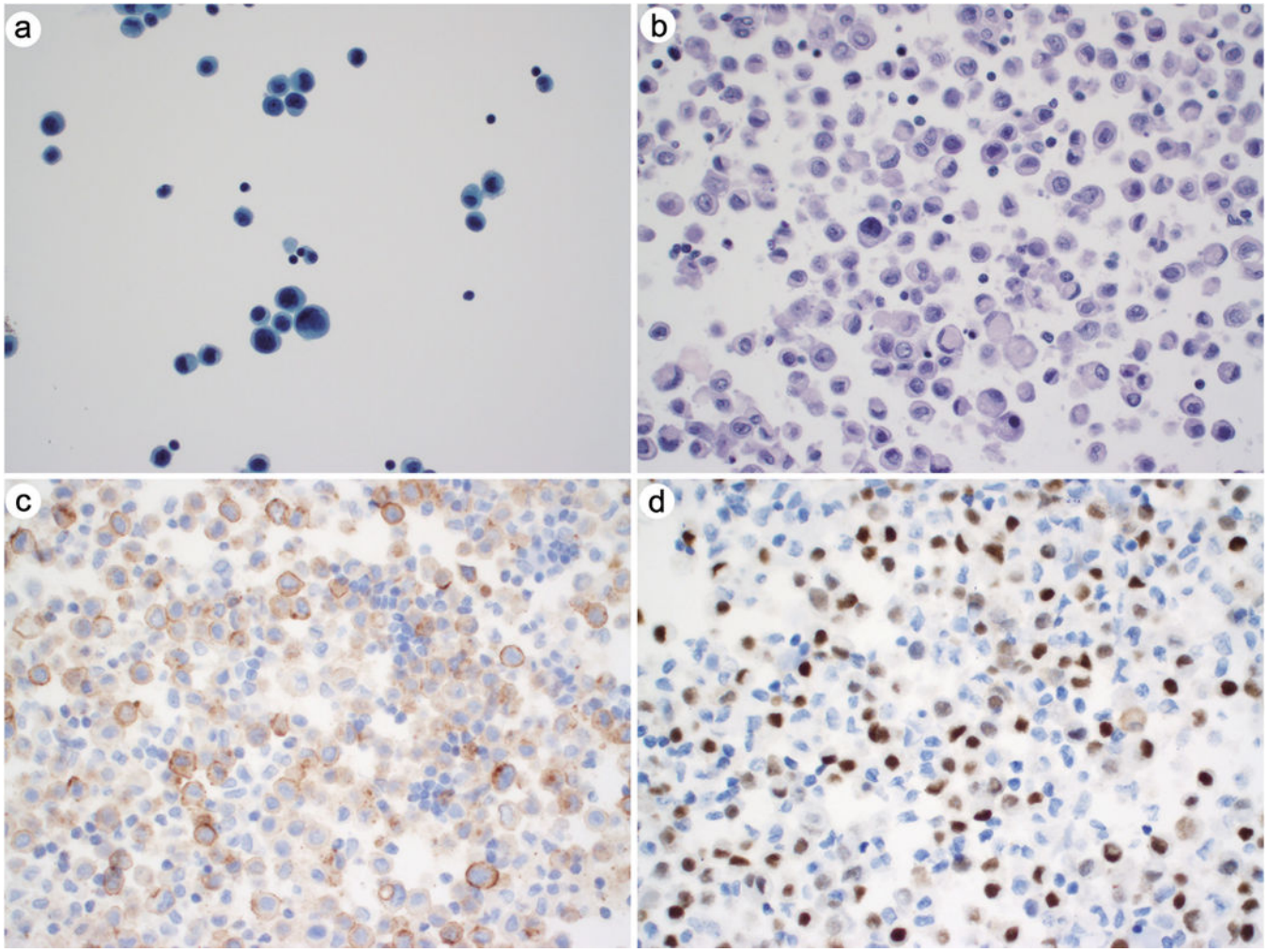

Fig. 6. Metastatic breast lobular carcinoma in pleural fluid.

(a) ThinPrep, 400×. (b) Hematoxylin and eosin stain, 400×. (c–d) Tumor cells show positivity for MOC31 (c) and GATA3 (d); all, 200×.

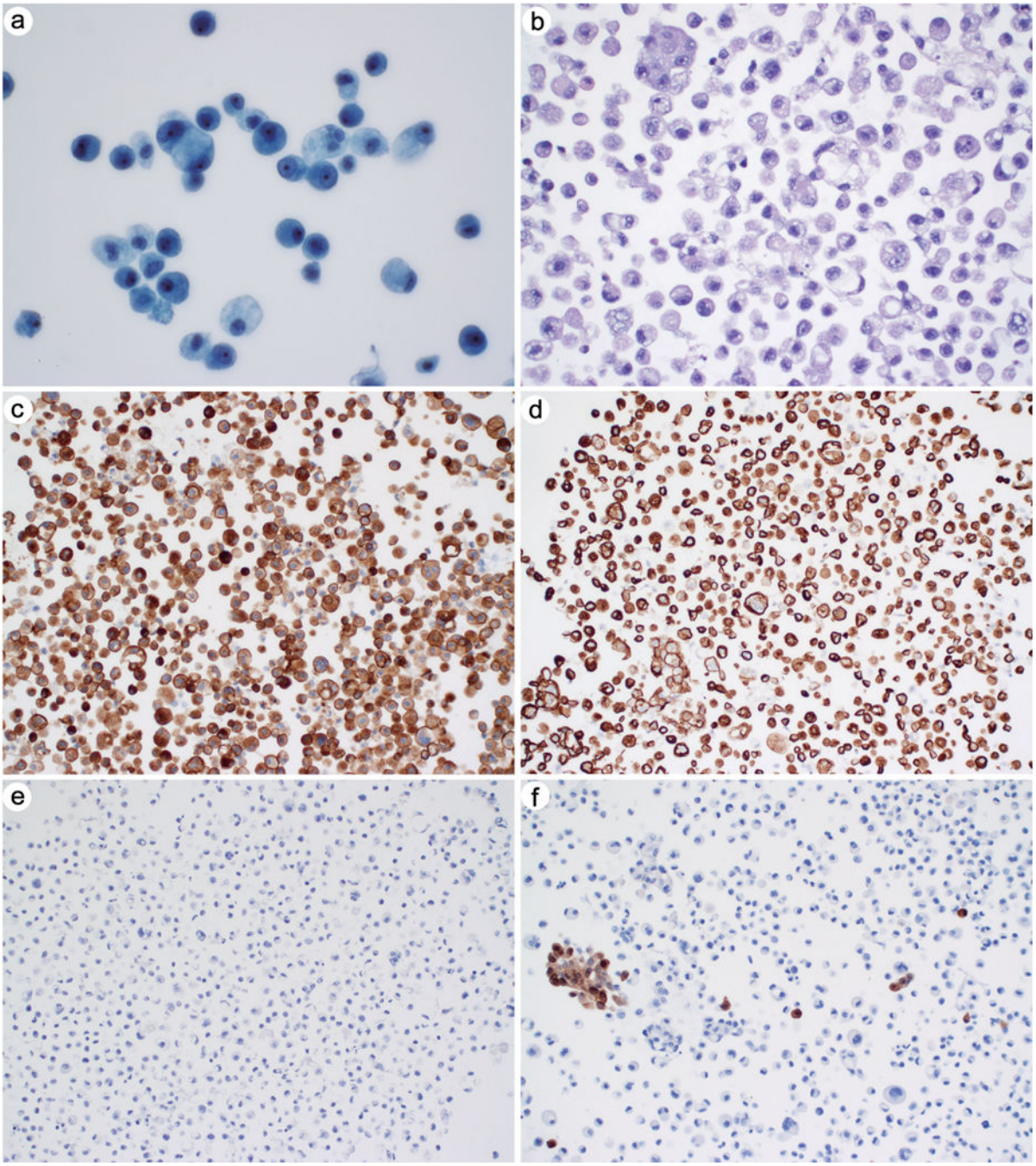

Fig. 7. Metastatic adenocarcinoma of gastrointestinal primary.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 400×. (c–f) Tumor cells show positivity for claudin-4 (c) and CK7 (d) but negativity for CK20 (e) and calretinin (f); all, 200×. Calretinin highlights reactive mesothelial cells.

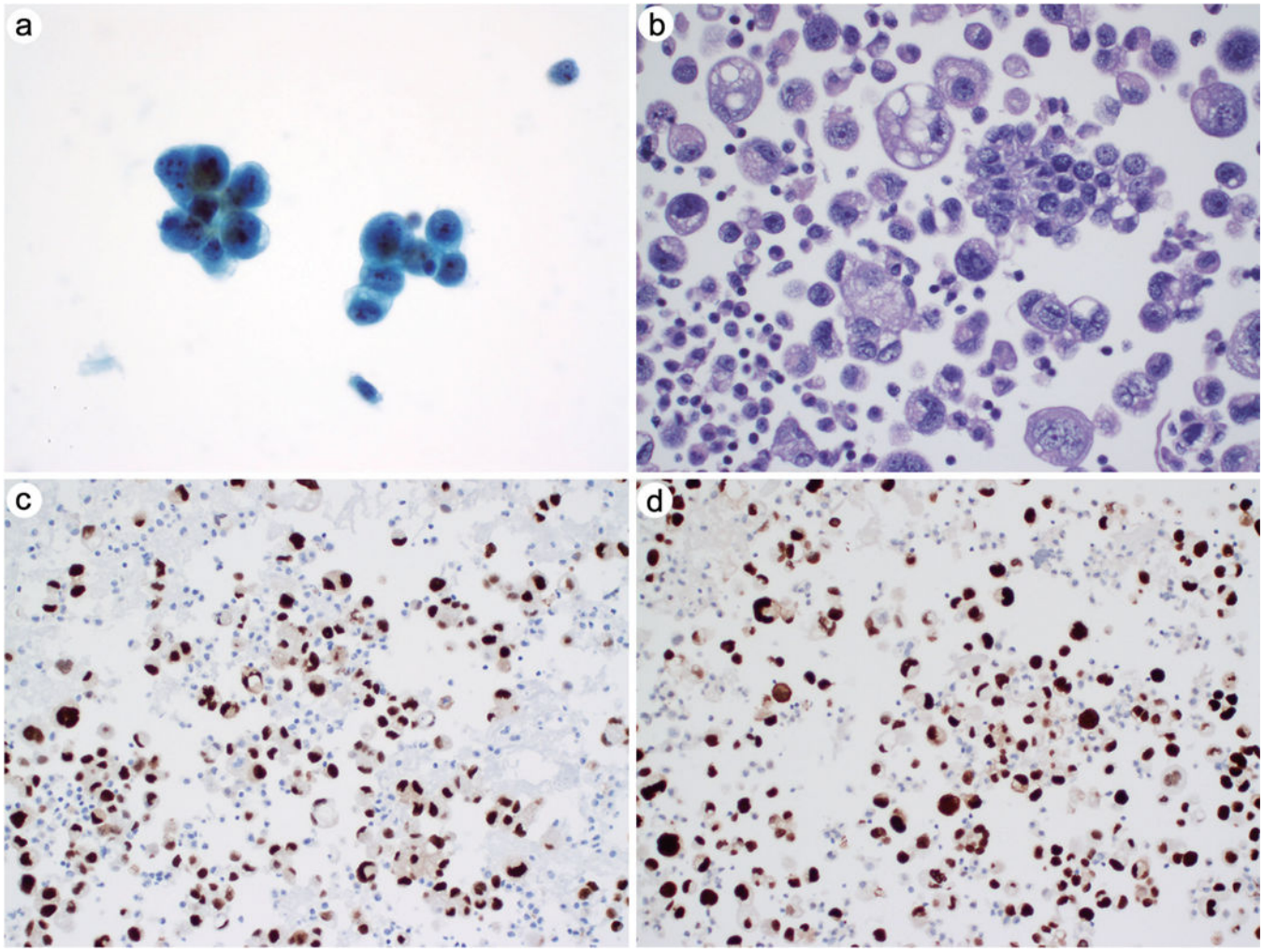

Fig. 8. Metastatic serous carcinoma in pleural fluid.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 400×. (c–d) Tumor cells show positivity for Pax8 (c) and diffuse and strong positivity for p53 (d); all, 200×.

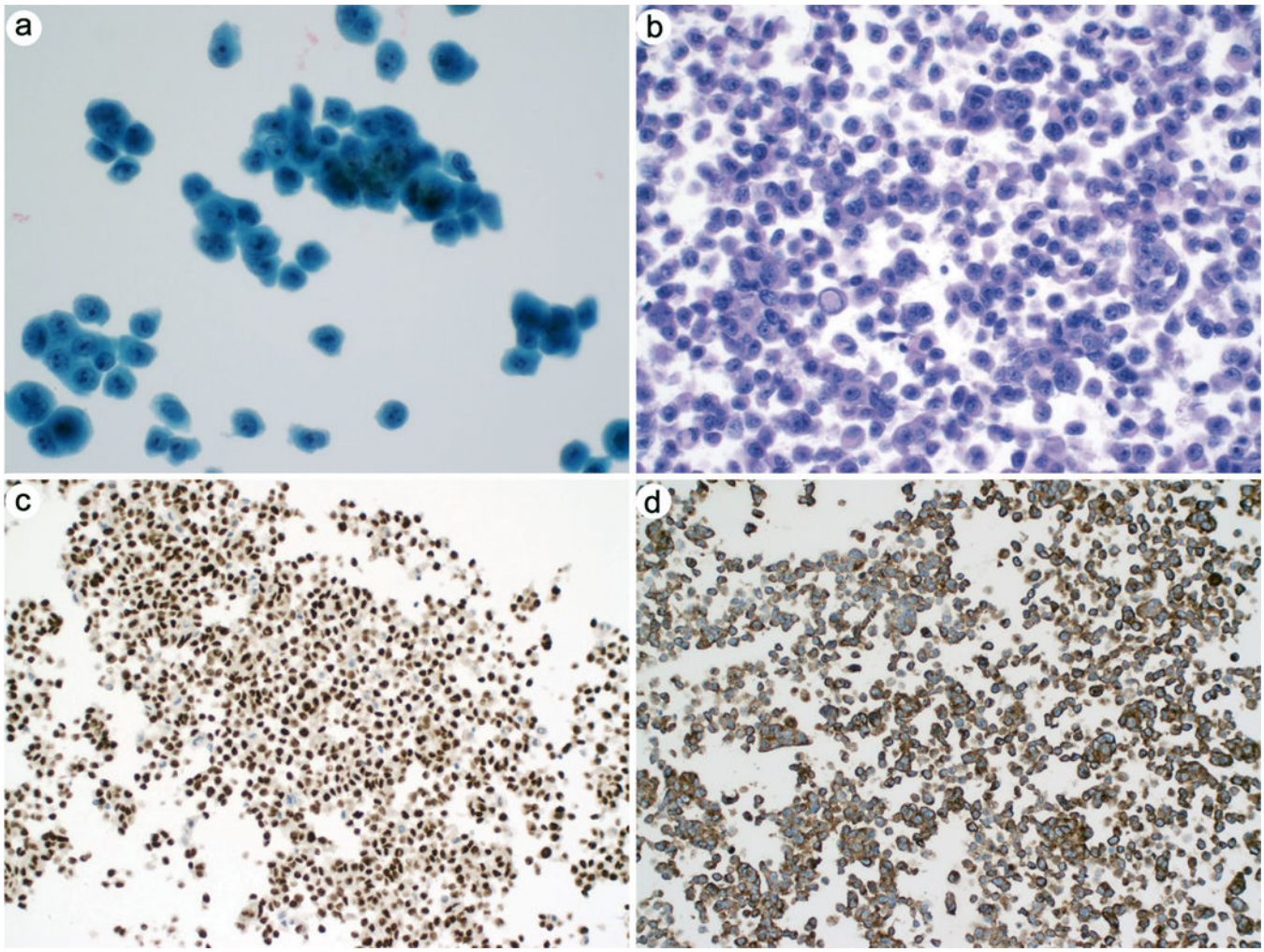

Fig. 9. Metastatic melanoma in pleural fluid.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 400×. (c–d) Tumor cells show positivity for SOX10 (c) and HMB45 (d); all, 200×.

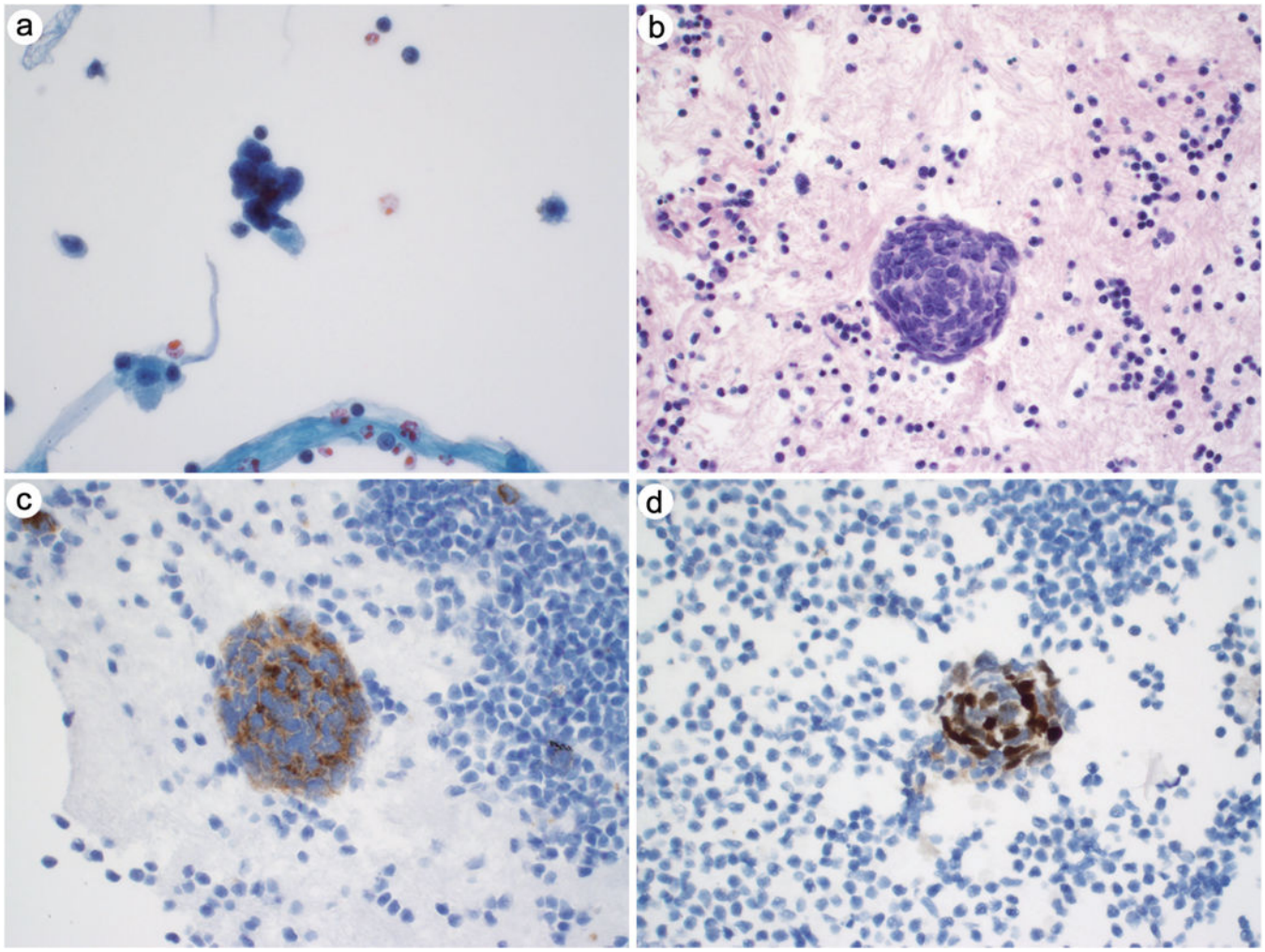

Fig. 10. Metastatic small cell carcinoma in pleural fluid.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 400×. (c–d) Tumor cells show positivity for synaptophysin (c) and INSM1 (d); all, 400×.

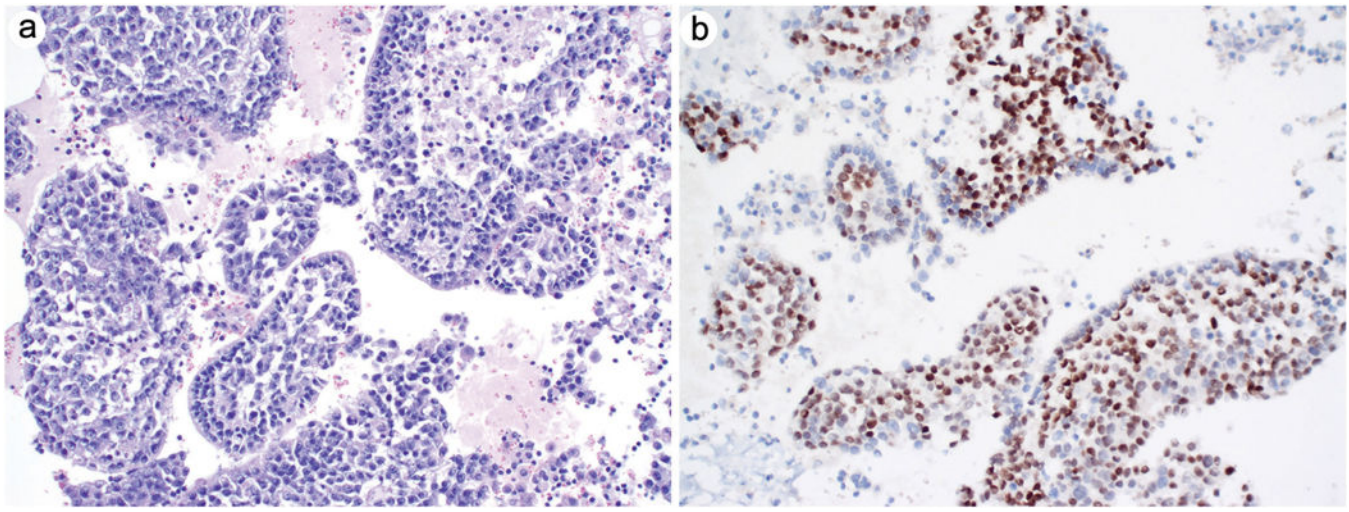

Fig. 11. Metastatic prostatic adenocarcinoma in pleural fluid.

(a) Hematoxylin and eosin stain, 200×. (b) Tumor cells are positive for NKX3.1, 200×.

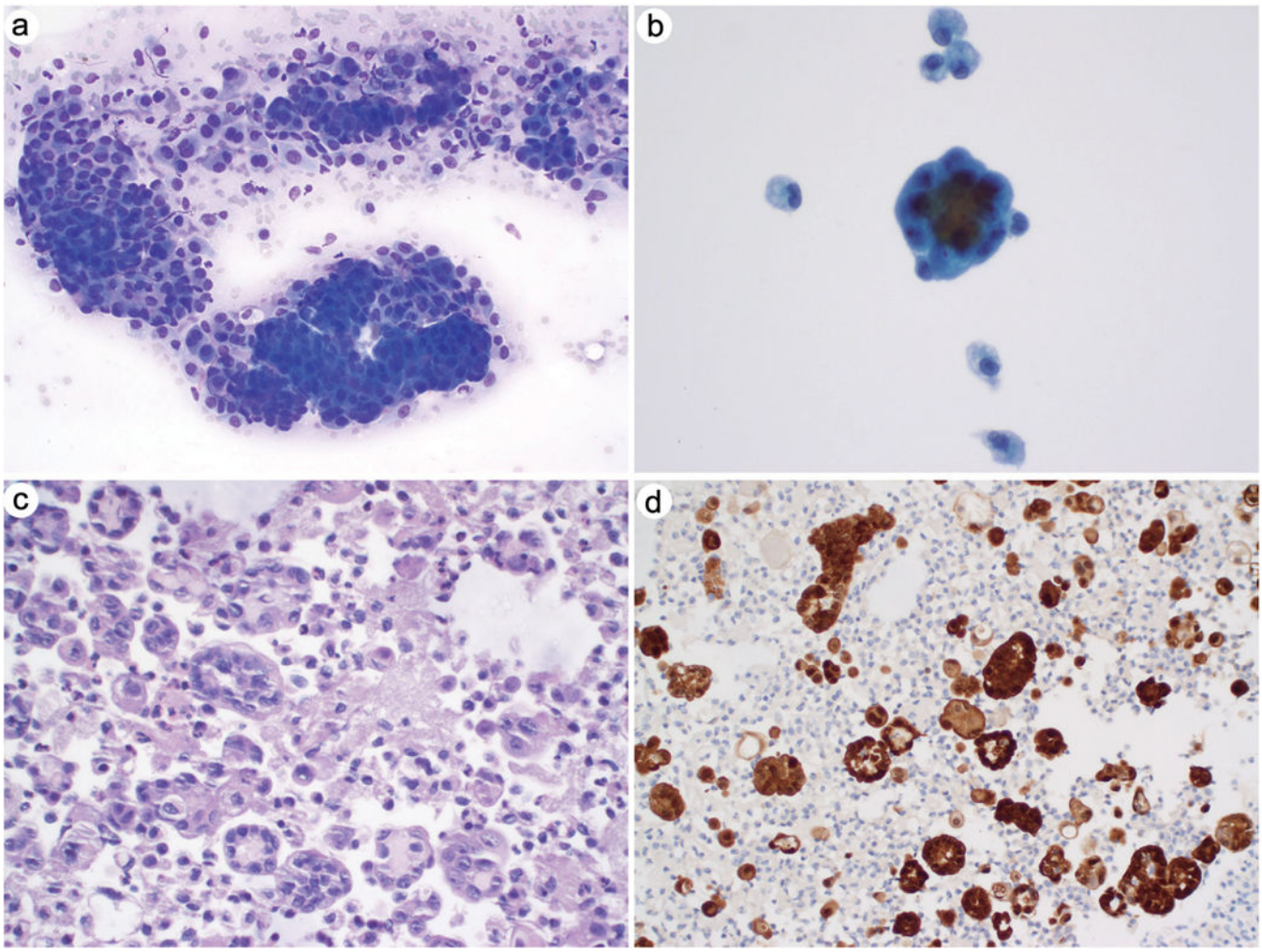

Fig. 12. Malignant mesothelioma.

(a) Touch prep with Diff-Quick stain, 200×. (b) ThinPrep, 600×. (c) Hematoxylin and eosin stain, 400×. (d) Tumor cells show positivity for calretinin; 200×.

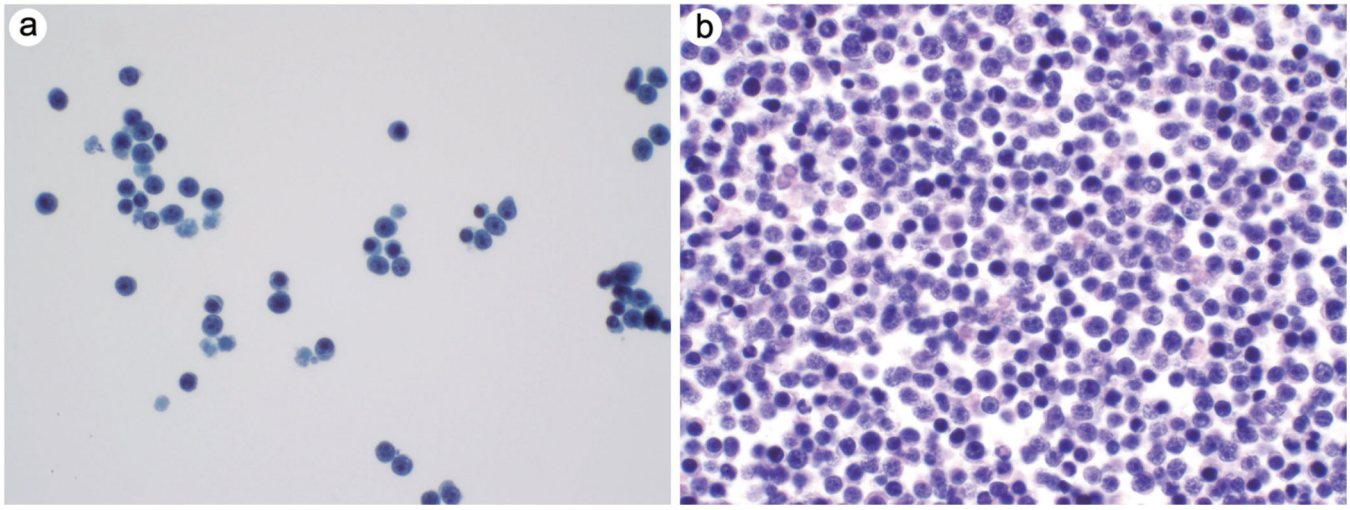

Fig. 13. Non-Hodgkin B cell lymphoma.

Medium to large lymphocytes with prominent nucleoli are in the pleural fluid. (a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 600×.

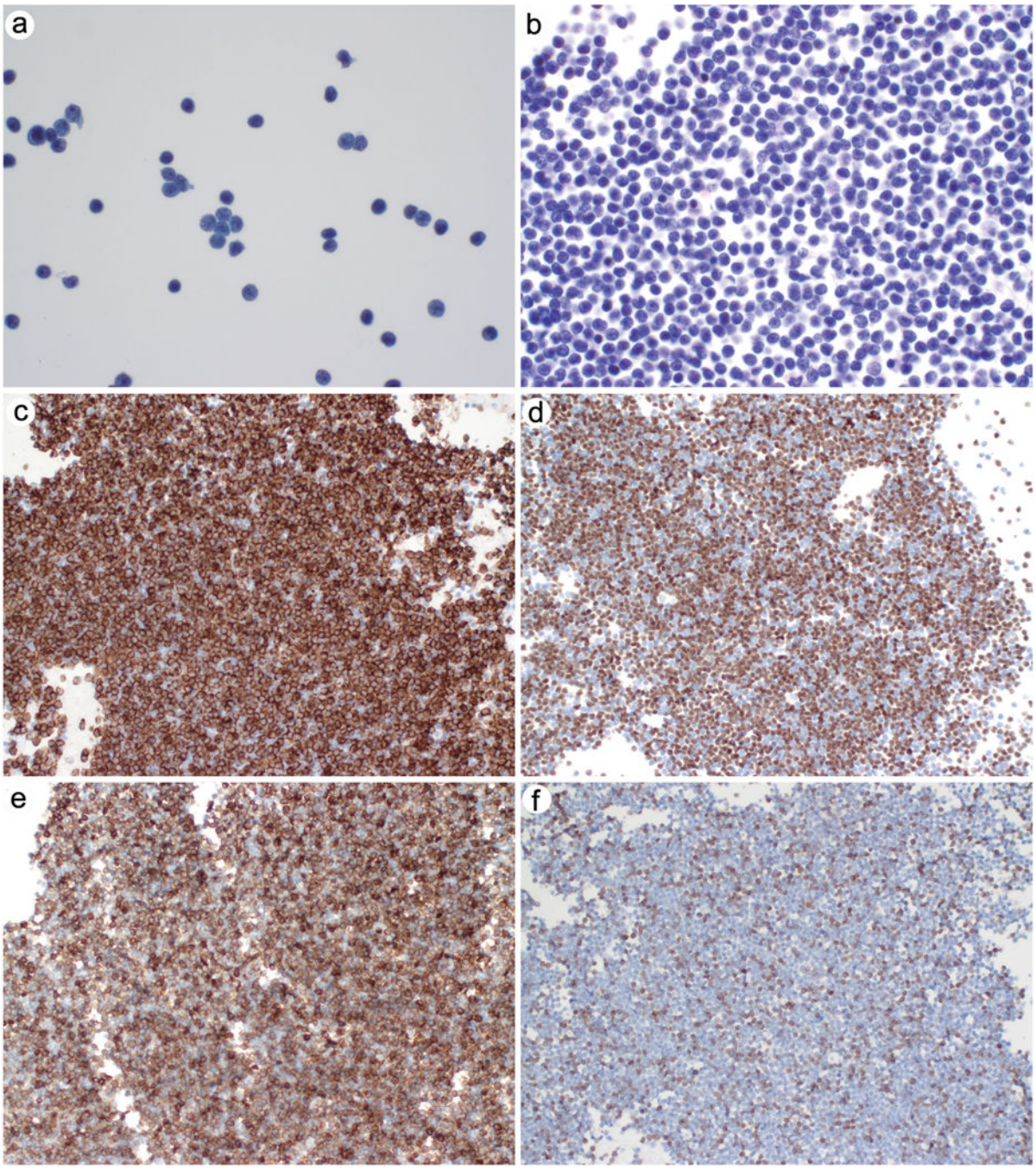

Fig. 14. Chronic lymphocytic leukemia in pleural effusion. Small to medium-sized lymphocytes are monotonous.

(a) ThinPrep, 600×. (b) Hematoxylin and eosin stain, 600×. (c–e) Neoplastic lymphocytes show positivity for CD20, PAX5 and CD5; all, 200×. (f) CD3 highlights scattered T-lymphocytes, 200×.

Table 1.

Diagnostic categories of TIS for reporting serous effusion cytopathology[6]

Diagnostic categories and definitions
I. Nondiagnostic (ND)
Specimens with insufficient cellular elements for a cytologic interpretation
II. Negative for malignancy (NFM)
Specimens with cellular changes completely lacking evidence of mesothelial or nonmesothelial malignancy
III. Atypia of undetermined significance (AUS)
Specimens showing limited cellular (nuclear) and/or architectural atypia (e.g., papillary clusters or pseudoglandular formations)
IV. Suspicious for malignancy (SFM)
Specimens showing features suspicious but not definitively diagnostic for malignancy
V. Malignant (MAL)
Specimens include those with definitive findings and/or supportive studies indicating mesothelial or nonmesothelial malignancies

AUS, Atypia of undetermined significance; MAL, Malignant; ND, Nondiagnostic; NFM, Negative for malignancy; SFM, Suspicious for malignancy.

Table 2.

International guidelines for reporting serous effusions (mesothelioma)[35]

Report as “mesothelioma” in the following conditions:
Morphologically malignant mesothelium (high cellularity and atypia) and supportive radiological findings. Additional biomarkers are optional. For pericardial fluids always confirm malignancy with biomarkers.
Morphologically malignant but of moderate cellularity and supportive radiological findings. Perform biomarker detection to support malignancy.
Morphologically malignant but radiological findings are not available. Perform biomarker detection to support malignancy.
Morphologically malignant but radiological findings are negative. Perform biomarker detection to support malignancy and include a comment to raise concern for MIS.
Report as “suspicious for mesothelioma” in the following conditions:
Morphologically malignant cases and negative radiology if biomarkers are not supportive of malignancy.
Morphologically malignant pericardial fluid if biomarkers are not supportive of malignancy.
Morphologically malignant but fluid is of low to moderate cellularity, positive radiological findings but negative for biomarkers.
Report as “atypical mesothelial cell proliferation” in the following conditions:
Only when the cytologic atypia exceeds that expected in reactive conditions or if reactive mesothelium cannot be supported by ancillary tests, but the findings are not enough to raise concern for mesothelioma. The term “atypical” should not be used in the context of reactive mesothelium, which instead should be diagnosed as negative for malignancy.

Note: Some mesotheliomas do not exhibit any abnormal biomarker results; consequently, negative biomarkers do not exclude the diagnosis of mesothelioma. MIS, Mesothelioma in situ.

Table 3.

Reactive mesothelial cells vs. mesothelioma

Feature	Reactive mesothelial cells	Mesothelioma
Cellularity	Low to moderate	High
Architecture	Individual, small clusters, flat sheets, and two-dimensional aggregates	Large clusters, complex papillary structures; Three-dimensional aggregates, irregular edge
Cytomorphology	Comparatively uniform; Small prominent nucleoli	Pleomorphic; Multiple macronucleoli; Marked cytologic atypia
EMA by immunohistochemistry	–	+ (membranous)
Desmin by immunohistochemistry	+ (cytoplasmic)	–
MTAP by immunohistochemistry	Nuclear and cytoplasm	Loss in 80–90% tumors with CDKN2A deletion
BAP1 by immunohistochemistry	Retained nuclear staining	–
Homozygous deletion of p16/CDKN2A by fluorescence *in situ* hybridization	–	+

BAP1, BRCA1-associated protein; CDKN2A, cyclin-dependent kinase inhibitor 2A; EMA, epithelial membrane antigen; MTAP, methylthioadenosine phosphorylase.

Table 4.

Immunohistochemical basic panel for small lymphocytes[49,61]

Type	Positive stains	Negative stains
Follicular lymphoma	Pan B cell markers (CD19, CD20, CD79a, PAX5), CD10, BCL6, BCL2	CD5, CD23, cyclin D1
Chronic lymphocytic leukemia/small lymphocytic lymphoma	Pan B cell markers (CD19, CD20, CD79a, PAX5), CD5, CD23, LEF1	CD10, cyclin D1, SOX11
Mantle cell lymphoma	Pan B cell markers (CD19, CD20, CD79a), CD5, cyclin D1, SOX11	CD10, BCL6, CD200, LEF1
Marginal zone lymphoma	Pan B cell markers (CD19, CD20, CD79a)	CD5, CD10, CD23, BCL6, cyclin D1, SOX1, LEF1

Table 5.

Immunohistochemical basic panel for intermediate to large-size lymphocytes[61]

Type	Positive stains	Negative stains
B-lymphoblastic lymphoma	CD19, CD79a, PAX5, CD10, TdT, CD34, CD99	
T-lymphoblastic lymphoma	CD7, CD3, CD5, TdT, CD34, CD99, CD1a, CD117	
Burkitt's lymphoma	Pan B cell antigens (CD19, CD20, CD79a, PAX5), CD10, BCL6, CD38; KI67 > 95%; MYC	CD5, CD23, CD138, BCL2, cyclin D1, TdT
Diffuse large B cell lymphoma	Pan B cell markers (CD19, CD20, CD79a, PAX5), variable expression of CD10, BCL6, MUM1, BCL2	CD3, cyclin D1, TdT
Plasmablastic lymphoma	CD138, CD38, IRF4/MUM1, CD79a (40%), CD10, CD56, CD30, EBER (*in situ* hybridization), MYC	CD20, PAX5, CD45
Primary effusion lymphoma	CD45, CD30 CD38, HHV8 by PCR or IHC, Epstein–Barr virus	CD20

Table 6.

Distribution of effusions following the International System for Reporting Serous Fluid Cytopathology

Reference	Specimen	ND, %	NFM, %	AUS, %	SUS, %	MAL, %
Zhu *et al.* 2022[14] (*n* = 2326)	Pleural	0.4	29.7	3.1	19	47.7
Sun *et al.* 2022[67] (*n* = 299)	Pleural	5	76	5	1.7	12.3
Xu *et al.* 2021[70] (*n* = 2454)	Pleural	1.2	68.1	6.2	2.2	22.4
Bharti *et al.* 2022[72] (*n* = 690)	Pleural	7.9	84.1	0.87	1.16	5.94
Straccia *et al.* 2022[73] (*n* = 1292)	Pleural	2.1	78.5	6.6	2.3	10.5
Ahuja *et al.* 2021[71] (*n* = 831)	Pleural	0.4	76.4	7.8	7.2	8.2
Jha *et al.* 2021[56] (*n* = 939)	Pleural	4.37	74.23	4.69	2.88	13.84
Pinto *et al.* 2021[65] (*n* = 350)	Pleural	1.43	72.29	2	4	20.57
Pergaris *et al.* 2021[69] (*n* = 528)	Pleural	0.57	81.44	2.84	2.84	12.31
Lobo *et al.* 2020[68] (*n* = 1496)	Pleural	0.8	63.1	0.6	3.6	31.9
Farahan *et al.* 2019[9] (*n* = 11799)	Pleural	0.3	69.2	0.6	3.5	26.4
Zhu *et al.* 2022[14] (*n* = 1145)	Peritoneal	0.2	28.6	2	19.4	49.9
Rakheja *et al.* 2022[74] (*n* = 818)	Peritoneal	15.2	72.7	3.2	1.5	7.3
Sun *et al.* 2022[67] (*n* = 446)	Peritoneal	11	77.1	4.1	0.9	12.4
Straccia *et al.* 2022[73] (*n* = 2257)	Peritoneal	1.3	84	1.7	2.3	10.5
Ahuja *et al.* 2021[71] (*n* = 457)	Peritoneal	1.1	80.5	4.2	3.7	10.5
Pergaris *et al.* 2021[69] (*n* = 500)	Peritoneal	1.2	69.4	2.6	2.4	24.4
Lobo *et al.* 2020[68] (*n* = 763)	Peritoneal	0.7	59.9	12	37	25.2
Farahani *et al.* 2019[9] (*n* = 3978)	Peritoneal	0.3	59.7	0.4	2.6	37
Li *et al.* 2023[75] (*n* = 358)	Pericardial	4.7	39.5	12.2	5.3	38.3
Wang *et al.* 2023[12] (*n* = 465)	Pericardial	4.1	71.4	4.5	2.4	17.6
Zhu *et al.* 2022[14] (*n* = 117)	Pericardial	0.9	39.3		11.1	48.7
Straccia *et al.* 2022[73] (*n* = 241)	Pericardial	1.6	71.7	4.1	3	19.5
Song *et al.* 2021[76] (*n* = 574)	Pericardial	0	66.6	9.4	1.7	22.3
Lobo *et al.* 2020[68] (*n* = 64)	Pericardial	–	35.9	1.6	6.3	56.2
Rodriguez *et al.* 2020[8] (*n* = 252)	Pericardial	0	84.3	4.3	1.3	10
Farahani *et al.* 2019[9] (*n* = 941)	Pericardial	–	70	0.2	1.9	27.9

Reference	Specimen	ND, %	NFM, %	AUS, %	SUS, %	MAL, %
Kolte *et al.* 2021[77] (n = 652)	Combined	2	71.6	2.4	4.7	19.3
Gokozan *et al.* 2021[11] (n = 911)	Combined	1	73.2	5.6	3	17.2
Hou *et al.* 2021[13] (n = 2405)	Combined	–	42	6	4	48

AUS, atypia of uncertain significance; MAL, malignant; ND, nondiagnostic; NFM, negative for malignancy; SFM, suspicious of malignancy.

Table 7.

Comparison of the ROM in previous studies

Reference	Specimen	ND, %	NFM, %	AUS, %	SUS, %	MAL, %
Farahani *et al.* 2019[a]9	Combined	17.4 ± 8.9	21 ± 0.3	66 ± 10.6	82 ± 4.8	99 ± 0.1
Zhu *et al.* 2022[14]	Pleural	40	29.8	49.3	99.3	100
Sun *et al.* 2022[67]	Pleural	0	1.8	37.5	83.3	100
Xu *et al.* 2022[70]	Pleural	26.7	12	62.3	77.8	100
Bharti *et al.* 2022[72]	Pleural	30.9	12.9	100	100	90.2
Straccia *et al.* 2022[73]	Pleural	18.5	15	45.3	93	100
Ahuja *et al.* 2021[71]	Pleural	0	2.1	33.3	94.1	100
Jha *et al.* 2021[56]	Pleural	87.5	51.61	88.23	87.5	100
Pinto *et al.* 2021[65]	Pleural	40	20.16	42.86	78.57	100
Pergaris *et al.* 2021[69]	Pleural	0	5.3	33.33	93.33	100
Lobo *et al.* 2020[68]	Pleural	57.1	23.9	50	76.2	100
Farahan *et al.* 2019[9]	Pleural	25.7*	22.3 ± 5.6	71.8 ± 17.7	75.9 ± 9.3	99.2 ± 0.2
Zhu *et al.* 2022[14]	Peritoneal	0	27.5	60.9	99.5	100
Rakheja *et al.* 2022[74]	Peritoneal	16.9	12.1	50	80	100
Sun *et al.* 2022[67]	Peritoneal	8.3	1.2	44.4	100	100
Straccia *et al.* 2022[73]	Peritoneal	19.3	10.4	43.5	100	100
Ahuja *et al.* 2021[71]	Peritoneal	50	4.8	22.2	83.3	100
Pergaris *et al.* 2021[69]	Peritoneal	16.66	9	38.46	83.33	100
Lobo *et al.* 2020[68]	Peritoneal	100	26.3	62.5	91.7	100
Farahani *et al.* 2019[9]	Peritoneal	0*	27.1 ± 7.2	40.5 ± 0.2	84.6 ± 3.8	98.4 ± 1.1
Li *et al.* 2023[75]	Pericardial	23.5	29.1	56.8	78.9	99.3
Wang *et al.* 2023[12]	Pericardial	0	1.3	20	100	100
Zhu *et al.* 2022[14]	Pericardial	100 *	17.4	0	100	100
Straccia *et al.* 2022[73]	Pericardial	25	13.2	35	100	100
Song *et al.* 2021[76]	Pericardial	–	3.7	20.9	57.1	89.3
Lobo *et al.* 2020[68]	Pericardial	–	0	0	No data	100

Reference	Specimen	ND, %	NFM, %	AUS, %	SUS, %	MAL, %
Farahani *et al.* 2019[9]	Pericardial	–	30.9 ± 9.2	100*	48.7 ± 0.1	99.1 ± 0.9
Kolte *et al.* 2021[77]	Combined	23	25	56	80.6	90
Hou *et al.* 2021[13]	Combined	–	–	39	64	–

[a] Proposed by the International System for Reporting Serous Fluid Cytopathology, from Farahani et al. 2019.

* There is only one study in this group. AUS, atypia of uncertain significance; MAL, malignant; ND, nondiagnostic; NFM, negative for malignancy; SFM, suspicious of malignancy.