Utility of flow cytometry analysis for pleural and peritoneal fluids

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Introduction
Pleural and peritoneal/ascites fluid samples with many lymphocytes are commonly received in the cytology laboratory. It is often difficult to distinguish reactive lymphocytes from hematopoietic malignancy based on morphology alone, however. Flow cytometry can be a useful adjunct in body fluids, although literature on this subject is limited.

Materials and methods
This study is a single-institution 5-year retrospective review of 377 fluid samples from 341 patients with corresponding flow cytometry analysis. The cytologic findings were correlated with the flow cytometry results and clinical data, as available.

Results
Of 4158 pleural fluids received over 5 years, 325 (7.8%) had corresponding flow cytometry analysis. Of these 325 samples, 57 (17.5%) were positive for hematopoietic malignancy by flow cytometry. Of the positive cases, only 24 (8.7%) represented a new diagnosis of hematopoietic malignancy (ie, did not have a known history). Of 3020 peritoneal/ascites fluids received over 5 years, 52 (2%) had corresponding flow cytometry. Of these, 8 were positive for hematopoietic malignancy, and only 2 represented a presumed new diagnosis.

Conclusions
Routine flow cytometry analysis for pleural and peritoneal/ascitic fluids is of limited utility, with only rare cases positive for hematopoietic malignancy without a known history. Of these cases, many had atypical cells that suggested a positive diagnosis. Conversely, in cases with a known history, about 75% were positive for hematopoietic malignancy. Our study suggests that the utility of flow cytometry for pleural and peritoneal/ascitic fluids is limited, and should be used sparingly in cases without atypical cytologic features, high clinical suspicion, or known history.

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malignancies based on morphology alone, however. Flow cytometry can be a useful test to identify NHL in body fluids, although literature on this subject is somewhat limited.1,6 The use of flow cytometry as an adjunctive test for other cytology specimens is fairly well established, especially for fine-needle aspiration (FNA) biopsies of suspected lymphoproliferative lesions. In these settings, flow cytometry performs well both in conjunction with cytology examination7-10 and independently.11

Low-grade lymphomas in particular may have a bland appearance similar to reactive lymphocytes, and cannot be reliably diagnosed using light microscopy alone. Flow cytometry has higher sensitivity than cytomorphology alone for detecting hematopoietic malignancies, especially for low-grade lymphomas.2 The combination of cytomorphology and flow cytometry has also been shown to have utility in lymphoproliferative lesions in the thyroid and salivary gland.12,13

Flow cytometry is an accepted adjunct method to aid the cytologic diagnosis of serous effusions with many lymphocytes, and is particularly useful to distinguish reactive effusions from low-grade lymphomas. As flow cytometry becomes used more routinely on cytology specimens, however, the utility of flow cytometry in serous effusions should be addressed. The clinical utility of flow cytometry in body fluid cytology has been discussed previously,14 and a recent publication15 reviews the use of flow cytometry in serous effusions over 4 years in a single academic institution. We aim to present an update of the utility of sending pleural and peritoneal fluid specimens for flow cytometry analysis.

Materials and methods

This study is a 5-year retrospective review of pleural fluid and peritoneal fluid samples with corresponding flow cytometry analysis, from a single institution. The cytologic findings were correlated with the flow cytometry results and clinical data, as available.

Case selection

The pathology database was searched for all pleural fluids, peritoneal fluids, and ascites fluids received in the cytology laboratory with a corresponding flow cytometry report mentioned over a 5-year period (January 1, 2008 to December 31, 2012). The cytology interpretation, flow cytometry interpretation, patient age, and any reported history of hematopoietic malignancy were recorded. The flow cytometry results were classified as “positive” when they were diagnostic of a hematopoietic malignancy or clonal cell population, and were classified as “negative” when no monoclonal B cell population or aberrant T cell population was noted.

Specimen processing

Pleural, peritoneal, and ascites fluids were initially received in the cytology laboratory. The appearance and total volume were noted, representative portions of the specimens were processed, and 5 cytospin slides were prepared. Four slides were alcohol-fixed and Papanicolaou-stained, and 1 slide was air dried and Diff-Quik-stained. Cell blocks were prepared for select cases per pathologist request, and were embedded in paraffin and stained with hematoxylin and eosin. Flow cytometry was usually requested by the pathologist following review of the prepared slides, but in some instances it was ordered by the requesting physician. Following cytologic analysis, the remainder of the specimen was sent to the flow cytometry laboratory for immunophenotypic analysis.

Flow cytometry

Eight-color flow cytometry was performed according to the manufacturer’s protocol on a BD FACSCanto machine (BD Biosciences, San Jose, Calif.). A standard screening panel using the following antibodies (BD Biosciences) was used: CD3, CD4, CD5, CD7, CD8, CD10, CD14, CD19, CD20, CD38, CD45, CD56, mKappa, mLambda, pKappa, and pLambda. Additional antibodies were added as per pathologist request. Data were analyzed using BD FACSDiva and BD Paint-A-Gate software (BD Biosciences).

Results

Pleural fluids

In a 5-year period (January 1, 2009 to December 31, 2013), 4,158 pleural fluid specimens were received in the cytopathology laboratory. The number of pleural fluid specimens received increased each year, from a total of 670 in 2009 to a total of 1019 in 2013: an overall 52% increase over 5 years. Of these specimens, 325 (7.8%) had corresponding flow cytometry analysis, from 298 patients. The number of pleural fluids sent for flow cytometry had a net increase of 67% over 5 years, roughly corresponding to the overall increase of pleural fluid specimens received. The age range was from 21 to greater than 90 years, with a mean age of 68 years. Nearly all (94%) of the pleural fluids were received from inpatients.

Of these 325 pleural fluid samples with corresponding flow cytometry analysis, 57 (17.5%) were positive for hematopoietic malignancy by flow cytometry. Despite an overall increase in both the number of pleural fluids received and the number of pleural fluids with flow cytometry, both the total number of positive cases and the total number of new diagnoses stayed roughly the same of the 5-year period (Table 1). The
average age for negative cases and positive cases was not significantly different (68 and 69 years, respectively).

There were 45 samples with a known history of NHL/leukemia, and 33 of these (73.3%) were positive for hematopoietic malignancy by flow cytometry. The remaining 280 samples had no reported history of NHL/leukemia, and 24 (8.7%) were positive for hematopoietic malignancy by flow cytometry (Fig. 1). These 24 cases represent a presumed first-time diagnosis of hematologic malignancy via the pleural fluid specimen, and represented 42% of the positive cases. Overall, 0.6% of pleural fluids received in a 5-year period reported a first-time diagnosis of hematologic malignancy. The most frequent diagnoses were large B cell lymphoma (7 cases), clonal B cell population that could not be more specifically diagnosed by flow cytometry alone (7 cases), and chronic lymphocytic leukemia/small lymphocytic lymphoma (5 cases). Other diagnoses included acute leukemia/myeloblasts, plasma cell neoplasm, mantle cell lymphoma, and T lymphoblastic lymphoma.

Of the cytology reports for these 24 cases with a positive flow cytometry result without a reported history of NHL/leukemia, 8 (33.3%) mention presence of cytologically atypical cells, and 9 (37.5%) were reported as consistent with NHL or leukemia. The remaining 7 cases (29.2%) describe a monotonous population of small lymphocytes (Fig. 2). These cases were reviewed, and no obvious uniting cytologic features were identified.

A small proportion (13.5%) of the pleural fluids with corresponding flow cytometry were recurrent. Of these recurrent pleural effusions, half (22 of 44) had repeatedly negative flow cytometry. About a third (14 of 44) had repeatedly positive flow cytometry. The remaining recurrent effusions either had a positive flow cytometry then a negative flow cytometry (4 of 44), or the opposite. Of note, only 4 of 44 (9%) of the recurrent pleural effusions in the study had an initial negative flow cytometry diagnosis with a repeat positive pleural effusion flow cytometry. However, we only examined those cases sent for flow cytometry, so recurrent effusions without a corresponding flow cytometry analysis were not included in this study. The general practice at our institution is to incorporate the flow cytometry findings into the cytology report, and reference the flow cytometry report number. We recommend this practice to help alleviate potentially redundant testing in recurrent effusions.

Information on the ordering physician, pathologist versus clinician, was available for 194 of the pleural fluids (60%). Of these cases, 155 (80%) were ordered by the pathologist and 39 (20%) were ordered by the clinician. The percent of positive cases was 15% for cases ordered by the pathologist and 36% for cases ordered by the clinician. However, the majority of the positive cases ordered by the clinician had a known history of hematopoietic malignancy (12 of 14 total cases). If the cases with a known history of hematopoietic malignancy are excluded, then the percent of positive cases was 8.4% for cases ordered by the pathologist and 7.4% for cases ordered by the clinician.

### Peritoneal fluids

In a 5-year period (January 1, 2009 to December 31, 2013), 3020 peritoneal/ascitic fluid specimens were received in the cytopathology laboratory. The number of peritoneal/ascitic fluid specimens received increased each year, from a total of 508 in 2009 to a total of 765 in 2013: an overall 51% increase over 5 years.

Of these specimens, 52 (2%) had corresponding flow cytometry analysis, from 43 patients. The age range was from 33 to 84 years, with a mean age of 60 years. A total of 71% of the peritoneal/ascitic fluid samples were from inpatients.

Of these 52 peritoneal/ascitic fluid specimens with corresponding flow cytometry, 8 (15%) were positive for hematopoietic malignancy by flow cytometry (Table 2). Of these 8 positives, 6 had a known history of hematopoietic malignancy, and two represented a presumed new diagnosis (6% of positive cases). Of interest, both of these diagnoses were of kappa light chain restricted plasma cell neoplasm.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total pleural fluids</th>
<th>Flow cytometry performed n (%)</th>
<th>Positive flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>670</td>
<td>46 (7)</td>
<td>14</td>
</tr>
<tr>
<td>2010</td>
<td>720</td>
<td>59 (8)</td>
<td>8</td>
</tr>
<tr>
<td>2011</td>
<td>819</td>
<td>79 (10)</td>
<td>13</td>
</tr>
<tr>
<td>2012</td>
<td>930</td>
<td>64 (7)</td>
<td>11</td>
</tr>
<tr>
<td>2013</td>
<td>1019</td>
<td>77 (7)</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>4158</td>
<td>325 (7.8)</td>
<td>57 (1.4%)</td>
</tr>
</tbody>
</table>
Six of 8 (75%) cases of peritoneal fluids with a known history of hematopoietic malignancy were positive.

Information on the ordering physician, pathologist versus clinician, was available for 16 of the peritoneal/ascitic fluid samples (31%). Of these cases, 15 were ordered by the pathologist and 1 was ordered by the clinician. Of the cases ordered by the pathologist, 1 was a new diagnosis and 2 were positive diagnoses in a patient with a known history. The case ordered by the clinician and the remainder of the cases ordered by the pathologist were negative.

Discussion

Rendering an accurate diagnosis in body fluid specimens with many lymphocytes can be challenging. It is now well established that flow cytometry is a useful adjunctive test in the examination of body fluid specimens sent for cytology analysis. Nevertheless, the question of which samples to send for flow cytometry remains. Our institution does not have any set criteria for ordering flow cytometry for body fluids, and it is up to the cytopathologist to decide on a case-by-case basis if flow cytometry is warranted. Clearly, sending every body fluid sample with lymphocytes for flow cytometry is excessive, and never sending any body fluid samples for flow cytometry would be ignoring a valuable adjunctive test. Flow cytometry is not an inexpensive test, however, and we should seriously consider the balance of over-utilization and rendering an accurate diagnosis.

In our single-institution study, flow cytometry analysis for pleural fluids and peritoneal/ascites fluids appears to be of limited utility, as we found only 8.7% and 6% of cases, respectively, without known histories were positive for hematopoietic malignancy. Of these positive cases, many had atypical cells that suggested a diagnosis of hematologic malignancy based on cytomorphology alone. Conversely, in cases with a known history, 73.3% of pleural fluids and 75% of peritoneal/ascites fluids were positive for hematopoietic malignancy. Our study suggests that the utility of flow cytometry for pleural fluids is low except in cases with atypical cytologic features or known history.

At our institution, flow cytometry for body fluids can be ordered directly by the clinician or can be requested by the cytopathologist after viewing the cytology slides. When pleural fluid cases ordered by the pathologist were compared with those ordered by the clinician, the rate of positive cases ordered by the pathologist is 15% versus 36% in cases ordered by the clinician. Most of the positive cases ordered by the clinician, however, had a known history of hematopoietic malignancy (12 of 14 total cases). The rates of positive diagnoses were similar in cases without a known history of hematopoietic malignancy (2.4% for cases ordered by the pathologist and 7.4% of those ordered by the clinician). Of the positive cases that were ordered by the pathologist, only half (12 of 24 total cases) had a known history of hematopoietic malignancy.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total peritoneal/ascitic fluids</th>
<th>Flow cytometry performed n (%)</th>
<th>Positive flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>508</td>
<td>13 (3)</td>
<td>4</td>
</tr>
<tr>
<td>2010</td>
<td>531</td>
<td>7 (1)</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>581</td>
<td>8 (1)</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>635</td>
<td>7 (1)</td>
<td>4</td>
</tr>
<tr>
<td>2013</td>
<td>765</td>
<td>17 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3020</td>
<td>52 (2)</td>
<td>8 (0.2%)</td>
</tr>
</tbody>
</table>

Figure 2 Representative cytomorphology from pleural fluids. A, Large B cell lymphoma with plasmablastic features, no known history. B, T lymphoblastic lymphoma, no known history. C, Negative for malignancy. D and E, Large B cell lymphoma, known history. F, Chronic lymphocytic leukemia/small lymphocytic lymphoma, known history.
Flow cytometry for fluids

So which cases should be sent for flow cytometry? There is not an easy cutpoint, but it is not unreasonable to send those cases with a clinical history or strong suspicion of hematologic malignancy, as well as those with atypical cytomorphology. In cases with many lymphocytes, but without significant atypia, additional investigation such as looking up the complete blood count test results and clinical documentation may be warranted. Additionally, consultation with the ordering physician may be helpful. Of note, we did not find a significant difference in the age of patients with negative pleural fluids and those with pleural fluids positive for hematopoietic malignancy, nor did we find an obvious trend of malignancy in recurrent effusions. This study included reports by 18 different cytopathologists. No appreciable difference in ordering patterns or rates of positive cases was identified.

As with the utilization of any clinical test, it is important to consider the diagnostic utility of flow cytometry for each case. In most situations, our data support the position that the pre-test probability of a positive result is low, and the additional expense may not be warranted. Ultimately, the decision should be left to the discretion of the cytopathologist handling each case, but this study provides some support to those who wish to avoid additional testing that is unlikely to benefit the patient.

A similar study by Yu et al6 emphasizes immunophenotypic analysis of hematopoietic cell populations by flow cytometry as a useful ancillary study in the diagnosis of lymphoid proliferations in fluid specimens. They reported flow cytometry performed in 4.8% of all pleural fluids received in the cytology laboratory, of which 38% were positive. Of all pleural fluids, they reported 1.8% with positive flow cytometry, which is comparable to our overall positivity rate of 1.3% of pleural fluids. In our study, a larger percentage of pleural fluids were sent for flow cytometry (7.8% versus 4.8%), with a similar overall positive flow cytometry percentage. This provides indirect support for the supposition that, outside of high-likelihood situations, flow cytometry has low yield. The data for peritoneal/ascites fluids are similar: we found that 2% of peritoneal/ascites fluids were sent for flow cytometry, with an overall 0.3% positive; Yu et al reported 1.1% of peritoneal fluids had flow cytometry performed, and overall 0.4% were positive. These data suggest only limited utility for flow cytometry in peritoneal/ascites fluids. The lower rate of positive flow cytometry in ascites fluids probably reflects the fact that many of these specimens derive from palliative fluid removal in the setting of cirrhosis and thus have a low likelihood of malignant findings.

Although the results of the two studies are similar, Yu et al have a somewhat different point of emphasis, focusing on the utility of flow cytometry in the small subset of cases where it can make a decisive difference. They make the excellent point that a definitive diagnosis of lymphoproliferative disorder in a body fluid “obviates the need for further costly diagnostic workup in order to establish an etiology for an effusion.” Although we agree with Yu et al that flow cytometry has great value in some cases, we have chosen to focus more attention on the problem of over-testing rather than under-diagnosis. This may reflect the larger proportion of negative flow cytometry tests that we found in our study. The cytopathologists at our institution appear to be ordering flow cytometry more often, with no appreciable increase in yield, relative to the cytopathologists involved in the study by Yu et al, raising more concern about excessive utilization. The reasons for increased utilization of flow cytometry are unclear, although possible reasons include increased availability, access, and acceptance of flow cytometry for body fluid specimens, as well as reassurance that a seemingly reactive increase of lymphocytes is nonclonal.

Of course, there will always be cases with unexpected findings. We report presumed new diagnosis of hematopoietic malignancy in 26 patients. This corresponds to an overall rate of about 3 per 1000 cases. Yu et al reported a similar overall rate of about 2 per 1000. Inevitably, reduction of the frequency of flow cytometric analysis will increase the likelihood of missing a low-grade lymphoma masquerading as benign T cells. Nevertheless, even among the few new diagnoses observed in our study, only 7 were morphologically bland and therefore likely to be missed with reduced flow cytometry utilization. Furthermore, if these 7 cases had not been detected in body cavity fluids, the diagnosis may still have been rendered by some other means before serious negative consequences befell the patient for many or all of these indolent lymphomas. Additionally, in patients with hematopoietic malignancy present in their blood, significant contamination of the fluid specimen by peripheral blood could cause a false positive interpretation of the body fluid.

We considered flow cytometry results as the final diagnosis, although false positive and negative flow cytometry analyses are possible. Of particular difficulty is the finding of a small aberrant lymphoid population in a body fluid, which should not always be interpreted as involvement by lymphoma. The risk of giving false positive results with extensive and frustrating additional workup must also be considered. The methods of this study only included cytology cases with an associated flow cytometry, and did not include any cases positive for hematologic malignancy based on cytology alone.

Flow cytometry is an established and useful adjunct for diagnoses of hematopoietic malignancy on body fluid specimens. Nevertheless, routine use of flow cytometry on body fluids is not indicated. In these days of increasingly stretched healthcare dollars, it is important to consider the utility of any test. We recommend use of flow cytometry in pleural and peritoneal/ascites fluid specimens with a known history or strong clinical suspicion of hematopoietic malignancy, as well as those with atypical cytogic features. Cases with many lymphocytes in the absence of cytologic atypia, clinical suspicion, or known history should be
carefully considered on an individual basis, keeping in mind the low likelihood of a positive finding.

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**References**

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