Original Article

# The Role of Liquid-Based Cytology and Ancillary Techniques in Pleural and Pericardic Effusions: An Institutional Experience

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**BACKGROUND:** Fine-needle aspiration cytology (FNAC) of serous membrane effusions may fulfil a challenging role in the diagnostic analysis of both primary and metastatic disease. From this perspective, liquid-based cytology (LBC) represents a feasible and reliable method for empowering the performance of ancillary techniques (ie, immunocytochemistry and molecular testing) with high diagnostic accuracy. **METHODS:** In total, 3171 LBC pleural and pericardic effusions were appraised between January 2000 and December 2013. They were classified as negative for malignancy (NM), suspicious for malignancy (SM), or positive for malignancy (PM). **RESULTS:** The cytologic diagnoses included 2721 NM effusions (2505 pleural and 216 pericardic), 104 SM effusions (93 pleural and 11 pericardic), and 346 PM effusions (321 pleural and 25 pericardic). The malignant pleural series included 76 unknown malignancies (36 SM and 40 PM effusions), 174 metastatic lesions (85 SM and 89 PM effusions), 14 lymphomas (3 SM and 11 PM effusions), 16 mesotheliomas (5 SM and 11 SM effusions), and 3 myelomas (all SM effusions). The malignant pericardic category included 20 unknown malignancies (5 SM and 15 PM effusions), 15 metastatic lesions (1 SM and 14 PM effusions), and 1 lymphoma (1 PM effusion). There were 411 conclusive immunocytochemical analyses and 47 molecular analyses, and the authors documented 88% sensitivity, 100% specificity, 98% diagnostic accuracy, 98% negative predictive value, and 100% positive predictive value for FNAC. **CONCLUSIONS:** FNAC represents a primary diagnostic tool for effusions and a reliable approach with which to determine the correct follow-up. Furthermore, LBC is useful for ancillary techniques, such as immunocytochemistry and molecular analysis, with feasible diagnostic and predictive utility. ***Cancer (Cancer Cytopathol)* 2015;000:000-000.** © *2015 American Cancer Society.*

**KEY WORDS:** pericardic effusions; pleural effusions; liquid-based cytology; immunocytochemistry.

## INTRODUCTION

The potential for cytologic evaluation of serous effusions has not been completely assessed to date, although there recently has been increasing and growing interest.[1–5] Recent data published by Lee et al indicate that 20% of body serous membrane effusions per year are malignant: approximately 50% are diagnosed as metastatic adenocarcinomas followed by pulmonary large cell carcinoma and lymphomas/leukemias (approximately 15% each).[1–3]

Considering the challenging implications of the presence of malignant cells in effusions, an accurate cytologic evaluation represents a critical and mainstream diagnostic tool, mostly because of its simplicity, safety, and

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cost effectiveness in reducing all of the possible consequences and complications of a more aggressive biopsy procedure, which often may fall short of obtaining adequate diagnostic material.[1–5] Furthermore, most pleural and pericardic effusions are associated with lung and ovarian cancers, and their cytologic evaluation may play an important role either in the initial diagnosis of patients with symptomatic cancers or in patient management and follow-up.[1–5]

The recognition of malignant cells as well as their discrimination from reactive mesothelial cells requires the ability to reproduce findings regardless of which methods of preparation and cytology are used, including conventional smears, cytospins, cell blocks, and liquid-based cytology (LBC).[1–8] The phenotype of reactive, nonmalignant mesothelial cells can be misclassified as cancer; accordingly, ancillary techniques are critical to obtain an accurate diagnosis. Although different groups have reported good results using each of these techniques, we extensively use LBC as the primary method for the morphologic evaluation of cytologic findings in effusions (which also permits the use of residual sample to perform complementary analyses) and fine-needle aspiration cytology (FNAC) samples, as discussed below.[1–8]

In addition, a strong diagnostic aid in assessing cytologic morphology is the increasing and accurate application of ancillary techniques (both molecular testing and immunocytochemistry [ICC]) to cytology, including malignant effusions, with diagnostic and predictive intent.[9–14] Several groups have attempted to define the best combination of immunomarkers for diagnosing effusions, which has resulted in a lucky application of these techniques for diagnostic, therapeutic, and prognostic purposes, especially when LBC is adopted.[5,15,16]

LBC, which has been approved by the US Food and Drug Administration since 1996 (and was originally developed for cervical samples), has gained popularity as an alternative technique for the collection and preparation of cytologic specimens from many different sites in the body, including effusions, with good results.[17,18] Conversely, some controversial data on the efficacy of LBC have been contradicted by the cost-effective, time-sparing, simple application of ancillary techniques (both ICC and molecular analysis) up to 3 or 4 months on stored LBC material.[1–7]

Herein, we describe our 13-year experience with cytologic samples of serous membrane effusions (pleural and pericardic samples) that were preserved and prepared using LBC, and we compare our results with data from the literature, including conventional cytology series. The objective of this study was to highlight the importance of a obtaining a correct cytologic diagnosis with LBC and of defining the exact, inherent feasibility of using FNAC for effusions, especially in the presence of positive malignant results. In addition, we assessed the validity of LBC as a reliable aid to the application of ancillary techniques (ICC and molecular analysis) in the analysis of both pleural and pericardic samples. To the best of our knowledge, this is the largest series to date of such analyses in pleural and pericardic effusions.

## MATERIALS AND METHODS

We included all pleural and pericardic FNAC samples that were obtained between January 2000 and December 2013 (N = 3171). All samples were recorded in the Division of Anatomic Pathology and Histology of the Catholic University, "Agostino Gemelli" Hospital (Rome, Italy). All FNAC procedures were carried out under ultrasound or computed tomography guidance, mostly by surgeons and radiologists, and the samples were processed using the ThinPrep method (ThinPrep 5000; Hologic Inc., Marlborough, Mass). All pleural and pericardic effusions were recorded. Our analyses included 2919 pleural effusions and 252 pericardic effusions, which are analyzed in detail below (see Results). Here, we focus our specific attention and discussion on the suspicious and positive results in both pleural and pericardic effusions.

The series included 1708 men and 1463 women, and the median patient age was 48 years (age range, 23-92 years). All aspirations (usually 2 passes for each lesion) were performed with 21-gauge needles, and no rapid on-site evaluations of the adequacy of material were done. All patients had been appropriately informed regarding use of the LBC method for processing their samples, and all signed a written informed consent form. Our study followed the tenants of the Declaration of Helsinki, and we received internal ethics approval for the study.

All aspirated material was fixed with the hemolytic and preservative solution Cytolyt (Hologic, Inc.) after rinsing the needle in this solution. The cells were spun at 1500 rotations per minute ($\times 0.289g$); then, the sediment was transferred in PreservCyt solution (Cytyc Corporation, Marlborough, Mass) to be processed with the T5000

automated processor according to the manufacturer's recommendation (Hologic Inc.). The resulting slide was fixed in 95% ethanol and stained with Papanicolaou, and the remaining material was stored in PreservCyt solution for possible later use in the preparation of additional slides for further investigations (including both ICC and molecular analyses).

Cytologic diagnoses were primarily considered as *adequate for a cytologic diagnosis* or *inadequate for a cytologic diagnosis*. The former group was subclassified as follows: 1) negative for malignancy (NM), 2) suspicious for malignancy (SM), and 3) positive for malignancy (PM). We also signed out cases with a finding of *positive for malignancy supporting/confirming the primary cancer diagnosis*.

Additional slides for ICC (307 pleural and pericardic malignant samples and all 104 pleural and pericardic suspicious samples) and/or molecular analysis (47 pleural samples) were obtained from the material stored in PreservCyt solution. In these slides, analyses could be performed even with only 2 mL of remaining material eluted in 5 mL of PreservCyt solution. The percentage of disease-specific cells for ICC analysis was at least 30% in all LBC samples.

### ICC

ICC staining was carried out with the avidin-biotin peroxidase complex using a variety of antibodies based on both clinical suspicions and the cytologic findings. The slides were washed 3 times in phosphate-buffered saline (PBS) and then preincubated in normal veal serum with PBS (1:50 dilution) for 20 minutes before an overnight incubation at 4°C with the primary antibody. Then, the slides were washed 3 times with PBS and incubated with biotinylated secondary antibody-conjugated avidin-biotin-peroxidase complex (Ventana Systems, Tucson, Ariz). The reaction was developed using 3-3′-diaminobenzidine as a chromogen. All slides were counterstained with hematoxylin for 5 seconds, rinsed in water 3 times, then mounted for microscopic examination. Each cytologic sample was assessed as positive if $\geq$50% of cells had strong cytoplasmic or nuclear positivity based on the specific immunomarker used. This stringent cutoff percentage was chosen to avoid false-positive results and was aligned with the reported staining results from histologic diagnoses. Positive and negative controls were selectively used according to each specific immunomarker. Our ICC evaluation was carried out in 411 of 450 samples (91.3%), including suspicious and malignant samples of both pleural and pericardic effusions (for details, see Results, below).

### Molecular Analysis

Genomic DNA was extracted from cytologic samples using a spin column extraction method (QIAamp DNA mini kit; QIAGEN, Milan, Italy). DNA concentration and purity were assessed using a NanoDrop 2000c Spectrophotometer (Thermo Scientific Inc., Wilmington, Del). Mutational analysis of epidermal growth factor receptor (EGFR) was performed using the Therascreen EGFR RGQ polymerase chain reaction kit (QIAGEN) in a Rotor-Gene Q 5plex HRM instrument according to the manufacturer's protocol (sensitivity, <1%). The mutation nomenclature used in this work follows the guidelines indicated by the Human Genome Variation Society.[19]

### Histology

All surgical specimens were fixed in 10% buffered formaldehyde and embedded in paraffin; then, 5-μm-thick sections were stained with hematoxylin and eosin. Concordance of ICC results was 100% between the cytologic and histologic samples.

### Statistical Analysis

Statistical analyses were performed using a commercially available statistical software package (SPSS 10.0; SPSS Inc., Chicago, Ill) for Windows (Microsoft, Redmond, Wash). Comparisons of categorical variables were performed using chi-square statistics with the Fisher exact test when appropriate. All $P$ values $< .05$ were considered significant.

## RESULTS

Table 1 provides data on the distribution of the 3171 benign and malignant cytologic diagnoses according to clinical and morphologic features. When matched for sex, no significant difference was observed, as indicated by the slightly higher number of men than women (1708 men vs 1463 women).

The samples were distributed based on their diagnoses—classified as NM, SM, or PM—for both pleural and pericardic effusions. The series included 2505 pleural and 216 pericardic NM samples, 93 pleural and 11 pericardic SM samples, and 321 pleural and 25 pericardic PM samples (Table 1). Furthermore, 20 samples produced

**Table 1.** Clinicomorphologic Evaluation in 3171 Pleural and Pericardic Effusions in Liquid-Based Cytology

| Variable | NM | SM | PM |
|---|---|---|---|
| Age range, y | 36-79 | 25-85 | 33-92 |
| Sex: No. of men/women | 1411/1310 | 67/37 | 230/116 |
| Group, no. of samples | | | |
| Pleural effusions | 2505[a] | 93 | 321 |
| Pericardic effusions | 216[b] | 11 | 25 |
| No. of additional cell-block slides | 582 | 104 | 356 |
| Histology, no. of samples | 943 | 63[c] | 296[d] |
| ICC, no. of samples | 0 | 104 | 307 |

Abbreviations: ICC, immunocytochemistry; NM, negative for malignancy; PM, positive for malignancy; SM, suspicious for malignancy.
[a] Forty-eight positive samples were included.
[b] One false-positive sample was included.
[c] Forty-one unknown samples (36 from the pleural group and 5 from the pericardic group) were excluded (see Table 2).
[d] Sixty unknown samples (40 from the pleural group and 20 from the pericardic group) were excluded (see Table 2).

inadequate results based on the use of LBC with the possibility of additional slides, so that the combined evaluation reached a diagnostic and descriptive sign-out in >98% of the samples analyzed.

The 2505 samples in the pleural NM category did not produce any false-positive results but did produce 48 false-negative results (1.9%). Samples in the pleural NM category were characterized by 980 with LBC slides only; 943 with histologic diagnoses, including 677 nonsmall cell lung carcinomas (NSCLCs), 65 ovarian carcinomas, and 201 breast carcinomas with negative histologic pleural involvement; and 582 that had an additional cell block to combine with the LBC slide for a more accurate morphologic diagnosis. None of these samples underwent any immunomarker or molecular testing (Table 1). The 216 pleural NM samples did not produce any false-positive results but did produce 1 false-negative result. Samples in the pericardic NM group were characterized by 186 with LBC slides only and 30 with histologic diagnoses, including 16 NSCLCs, 5 ovarian carcinomas, and 9 breast carcinomas (Fig. 1) with negative histologic pleural involvement.

Among the 414 pleural SM and PM samples, 76 unknown primary malignancies and 338 known primary carcinomas were reported; whereas, among the 36 pericardic SM and PM samples, 20 unknown primary malignancies and 16 known primary carcinomas were reported, as detailed for pleural and pericardic effusions in Table 2 and Figure 2.

These latter interesting and challenging categories are the focus of our discussion and evaluation, as also supported by the application of ancillary techniques (Table 3).

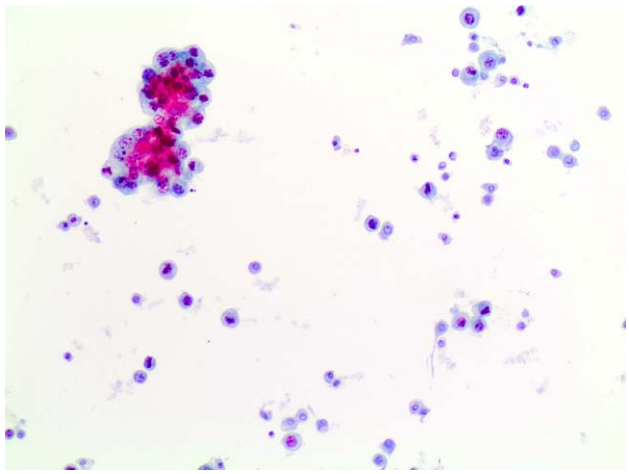

**Figure 1.** This photomicrograph shows the details of a malignant pericardic effusion from a breast carcinoma (Papanicolaou stain; original magnification ×400).

**Table 2.** Distribution of Pleural and Pericardic Diagnoses With Histological Findings

| | Cytologic Diagnoses, No. of Samples | | | | |
|---|---|---|---|---|---|
| Histologic Findings | Inadequate | NM[a] | SM | PM | ICC and Cell-Block Samples |
| Pleural diagnoses | | | | | |
| Unknown | 0 | 0 | 36 | 40 | 76 |
| Lung Ca | 0 | 24 | 21 | 133 | 128 |
| Ovarian Ca | 0 | 3 | 13 | 62 | 73 |
| Breast Ca | 0 | 11 | 4 | 31 | 32 |
| Gastrointestinal Ca | 0 | 6 | 9 | 22 | 27 |
| Mesothelioma | 0 | 0 | 5 | 11 | 16 |
| Myeloma | 0 | 0 | 0 | 3 | 0 |
| Kidney/urologic Ca | 0 | 4 | 0 | 5 | 5 |
| Lymphoma | 0 | 0 | 3 | 11 | 14 |
| Head and neck Ca | 0 | 0 | 1 | 4 | 4 |
| Pericardic diagnoses[b] | | | | | |
| Unknown | 0 | 0 | 5 | 15 | 20 |
| Lung Ca | 0 | 1[c] | 0 | 9 | 9 |
| Ovarian Ca | 0 | 0 | 0 | 4 | 4 |
| Breast Ca | 0 | 0 | 0 | 1 | 1 |
| Lymphoma | 0 | 0 | 0 | 1 | 1 |
| Head and neck Ca | 0 | 0 | 1 | 0 | 1 |

Abbreviations: Ca, carcinoma; ICC, immunocytochemistry; NM, negative for malignancy; PM, positive for malignancy; SM, suspicious for malignancy.
[a] None of the 48 false-negative pleural samples had ICC.
[b] Data for gastrointestinal, mesothelioma, myeloma, and urologic Ca were omitted because of the absence of samples.
[c] The 1 false-negative pericardic sample had no ICC.

Considering the application of ICC, which we performed as an immunopanel rather than as a single immunomarker, in total, we yielded 375 pleural effusions (all 93 SM samples and 282 of 321 pleural PM samples) and all SM and PM pericardic effusions. The ICC panels were carried out on stored LBC material with 100% conclusive results. ICC was performed both on stored LBC slides and on cell

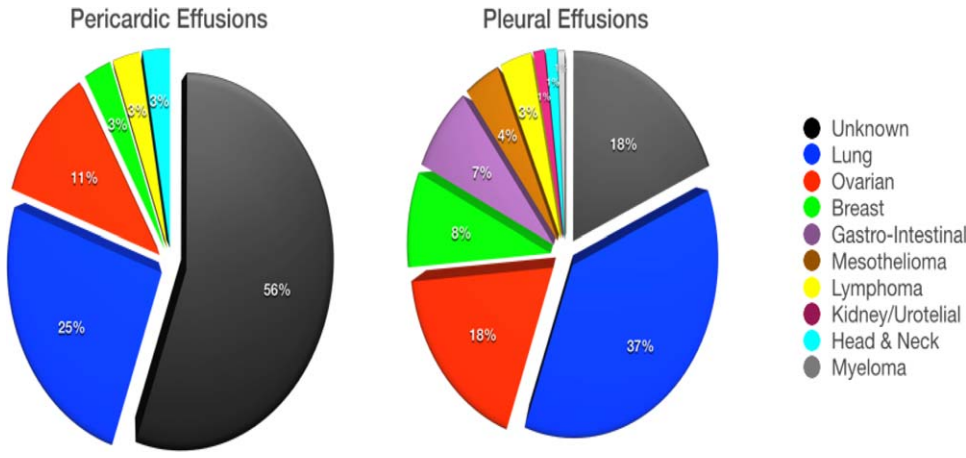


**Figure 2.** The data concerning diagnoses of suspicious for malignancy and positive for malignancy are summarized in patients who had pleural and pericardic effusions.

blocks that were obtained from stored LBC material, and no significant differences in expression were observed.

Table 3 provides data on the distribution of immunomarkers in the SM and PM pleural and pericardic effusions (Figs. 3 and 4). The same table provides an evaluation of the number of cell-block slides performed on stored LBC material. In all of our known cases, we combined LBC slides and cell blocks obtained from stored LBC material and performed ICC on both. Furthermore, we reached a conclusive diagnostic report in all 96 unknown cases (70 pleural effusions and 20 pericardic effusions), although we did not have histologic follow-up.

In addition, we used an extensive ICC panel made up of epithelial markers, including, ie, keratins 7 and 20, AE1/AE3 (antipan-cytokeratin antibody), CAM 5.2 (cytokeratin antibody), epithelial-specific antigen, thyroid trascriptor factor-1 (TTF-1), E-cadherin, and others (see Table 3); mesenchymal markers (vimentin, desmin, neurofilaments); cluster of differentiation (CD) markers (CD10, CD15, CD30; and CD45); or other markers (S100 [calcium-binding protein], carcinoembryonic antigen messenger RNA [mCEA], neuron-specific enolase, calcitonin, and HMB45 [human melanoma black 45]). The ICC panels were carried out both in unknown primary tumor samples and in samples from patients who had a clinical history of neoplasia.

The assessment of specificity, sensitivity, diagnostic accuracy, positive predictive value (PPV), and negative predictive value (NPV) is provided in Table 4. We did not observe any false-positive results in any of the categories analyzed; however, there were 49 false-negative results, including 48 in the NM pleural effusions and 1 in a pericardic effusion. When the pleural and pericardic effusions were considered together, the results demonstrated 88% sensitivity, 100% specificity, 98% diagnostic accuracy, 98% NPV, and 100% PPV. Table 4 also reports separate data for the pleural and pericardic effusions. Moreover, we used 47 pleural PM effusions for *EGFR* mutational analysis based on the diagnosis of NSCLC and identified 10 with mutations (6 with short in-frame deletions of exon 19 and 4 with single-nucleotide substitutions in exon 21 characterized by the missense leucine to arginine substitution at codon 858 [p.L858R] mutation; data not shown).

## DISCUSSION

The results reported here demonstrate the great efficacy of using cytologic evaluation to ascertain precise diagnoses in both technical types of sample preparation: smeared sediment and LBC. Not only does LBC offer some additional morphologic advantages (clearer background, cell enrichment, and better nuclear details), but residual LBC material is also very useful for ancillary methodologies, including ICC evaluation and molecular tests.[5–7,15,16,19] It is important to highlight this point, because LBC diagnoses are comparable to those obtained with traditional smears and offer a value added in terms of potential use for additional methodologies, including receptor gene rearrangements by polymerase chain reaction analysis or

**Table 3.** Correlation Between Primary Tumors and Pleural Effusions: Morphologic and Immunocytochemical Profiles

| Primitive Cancers | No. With Suspicious/ Malignant Effusions | Antibody Tested on Cytologic and Cell-Block Samples[a] |
|---|---|---|
| Pleural effusions | | |
| Unknown | 30/46 | Positive: 5 vimentin, 5 calretinin, 10 TTF-1, 2 CEA, 10 CAM 5.2M, 6 BER-EP4, 5HBME-1, 15 CD20, 10 CK7, 5 CK20, 1 p63, 2 CK5/6[b] |
| Breast Ca | 4/28 | Positive:CK7, GCDFP15, ER, PR, E-cadherin; negative: TTF-1,calretinin, CK5/6 |
| Lung Ca | 21/107 | Positive:TTF-1, CAM 5.2, CK7, p63; negative: S100, calretinin, CK5/6 |
| Ovarian Ca | 13/60 | Positive: CA125, WT1, CAM 5.2, CK7, ER, PR, CEA; negative: S100, CK5/6, calretinin, CK20, CDX2 |
| Mesothelioma | 5/11 | Positive: CAM 5.2, calretinin, HBME-1, podoplanin; negative: TTF-1 |
| Gastrointestinal Ca | 9/18 | Positive: CAM 5.2,CK20, CDX2; negative: TTF-1, calretinin, CK5/6 |
| Lymphoma | 3/11 | Positive: LCA, CD20, CD79A, CD30, CD15; negative: TTF-1, CAM 5.2, CK20, CDX2, CK5/6, calretinin |
| Head and neck Ca | 1/3 | Positive: EMA, CAM 5.2, AE1/AE3; negative: thyroglobulin, TTF-1, calcitonin, HBME-1, galectin3 |
| Urogenital Ca | 0/5 | Positive: EMA, CAM 5.2, vimentin, CD10; negative: thyroglobulin, TTF-1 |
| Pericardic effusions | | |
| Unknown | 5/15 | Positive: 5 vimentin, 1 desmin, 5 calretinin, 10 TTF-1, 2 CEA, 10 CAM 5.2M, 6 BER-EP4, 5 HBME-1, 15 CD20, 10 CK7, 5 CK20, 1 p63, 2 CK5/6[b] |
| Breast Ca | 0/1 | Positive:CK7, GCDFP15, ER, PR, cadherin; negative: TTF-1 |
| Lung Ca | 0/9 | Positive:TTF-1, CAM 5.2, CK7, p63; negative: S100, calretinin, CK5/6 |
| Ovarian Ca | 0/4 | Positive: CA 125, WT1, CAM 5.2, CK7, ER, PR; negative: S100 |
| Lymphoma | 0/1 | Positive: LCA, CD20, CD79A, CD30, CD15; negative: TTF-1, CAM 5.2, CK20, CDX2, CK5/6, calretinin |
| Head and neck Ca | 1/0 | Positive: EMA, CAM 5.2, AE1/AE3; negative: thyroglobulin, TTF-1, calcitonin, HBME-1, galectin3 |

Abbreviations: AE1/AE3, antipan-cytokeratin antibody; BER-EP, antiepithelial antigen antibody; CA 125, cancer antigen 125; Ca, carcinoma; CD15, cluster of differentiation 15, 3-fucosyl-N-acetyl-lactosamine; CD20, cluster of differentiation 20, B-lymphocyte antigen; CD30, cluster of differentiation 30, cell membrane protein of the tumor necrosis factor receptor family; CD79K, cluster of differentiation 79A molecule, immunoglobulin-associated α; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen; CK, cytokeratin; CAM 5.2, cytokeratin antibody; EMA, antiendomsial antibody; ER, estrogen receptor; GCDFP-15, gross cystic disease fluid protein 15; HBME-1, mesothelioma marker antibody; LCA, leukocyte common antigen; p63, tumor protein 63; PR, progesterone receptor; S100, S100 calcium-binding protein; TTF-1; thyroid transcription factor-1; WT1, Wilms tumor 1.

[a] Values for positive results are reported, so that the remaining values reflect the corresponding negative values.

[b] Immunocytochemistry was performed both on stored liquid-based cytology slides and on cell blocks derived from stored liquid-based cytology material, and there was no significant difference in expression.

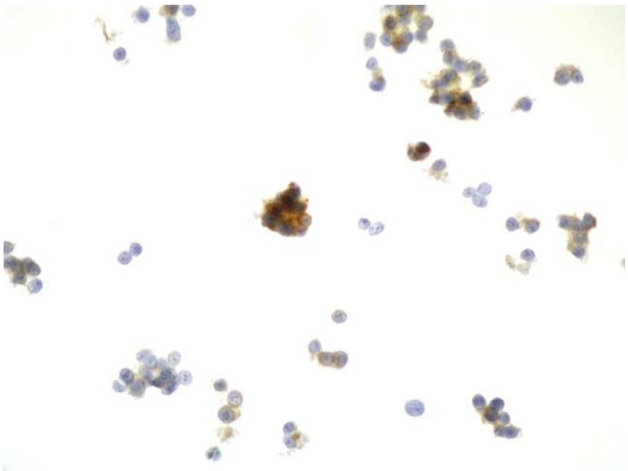

**Figure 3.** This photomicrograph shows a thyroid transcription factor 1 (TTF-1)-positive pleural effusion from a lung carcinoma (avidin-biotin-peroxidase complex; original magnification 3400).

chromosome translocation by fluorescence in situ hybridization analysis. All this is critical, because mesothelial cells can be misinterpreted as neoplasia in certain conditions.

On the basis of our previous, large experience in the field of FNAC with LBC, we conducted the current analysis in a large series of 3171 pleural and pericardic effusions that were processed with LBC. The results underscore the reliability of using cytology to identify positive and suspicious effusions, in which cytology can provide essential clues for diagnosis and staging regardless of body site.

Although no specific guidelines for the optimal or best processing of FNAC effusions have been proposed in recent years, several authors have reported reliable data with the application of conventional cytology, which has been the cornerstone of cytologic preparations for many years.[1–8] With this perspective, we have analyzed our 13-year experience using LBC preparations, underscoring the pros and cons of this method reported in the recent literature.[1–3,9,13]

The enthusiasm for LBC has been because of its different advantages, identified mainly as a uniform collection procedure, avoiding the hazards of needles during conventional preparations, standardized processing

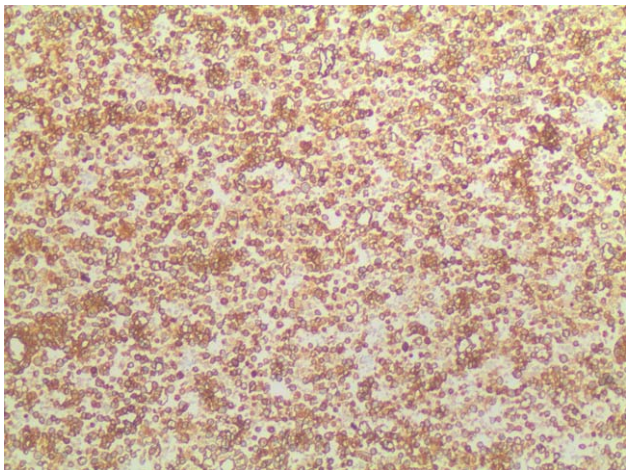

**Figure 4.** Positivity for E-cadherin in the same sample (pericardic effusion from a breast carcinoma) from Figure 1 is observed in a cell-block preparation (avidin-biotin-peroxidase complex; original magnification ×400).

**Table 4.** Descriptive Statistics for Morphologic Yield in the Pleural and Pericardic Groups

| | Percentage of Samples | | |
|---|---|---|---|
| Variable | Pleural and Pericardial Effusions | Pleural Effusions | Pericardial Effusions |
| Sensitivity | 88 | 87 | 97 |
| Specificity | 100 | 100 | 100 |
| Diagnostic accuracy | 98 | 98 | 99 |
| NPV | 98 | 98 | 99 |
| PPV | 100 | 100 | 100 |

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Fine-needle aspiration cytology of serous membrane effusions may fulfill a challenging role in the diagnostic analysis of both primary and metastatic disease. In addition, liquid-based cytology is useful for ancillary techniques, with reliable and feasible yields demonstrated in 3171 cytologic samples.

techniques, the availability of residual material for additional slides or cell blocks, and the easier application of ancillary techniques (ICC and molecular testing).[1–3,5,6,15,19] Conversely, the limits of LBC may be represented by the impossibility of assessing samples for adequacy or triage, including culture and flow cytometry, which may represent critical points in some diagnostic situations.[6] In addition, Hoda[3] reviewed all of the published literature on the morphologic aspects of LBC for nongynecologic cytology and underscored the accuracy of both ThinPrep and SurePath for the interpretation of effusions, in alignment with data published by Ylagan et al.[3,10]

The first critical role of FNAC in effusions is to discriminate between benign and malignant disease and to rule out a diagnosis of benign reactive mesothelial cells versus adenocarcinoma and epithelial mesothelioma.[9,12,13,18,20,21] Indeed, as highlighted by Ylagan et al, the overlapping cytologic features in some samples may prevent a more definitive diagnosis, and a panel of immunomarkers is strongly suggested when a cytopathologist faces this issue.[10]

Moreover, when analyzing other possible malignant neoplasms diagnosed in pleural effusions, many associations may be observed between malignant mesothelioma and serous carcinoma because of the common histogenesis and coexpression of the immunomarkers. In our series, we reported 72 ovarian metastatic carcinomas in which Wilms tumor-1 was not specific for ruling out the correct diagnosis, whereas CA 125 combined with other markers (including keratin 7 and calretinin) had good specificity for discriminating between ovarian metastatic carcinomas and mesotheliomas.

In our experience, the hotspot evidence that only 49 negative pleural and pericardic effusions were diagnosed as malignant (1.5%) underlines the high NPV of cytologic effusions. This relevant statistical significance maximizes the role of LBC. In fact, in 572 of 2505 pleural NM effusions that had unequivocal interpretations, we combined the morphologic evaluations of LBC slides and cell blocks obtained from stored LBC material, and the results were concordant in all of the 572 samples that were analyzed; therefore, in those samples, we did not perform any ICC. Nevertheless, we are conscious that, according to data published by Sun et al, results from an immunopanel may be useful in supporting some "critical" morphologic findings, which can overlap between reactive mesothelial cells and mesothelioma even among experienced cytopathologists.[13] In addition, although we did not use any immunomarkers, a growing body of literature is encouraging the diagnostic value of some immunomarkers (ie, claudin-4 or mucin-1) with high sensitivity and specificity for discriminating between reactive mesothelial cells and metastatic adenocarcinomas.[12,13,22,23]

It is important to point out the inadequate rate in our current results. Our 0.6% nondiagnostic rate for the pleural NM samples was very low, probably because LBC offered the ability to prepare additional slides and cell blocks, with a consequent decrease in the inadequate rate. Likewise, the nondiagnostic samples were ascribed to the scant material obtained from aspiration, which also

rendered it impossible to prepare a second LBC slide or cell block or to apply any of the ancillary techniques.

Therefore, our finding of a 14.8% malignant rate for LBC slides is in perfect agreement with the 15% malignant rate reported by Lee et al on LBC, whereas the discrepancies with the results from other series are a consequence of differences in preparation and cytohistologic bias.[1,2] Our data on the LBC method are in keeping with a report by Gabriel et al, who compared 2 series (an LBC series and a conventional cytology series) without observing any discrepancies in terms of sensitivity, specificity, or diagnostic accuracy and concluded that in, body fluid cytology, LBC may replace other types of preparations with a lower false-negative rate.[8]

The well known assessment that malignant effusions in the pleura and peritoneum are frequently signs of the metastatic involvement of primary adenocarcinomas, particularly among women with breast and ovarian cancers and among both men and women with lung carcinoma, is in keeping with the current findings.[1,2,18,20,21] In fact, ovarian and breast carcinomas accounted for 72 and 34 malignancies, respectively, in pleural PM and SM samples and also represented the most frequent primary malignancies in the pericardic samples.

Overall, some original and review articles have pointed to the application of ICC as well as fluorence in situ hybridization, comparative genomic hybridization, and other molecular techniques mainly for 2 clinical scenarios: searching for metastatic cells and characterizing mesothelioma.[12–16] Although the application for ancillary techniques represented a valid aid even in our study, we assessed the central role of morphology in FNAC for drawing attention to the performance of a specific useful panel unless we were testing unknown malignancies. Although the majority of articles we reviewed produced feasible results with the application of ICC on conventional cytology or cell-block slides, we encourage the use of LBC as a valid alternative method for the application of different, specific ICC panels to additional slides obtained from the material stored in PreservCyt solution.[3,5,6,15,16] Indeed, the findings for >66% of our SM and PM effusions (441 of 450 total malignant effusions) were supported by ICC applications, including the use of specific diagnostic immunomarkers in 96 samples from unknown primary neoplasms, resulting in 100% conclusive results. Therefore, in the latter 96 samples, the use of an LBC preparation offered the opportunity to refine and polish the morphologic description of samples in which morphology alone could no longer achieve a conclusive and specific malignant diagnosis.

We carried out ICC analyses on both LBC slides and cell blocks obtained from stored LBC material without observing any discrepancies, in keeping with data reported by Jing et al, who also explored the role of collecting media in cell block preparations without observing any interference in the performance of immunostaining.[17] Hence, in contrast to the central role played by ICC in recent years, the emerging role of molecular testing of effusions has gained new enthusiasm.[14]

Different cytologic methods did not affect DNA quality or molecular tests, enforcing the use of LBC for identifying *EGFR* mutational expression (including all mutations in exons 18-21) or anaplastic lymphoma kinase (*ALK*) rearrangements in 39 metastatic pleural effusions from patients with NSCLC, as noted in some previous reports, including ours.[24,25] The increasing use of LBC for ancillary techniques and in effusions has widened the classic role of morphologic distinction between small cell lung carcinomas and NSCLCs without any possible further diagnostic or prognostic implications. Our current investigation demonstrates the outstanding role of cytologic specimens in supplying adequate material not only for diagnosis but also for gene mutation assays, essentially in *EGFR* and other gene mutations or rearrangements (ie, *KRAS* gene mutations or *ALK* rearrangements).[25]

In the current series, and in agreement with the literature, we observed that LBC preparations in pleural and pericardic effusions had 87% sensitivity, 100% specificity, 89% diagnostic accuracy, 98% NPV, and 100% PPV, which undoubtedly exceeded the average values underscored in several series that used conventional cytology.[2,9,10] In conclusion, to date, this is the largest series of pleural and pericardic effusions in LBC preparations; and, based on our findings, we believe that the combined use of morphologic FNAC evaluation and the application of both ICC and molecular analyses on LBC preparations is feasible and reliable and that the combination of morphology and ancillary techniques represents the best strategy, particularly in patients for whom morphology alone cannot achieve a conclusive diagnosis.

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## CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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