Quantitation of Plasma Cells in Bone Marrow Aspirates by Flow Cytometric Analysis Compared With Morphologic Assessment

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● Context.—Accurate quantitation of bone marrow plasma cells is an important component in the diagnosis and post-treatment assessment of plasma cell dyscrasias. Although flow cytometry is sometimes used for this purpose and can rapidly evaluate many cells, the accuracy of flow-based plasma cell quantitation compared with morphologic assessment (currently the gold standard) is uncertain as direct comparison studies have not been previously reported.

Objective.—To determine how percentages of plasma cells in diagnostic aspirate smears quantitated by morphologic assessment relate to percentages of plasma cells quantified by flow cytometry.

Design.—Thirty bone marrow cases with 10% or more plasma cells and leukemia/lymphoma flow cytometry immunophenotyping studies were identified from our hematology database. The Wright-stained aspirate smears, marrow biopsy sections, and flow cytometry histograms were reviewed.

Results.—Morphologically determined plasma cell percentages from the diagnostic aspirate smears were consistently higher than those determined by flow cytometry. Much of this difference appeared to be related to differences in sample quality. However, the cellular processing involved in performing flow cytometry also appeared to reduce plasma cell percentages in many cases.

Conclusions.—This study helps define the limitations of flow cytometry for quantitating plasma cell loads in marrow aspirate specimens that may significantly affect the diagnosis or assessment of treatment response.

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flow cytometry in identifying and quantifying neoplastic plasma cells. In most cases, the percentages of plasma cells quantitated by flow cytometry were markedly lower than those determined by morphologic analysis.

MATERIALS AND METHODS

Case Selection and Demographic Characteristics

The ARUP Laboratories’ flow cytometry and hematopathology databases were searched for recent bone marrow cases submitted for both morphologic review and leukemia/lymphoma phenotyping by flow cytometry in which plasma cells accounted for 10% or more of the leukocytes by morphologic assessment of the diagnostic aspirate smears. The 30 cases selected for this study were obtained from 18 men and 12 women (mean age ± SD, 64 ± 12; range, 39–83 years). The research use of these materials was approved by the University of Utah institutional review board (No. 18325).

Morphologic Analysis

All the original diagnostic material, including Wright-stained bone marrow aspirate smears and paraffin-embedded, hematoxylin-eosin–stained sections of bone marrow cores, were reviewed. The morphologically determined plasma cell values from the diagnostic aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the original surgical pathology reports appeared to be accurate. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells. In 28 of 30 cases, the percentages of plasma cells listed on the aspirate smears were based on counts of 500 nucleated cells.

Flow Cytometric Analysis

Routine flow cytometric immunophenotyping studies were performed on all cases at the time of diagnosis, as described elsewhere. More specifically, erythrocytes and erythrocyte precursors in the bone marrow specimens were lysed by incubating at 37°C for 10 minutes in a 10-fold excess of ammonium chloride (0.15M with 0.01M sodium bicarbonate and 0.1M disodium EDTA). Following centrifugation (for 5 minutes at 400g), liquid was removed by decanting and blotting (procedure following all centrifugation steps). The leukocytes were manually resuspended by using a plastic bulb pipette, washed with 15 mL of RPMI medium 1% bovine serum albumin (BSA), and resuspended with a plastic bulb pipette in 2 mL of RPMI medium 1% BSA. For surface staining, 10 μL or 20 μL of antibodies was added and washed with 3 mL of phosphate-buffered saline (PBS) and resuspended in 0.5 mL of 0.5% paraformaldehyde before analysis by gently running the tubes over an empty tube rack (procedure used for all subsequent re-suspension steps). Cytoplasmic staining was performed by using the IntraPrep permeabilization reagents according to the manufacturer’s directions (Beckman Coulter, Miami, Fla). More specifically, approximately 1 million erythrocyte-depleted leukocytes were incubated in 100 μL of fixative solution at room temperature for 15 minutes in the dark, washed with 3 mL of PBS, and resuspended in 100 μL of permeabilization solution. After 5 minutes, 10 μL or 20 μL of antibodies was added and washed with 3 mL of PBS following 15 minutes of incubation in the dark, and resuspended in 0.5 mL of 0.5% paraformaldehyde before analysis. Data acquisition and analysis were performed with an EPICS XL cytometer and EXPO 32 software (Beckman Coulter). Antibodies used to identify plasma cells included anti-CD38 PE (Leu-17), anti-CD56 FITC (NCAM16.1), anti-k FITC (TB28-2), and anti-λ PE (1-155-2), purchased from BD Biosciences (San Jose, Calif), along with anti-CD45 PC5 (HE30), purchased from Caltag Laboratories (Burlingame, Calif). Plasma cell quantitation was performed by surface staining (using the combination of CD56 FITC/CD38 PE/CD45 PC5) and by cytoplasmic staining (using the combination of k FITC/CD38 PE/CD45 PC5). With use of our procedure, plasma cells displayed cytoplasmic light-chain levels 1 to 2 log higher than mature B cells. Flow cytometric plasma cell percentages were determined by quantitating those cells with dim to negative CD45 that had bright (fourth decade) surface expression of CD38. Plasma cell percentages were also determined by quantitating cells with bright cytoplasmic light-chain expression that were usually located in the CD45 dim to negative gate. Ungated k histograms were reviewed in all cases to ensure that rare neoplastic plasma cells that may express CD45 could also be detected. An average of these 2 flow cytometry–derived plasma cell values was used, but in all cases these methods gave values within 10% or less of one another.

RESULTS

Percentages of plasma cells were determined morphologically and by flow cytometry for the 30 cases (Figures 1 and 2). The flow cytometry–determined plasma cell percentages were typically markedly lower than those determined morphologically from the diagnostic aspirate specimens (mean difference ± SD, 78% ± 20%; mean fold-reduction, 10 ± 10), with only 2 cases (cases 17 and 18) showing similar, but still slightly lower, values by flow cytometry (Table; Figure 3, A). Morphologic analysis indicated that the specimens submitted for flow cytometry usually had markedly reduced plasma cell percentages relative to the diagnostic aspirate specimens (mean difference ± SD, 69% ± 28%; mean fold-reduction, 9 ± 14), with only 3 cases having similar values (cases 17, 18, and 24). Plasma cell percentages determined by flow cytometry correlated better with morphologically determined plasma cell percentages in the flow cytometry specimens (Figure 3, B), although many of the flow cytometry values were lower. Of the 11 cases with both more morphologically determined plasma cells in the flow cytometry specimens, 5 had values similar to the flow percentages, whereas 6 had markedly lower plasma cell percentages by flow cytometry (mean difference ± SD, 48% ± 16%; mean fold-reduction, 2 ± 1).

COMMENT

It is commonly assumed that the accuracy of flow cytometry in quantitating plasma cell numbers in bone marrow aspirates may be problematic because of hemodilution or increased plasma cell fragility relative to other leukocytes that are typically present. However, to our knowledge, studies that specifically address the accuracy of routine flow cytometric immunophenotyping in quantitating marrow aspirate plasma cell values have not yet been published. In this study, we document the frequency and magnitude by which plasma cell percentages determined by flow cytometry typically underestimate percentages determined morphologically from diagnostic aspirate
smears. In addition, our data also support the hypothesis that the process of performing flow cytometric immunophenotyping may also reduce plasma cell numbers in some cases.

Based on morphologic analysis of the flow cytometry specimens, most of the underestimation of plasma cell percentages by flow cytometry appears to be due to hemodilution or poorer quality of the flow cytometry specimens compared with the aspirate smears used for diagnosis. At our institution and most others, the aspirate material used to make smears for diagnostic morphologic evaluation is obtained before any others and is often designated as first pull. Aspirate material obtained for ancillary studies, such as flow cytometry, is typically obtained from later aspirations or pulls and can often be of poor quality and nonrepresentative because fewer marrow cells were aspirated and/or substantial peripheral blood contamination was present. An interesting finding from our study was the large magnitude of this factor (mean percentage difference, 69% ± 28%), with some flow cytometry specimens having almost no morphologically detectable plasma cells and diagnostic smears having abundant plasma cells above 30%. In only 10% of cases were the morphologically determined plasma cell percentages in the flow cytometry specimens similar to the diagnostic aspirate smears.

Flow cytometrically determined plasma cell percentages correlated better with the morphologically determined plasma cell percentages from the specimens submitted for flow cytometric analysis. However, in the 11 cases containing more than 10% plasma cells by morphologic analysis of the flow cytometry specimens, which represent more reliable values, slightly more than half (6/11) had markedly lower plasma cell values by flow cytometry (mean difference, 48% ± 16%; range, 36%–76%). These data support the common assumption that the process of performing routine flow cytometric immunophenotyping also contributes to reduction of reported plasma cell values in many cases. The mechanism whereby plasma cells are sometimes depleted by routine flow cytometric immunophenotyping is unclear but in our cases did not appear to be related to differences in specimen viability or specimen age. Perhaps the malignant plasma cells in some cases are more susceptible to ammonium chloride lysis than others or are less able to survive resuspension after centrifugation. Others have reported that ammonium chloride leaves the greatest number of plasma cells intact relative to other processing agents or methods, and this method is also used for sensitive nonroutine flow cytometric tests that reportedly can detect 1 plasma cell in 10 000 leukocytes. However, few if any data have been published concerning loss of plasma cells related to specimen processing, and ammonium chloride may decrease the expression levels of some antigens, such as CD138, that are sometimes used to identify plasma cells by flow cytometry. It appears, therefore, that additional study of this issue is warranted.

Our data suggest that there are several reasons why...
Routine flow cytometric immunophenotyping studies are unlikely to generate accurate plasma cell percentage values in most cases, including differences in plasma cell content between samples submitted for morphologic review and samples submitted for flow cytometry, and plasma cell loss during flow cytometry sample processing steps. Although we used only 3-color analysis to identify plasma cells and did not use antibodies against CD138, it is unlikely that substantial numbers of plasma cells were missed because of this technical approach. Our method of identifying plasma cells as those cells with dim to negative expression of CD45 that also expressed bright CD38 is used by other laboratories, and also gave excellent agreement with the percentages of plasma cells independently determined by quantitating cells that expressed bright monoclonal cytoplasmic light chains. Moreover, our analysis of cytoplasmic light-chain expression can also easily identify plasma cells that may express CD45, although all the cases in this study were CD45 negative. In addition, variable expression of CD138 on plasma cells has been reported, and most participants in an international flow cytometry consensus meeting felt that the use of CD138 was not essential in identifying plasma cells, whereas the markers we evaluated (cytoplasmic light chains, CD38, CD45) were the most important.

In conclusion, this study provides considerable evidence that routine flow cytometric immunophenotyping is not a good method for generating accurate bone marrow plasma cell load values. Moreover, the large discrepancies between morphologically determined plasma cell values of the diagnostic aspirates and the flow cytometry-determined values suggest that flow cytometry may also easily miss plasma cell neoplasms in many cases unless antibodies specific for plasma cell identification are requested or relevant history is provided. Although flow cytometry typically underestimates the bone marrow plasma cell load, it appears to provide a lower limit estimate and is also useful in distinguishing between normal and malignant plasma cells, which can be very important in evaluating posttreatment follow-up specimens for residual disease.

We thank Ms Jackie McCowen-Rose for her help in producing Figure 1.

References

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* Values are the percentages of leukocytes.


