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Use of Automated Microscopy for the Detection of Disseminated Tumor Cells in Bone Marrow Samples

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The use of automated microscopy has reached the maturity necessary for its routine use in the clinical pathology laboratory. In the following study we compared the performance of an automated microscope system (MDS™) with manual method for the detection and analysis of disseminated tumor cells present in bone marrow preparations from breast carcinoma patients. The MDS System detected rare disseminated tumor cells among bone marrow mononuclear cells with higher sensitivity than standard manual microscopy. Automated microscopy also proved to be a method of high reproducibility and precision, the advantage of which was clearly illustrated by problems of variability in manual screening. Accumulated results from two pathologists who had screened 120 clinical slides from breast cancer patients both by manual microscopy and by use of the MDS System revealed only two (3.8%) missed by the automatic procedure, whereas as many as 20 out of 52 positive samples (38%) were missed by manual screening. Cytometry (Comm. Clin. Cytometry) 46:215–221, 2001. © 2001 Wiley-Liss, Inc.

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The new economic realities of the clinical pathology laboratory and the increasing number of new markers being discovered through the Human Genome Project have created an urgent need for test automation. Cytopathology tests in particular can be time consuming, and when performed in high volumes, are susceptible to human error. In recent years the development of automated microscopy has been refined in performance and analytical clarity. Previously difficult tasks such as morphology recognition and DNA probe analysis can now be reliably performed by automated microscopy (1–6). Among the new cytopathology assays where automation can be applied is the identification of disseminated ("isolated" or "occult") tumor cells in patients suffering from carcinomas (7–14).

During the past 10 years, the prognostic impact of the presence of immunocytochemically detectable disseminated tumor cells in the bone marrow has been reported for various cancer types (15–29). Recently Braun et al. (30), investigating bone marrow aspirates from 552 breast cancer patients for cytokeratin-positive tumor cells, demonstrated an independent prognostic impact for the risk of death from cancer. The same author finds that identification of occult metastatic cells in the bone marrow predicts poor prognosis better than either Her-2/*neu* status or angiogenesis in breast carcinoma (12).

These studies indicate that the detection of occult tumor cells will influence therapeutic strategies and can become part of routine pathology. The detection of isolated tumor cells may also become a valuable tool for monitoring the effectiveness of cancer treatment and early predicition of cancer relapse, or may contribute to the development of cancer immunotherapy.

The identification of occult tumor cells in bone marrow using immunocytochemistry requires careful evaluation (31). Because of the small numbers of tumor cells present in these preparations, the screening is a long and tedious process. Consequently, automation would be of great value given the large number of tests generated by the high incidence of carcinomas.

In the following study we performed an exhaustive comparison of manual and automated microscopy showing that the automatic screening instrument simplifies the

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analysis and provides more sensitive, reproducible, and accurate results.

MATERIALS AND METHODS

Bone Marrow Processing

The bone marrow was processed according to a method described previously (31). Briefly, heparinized bone marrow aspirates from human subjects were enriched for bone marrow mononuclear cells (BMMNC) by centrifugation over Lymphoprep density media (Nycomed, Oslo, Norway). Sample deposition of the BMMNC was done by cytospin methodology onto polylysine-coated glass slides (0.5×10^6 BMMNC/spot), air-dried overnight and immunostained, or stored at –80°C before immunostaining. For the sensitivity, specificity, and reproducibility testing of automated microscopy, several sets of cytospins of spiked samples were prepared from normal human bone marrow and breast carcinoma cells (cell line SK-BR-3, American Type Culture Collection, Rockville, MD). Normal BMMNC suspensions were spiked with approximately 5–50 carcinoma cells per 500,000 bone marrow cells. Clinical slides from a large collection of bone marrow cytospins from breast cancer patients, Stage I–III from the Norwegian Radium Hospital, were used for both the sensitivity, specificity, and reproducibility testing and the clinical testing of the automatic scanning.

For the clinical testing, 120 slides were selected from consecutive patients in such a way that one out of four slides had shown to contain at least one positive cell, and the other three had been scored negatively by previous manual screening. Five of the slides contained more than five tumor cells per slide; the remaining 115 slides contained one to five tumor cells per slide (mean 1.3 tumor cells/slide). The definition of a positive slide was the presence of tumor cell(s) detected by either of the screenings in the present study. No isotype-specific negative control slides were included.

Immunostaining of Cytospins

Cytospins were immunostained according to a method described previously (31). Briefly, the air-dried slides were fixed in acetone, then incubated with pan-anticytokeratin monoclonal antibodies AE1 and AE3 (Monosan or Signet, Sanbio, Uden, The Netherlands). This was followed by incubation with polyclonal rabbit anti-mouse antibody and then with alkaline phosphatase/mouse anti-alkaline phosphatase complex (both from Dako, Copenhagen, Denmark). Color development was achieved by an enzymatic reaction with New Fuchsin solution (Aldrich Chemicals, Milwaukee, WI) containing levamisole and then counterstained with hematoxylin to visualize nuclear morphology.

Cytospin Analysis

Manual screening. The manual screening of cytospins was performed by two pathologists (K.W.N. and E.B.) using conventional light microscopy (10× lens for screening, up to 60× lens for closer examination of individual cells). The coordinates of all cells evaluated as cytokeratin-positive tumor cells were recorded using an England Finder slide (Graticules, Pyser-SGI Ltd., UK).

Automated screening. All slides were coded using blinded experimental methods and were scanned with a MDS™ System (Applied Imaging Corp., Santa Clara, CA) by independent operators. Briefly, the MDS System consists of an epifluorescence microscope with computer-controlled stage movements, autofocus mechanism, two filter wheels for the detection of multiple chromogen/fluorochromes (one with 10 filter positions for fluorochrome detection and one with seven filter positions for brightfield transmitted light chromogen detection), a black-and-white CCD camera, computer, monitor, and proprietary scanning and analysis software. For the detection of red immunostained cells as in this material, the camera takes two pictures of each scanning field, one through a brightfield red filter and one through a brightfield green filter. Objects producing approximately the same grey value through both filters, i.e. hematoxylin-stained BMMNC nuclei, are below the detection threshold. Red cells, clearly visible through green filter but close to invisible through red filter are detected and captured, as the difference in grey value seen through green and red filter is above the set threshold. Various thresholds and finding parameters, for the measurement of stain intensity, size, and shape morphology, may be adjusted to obtain optimal sensitivity and specificity in a given sample type using different chromogens and filter sets. In this study the system's finding parameters were set to obtain a high sensitivity in order to detect all tumor cells in both clinical and spiked samples, including weakly/heterogeneously stained cells and badly conserved cells lacking nuclei.

The review process for the automatic scanning procedure, consisting of visual examination of detected objects, was performed by a pathologist (K.W.N. and/or E.B.). Initially the pathologist marked all candidate objects in the picture gallery on the screen, then looked into the instrument's microscope (Olympus BX60, Olympus Optical, Tokyo, Japan) where the selected candidates were presented successively and examined one-by-one in conventional light microscopy. Alternatively, the pathologist selected a specific cell for examination from the picture gallery by a simple mouse click over the desired image, causing the system to relocate that cell instantly to the center of the microscope field for examination. The England Finder coordinates of all objects were registered by the system. Classification of the detected elements was accomplished by selecting one of the predetermined diagnostic categories (i.e., tumor cell, haematopoietic cell, etc.) via the user interface. To facilitate the review process the instrument's sorting function (a mathematic formula using the object's various finding parameter values) was used, by which the elements most likely to be tumor cells are presented first in the gallery and debris last.

Morphological Evaluation

Slides with one or more cytokeratin-positive tumor cells present were scored as positive samples. The morpholog-

Table 1
*Analysis of Inter- and Intra-instrument Variation in the MDS™ Detection of Tumor Cells/Stained Objects, in Comparison to Manual Screening**

	Manual	Instrument 1		Instrument 2		Instrument 3	
Slide 1	Tumor cells[a]	Collected objects[b]	Tumor cells	Collected objects	Tumor cells	Collected objects	Tumor cells
Day 1	2	88	2	169	2	134	2
Day 2		93	2	162	2	136	2
Day 3		96	2	137	2	144	2
Mean		92	2	156	2	138	2
SD		4,0	0	16,8	0	5,3	0
% CV		4,4%	0%	10,8%	0%	3,8%	0%
Slide 2							
Day 1	7	91	8	151	8	147	8
Day 2		99	8	208	8	174	8
Day 3		108	8	191	8	155	8
Day 4		110	8	149	8	171	8
Day 5		116	8	176	8	176	8
Mean		104,8	8	175,0	8	164,6	8
SD		9,8	0	25,5	0	12,9	0
% CV		9,4	0%	14,6%	0%	7,8%	0%

*Manual screening of two cytospins (Slide 1 and 2) with bone marrow mononuclear cells harbouring disseminated breast carcinoma cells, compared to the results of automatic screenings of the same slides on three different instruments, performed over three days (Slide 1) and five days (Slide 2).
[a]Tumor cells = cells among collected objects identified as carcinoma cells by morphpological evaluation.
[b]Collected objects = total number of red objects collected by the instrument.

ical criteria used for scoring a cell as a tumor cell were described previously (31). The intention was to register only true tumor cells, both by manual and automatic screening, but a few cells with indeterminate appearance were evaluated differently from one procedure to the other. The final number of positive samples, together with the number of detected and missed samples by the different screenings, were finally defined (by Pathologist 1) and corrected for interpretational discrepancies on the level of individual cell morphology.

Performance of the Testing of Automated Microscopy

A series of four studies were conducted to evaluate automated microscopy vis-à-vis manual screening. These studies included: scanning the same slides on different instruments in multiple runs over several days, scanning 30 slides with varying number of spiked tumor cells on one instrument, and analysis of 120 clinical patient slides, by both pathologists, manually and by automated microscopy.

RESULTS

Study of Sensitivity, Reproducibility, and Specificity of Automated Microscopy

Three representative BM cytospins from breast carcinoma patients were screened manually by Pathologist 1, recording the coordinates of all cytokeratin-positive tumor cells and thus establishing the known reference locations. The slides were then scanned automatically on three different instruments over three days (nine runs/slide), and the scanning images were evaluated by Pathologist 2. The total number of red objects detected by the machine ("collected objects") and, among these, the number of cells identified as tumor cells by Pathologist 2, were registered. Objects collected by the automated scan were determined by the system's finding parameter settings (based on red object stain intensity, size, and shape morphology). In another experiment, two clinical BM cytospins from breast carcinoma patients and two BM cytospins with spiked tumor cells were analyzed both manually and by the automatic procedure by Pathologist 2. These four slides were scanned on three different instruments over five separate days (15 runs/slide). Table 1 shows results from two representative slide analyses. As it can be observed, the same number of tumor cells were detected in every run. Similar results were observed for the other slides (data not shown). The total number of collected objects varied slightly from run to run (CV between 3.8% and 14.6% for the slides in Table 1, and between 2.4% and 15.5 % CV for the other slides (data not shown)). In some instances, as shown in Table 1, the instruments detected additional tumor cells that had been missed by manual screening. Comparisons of these tumor cell's coordinates showed that it was the same tumor cell that was identified for all three instruments among the various runs. In one of the slides (data not shown), one tumor cell was missed by the pathologist during the review process of one of the automatic scans. All tumor cells, however, were detected by all instruments in every run.

In a third experiment a set of 30 BM cytospins were analyzed, 20 of which contained spiked tumor cells and 10 containing only normal BM cells. Ten of the spiked slides had high tumor cell load (40–67 tumor cells/slide) and 10 slides had medium/low tumor cell load (1–10 tumor cells/slide). After Pathologist 2 performed the man-

Table 2
Comparison of Automated Microscopy with Manual Screening for Detection of Tumor Cells in Experimental Samples

	Automated scan		Manual scan		Instrument vs. manual
Spike	Code	# Tumor cells	Code	# Tumor cells	
H	E3-10	67	E3-03	64	>
H	E3-14	66	E3-13	55	>
H	E3-18	65	E3-04	58	>
H	E3-25	62	E3-24	55	>
H	E3-21	60	E3-28	53	>
H	E3-08	54	E3-18	44	>
H	E3-04	52	E3-08	41	>
H	E3-22	42	E3-17	33	>
H	E3-13	41	E3-09	41	=
H	E3-12	40	E3-22	40	=
M	E3-27	15	E3-11	15	=
M	E3-29	10	E3-12	10	=
M	E3-15	10	E3-30	9	>
M	E3-11	7	E3-27	7	=
M	E3-07	6	E3-15	4	>
M	E3-23	6	E3-20	6	=
M	E3-28	5	E3-21	4	>
M	E3-02	3	E3-01	2	>
M	E3-16	3	E3-26	4	<
M	E3-20	1	E3-06	0	>
N	E3-03	0	E3-02	0	=
N	E3-19	0	E3-05	0	=
N	E3-01	0	E3-07	0	=
N	E3-24	0	E3-10	0	=
N	E3-09	0	E3-14	0	=
N	E3-06	0	E3-16	0	=
N	E3-17	0	E3-19	0	=
N	E3-30	0	E3-23	0	=
N	E3-05	0	E3-25	0	=
N	E3-26	0	E3-29	0	=
Total number of tumor cells		615		545	

Manual and automatic screening of 30 BM cytospins. Twenty of the slides contained spiked tumor cells and 10 contained normal BM cells only. Automated microscopy detected a higher number of tumor cells than manual screening in 13 cases and an equal number in six cases. One positive sample (automated code E3-20) was missed by manual screening but identified by the automatic scan. No positive samples were missed by the scan, but one of a total of four tumor cells was missed during the review phase of one of the samples (automated code E3-16); the cell had, however, been detected by the instrument.

H = samples spiked with high number of tumor cells; M = samples spiked with medium/low number of tumor cells; N = normal, unspiked samples.

ual screening the slides were recoded and scanned on one instrument. The same pathologist reviewed the automatically collected objects and the two sets of data (manual and automated) were compared and the concordance between them was determined. The data are presented in Table 2 and Figure 1. Among the 20 tumor-containing slides, automated microscopy detected a greater number of tumor cells in 13 cases, and an equal number in six cases. Overall, 615 tumor cells were detected with the instrument and 545 were detected with manual screening (Fig.1). One positive sample was missed, i.e. diagnosed as negative by manual screening but correctly identified as positive by the automatic procedure (sample E3–20, Table 2). No positive samples were missed by the automatic procedure, but in one sample (E3–16) one cell (of a total of four tumor cells) was missed during the review phase of the automatic screening procedure after the cell had been correctly detected by the instrument. Furthermore, no false positive results were obtained by either procedure in the 10 negative cases.

Testing of Automated Microscopy on Clinical Samples

To evaluate automated microscopy on routine clinical slides, 120 BM cytospins from patients with breast cancer, both with and without evidence of metastatic tumor cells, were analyzed. The slides were screened manually by the two pathologists in their own laboratories and then submitted to the automated scan procedure two times, one time for each of the two pathologists. The concordances between manual and automated screenings for each pathologist were recorded. As shown in Table 3, manual screening revealed interpathologist variation in the detection of tumor cells. The number of positive samples found manually by the two pathologists were 26 and six, respectively, out of a total of 26 positive samples. With the aid of automated screening, however, both pathologists de-

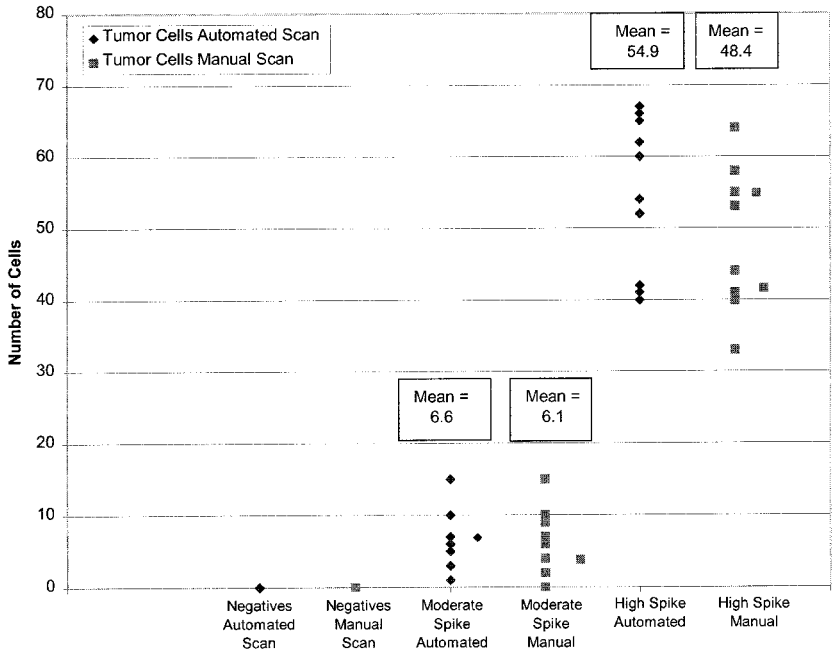

FIG. 1. An illustration of the tumor cell numbers listed in Table 2. The numbers of tumor cells detected by the automated scan are shown as diamonds; the numbers of manually detected tumor cells are shown as squares.

tected 25 positive samples. Pathologist 1 missed one positive sample by the automated procedure because the single tumor cell present was located outside the main deposition area and was therefore not included in the area that was preset for scanning. The single tumor cell present in the positive sample missed by the automated scan by Pathologist 2 was actually detected by the instrument and presented in the picture gallery but missed during the review phase of the detected objects. The accumulated results from both pathologists show that the automated procedure detected 50 of 52 (96%) possible positive samples, whereas totally only 32 (62%) were detected by manual screenings.

DISCUSSION

The presence of disseminated tumor cells in bone marrow may have important implications for clinical outcome and for choice and monitoring of therapy in the individual cancer patient (12,30,32–34). However, for most samples, the number of tumor cells in positive bone marrows is low and often only a single tumor cell is detected in 2×10^6 BMNNC analyzed (30,35). This indicates that in order to catch positive samples, careful screening of high amounts of BMMNC is necessary. The optimal methods for bone marrow processing and tumor cell detection are still under investigation, as well as the number of hematopoietic cells necessary for analysis. In our ongoing studies at The Norwegian Radium Hospital, we have investigated 2×10^6 mononuclear cells from both bone marrow and peripheral blood by the standard immunocytochemical method (31). An equivalent number of cells were submitted to an isotype-specific negative stain control. Thus 16 cytospins (0.5×10^6 mononuclear cells per spin) or more have been screened for each patient sample and the manual screening of these represented a considerable burden of work.

The results from the present study show that automated microscopy is able to detect disseminated tumor cells in BMMNC with a sensitivity that is equal to or above the sensitivity of parallel manual screening, both for spiked samples and for clinical samples from breast carcinoma patients. Furthermore, the screening reliability of the automatic procedure is stable from day to day for a single machine and is consistent from one machine to the other (Table 1).

Table 3
*Comparison of Two Pathologist's Results from the Analysis of 120 Clinical Cytospins from Breast Cancer Patients, Both by Manual Microscopy and by Automated Microscopy**

	Manual screening		Automated screening	
	Pathologist 1	Pathologist 2	Pathologist 1	Pathologist 2
Positive samples found	26	6	25	25
Positive samples missed	0	20	1	1

*Number of positive samples scored by the two different procedures by the two investigators. The total number of positive (i.e., tumor-containing) cytospins was 26.

The analysis of the 120 clinical slides revealed a marked manual screening interobserver variation (Table 3). Differences in manual screening is in accordance with the results from a previous interlaboratory study comparing screening for micrometastases among seven European laboratories (31). In contrast, our concordant results using automated microscopy show that the automatic procedure can produce reproducible and sensitive results. Furthermore, the isolated samples where tumor cells were missed by the automated method were caused by the inappropriate setting of the scan area or by not recognizing a tumor cell as such during the review phase of the objects presented by the instrument.

One cannot exclude the possibility that excessively bad technical quality of the preparations might interfere with automated microscopy cell detection. The manual method might catch more positives in such slides, but bad technical quality may also cause missed cells by manual screening. However, a measure of the robustness of the automated method is illustrated by the fact that the 120 clinical slides included in this study were selected randomly, without taking into account the quality of the preparations. Hence, the high quality of data collected with the instrument was derived from samples that represent a spectrum of quality one may expect in a clinical laboratory.

The number of automatically detected objects in each slide may vary from a few to several hundred objects. The total number of stained objects in a slide depends on the biological material to be analyzed, on the cell processing methods, and the immunocytochemical staining protocol in use. For example, some anti-cytokeratin antibodies, like AE1AE3 used in the present study, also stain normal squamous skin epithelial cells, which are present in most slides either as contamination from the laboratory staff or, possibly, from the patient skin during bone marrow aspiration. Other anti-epithelial antibodies stain these cells to a lesser extent (E.B., unpublished observation). Many stained objects are merely debris, not originating from skin cells. Disseminated tumor cells may exhibit a strong variability in their morphological appearances, even within the same patient sample, and some tumor cells are destroyed or degenerated (36).

The optional setting of the computer's finding parameters, i.e. the choice of level of sensitivity and specificity, also influence the number of detected events. In the present study, the instrument's finding parameters were set to obtain a high sensitivity in order to detect all tumor cells, including degenerated or heterogenously stained tumor cells. Table 1 shows a considerable variation in the total number of collected objects by Instrument 1 compared to Instruments 2 and 3, in addition to a day-to-day variation for each instrument. As identical finding parameters were used for all instruments, these variations were most likely a result of slightly different settings of the scan area and of calibration in the different instruments. Red debris or contaminating squamous skin cells located in the cytospin periphery, or objects with staining features near the set threshold limits for detection, could therefore be included in one scan but excluded in other scans.

During the review phase of the automatic screening procedure the observer examines the images of all detected events, consisting of stained tumor cells, normal squamous skin cells, debris, and, in some patients, stained ("false positive") hematopoietic cells. This visual examination of the picture gallery during the review phase is a crucial part of the automatic screening procedure. The MDS System uses a black-and-white video camera. The composite image presented from the red and green filter images is a color-rendered image. All objects representing potential immunostained cells may be examined using the system's integrated microscope. Only objects that are easily recognized as debris or squamous skin cells were classified solely on the screen image. To assure 100% sensitivity of the review procedure, including small damaged cells, between one-third and one-half of the automatically detected objects were checked in the microscope (by Pathologist 1). The mean number of detected stained objects per clinical slide in the present study was 162 objects (data not shown). Average time for detailed review, using these high-sensitivity standards, was 6.7 min (Pathologist 1). The time necessary to set up a scan was 2.5 min on average. Total labor time, per slide, for the whole automatic scan procedure was thus 9.2 min when the pathologist followed this 100% sensitivity review procedure. In comparison, manual screening time on a day-to-day basis is, on average, a minimum of 10 min per slide, if performed by an experienced screener (E.B., unpublished observation). Manual screening of cytospins for the detection of isolated tumor cells is strenuous, requires dedicated time, usually more than six months of training, and may also be subject to considerable intra- and interobserver variation.

Reproducible automated scanning systems, such as the MDS, offer the performance needed to modify laboratory screening practices for clinically significant rare cellular events, such as disseminated tumor cell detection, in order to reduce pathologist time requirements. As an example of the potential impact of automated microscopy on the laboratory workflow, a laboratory technologist trained to differentiate between debris and stained cells could set up the scans and do an initial assessment of the scan results, selecting only candidate cells for the final review. Subsequently, the pathologist or responsible investigator would look only at these selected candidates and make the final diagnosis. As all initial images are digitally stored, the investigator may, if needed, easily repeat the entire review of the picture gallery. The MDS System thus has high potential impact on the laboratory workflow by reducing specialized labor time to a minimum. By relieving a common bottleneck and by increasing the objectivity of the analysis, automated screening may bring immunocytochemical detection of isolated tumor cells closer to routine clinical use.

The automatic collection and archiving of tumor cell images in a picture gallery on the computer screen has several advantages. The investigator can compare the morphology of different tumor cells within one patient, or among different patients and carcinoma types. The picture galleries may serve as valuable tools for standardization of diagnostic decisions and for quality control for inter- and intralaboratory procedures. The registration of

the tumor cell count per slide is advantageous, as several reports indicate that not only the mere presence but also the number of tumor cells detected in the bone marrow may be clinically relevant (15,18,25,29).

In summary, the use of automated microscopy, both qualitatively and quantitatively, was demonstrated to provide results equal to, and in many cases better than, the use of manual screening for the detection of isolated tumor cells in bone marrow specimens. Because of the low prevalence of tumor cells in most clinical samples, manual screening may potentially miss a substantial number of the positive samples. In the present study, the use of automated microscopy increased the percent of detected positive samples from 62% to 96%. This is a large proportion of patients that would have been otherwise considered negative and, potentially, might have received inadequate therapy.

LITERATURE CITED

1. Birdsong GG. Automated screening of cervical cytology specimens. Hum Pathol 1996;27:468–481.
2. Netten H, Young IT, van Vliet LJ, Tanke HJ, Vroljik H, Sloos WCR. FISH and chips: automation of fluorescent dot counting in interphase cell nuclei. Cytometry 1997;28:1–10.
3. Ravkin I, Temov V. Automated microscopy system for detection and genetic characterization of fetal nucleated red blood cells on slides. SPIE 1998;3260:180–191.
4. Boland MV. Automated recognition of patterns characteristic of subcellular structures in fluorescence microscopy images. Cytometry 1998;33:366–375.
5. Ravkin I, Temov V. Automated counting of FISH spots in interphase cells for prenatal characterization of aneuploidies. SPIE 1999;3604: 208–217.
6. Kozubek M, Kozubek S, Lukášová E, Marĉková A, Bártová E, Skalníková M, Jergová A. High-resolution cytometry of FISH dots in interphase cell nuclei. Cytometry 1999;36:279–293.
7. Mansi JL, Mesker WE, McDonnell T, Van Driel-Kulker AMJ, Ploem JS, Coombes RC Automated screening for micrometastases in bone marrow smears. J Immunol Meth 1988;112:105–111.
8. Mesker WE, v.d. Burg MJM, Oud PS, Knepflé CFHM, Ouwerkerk-v.Velzen MCM, Schipper NW, Tanke HJ. Detection of immunocytochemically stained rare events using image analysis cytometry 1994; 17:209–215.
9. Cote RJ, Shi S-R, Beattie EJ, Makarewicz K, Chaiwun B, Yang C, Groshen S, Taylor CR. Automated detection of occult bone marrow micrometastases in patients with operable lung carcinoma [abstract]. Proc ASCO 1997;16:1645.
10. Makarewicz K, McDuffie L, Shi S-R, Chatterjee S, Yang C, Taylor C, Cote RJ. Immunohistochemical detection of occult micrometastases using an automated intelligent microscopy system [abstract No. 1805]. Proc Am Assoc Cancer Res 1997;38:269.
11. Bauer KD, de la Torre-Bueno J, Diel IJ, Hawes D, Decker WJ, Priddy C, Bossy B, Ludmann S, Yamamoto K, Masih AS, Espinoza FP, Harrington DS. Reliable and sensitive analysis of occult bone marrow metastases using automated cellular imaging. Clin Cancer Res 2000; 6:3552–3559.
12. Braun S, Schaffer P, Atkinson R. Identification of occult metastatic cells in bone marrow predicts poor diagnosis better than Her-2/neu status and angiogenesis in breast carcinomas. Breast Cancer Res Treat 2000;64:26.
13. Kraeft S-K, Gravelin L, Ferland LH, Richardson P, Elias A, Chen LB. Detection and analysis of cancer cells in blood and bone marrow using a rare event imaging system. Clin Cancer Res 2000;6:434–442.
14. Mehes G, Witt A, Kubista E, Ambros PF. Classification of isolated tumor cells and micrometastasis. Cancer 2000;89:709–711
15. Cote RJ, Rosen PP, Lesser LM, Old LJ, Osborne M. Prediction of early relapse in patients with operable breast cancer by detection of occult bone marrow micrometastases. J Clin Oncol 1991;9:1749–1756.
16. Schlimok G, Funke I, Pantel K, Strobel F, Lindemann F, Witte J, Riethmüller G. Micrometastatic tumor cells in bone marrow of patients with gastric cancer: methodological aspects of detection and prognostic significance. Eur J Cancer 1991;27:1461–1465.
17. Dearnaley DP, Ormerod MG, Sloane JP. Micrometastases in breast cancer: long-term follow-up of the first patient cohort. Eur J Cancer 1991;27:236–239.
18. Moss TJ, Reynolds CP, Sather HN, Romansky SG, Hammond GD, Seeger RC. Prognostic value of immunocytologic detection of bone marrow metastases in neuroblastoma N Engl J Med 1991;24:219–226.
19. Schlimok G, Lindemann F, Holzmann K, Witte J, Renner D, Riethmüller G. Prognostic significance of disseminated tumor cells detected in bone marrow of patients with breast and colorectal cancer: a multivariate analysis. Proc Am Soc Clin Oncol 1992;11:102.
20. Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmüller G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 1992;340:685–689.
21. Harbeck N, Untch M, Pache L, Eiermann W. Tumor cell detection in the bone marrow of breast cancer patients at primary therapy: results of a 3-year median follow-up. Br J Cancer 1994;69:566–571.
22. Cote RJ, Beattie EJ, Chaiwun B, Shi S-R, Harvey J, Chen S-C, Sherrod AE, Groshen S, Taylor CR. Detection of occult bone marrow micrometastases in patients with operable lung carcinoma. Ann Surg 1995; 222:415–425.
23. Diel IJ, Kaufmann M, Costa SD, Holle R, von Minckwitz G, Solomayer EF, Kaul S, Bastert G. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. J Nat Cancer Inst 1996;88:1652–1658.
24. Pantel K, Izbicki J, Passlick B, Angstwurm M, Häussinger K, Thetter O, Riethmüller G. Frequency and prognostic significance of isolated tumor cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. Lancet 1996;347:649–653.
25. Jauch KW, Heiss MM, Gruetzner U, Funke I, Pantel K, Babic R, Eissner HJ, Riethmüller G, Schildberg FW. Prognostic significance of bone marrow micrometastases in patients with gastric cancer. J Clin Oncol 1996;14:1810–1817.
26. Roder JD, Thorban S, Pantel K, Siewert JR. Micrometastases in bone marrow: prognostic indicators for pancreatic cancer. World J Surg 1999;23:888–891.
27. Mansi JL, Gogas H, Bliss JM, Gazet JC, Berger U, Coombes RC. Outcome of primary-breast-cancer patients with micrometastases: a long-term follow-up study. Lancet 1999;354:197–202.
28. Thorban S, Rosenberg R, Busch R, Roder RJ. Epithelial cells in bone marrow of esophageal cancer patients: a significant prognostic factor in multivariate analysis Br J Cancer 2000;83:35–39.
29. Janni W, Gastroph S, Hepp F, Kentenich C, Rjosk D, Schindlbeck C, Dimpfl T, Sommer H, Braun S. Prognostic significance of an increased number of micrometastatic tumor cells in the bone marrow of patients with first recurrence of breast carcinoma. Cancer 2000;88:2252–2259.
30. Braun S, Pantel K, Müller P, Janni W, Hepp F, Kentenich CRM, Gastroph S, Wischnik A, Dimplf T, Kindermann G, Riethmüller G, Schlimok G. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II or III breast cancer. N Engl J Med 2000;342:525–533.
31. Borgen E, Naume B, Nesland JM, Kvalheim G, Beiske K, Fodstad Ø, Diel I, Solomayer E-F, Theocharous P, Coombes RC, Smith BM, Wunder E, Marolleau J-P, Garcia J, Pantel K. Standardization of the immunocytochemical detection of cancer cells in BM and blood: I. Establishment of objective criteria for the evaluation of immunostained cells. Cytotherapy 1999;1:377–388.
32. Pantel K, Cote R, Fodstad Ø. Detection and clinical importance of micrometastatic disease. J Nat Cancer Inst 1999;91:1113–1124.
33. Pantel K, von Knebel Doeberitz M. Detection and clinical relevance of micrometastatic cancer cells. Curr Opin Oncol 2000;12:95–101.
34. Müller P, Schlimok G. Bone marrow micrometastases of epithelial tumors: detection and clinical relevance J Cancer Res Clin Oncol 2000;126:607–618.
35. Pantel K, Schlimok G, Angstwurm M, Weckermann D, Schmaus W, Gath H, Passlick B, Izbicki JR, Riethmüller G. Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematotherapy 1994;3:165–173.
36. Naume B, Borgen E, Nesland JM, Beiske K, Gilen E, Renolen A, Ravnås G, Qvist H, Kåresen K, Kvalheim G. Increased sensitivity for detection of micrometastases in bone-marrow/peripheral-blood stem-cell products from breast-cancer patients by negative immunomagnetic separation. Int J Cancer 1998;78:556–560.