Journal of the American Society of Cytopathology (2019) 8, 362–368

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SHORT COMMUNICATION

Proceedings of the American Society of Cytopathology Companion Session at the 2019 United States and Canadian Academy of Pathology Meeting Part 1: towards an International System for Reporting Serous Fluid Cytopathology

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Received 1 June 2019; received in revised form 3 August 2019; accepted 16 August 2019

KEYWORDS
Cytology;
Terminology;
Serous;
Body fluid;
Effusions

Introduction The International System for the Reporting of Serous Fluid Cytopathology was initiated to provide a common language for cytopathology reports on body fluids.
Materials and methods The International Academy of Cytology and the American Society of Cytopathology collaborated to provide evidence-based and expert-driven terminology for reporting serous fluids. Lead editors were selected and expert authors invited to form working groups for the diagnostic categories and special sections.
Results Preliminary results of the collaboration include the 6 diagnostic terminology categories: non-diagnostic, negative for malignancy, atypia of undetermined significance, suspicious for malignancy, malignant-primary, and malignant-metastatic. Four special sections on ancillary testing, peritoneal washings, cytotechnical aspects, and quality assurance will appear in the final text. Initial results of an international survey indicate strong support for a uniform terminology for reporting serous fluids.
Conclusions This article outlines the initial findings of the collaboration as presented to the United States and Canadian Academy of Pathology at the 2019 annual meeting at the National Harbor, MD.

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2213-2945/$36
https://doi.org/10.1016/j.jasc.2019.08.006

Introduction

Cytopathology has always been on the forefront of standardized reporting terminology, beginning with The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses in 1988.[1] Other cytology terminologies have been sequentially introduced into the lexicon, including pancreaticobiliary, thyroid, urine, salivary gland, and breast. One of the last and yet most common specimen types to yield to standardization is serous effusions. Resistance to creating terminology for this specimen may be due to the perceived simplicity of reporting for this system. One might ask, is it truly necessary to standardize this specimen type, when reporting it as unsatisfactory, benign, atypical, suspicious, or malignant seems sufficient? Nevertheless, there are inconsistencies in the use of terms such as "atypical", "suggestive of", "suspicious for", and "consistent with"—pathologists tend to use their own terms or local terms that are not always translatable between institutions. The role of fluids has been changing as patients live longer with cancer and have multiple cancers. Identifying tumor type and targetable proteins for therapy from metastatic sites is paramount to patient survival. Fluids are sources of easily obtainable tumor for ancillary tests and can serve as cell cultures for biobanking or research. Finally, the need for portability of information across digital platforms demands common language to synchronize systems.

The proposal to create a system of terminology for serous fluids originated in Porto, Portugal, at the 2018 International Academy of Cytology Tutorial Conference, and the American Society of Cytopathology was invited to participate, culminating in a joint venture intended to serve an international audience. The executive boards of the respective professional organizations approved the collaboration, which kicked off in June 2018 at the European Congress of Cytopathology in Madrid and was initiated with several guiding principles. First, proposed terminology should reflect common current practices and parallel existing cytology terminology systems as much as possible. Second, guidelines for practice, such as a definition of an adequate serous sample, should be supported by research studies where available, or expert consensus, if necessary, and both should be documented. Third, the proposed system should be simple, efficient, and effective, allowing for accurate correlation between institutions and research efforts. The International System for Reporting Serous Cytopathology was born. There were important practice questions to be considered, such as evaluating existing evidence for use of volume and/or cellularity in determining adequacy, defining a true negative sample, use of "atypia" versus "suspicious for" terminology, and reporting the presence of epithelial cells in peritoneal washings. It was also an opportunity to revisit the value of cytology in the diagnosis of malignant mesothelioma. Ultimately, the system should result in practice improvement with greater interpretive concordance between pathologists, and use of the terminology to establish clinical practice guidelines and monitor patient outcomes. Common terminology permits greater patient engagement by providing common searchable terms so that patients can be prepared with questions at clinical visits. It facilitates communication with external consultants of all specialties and enhances teaching. Importantly, the use of established terminology enhances comparison between published studies: my atypia is now morphologically similar to your atypia. Data become more easily accessible due to categorization and can be harvested for population studies. All of these outcomes have been proven effective through the implementation of The Bethesda System for Cervical Cytology and can be mirrored with the introduction of standardized terminology for all cytology specimens.

The lead editors (Ashish Chandra, MD; Barbara A. Crothers, DO; Daniel I. Kurtycz, MD; and Fernando Schmitt, MD) proposed and invited internationally recognized cytology experts to participate as chapter authors. Each chapter lead author approved the list of authors or suggested other contributors and each editor was assigned to chapters for editorial oversight. Chapter lead authors formulated a list of critical questions to answer and initiated a literature search for existing studies on those questions or on existing practices. Several chapter working groups created separate practice surveys. An international survey was initially promulgated by the editors and deployed online for 6 months to members of the International Academy of Cytology and the American Society of Cytopathology to determine current practice patterns. There were 512 respondents, primarily students or practitioners of cytology, representing most of the world's continents. A complete report of those survey results will be published at a future date.

Initial results suggested that many cytologists use the same terminology differently and do support guidance in reporting. Most respondents already provided a general category akin to those listed above when reporting serous cytology (385 of 429; 89.74%) and 78.76% (330 of 419) would prefer to issue this type of report. Reporting adequacy, however, was highly variable. Only 32.23% (136 of 422) reported adequacy on all specimens, 31.99% (135 of 422) reported on some specimens, and 35.78% (151 of 422) never reported adequacy. This is likely due to lack of consensus and scientific evidence on what constitutes an adequate fluid specimen. Adequacy is contentious in many body systems in cytology. How many cells are required for an adequate specimen? Are mesothelial cells required? In fluids, adequacy is especially perplexing, since every excessive accumulation of body fluid is abnormal, and cytologists can only evaluate what is exfoliated into that fluid. The presence of tumor cells would imply an adequate sample even with scant cellularity. Other unresolved questions regarding adequacy involve the submission of a sufficient fluid volume and ideal processing techniques to capture sufficient cells for interpretation.

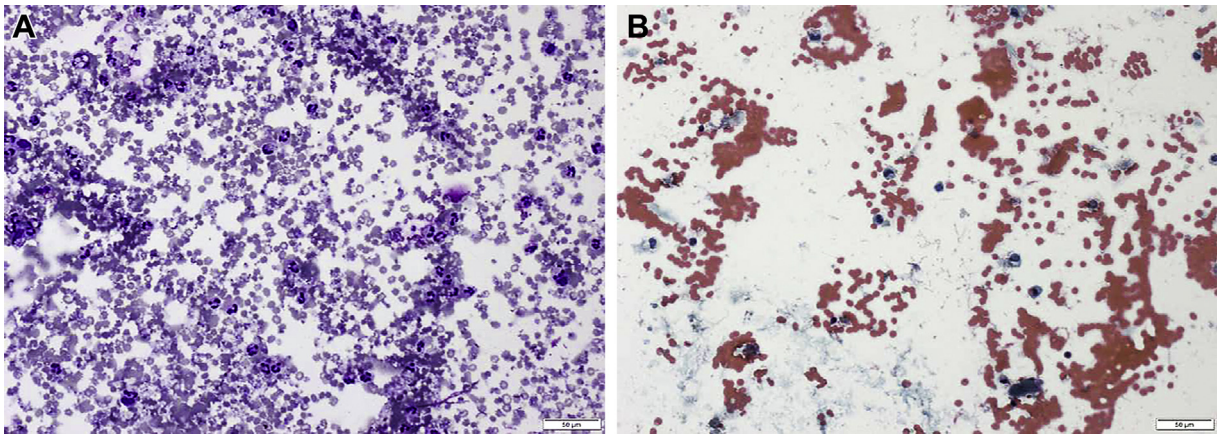

Figure 1 Non-diagnostic (ND). The cytospins (A, modified Giemsa; B, Papanicolaou ×10) consist of blood only.

Respondents were strongly in favor (77.73%; 335 of 431) of a 2-step diagnostic reporting system, with a preliminary report issued while awaiting confirmatory studies for uncertain or malignant cases, and most (64.44%; 212 of 329) already did so. Most institutions regularly performed ancillary studies on serous fluid samples (71.37%; 329 of 461), usually on the cell block (54.30%; 303 of 558), and used ancillary studies on both atypical and suspicious initial findings (74.34%; 252 of 339). Of these, more suspicious cases were converted to malignant (mean: 54.36) than were atypical cases (mean: 34.08). The majority of respondents (89.74%; 385 of 429) also report a general category for serous cytology and agree that this trend should continue (78.76%; 330 of 419). Other questions related to suggested diagnostic terms and current interpretation practices that will be incorporated into a consensus decision for final terminology.

As an abnormal accumulation of fluid in body cavities, serous fluids are a common and important clinical specimen that often implies metastatic disease and advanced tumor progression. Serous effusions include those from the pleura, pericardium, and peritoneum, whether obtained by washing or passive collection. Clinicians may require education on appropriate submission to ensure specimens are routed to the correct laboratory section. Collected samples should be divided and separated for submission for specific desired tests—biochemical assays, microbiology, or cytology or molecular tests. Ideally, cytopathology should receive the entire remainder of the fluid after distribution for other tests. Biochemical assays facilitate differentiation between transudates and exudates; microbiologic tests detect organisms; cytology identifies tumor cells; and molecular tests can be diagnostic, prognostic, or theranostic. Findings from the macroscopic examination of the specimen can anticipate the disease process. Benign processes are often straw-colored. Malignant fluids tend to be bloody and turbid. Milky effusions indicate chylous contamination, and viscous fluids are often rich in hyaluronic acid. Clear and chylous fluids can be processed by cytospin to produce slides to be stained with modified Giemsa and Papanicolaou; processed for Papanicolaou stains using liquid-based preparations (eg, ThinPrep; SurePath) or centrifuged and processed to form a clot or button for cell block and hematoxylin and eosin

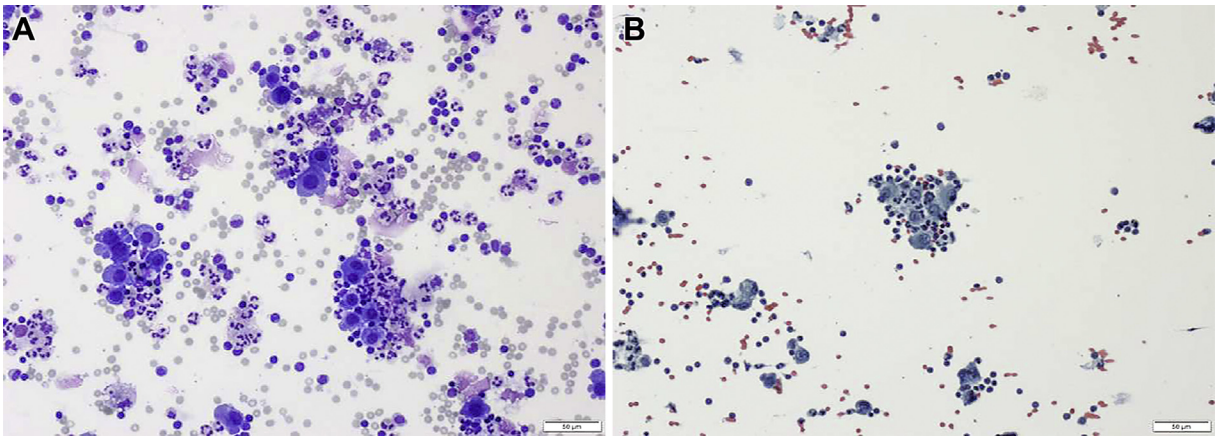

Figure 2 Negative for malignancy (NFM). The cytospins (A, modified Giemsa; B, Papanicolaou ×10) show a mixed population of mesothelial cells, neutrophils, and a few lymphocytes and macrophages. No malignant cells are seen.

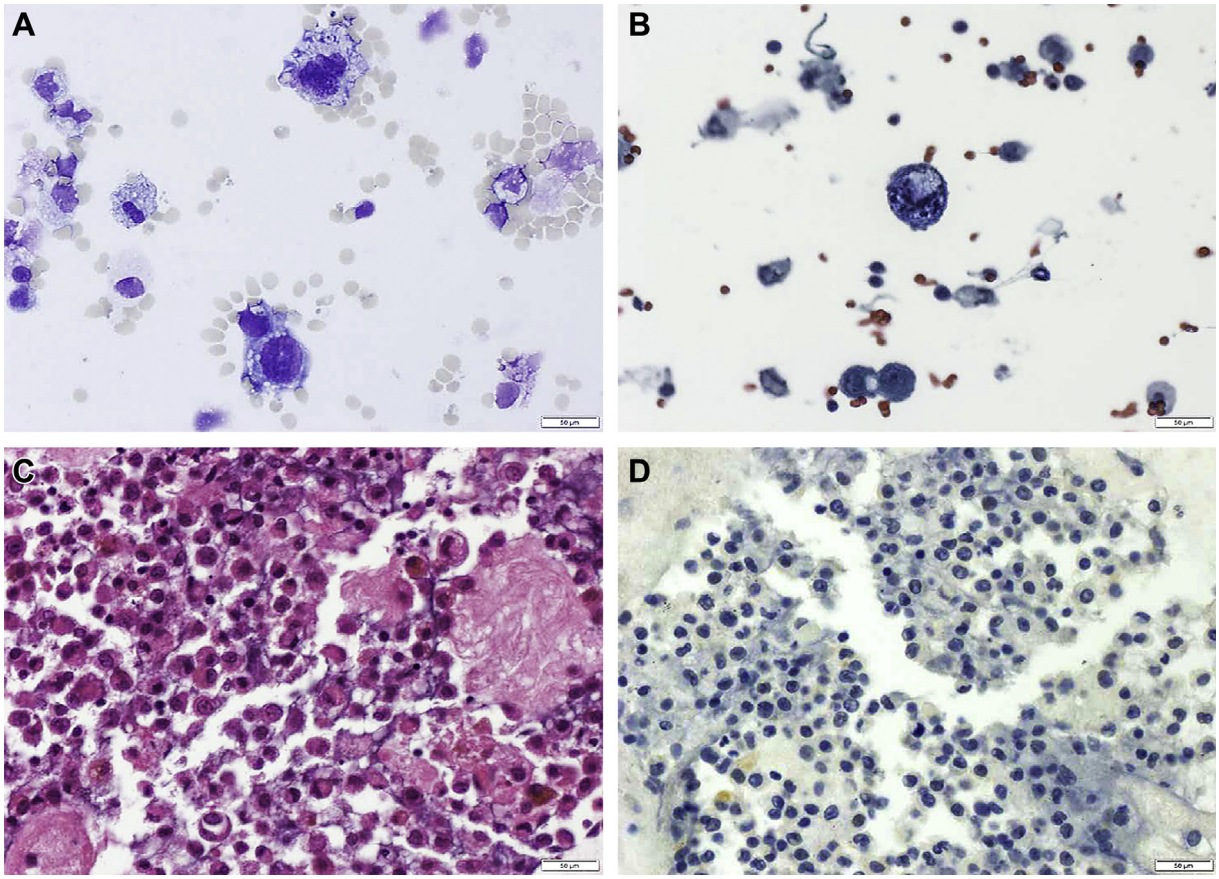

Figure 3 Atypia of undetermined significance (AUS). The cytospins (A, modified Giemsa; B, Papanicolaou ×20) show occasional poorly preserved cells, probable mesothelial cells or macrophages but confirmation may be needed to exclude infiltration by small numbers of malignant epithelial cells. A provisional impression of AUS may be rendered. In this case, the cell block (C) showed degenerative changes in mesothelial cells and epithelial markers including BerEP4 (D) did not highlight any malignant cells. The specimen may be signed out as negative for malignancy (NFM). A further sample of sufficient volume may be considered if clinically indicated.

stains. Turbid or hemorrhagic samples may require additional processing to remove blood, such as dilution or the addition of acetic acid. After centrifugation, direct smears can be made from the sediment for modified Giemsa and Papanicolaou stains, or specimen processed for cell block. After dilution, the specimen can be processed with liquid-based preparations. Optimal processing is essential for correct interpretation, but, in addition, pathologists must have relevant clinical information available when reviewing cytology, including results of imaging studies, clinical differential diagnosis, prior history of malignancy and type(s), and current clinical findings.

The proposed terminology does not deviate far from current reporting practices and is intended to mirror that of previously proposed cytopathology terminology in current use.[1-7] The proposed diagnostic categories are as follows:

- Non-diagnostic (ND)
- Negative for malignancy (NFM)
- Atypia of undetermined significance (AUS)
- Suspicious for malignancy (SFM)
- Malignant (MAL): primary (mesothelioma) or secondary (indicate cell type and possible site of origin)

ND specimens could be either non-representative for the site (eg, peripheral blood only) or insufficient for interpretation in other ways, as yet to be determined. Criteria for adequacy to be considered include sample volume, cellular content, and cellular preservation. A recent study[8] suggests that 75 mL of fluid for cytology or more is required to confirm that a benign fluid is truly benign (negative predictive value). Some practices require the presence of mesothelial cells for adequacy, but the reliability of that metric is unclear. The sample may consist of blood only (Fig. 1A, B) or cellular preservation can be a major limiting factor: a specimen with excessive cell degeneration, bacterial overgrowth or other contaminants, or processing and staining artefacts, may be uninterpretable.

NFM was the term most commonly used by survey respondents (45.85%; 188 of 410) when reporting negative serous fluid specimens. NFM implies that certain non-malignant cell populations may be present in variable numbers (Fig. 2A, B); their presence may still indicate disease, but not malignancy. Although this seems like a straightforward category, it can be quite complex. One must first determine that the sample is representative of the

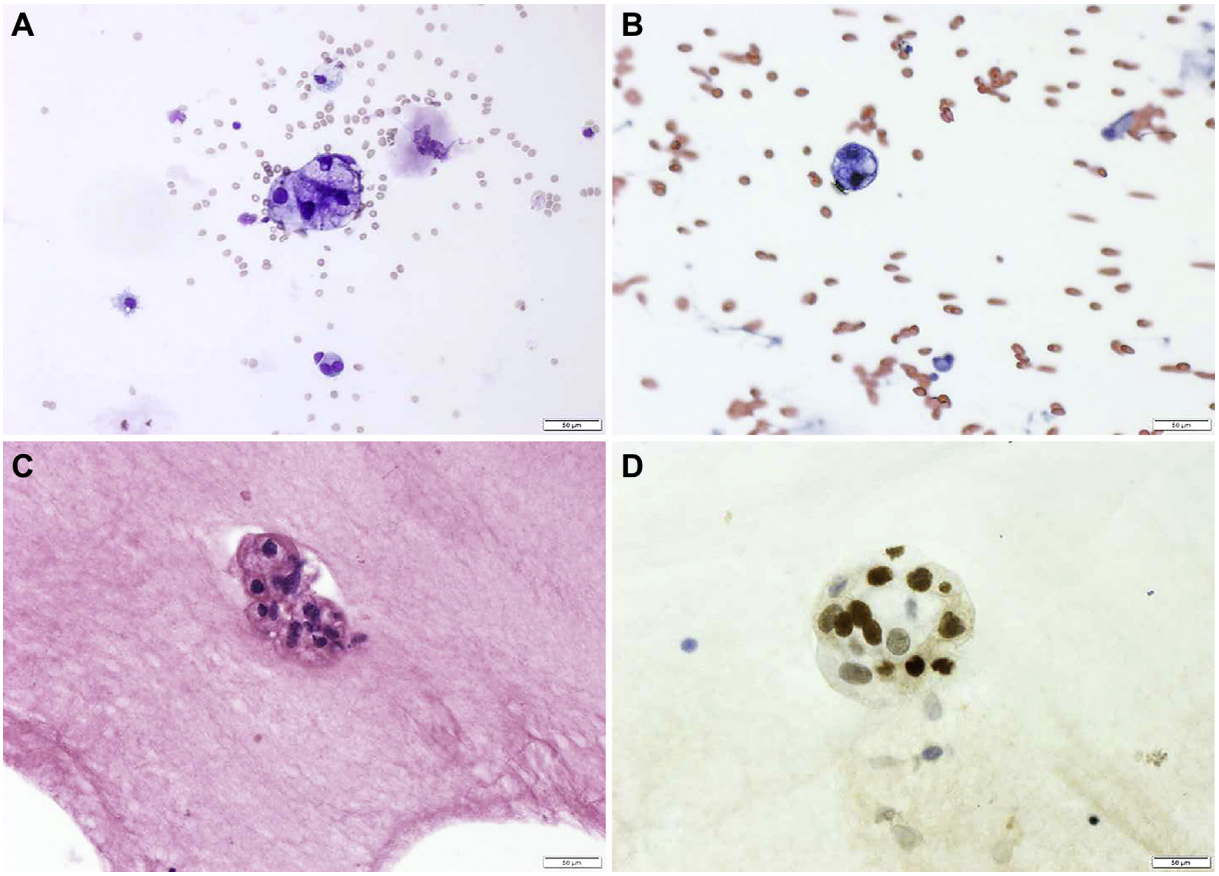

Figure 4 Suspicious for malignancy (SFM). The cytospins (A, modified Giemsa; B, Papanicolaou ×20) and cell block (C) show an occasional 3-dimensional group of cells or singly dispersed cells with cytoplasmic vacuolation and nuclear abnormalities such as hyperchromasia and coarse chromatin. The appearances may be regarded as SFM (metastatic carcinoma). Immunostain CDX2 on the cell block (D) confirms their epithelial nature and the final report may be signed out as Malignant (MAL-secondary) from known colorectal carcinoma in this case.

potential disease state, and whether the cells present are truly "normal". At present, the working group is developing an algorithmic approach to determining an appropriate diagnostic category where there is morphologic overlap between benign and malignant cells.

The term AUS was supported by 63.47% (245 of 386) of survey respondents. As with other cytopathology terminology systems, the working group for AUS is proposing that this category be used judiciously as opposed to a wastebasket for suboptimal cases. Suboptimal specimens, processing, or staining often result in frustrating interpretation problems for pathologists. In the ideal setting, additional information, tests, or studies should resolve diagnostic uncertainty. For this reason, a 2-step reporting process is proposed, where a preliminary AUS report or even verbal communication with the clinical team may be followed by a more definitive final report. Most survey respondents relied on an atypical reporting terminology when the numbers of cells or groups of cells was small and there was cell and nuclear enlargement along with hyperchromasia (Fig. 3A-D). There may be particular settings where use of AUS is more appropriate than a definitive report, such as cases of ovarian borderline tumors. Criteria for atypia are controversial, but many atypical cytological findings can be resolved with the use of immunochemical studies. If there is insufficient specimen for those studies, or the results are inconclusive, the final report can be AUS. For the majority of these cases, the anticipated clinical outcome would be negative for malignancy.

There is some overlap between the categories of SFM and AUS, but the primary determining factor between the 2 categories will usually depend on the number of abnormal cells present and the clarity of malignant morphologic features (Fig. 4A-D). It is, as yet, unknown how many malignant-appearing cells are necessary before imparting a conclusive interpretation of malignancy. As with AUS, a more definitive interpretation may be possible after application of immunochemistry, flow cytometry, or molecular assays. For the majority of these cases, the anticipated clinical outcome would be detection of or confirmation of malignancy (high positive predictive value).

The MAL category is divided into primary (mesothelioma) and secondary (other) malignancies. A diagnosis of malignancy implies sufficient morphologically recognizable

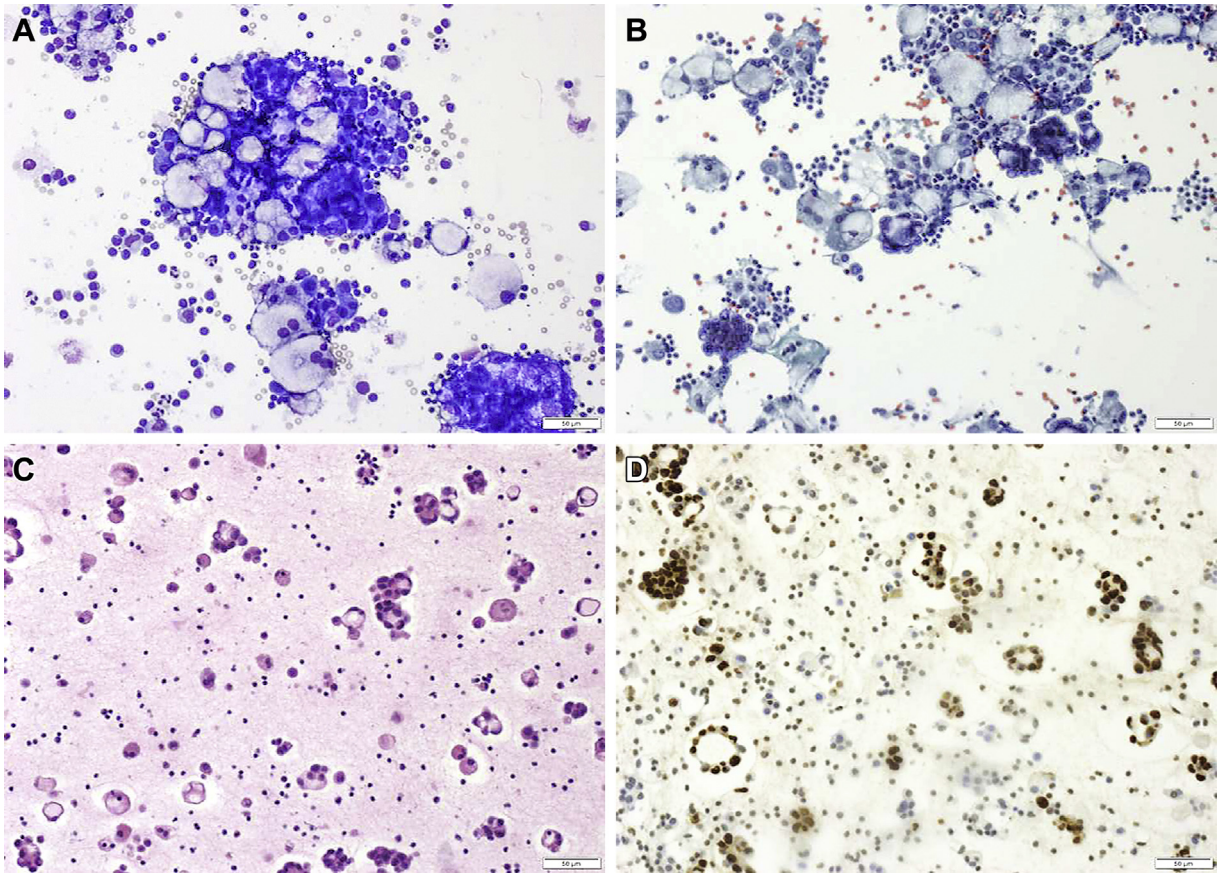

Figure 5 Malignant (MAL-secondary). The cytospins (A, modified Giemsa; B, Papanicolaou ×10) contain numerous groups and dispersed malignant cells with vacuolated cytoplasm, nuclear enlargement and hyperchromasia. The appearances are consistent with metastatic adenocarcinoma. The cell block (C, hematoxylin and eosin ×10) contains similar malignant cells and immunostain TTF1 (D) confirms the site of origin to be the lung as clinically suspected.

malignant cells in an appropriate clinical setting, supported by immunochemical (IHC) studies as necessary. For secondary tumors, an algorithmic approach first necessitates the division of tumor cells into broad categories: carcinoma, lymphoma/leukemia, melanoma, or sarcoma. Most metastatic tumors are adenocarcinomas, which are further investigated with IHC, in conjunction with clinical and radiographic information, to identify the primary site. There is strong agreement with this approach; 95.89% (327 of 341) respondents agree that when malignant cells are identified in a fluid, they should be specifically characterized and a likely primary site identified as much as possible (Fig. 5A-D). It is important to check the patient's record for prior cytology or other surgical specimen reports to avoid repeating tests that have already been performed and to direct the choice of IHC panel. Where sample material is limited, it should be handled conservatively so that sufficient tissue is retained for molecular studies. The collection site along can direct IHC choices. Pleural fluid panels would likely include a breast marker, such as GATA-3; lung markers such as TTF-1 and Napsin-A; thyroid markers such as thyroglobulin; and mesothelial markers, discussed below. Peritoneal fluid panels would include gynecologic tumor markers, such as CK7, PAX8, CA125, and p53; gastrointestinal markers such as CK20, CDX2, and SATB-1; renal markers such as PAX8 and RCC; urothelial markers such as GATA-3 and p40; and prostate markers such as PSA, PSMA, and NKX3.1. Other choices would be driven by the morphology, such as markers for small cell carcinoma, melanoma, and specific sarcomas, as well as markers for benign mimics such as histiocytes.

The diagnosis of primary mesothelioma in fluids has both supporters and detractors. Many, if not most, florid mesothelial proliferations will fall into the AUS or SFM categories, depending on the clinical history, imaging findings, and degree of suspicion for mesothelioma. Of interest, the majority of respondents (81.20%; 313 of 385) will make a diagnosis of mesothelioma on cytology alone, if the clinical/radiographic and IHC all support the interpretation (62.08%; 239 of 385); in the absence of clinical information if the cytology and IHC are supportive (16.10%; 62 of 385); and, in some cases, based on the cytology features alone (3.12%; 12 of 385). Multiple responses were allowed for this question on the survey. Nearly all respondents (97.08%;

332 of 342) wanted a special section on the diagnosis of mesothelioma in the upcoming textbook. Investigating the criteria necessary for a definitive diagnosis of mesothelioma will be the major challenge facing this working group. Proposals for discriminating IHC or molecular panels would be welcome, especially when the differential diagnosis includes benign entities along with primary and metastatic malignancy. Importantly, guidance on downgrading mesothelial AUS cases to negative and understanding the vast array of reactive changes possible in mesothelial cells could prevent unnecessary procedures.

Other proposed special sections include entities found in peritoneal washings, such as endometrial cells or borderline ovarian tumors, and sections on specimen processing and quality improvement. The 2014 FIGO ovarian cancer staging system confines peritoneal or ascitic fluid ovarian tumor cells to stage IC9 but subdivides the category into IC1-surgical spillage; IC2-capsule rupture prior to surgery or tumor on the ovarian surface, and IC3- malignant cells in the fluid. Only malignant cells in pleural fluid upstage the ovarian carcinoma patient to stage IVA. Other entities that occur in peritoneal fluid washings can be mistaken for tumor cells or remain unidentified, so the working group is investigating the significance of reporting those cells. Quality improvement is a critical activity in diagnostic cytology to prevent patient harm from misdiagnosis, but there are scant data on the impact of these activities for serous cytopathology. Clinical guidance on practical approaches to quality projects might prompt pathologists to collect practice data that can be compared between institutions and used to leverage improvements in care.

Serous fluid cytopathology, as a specimen type, lacks robust clinical investigation regarding the impact of cytology terminology, what constitutes adequacy, the diagnosis of mesothelioma, and significance of reporting non-malignant peritoneal fluid findings. This collaboration is the starting point required to align future research and to validate proposed terminology or to make changes in terminology.

Conflict of interest disclosures

The authors have no conflicts of interest to declare.

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