Reliable and Sensitive Analysis of Occult Bone Marrow Metastases Using Automated Cellular Imaging


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ABSTRACT

The presence of occult bone marrow metastases (OM) has been reported to represent an important prognostic indicator for patients with operable breast cancer and other malignancies. Assaying for OM most commonly involves labor-intensive manual microscopic analysis. The present report examines the performance of a recently developed automated cellular image analysis system, Automated Cellular Imaging System (ACIS) (ChromaVision Medical Systems), for identifying and enumerating OM in human breast cancer specimens.

OM analysis was performed after immunocytochemical staining. Specimens used in this study consisted of normal bone marrow \( n = 10 \), bone marrow spiked with carcinoma cells \( n = 20 \), and bone marrow obtained from breast cancer patients \( n = 39 \).

The reproducibility of ACIS-assisted analysis for tumor cell detection was examined by having a pathologist evaluate montage images generated from multiple ACIS runs of 5 specimens. Independent ACIS-assisted analysis resulted in the detection of an identical number of tumor cells for each specimen in all instrument runs. Further studies were performed to analyze OM from 39 breast cancer patients, with 2 pathologists performing parallel analysis using either manual microscopy or ACIS-assisted analysis. In 7 of the 39 cases (44%), specimens were classified by the pathologist as positive for tumor cells after ACIS-assisted analysis, whereas the same pathologist failed to identify tumor cells on the same slides following analysis by manual microscopy.
These studies indicate that the ACIS-assisted analysis provides excellent sensitivity and reproducibility for OM detection, relative to manual microscopy. Such performance may enable an improved approach for disease staging and stratifying patients for therapeutic intervention.

**INTRODUCTION**

A substantial body of literature exists evaluating the biologic significance of OM from patients with operable breast cancer and a spectrum of other solid tumor types. Many studies have concluded that the presence of OM provides important prognostic information predictive of disease-free and overall survival in both locally recurring and advanced breast cancer.1–5 Similar conclusions have been made following the analysis of other human solid tumors including non–small cell lung carcinoma, colorectal carcinoma, and esophageal carcinoma.6–8

In contrast, other reports have found no statistically significant relationship between OM and prognosis.9,10 The issue of varying conclusions regarding the prognostic significance of OM was recently investigated by Funke and Schraut.11 These authors performed a meta-analysis of 20 published reports, including the analysis of approximately 2500 patients. Although significant, the impact of this study was diminished by the fact that the authors compared a range of carcinoma types and included studies with highly variable staining and analysis methods, along with substantial variation in the duration of clinical follow-up. Despite these caveats, there is no doubt that a key conclusion of the authors is correct: There remains the need for improved standardization of OM assay methods before the prognostic significance of OM can be substantiated.

Surprisingly, large (greater than 10-fold) differences in the number of bone marrow cells analyzed for OM classification are evident in previously published reports. Most studies evaluated between 10^5 and 10^6 normal bone marrow cells, but in some cases the number of cells analyzed was not specified. Such variation clearly affects the sensitivity of the OM assay, and could affect the prognostic significance of the results obtained.12 The importance of standardizing the number of cells assayed is further underscored from studies of Cote et al.1 These studies suggest that the prognostic predictability of the OM assay is affected by the number of tumor cells in the specimen, as opposed to simply whether a specimen is positive or negative for the presence of tumor cells.

The analytical requirement for identifying and enumerating very rare tumor cells in the OM assay is highly laborious, with the accuracy and sensitivity of the result potentially affected by the fatigue of the reviewer. Variation in results between laboratories is further complicated by the use of varying criteria for the classification of cells as tumor or nontumor.13,14 By providing objective computer-based analysis that can reduce the subjectivity inherent in manual microscopic
interpretation, along with images of cells that are classified, automated cellular imaging holds considerable potential for improving both the sensitivity and the interlaboratory consistency of the OM assay.

Previous reports\textsuperscript{15–17} have provided initial proof-of-concept regarding the use of image analysis for rare tumor cell detection. Mansi et al.\textsuperscript{15} analyzed bone marrow specimens that were spiked with human carcinoma cells. In that study, image analysis results agreed with those of manual microscopy in only 11 of 20 cases, and image analysis was reported to be considerably slower than analysis by manual microscopy. Mesker et al.\textsuperscript{16} used a cell model system in which carcinoma cells were spiked into peripheral blood. The results of that study suggested that the image analysis–based detection of rare SKBR3 tumor cells could provide good correlation with results from manual microscopy when both specimen preparation and immunocytochemical staining were optimized. Kraeft et al.\textsuperscript{17} recently described a fluorescence-based imaging system capable of identifying rare carcinoma cells in blood, bone marrow, and stem cell–enriched products. This system was reported to perform sensitive analysis of rare tumor cells from these specimens, although the consistency of tumor cell detection using the instrument was not addressed.

In the present study, the performance of a recently developed automated cellular imaging system (ACIS; ChromaVision Medical Systems) as a tool for the evaluation of OM is explored. Results are described following the analysis of normal human bone marrow, bone marrow specimens spiked with breast carcinoma cells, and bone marrow specimens from 39 breast cancer patients. OM assay sensitivity by ACIS-assisted analysis is compared with parallel analysis of the same specimens using manual microscopy. The performance of the imaging system in terms of OM assay reproducibility is also examined.

**METHODS**

**Bone Marrow Specimens**

Bone marrow specimens from breast cancer patients in this study were provided by Dr. Ingo Diel. They were obtained from patients with primary operable breast cancer, stage T1–4, N0–2, and M0 (International Union Against Cancer criteria).\textsuperscript{18} Bone marrow puncture and aspiration were performed as detailed in Diel et al.\textsuperscript{3} Bone marrow aspirate (10–12 mL) was collected from 2 puncture sites on each anterior iliac crest (total, 40–50 mL) and stored in heparinized tubes with Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Paisley, UK). Components of the aspirate were separated by density centrifugation through Ficoll-Hypaque (density = 1.077 g/mL; Biochrom, Berlin, Germany). After separation, cells were washed twice and resuspended with DMEM. Subsequently,
the cell suspension was transferred onto microscope slides using a cytocentrifuge (Universal 16A; Hettich, Tuttingen, Germany). The slides were suspended in a conventional freezing medium and stored in a freezer at –80°C before immunocytochemical staining and analysis.

For normal human bone marrow or bone marrow spiked with carcinoma cells, the following procedure was employed. Mononuclear cells were isolated from human bone marrow aspirates using standard protocols (Poietics, BioWhittaker, Gaithersburg, MD). Briefly, a nucleated cell count was performed on bone marrow aspirates using a Coulter counter with Zap-globin-II for red cell lysis, according to manufacturer’s directions. The bone marrow was diluted to 5 million nucleated cells/mL with 5 mM EDTA (Sigma), pH 7.4. Fifteen milliliters of Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, NJ) was overlaid with 35 mL of the diluted bone marrow in a 50-mL conical tube and centrifuged at 400g for 30 minutes at room temperature. The mononuclear cell layer was harvested from each tube, combined, diluted 1:4 with Hanks’ balanced salt solution and EDTA, and centrifuged at 300g for 15 minutes at room temperature. The cells were resuspended in phosphate-buffered saline (PBS) (Dako, Carpinteria, CA), and an aliquot was counted. The cells were centrifuged at 250g for 10 minutes at room temperature. The mononuclear cells were resuspended in PBS at 5 million cells/mL and maintained on ice. Two hundred microliters of the cell suspension (~1 million cells) was cytocentrifuged (Hettich Universal 16A) onto silanized slides (Dako) at 500 rpm for 5 minutes at room temperature. The supernatant was carefully removed from each slide after cytocentrifugation, and the slides were allowed to air-dry overnight. For spiked slides, MDA-MB-468 breast carcinoma cells (ATCC, Manassas, VA) were harvested, resuspended in PBS, and counted. The appropriate number of cells was added to the PBS-resuspended bone marrow mononuclear cells to give a final count of 1.5 or 40 cells in 200 µL. Slides were prepared by cytocentrifugation as described above.

**Immunocytochemical Staining**

Details of the procedure used for the staining of bone marrow specimens from breast carcinoma patients are presented in Diel et al. Briefly, the monoclonal antibody 2E11 (BM2), which recognizes a carcinoma-associated epithelial mucin MUC-1, was used.

Before staining, the cells were fixed with 3.7% formalin in PBS for 15 minutes, rinsed 3 times with PBS, and postfixed with absolute methanol (–20°C) for 5 minutes. Endogenous alkaline phosphatase was next blocked with 20% acetic acid, 2.28% periodic acid, and 2% levamisole. After blocking, the slides were incubated with biotinylated BM2 antibody (2 µg/mL) in PBS that contained 1% bovine serum albumin (Boehringer Mannheim, Mannheim, Germany) for 1 hour at
room temperature. Immune complexes were made visible by use of the avidin-
biotin-alkaline phosphatase complexes (ABC test; Vectastain, Camon, Wiesbaden,
Germany) and fast red as substrate. The cells were counterstained with 10% Gill 2
hematoxylin (Dako) in distilled water for 30 seconds. The specimen was next
rinsed under running water for 2 minutes then coverslipped using Aquatex
mounting medium (Merck, Darmstadt, Germany).

Normal marrow or bone marrow specimens spiked with human breast
carcinoma cells were immunocytochemically stained using the EPiMET Epithelial
Cell Detection Kit (Baxter Europe, Micromet, Martinsried, Germany) with minor
modifications to the manufacturer’s recommendations. Briefly, slides were fixed in
0.5% neutral buffered formalin (Sigma, St. Louis, MO) diluted in PBS (Dako) for
10 minutes at room temperature. Samples were gently washed with PBS and
permeabilized according to the manufacturer’s recommendations. Cytokeratins 8,
18, and 19 were stained with A45-B/B3 (conjugate of Fab-fragment of antibody
A45-B/B3 with alkaline phosphatase), washed, and detected with chromogen (New
Fuchsin) according to the manufacturer’s recommendations. The slides were
counterstained by incubating in undiluted hematoxylin stain (Dako) for 4 seconds
and rinsed with deionized water. The slides were dried at 70°C in a drying oven for
20 minutes. The dried slides were coverslipped with a cellulose film using a
Tissue-Tek SCA automated coverslipper (Sakura Fineteck) and xylene.

**ACIS SYSTEM OVERVIEW**

The ACIS consists of 2 major subassemblies. The first is the microscope with
its associated electromechanical hardware, and the second is a computer with a
frame grabber and image processing system.

The microscope subsystem includes the components of a standard microscope
(lamp, condenser, turret, etc) mounted in a special shock-resistant frame with a
video camera. The camera has a 60-frame-per-second, 640-by-480-pixel, 3-chip
camera (Sony DX9000). Also included in the microscope assembly is an infeed
hopper, a stage, motors that provide X, Y, and Z (focus) translation, and an outfeed
drawer. The system uses a carrier system, with 4 slides per carrier, and the input
hopper is capable of holding 25 carriers. The computer subsystem consists of a dual
450 MHz Pentium II computer running Microsoft Windows NT. It has 512
megabytes of memory, 27 gigabytes of hard disk storage, and 25 gigabytes of tape
backup, compressible to 50 gigabytes.

For rare event detection, the ACIS makes use of proprietary software allowing
for fast and highly sensitive color detection, along with the capability for the
analysis of a variety of morphometric features. The application software available
on the ACIS for OM detection involves first scanning a microscope slide at low
magnification ($\times 10$). The system next returns to objects that were originally
identified for a second analysis at higher magnification (×40 or ×60). In this case, more comprehensive image analysis of color and morphometric characteristics (nuclear size, nuclear shape) is undertaken in an effort to exclude cellular debris, large clumps, and cells with morphological features typical of normal hematologic mononuclear cells, as opposed to carcinoma cells. At the same time, objects that meet color- and morphometry-based criteria as likely tumor cells are collected and presented as montage images for review and classification by a pathologist or other laboratory professional. In the data file generated after specimen analysis, the x and y coordinates of the object within a framelet are stored using location data results in powerful sample navigation features. A revisit capability allows the user to double-click on framelets of interest to return to the proper location on the specimen slide for further review under manual control of the microscope. In this mode, it is possible to navigate across the slide, adjust focus, and change microscope objectives. Comparison of the montage images that result from each repeated run is another feature that uses location data. Tumor cells or cell clusters found multiple times by the system can be identified by highlighting framelets with proximate locations as suspected duplicates.

RESULTS

Figure 1 illustrates an example of a carcinoma cell identified from a bone marrow preparation obtained from a breast cancer patient. The specimen was immunocytochemically stained using the BM2 monoclonal antibody and enzymatically visualized in combination with fast red chromogen. The upper panel illustrates an example of positive immunocytochemical staining of a single tumor cell. Staining is evident on the plasma membrane and cytoplasm of the tumor cell but is absent in surrounding normal mononuclear bone marrow cells, which demonstrate only the blue hematoxylin nuclear counterstain. Using manual microscopy, OM analysis is conventionally performed following the laborious process of examining approximately $10^5$ to $10^6$ normal bone marrow mononuclear cells for the presence of 1 or more carcinoma cells.

In an effort to examine the feasibility for automating the OM assay, ACIS analysis was performed on the same bone marrow specimen. The lower panel of Figure 1 illustrates the result of ACIS analysis using an application that detects likely tumor cells based on the combination of presence of the red chromogen immunocytochemical staining and preset morphological characteristics (eg, range of nuclear size, nuclear shape). A laboratory professional next reviews the collected framelets to classify them as tumor cells or nontumor cells. In the case illustrated, 8 collected objects were reviewed and classified as tumor cells, representing the result following analysis of the entire bone marrow specimen. In addition, the ACIS counted approximately $1.26 \times 10^6$ total mononuclear cells on
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The specimen slide. ACIS analysis of this specimen required approximately 18 minutes.

To evaluate the sensitivity and specificity of the ACIS for tumor cell detection, known control populations were next evaluated. Bone marrow specimens were
obtained from normal individuals ($n = 10$), specimens into which 1–7 in vitro human breast carcinoma cells (MDA-MB-468) were spiked ($n = 10$), and specimens spiked with 43–59 breast carcinoma cells ($n = 10$). Each of these specimens also included ~500,000 normal bone marrow mononuclear cells. The results of this study are shown on Table 1. No tumor cells were identified in any of the 10 normal bone marrow specimens analyzed. In contrast, 1 or more tumor cells was identified in each of the 20 specimens that were spiked with breast carcinoma cells.

Further studies were undertaken to evaluate and compare the OM review time as performed using the ACIS analysis vs. manual microscopy using these same specimens. For ACIS-assisted analysis, 2 elements contribute to the total analysis time: ACIS image scan and collect (in which the instrument performs analysis in an unattended fashion) and the ACIS-assisted review of montage images, which is performed by a laboratory professional. The overall ACIS scan and collect time was an average of 17.75 minutes (2.69 standard deviation [SD]), with a range of 14 to 25 minutes for the 30 specimens. The automated portion of the ACIS analysis for all specimens, therefore, was accomplished in a total of 8.9 hours. The total pathologist-assisted ACIS review of all montage images from the 30 specimens was accomplished in slightly less than 1 hour. This leads to a total ACIS analysis time (unattended scan and collect along with pathologist montage review) of 9.9

Table 1. Specificity for Tumor Cell Detection Using ACIS-Assisted Analysis (Normal Bone Marrow vs. Bone Marrow Spiked With MDA-MB-468 Breast Carcinoma Cells)*

<p>| Tumor Cell– Tumor Cell– | Tumor Cell– | Total Cases |</p>
<table>
<thead>
<tr>
<th>Positive Cases</th>
<th>Negative Cases</th>
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<tbody>
<tr>
<td>Bone marrow spiked with 4 + 3 breast carcinoma cells ($n = 10$)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow spiked with 51 + 8 breast carcinoma cells ($n = 10$)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Normal bone marrow ($n = 10$)</td>
<td>0</td>
<td>10</td>
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<tr>
<td>Total</td>
<td>20</td>
<td>10</td>
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</tbody>
</table>

*Mononuclear cell preparations were from normal bone marrow specimens and bone marrow to which varying numbers (range, 1–59) of MDA-MB-468 breast carcinoma cells were added and immunocytochemically stained using an alkaline phosphatase–conjugated anti–pan-cytokeratin antibody (A45/BB3). Positive cells were visualized following incubation with the new fuchsins chromogen. The slides, each consisting of ~500,000 cells, were then analyzed using the ACIS. Specimens with 1 or more carcinoma cells were classified as tumor cell–positive.
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Table 2. Reproducibility Within and Between ACIS Instruments for Tumor Enumeration (Bone Marrow Specimens From Breast Cancer Patients)*

<table>
<thead>
<tr>
<th>Instrument and Run Number</th>
<th>Specimen A, No. Positive Tumor Cells</th>
<th>Specimen B, No. Positive Tumor Cells</th>
</tr>
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<tbody>
<tr>
<td>Instrument 1</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<tr>
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<td>18</td>
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<tr>
<td>5</td>
<td>7</td>
<td>18</td>
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</table>

*Bone marrow mononuclear cell preparations from 2 breast cancer patients were fixed and immunoenzymatically stained using the BM2 monoclonal antibody and fast red chromogen. To examine the consistency of tumor cell detection and enumeration, each specimen was evaluated following analysis in 5 separate runs on each of 3 different ACIS instruments, for a total of 15 independent analyses per specimen.

hours. By contrast, manual microscopic evaluation of the 30 specimens required 11.9 hours, or an average of 23.8 minutes for each of the 30 specimens. Thus, using ACIS-assisted analysis, a reduction of approximately 11.9-fold in pathologist review time was accomplished, relative to analysis using manual microscopy.

Bone marrow specimens obtained from 5 breast cancer patients were subsequently analyzed in an effort to assess the reproducibility of ACIS-assisted analysis results. Table 2 illustrates the result of this analysis for 2 specimens in which 15 separate analyses were performed for each specimen. Each of 3 separate instruments was used to analyze each specimen, with 5 separate analyses per instrument. The data indicate that the ACIS identifies tumor cells in a highly reproducible fashion. Exactly the same number of tumor cells (as classified by the pathologist following the review of montage images) was identified for each specimen.
Three additional breast cancer bone marrow specimens were next analyzed on 3 separate ACIS instruments, with 3 independent runs per instrument. This analysis (data not shown) again revealed that exactly the same number of tumor cells was identified for each specimen in each run, with 23, 7, and 50 cells, respectively, consistently identified from each of 3 specimens.

The “montage compare” feature within the ACIS instrument displays multiple montage images of collected cells from separate instrument runs for review and comparison. Using this analysis tool, the reviewer determines whether identical cells are detected in different instrument runs. Reviewing the individual framelet images of all of the collected tumor cells from these 3 specimens revealed that, in all cases, not only the same number of tumor cells but also exactly the same tumor cells, were collected.

Additional studies were next performed using bone marrow specimens obtained from 39 breast cancer patients to further examine the performance of ACIS-assisted OM analysis vs. OM analysis by manual microscopy. For these studies, the specimens were evaluated by either of 2 pathologists, with independent evaluation performed using manual microscopy and ACIS-assisted analysis. The results of this study are shown in Table 3. In 7 of the 39 specimens (44%), the pathologist detected 1 or more tumor cells after ACIS-assisted analysis that were not detected by the same pathologist using manual microscopy.

Based on the fact that the ACIS data file includes x and y coordinates of each object that was classified in the original ACIS-assisted analysis, it was possible for the pathologist to recall all tumor cells for further studies. Each of the tumor cells

Table 3. Tumor Cell Detection: ACIS-Assisted Analysis vs. Manual Microscopy
(Specimens From Breast Cancer Patients, n = 39)*

<table>
<thead>
<tr>
<th>ACIS-Assisted Analysis</th>
<th>Manual Microscopy</th>
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<tr>
<td></td>
<td>Tumor Cell–Positive Cases</td>
<td>Tumor Cell–Negative Cases</td>
</tr>
<tr>
<td>Tumor cell–positive cases</td>
<td>9</td>
<td>17</td>
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<tr>
<td>Tumor cell–negative cases</td>
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<td>10</td>
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<tr>
<td>Total</td>
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<td>27</td>
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</table>

*Bone marrow specimens from 39 breast cancer patients were evaluated using both manual microscopy and ACIS-assisted analysis by 2 pathologists following immunoenzymatic staining with the BM2 monoclonal antibody and fast red chromogen. Manual microscopy and ACIS-assisted analysis agreed on 9 positive and 10 negative cases. In 20 cases, manual microscopy and ACIS-assisted analysis disagreed. In 17 of these cases, the specimen was classified as positive for tumor cells by ACIS-assisted analysis but negative for tumor cells by manual microscopy. (These discrepant cases were further investigated as described in Table 4.)
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Table 4. Tumor Cell Detection Reproducibility: ACIS-Assisted Analysis vs. Manual Microscopy (Specimens From Breast Cancer Patients, n = 21)*

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<td></td>
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*The number of tumor cells counted from a total of 21 bone marrow specimens from breast cancer patients, which had initially been analyzed by both ACIS-assisted analysis and manual microscopy, were reevaluated. For 17 specimens that had originally been classified as tumor cell–negative by manual microscopy, a total of 37 objects were originally classified as tumor cells by ACIS-assisted analysis. These cells, along with 74 nontumor cells from the same 17 slides and 5 cells from each of 4 additional specimens (for a total of 131 cells), were recalled and reexamined by a pathologist who was blinded to the original ACIS-assisted cellular classification. Finally, the pathologist reviewed ACIS montage images a second time, again blinded to the original classification.

from the 17 disparate specimens (described above) was manually reevaluated using the binoculars available on the ACIS. To ensure objectivity, additional nontumor cells were also examined with all determinations blinded to the original cellular classification. The results of these studies are shown in Table 4. For all but 1 cell
(specimen 10), manual microscopic review led to findings that agreed with those obtained following the original ACIS-assisted analysis. For the 1 cell where a disparity was noted, the pathologist again reviewed both the original ACIS montage image and the specimen slide. The pathologist’s conclusion, consistent with the original ACIS-assisted analysis, was that the disparate cell was not a tumor cell.

Finally, to verify the consistency of the ACIS-assisted analysis, the pathologist independently reviewed all ACIS montage images a second time, blinded to the original classification. The second ACIS-assisted analysis led to results that were identical to the original ACIS-assisted analysis (Table 4). From these findings, we conclude that the original discrepancy between ACIS-assisted analysis and manual microscopy reflected tumor cells that were not detected (ie, false negatives) by manual microscopy.

In 3 specimens, the original manual microscopic analysis (Table 3) led to the conclusion that tumor cells were present, whereas ACIS-assisted automated analysis revealed no detectable tumor cells. At a later date, the pathologist reanalyzed the specimen, blinded to the original interpretation. In an effort to optimize the reliability of the second read, the pathologist further reviewed the specimen slide for possible tumor cells at both conventional (×200) and higher resolution (×400) magnification. Following reanalysis, the pathologist concluded that none of the specimens included even 1 tumor cell—consistent with the original ACIS analysis but contrasting with the original analysis by manual microscopy. These findings indicate that the original manual microscopic analysis resulted in false-positive findings that were not observed by ACIS-assisted analysis.

**DISCUSSION**

This study demonstrates that analysis of OM using immunocytochemical staining and analysis by the ACIS provides a sensitive, highly reproducible, and efficient means for OM detection and enumeration. Superior sensitivity for OM detection was demonstrated using ACIS-assisted analysis relative to manual microscopy. Specifically, in bone marrow specimens from 17 of 39 breast cancer patients in which the pathologist identified the presence of OM by ACIS-assisted analysis, the same pathologist incorrectly classified the specimen as negative after initial analysis by manual microscopy (Table 4). Analysis of the same specimen from breast cancer patients on multiple occasions, across multiple ACIS instruments, revealed excellent assay consistency (Table 2). Finally, an approximately 11.9-fold reduction in pathologist review time was achieved for analysis of 30 bone marrow specimens for OM using ACIS-assisted analysis relative to manual microscopy.

In addition to analysis by manual microscopy, 2 other methods are commonly employed for the analysis of OM today: flow cytometry and nucleic acid–based methods including reverse transcriptase–polymerase chain reaction (RT-PCR).
Flow cytometry represents an alternative technology that allows for the rapid analysis of tens of thousands of cells. The detection sensitivity of this methodology varies in published reports, but most commonly is reported to be in the range of approximately 1 cell in 10,000. Other reports suggest a theoretical detection sensitivity in the range of approximately 1 cell in 200,000; even rarer cell types may be detectable. The results of Gross et al., however, suggest that flow cytometric analysis of rare tumor cells (<1 cell in 100,000) leads to a detection sensitivity of only 10–40%. One key advantage for analysis by automated cellular imaging as opposed to flow cytometry is the fact that the analysis result is a cellular image. The use of a conventional counterstain (eg, hematoxylin) facilitates interpretation by a pathologist or other laboratory professional, leading to enhanced diagnostic certainty of the assay. Only with the use of tedious cell sorting methods (available on flow cytometry research instruments) can this type of interpretation be made using flow cytometry.

DNA-based nucleic acid methods for detecting OM analyze specific gene mutations, sequences of carcinogenic viruses, or other alterations specific to neoplastic cells. This method holds considerable potential, but its true clinical relevance awaits confirmation in larger prospective studies. An alternative strategy for detecting OM in bone marrow specimens involves the use of RT-PCR to amplify specific mRNA molecules that are expressed in carcinoma cells but absent in normal cells. Although this approach holds considerable promise, it suffers from the fact that, for most cancer types, true tumor-specific mRNA molecules have not yet been identified. Inherent biological factors including the presence of pseudogenes or low-level expression of the targeted mRNA in normal cells can lead to false-positive test results using this method. For example, “illegitimate expression” of cytokeratin 19 mRNA has been reported in peripheral blood. In another study, 7 epithelial- and tumor-associated markers, including cytokeratin 18 mRNA, were amplified by RT-PCR in a significant percentage of bone marrow samples from noncancer patients, illustrating the risk of false-positive results by this method. On the other hand, downregulation of a more specific carcinoma cell marker, such as prostate-specific antigen (PSA), in tumor cells may limit the sensitivity of RT-PCR in bone marrow from breast cancer patients, possibly leading to false-negative interpretations.

One assay component of critical importance for the immunocytochemical detection of OM is the antibody reagent used for immunocytochemical staining. To date, a variety of antibodies have been used for the OM assay, which vary substantially in terms of sensitivity and specificity for carcinoma cell detection. Antibodies used for OM detection have been reported to bind both specifically (eg, Fc-receptor–bearing leukocytes, illegitimate expression of epithelial antigens in normal hematopoietic cells) and nonspecifically in cells including macrophages, plasma cells, and nucleated erythroid precursors. The use of antibodies with
well-documented specificity for carcinoma cells, as opposed to normal hematologic mononuclear cells (eg, A45/BB3, A45/BB5), is highly advantageous for a robust OM assay. In addition, careful morphologic assessment, along with proper interpretation of immunocytochemical staining, appears imperative to ensure that immunocytochemically stained cells are, in fact, tumor cells,34 as opposed to leukocytes and other nonneoplastic cells. By providing cellular images with counterstains conventionally used for cellular diagnostic purposes, along with the capability to revisit the cell on the microscope slide, the ACIS provides the opportunity for cellular classification with increased diagnostic certainty relative to the alternative methods discussed above.

In conclusion, these studies demonstrate that ACIS-assisted analysis combined with immunocytochemical staining offers the possibility of sensitive and reliable assessment of OM. Prospective studies now appear important to further explore the true clinical significance of this assay.

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