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Improving Malignancy Detection Rates in Body Fluids Submitted to the Hematology Laboratory for Nucleated Cell Count and Differential

A Quality Improvement Study

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Context.—Body fluid specimens are regularly submitted to the hematology laboratory for cell count and differential. Unless there is high clinical suspicion for malignancy, most cases lack concurrent cytology review and may not benefit from more focused examination for malignancy.

Objective.—To compare rates of malignancy detection before and after fluid-focused training for hematology technologists as part of a quality improvement initiative.

Design.—During an 8-week pretraining period, body fluids submitted to the cytology laboratory were correlated with concurrent hematology specimens. After slide review and training sessions for the hematology technologists, the same data were collected for a 4-week period. Discrepant cases were reviewed by hematology laboratory supervisors and pathologists.

Results.—We collected 465 pretraining and 249 posttraining body fluids with concurrent cytology and hematology evaluation. In the pretraining cohort, 48 cases (10.3%) were diagnosed as malignant by cytology; of those, 33 were detected by hematology. In the posttraining cohort, 30 cases (12.0%) were diagnosed as malignant by cytology of which 27 were detected by hematology. Of the 18 discrepant cases (all carcinomas), hematology slide review showed definite features of malignancy in 15 and no tumor cells in 3. The malignancy detection rate by the hematology laboratory significantly improved after training (68.8% versus 90.0%, \( P = .01 \)).

Conclusions.—We demonstrate the comparatively lower malignancy detection rate for body fluid specimens processed in our hematology laboratory, particularly for carcinomas. Hematology technologist education/training improved the malignancy detection rate, an important quality improvement given the large proportion of body fluids undergoing hematology evaluation without concurrent cytology reviews.

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the hematology laboratory for appropriate specimen triaging and prompt patient care.

As part of a quality improvement initiative to enhance our hematology laboratory’s ability to detect malignancy in body fluid specimens, we compared the rates of malignancy detection, based on review of the hematology laboratory’s fluid cytospin and the cytology laboratory’s concurrent specimen, before and after training sessions with hematology technologists.

**MATERIALS AND METHODS**

**Study Design**

Data were prospectively collected for body fluids submitted to the cytology laboratory during an 8-week pretraining period (July 2016–August 2016) with concurrent hematology specimens. All cytology cases were signed out by a cytopathologist. Cases classified as positive for malignancy were those signed out as “positive” by a cytopathologist and those flagged with “unclassified cells” by a hematology technologist. Discrepant cases were reviewed by hematology laboratory supervisors, hematopathologists, and cytopathologists during consensus slide review sessions. Factors contributing to the discrepancies were noted. Afterwards, multiple mandatory body fluid slide review and training sessions were held for all 26 hematology technologists involved in evaluating fluid specimens as part of a laboratory quality improvement initiative. These sessions consisted of an introductory lecture, review of body fluid cytology, and practical slide review sessions featuring the actual discrepant cases identified during the pretraining period. To monitor and assess improvement after the training sessions, data were again prospectively collected for body fluids with concurrent cytology and hematology specimens during a 4-week posttraining period (January 2017) (Figure 1). Since this study was conducted as part of a laboratory quality improvement initiative, institutional review board approval was waived; however, all patient information and materials were handled according to the principles outlined in the Declaration of Helsinki.

**Sample Processing**

All hematology specimens underwent automated or manual nucleated cell counts in the hematology laboratory in accordance with laboratory protocol. Red blood cell counts were performed on cerebrospinal fluids. An air-dried Wright-Giemsa–stained cytospin was made from all samples regardless of the total nucleated cell count and was used for manual nucleated cell differential counting. If abnormal cells were present, the slides were flagged as containing unclassified cells and referred to a hematopathologist for review and interpretation. Cytology specimens were processed in accordance with laboratory protocol. For all sample types, liquid-based (ThinPrep, Hologic, Inc, or SurePath, BD Life Sciences) Papanicolaou-stained cytology slides were prepared for evaluation and diagnosis. Cerebrospinal fluid samples additionally had paired cytopspins prepared, one fixed in ethanol after centrifugation for Papanicolaou stain and the other air-dried for Giemsa stain.

**Statistical Analysis**

Categorical variables were assessed by using the Fisher exact test. *P* values of <0.05 were considered statistically significant. Statistical analyses were performed with R version 3.4.1.

**RESULTS**

**Pretraining Cohort**

During the 8-week pretraining period, a total of 1390 body fluid specimens were submitted to the hematology laboratory and 610 were submitted to the cytology laboratory. Of those, 465 specimens (33.5% [465 of 1390] of hematology samples and 76.2% [465 of 610] of cytology samples) had concurrent cytology and hematology evaluation (Table 1). These included 137 (29.5%) pleural, 124 (26.7%) bronchoalveolar lavage, 97 (20.9%) cerebrospinal, 87 (18.7%) peritoneal, 18 (3.9%) pericardial, and 2 (0.4%) miscellaneous fluids. In this cohort, 48 of 465 cases (10.3%) were diagnosed as malignant by cytology. Of those, 33 (68.8%) were detected by hematologist and flagged for hematopathologist review. Of the 15 discrepant cases, all diagnostic of carcinoma, 13 showed definite features of malignancy on the hematology slides, while 2 did not contain tumor cells (Table 2). The major factors related to the hematology cytospin that contributed to the discrepancies in malignancy detection included scant tumor cellularity in conjunction with incomplete scan of the cytospin area by the technologist and performing the nucleated cell differential on smears that were too thick, which affected cellular morphology and staining process and quality (Figures 2; A through D, and 3; A through D; Table 3).

There were 8 hematologic malignancies detected during the pretraining period, 2 of which were chronic lymphocytic leukemias. Given that the neoplastic B cells were morphologically indistinguishable from small, benign lymphocytes, and flow cytometry was needed to confirm this diagnosis, these 2 cases were excluded from the morphologic concordance review. The other 6 cases (4 plasma cell myelomas, 1 diffuse large B-cell lymphoma, and 1 peripheral T-cell lymphoma, not otherwise specified) were all detected by both the cytology and hematology laboratories. In the pretraining cohort, 7 cases were signed out as “suspicious” on cytology; all were from patients with a known history of malignancy, but there was not enough material to make a definitive diagnosis. Of these 7 cases, 6 were negative and 1 showed unclassified cells on the corresponding hematology specimens. Seventeen cases were signed out as “atypical” on cytology; of these, 15 were negative and 2 showed unclassified cells on the corresponding hematology specimens. In addition, 12 hematology specimens were referred to the hematopathologist for review of possible malignant cells that were classified as negative on cytology in this study. One of these cases was signed out as “atypical” by cytology, but confirmed to
represent plasma cell myeloma by flow cytometry, and was therefore considered a false-negative case from the cytology perspective. Another was signed out as ‘‘suspicious’’ by cytology in a patient with known metastatic colonic adenocarcinoma, but the cytology preparation lacked sufficient material to make a definitive diagnosis. The remaining 10 cases were signed out as ‘‘negative’’ by cytology and therefore considered as false-positive from the hematology perspective (10 of 465, 2.2%). An additional 8 cases were flagged for pathologist review that lacked concurrent cytology evaluation during the pretraining period; of these cases, 3 were diagnosed as malignant upon hematopathologist review, while the remaining 5 were negative. This yielded a total of 15 false-positive cases from the hematology perspective (15 of 1390, 1.1%): most (14 of 15, 93.3%) were serous effusion specimens containing reactive mesothelial cells and/or vacuolated macrophages; the remaining case (1 of 15, 6.7%) was a cerebrospinal fluid containing ependymal lining cells. None had features concerning for malignancy, and further follow-up of these cases did not reveal malignancy on subsequent specimens.

Posttraining Cohort

During the 4-week posttraining period, a total of 748 body fluid specimens were submitted to the hematology laboratory and 304 were submitted to the cytology laboratory. Of those, 249 specimens (33.3% [249 of 748] of hematology samples and 81.9% [249 of 304] of cytology samples) had concurrent cytology and hematology evaluation (Table 1). Sixty-nine (27.7%) were pleural, 85 (34.1%) bronchoalveolar lavage, 70 (28.1%) cerebrospinal, 16 (6.4%) peritoneal, 4 (1.6%) pericardial, and 5 (2.0%) miscellaneous fluids. The distribution of specimen types was significantly different between the pretraining and posttraining cohorts (P < .001), with a greater proportion of bronchoalveolar lavages and cerebrospinal fluids and a smaller proportion of peritoneal fluids in the posttraining cohort. Among this cohort, 30 of 249 cases (12.0%) were diagnosed as malignant by cytology, of which 27 (90.0%) were flagged by the hematology

| Case | Specimen Type   | Cytology Result       | Hematology Result | Hematology Slide Quality Review and Assessment |
|------|----------------|-----------------------|-------------------|-----------------------------------------------|
| 1    | Pericardial fluid | Positive: lung adenocarcinoma | Negative | Hemodilute smear; focal area with clusters of tumor cells |
| 2    | Ascitic fluid    | Positive: gastric adenocarcinoma | Negative | Thick/cellular and understained smears; deceptively bland, small, and discohesive tumor cells |
| 3    | Ascitic fluid    | Positive: colonic adenocarcinoma | Negative | Scant tumor cells |
| 4    | Pleural fluid    | Positive: breast (ductal carcinoma) | Negative | Scant tumor cells; understained smears |
| 5    | Pleural fluid    | Positive: colonic adenocarcinoma | Negative | No tumor cells |
| 6    | Cerebrospinal fluid | Positive: lung adenocarcinoma | Negative | Scant tumor cells |
| 7    | Pleural fluid    | Positive: colonic adenocarcinoma | Negative | Scant tumor cells |
| 8    | Pleural fluid    | Positive: breast (dultal carcinoma) | Negative | Understained smear; thick tumor clusters |
| 9    | Pleural fluid    | Positive: lung adenocarcinoma | Negative | Numerous tumor cells; technologist did not review specimen |
| 10   | Ascitic fluid    | Positive: cholangiocarcinoma | Negative | No tumor cells |
| 11   | Pleural fluid    | Positive: lung adenocarcinoma | Negative | Scant tumor cells |
| 12   | Ascitic fluid    | Positive: breast (lobular carcinoma) | Negative | Deceptively bland, small, and discohesive tumor cells |
| 13   | Pleural fluid    | Positive: gastric adenocarcinoma | Negative | Thick and understained smears |
| 14   | Ascitic fluid    | Positive: breast (lobular carcinoma) | Negative | Scant tumor cells |
| 15   | Pericardial fluid | Positive: squamous cell carcinoma | Negative | Scant tumor cells |
laboratory for hematopathologist review. Compared to the pretraining cohort, a significantly greater proportion of cases was appropriately flagged by the hematology laboratory as containing malignant cells in the posttraining cohort (33 of 465 [7.1%] versus 27 of 249 [10.8%], \( P = .048 \)). Of the 3 discrepant cases—all examples of carcinoma—tumor cells were not seen on review of the hematology fluid slide in 1 case; the other 2 cases showed definite features of malignancy on the hematology slide, but tumor cells were focal or scant (Table 4). The improvement in rate of malignancy detection by the hematology laboratory before (33 of 48, 68.8%) versus after (27 of 30, 90.0%) training was statistically significant (\( P = .01 \)).

There were 4 hematologic malignancies detected during this period, one of which was chronic lymphocytic leukemia and excluded from the morphologic concordance review for the reason stated previously. The remaining 3 cases were all anaplastic large cell lymphomas involving cerebrospinal fluid and were all detected by both the cytology and hematology laboratories.

In the posttraining cohort, 1 case was signed out as “suspicious” on cytology and the concurrent hematology specimen was negative; the patient did not have a history of malignancy and died during the hospital course from medical complications before any further workup could be performed. No cases were signed out as “atypical” on cytology during this period. Ten hematology specimens were referred to the hematopathologist for review of possible malignant cells that were signed out as “negative” by cytology (ie, false-positive from the hematology perspective, 10 of 249 [4.0%]). An additional 15 cases were flagged for pathologist review that lacked concurrent cytology evaluation; of these, 4 were diagnosed as malignant upon hematopathologist review, while the remaining 11 were negative. This yielded a total of 21 false-positive cases from the hematology perspective (21 of 748, 2.8%); most (13 of 21, 61.9%) were serous effusion specimens found to contain reactive mesothelial cells and/or vacuolated macrophages; 7 (7 of 21, 33.3%) were bronchioloalveolar lavages with bronchial lining cells and reactive histiocytes or other inflammatory cells, and the remaining case (1 of 21, 4.8%) was a synovial fluid with reactive lymphocytes. Follow-up of these cases did not reveal malignancy on subsequent specimens.

Figure 2. Ascitic fluids interpreted as negative by the hematology laboratory and positive by cytopathologist review. A and C, Hematology slides showing thick and understained smears. B and D, Corresponding cytology slides to (A) and (C), respectively. Note the uniform, bland appearance and the discohesiveness of the malignant cells (Wright-Giemsa, original magnification ×20 [A and C]; Papanicolaou, original magnification ×40 [B and D]).
DISCUSSION

In the hematology laboratory, the main objective of body fluid analysis is to provide a nucleated cell count and differential. In addition, determination of a red blood cell count is important for certain samples, namely cerebrospinal fluids. The cytology literature reports that almost 20% of examined serous cavity effusions are related to the presence of malignant disease, and that cytology has sensitivities ranging from 58% to 71% and very high specificity for the diagnosis of malignancy in serous fluids. Given that malignancy may initially present in a body fluid specimen, recognition of cells that may be suggestive of malignancy is an important part of the hematology technologist’s task. If abnormal cells are found, the sample should be reviewed by a pathologist to triage the specimen appropriately. Subsequently, the clinical provider should be informed to ensure appropriate patient management.

Our study demonstrates the comparatively lower rate of malignancy detection for body fluid specimens processed in our hematology versus cytology laboratories and highlights certain opportunities for improvement. Of note, all the discrepant cases were carcinomas. On subsequent review,
many of the discrepant cases in our pretraining cohort revealed suboptimal slide preparation and staining, which rendered evaluation for atypical cells challenging. Slide quality is important in assessing for malignancy, especially in carcinoma cases that are air-dried and stained with Wright-Giemsa or similar Romanovsky-type methods.\(^8\) Low tumor cellularity in the hematology specimens, a known pitfall in the evaluation of body fluids on conventional cytology preparations, was also a contributing factor in a subset of specimens.\(^9\) Despite their utility for nucleated cell differential counts, cytocentrifuged cytology cytospins are similar to conventional cytology smears as both lack advantages offered by liquid-based cytology, including better methods for cell concentration, optimal cellular preservation and distribution, consistency of staining, minimization of obscuring artifacts, and enhanced visibility of abnormal nuclear features.\(^10,11\) It is important for hematology technologists to be aware of the inherent limitations of cytocentrifuged preparations in order to carefully scan the entirety of the cytospin area, to appropriately assess slide and stain quality and overall cellular preservation, and to maintain a low threshold for making additional well-stained smears for proper morphologic evaluation when performing nucleated cell differential counts.

Additionally, our study showed that quality improvement measures in the form of educational sessions and/or periodic consensus review of morphology for the hematology technologists could significantly improve the rate of malignancy detection in our hematology laboratory. Education and training are integral parts of laboratory quality improvement.\(^12\) The College of American Pathologists requires laboratories to have a mechanism in place to compare data and interpretation if a fluid specimen is submitted concurrently to different parts of the laboratory for morphologic review.\(^7\) Our study not only illustrates the value of correlating the hematology review with the cytology diagnosis, but also highlights the potential role of hematology cytospin review in informing cytologic evaluation.\(^13\) Although rare, there were 2 instances in the pretraining component of our study where the cytology diagnosis was negative or lacked definite features of malignancy, but abnormal cells were seen on the hematology cytospin slide, including a case of plasma cell myeloma. Similar findings were seen in prior studies comparing malignancy detection rates by the 2 laboratories for all fluids and for cerebrospinal fluids.\(^14,15\) While cytology evaluation is generally considered the ideal detection method for carcinoma, the hematology preparation may be more optimal for the identification of hematologic malignancies. Currently, in our laboratory, hematology cytospins flagged for pathologic review are sent to our hematopathology service for evaluation with cytology consultation sought on an ad hoc basis. Although infrequent, cases of malignancy that are correctly identified by the hematology laboratory but missed on cytology underscore the potential value of a broader consensus approach. This could be achieved by developing a workflow that ensures routine incorporation of relevant information from the hematology smear into the cytologic diagnosis.

Fluid microscopy proficiency surveys include identification of malignant cells,\(^6\) making it imperative for hematology technologists to be proficient in detecting these cells. We continue to monitor correlation data between hematology and cytology evaluations of fluid specimens in the period following this study. The monthly rates of malignancy detection by the hematology laboratory (using the cytology diagnosis as the gold standard) have ranged from 70% to 85%, which represents a decline from the immediate posttraining malignancy detection rate of 90% found in our study. This illustrates the need for regularly scheduled, ongoing educational review sessions that directly involve hematology laboratory technologists. Currently, at our institution, case-based training sessions are being held for the hematology technologists on a quarterly basis, with the goal of maintaining high rates of malignancy detection.

One potential effect of increased sensitivity of malignancy detection by the hematology laboratory may be increased referral of “atypical” or abnormal-looking cells by the hematology technologists for cases containing reactive cells with no definite features of malignancy, thus potentially increasing the workload for pathologist slide review. In our study, referrals of false-positive cases for hematopathologist review increased between the pretraining and posttraining periods when considering the false-positive rate as a proportion of all fluids evaluated by the hematology laboratory (15 of 1390 [1.1%] versus 21 of 748 [2.8%], \(P = .004\)). Although statistically significant, this increase in the false-positive rate is relatively modest in practical terms, representing an increase from 2 cases per week to 5 cases per week submitted for pathologist review. Given the overall improvement of the malignancy detection rate and associated clinical benefits, this increase represents an acceptable and potentially desirable outcome for test performance. In addition, this issue can be mitigated by incorporating review of such cases into ongoing educational sessions for hematology technologists, focusing on cell populations that are common sources of concern such as vacuolated macrophages or reactive mesothelial, epithelial, or inflammatory cells, with emphasis on appropriate cytologic criteria for distinguishing reactive from malignant cell populations.\(^9\)

Improving the rate of malignancy detection by the hematology laboratory is especially important for institutions that process a large volume of body fluids, most of which may not have concurrent cytology specimens. Though clinicians make the initial decision to send a specimen to cytology if malignancy is suspected, there may be rare instances where an unknown malignancy may be detected incidentally in a body fluid specimen. During the pretraining and posttraining periods at our institution, two-thirds of fluid specimens processed by the hematology laboratory did not have concurrent cytology specimens. Although we do not have follow-up data for most of these

| Case | Specimen Type | Cytology Result | Hematology Result | Hematology Slide Quality Review and Assessment |
|------|---------------|-----------------|-------------------|-----------------------------------------------|
| 1    | Pericardial fluid | Positive: lung adenocarcinoma | Negative | Hemodilute smear; focal area with clusters of tumor cells |
| 2    | Ascitic fluid | Positive: gastric adenocarcinoma | Negative | No tumor cells |
| 3    | Bronchoalveolar lavage | Positive: colonic adenocarcinoma | Negative | Scant tumor cells |

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cases, improving the ability of hematology technologists to detect malignancy in fluid specimens may help identify cases that would benefit from further cytologic evaluation. Such quality improvement measures could empower the hematology laboratory to provide accurate and valuable information to clinicians, thus facilitating prompt and appropriate patient management.

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