Comparison of Ancillary Studies in the Detection of Residual Disease in Plasma Cell Myeloma in Bone Marrow

Xiaohui Zhao, MD, PhD,1 Qin Huang, MD, PhD,2 Marilyn Slovak, PhD,2 and Lawrence Weiss, MD2

Key Words: Bone marrow; Plasma cell myeloma; Residual disease; Ancillary studies

Abstract

Morphologic determination of residual plasma cell myeloma is often difficult. This study was conducted to evaluate the diagnostic value of several ancillary methods. A total of 121 bone marrow specimens from patients with myeloma after bone marrow transplantation or chemotherapy were studied. Of those, 49 cases were studied by conventional cytogenetics (CC), 86 by flow cytometry (FC), 50 by immunohistochemical analysis, 72 by molecular studies (MS) for immunoglobulin gene rearrangements, and 18 by standard fluorescence in situ hybridization (FISH) study. Of the patients, 70 were found to have residual myeloma. Residual disease was detected in 58 of 70 patients by morphologic examination (detection rate, 83%), 25 of 26 by immunohistochemical analysis (96%), 39 of 54 by FC (72%), 6 of 40 by CC (15%), 6 of 12 by FISH (50%), and 24 of 40 by MS (60%). Among the ancillary studies, immunohistochemical analysis and FC were the most helpful ancillary studies for detection of residual disease in myeloma, with immunohistochemical analysis the most effective single method.

Multiple myeloma is a B-cell malignancy characterized by a clonal expansion of plasma cells. Although many patients with multiple myeloma achieve complete remission by conventional criteria, only a minority achieve molecular remission. With current therapies, including chemotherapy and bone marrow transplantation, all patients eventually experience relapse as a consequence of residual disease. At present, the methods for assessment of residual disease require bone marrow examination. It is, therefore, important to choose a relatively sensitive ancillary method to detect residual disease.

Current approaches for the measurement of residual disease in a bone marrow specimen usually are based on the morphologic assessment of bone marrow aspirate and biopsy specimen, immunohistochemical analysis, flow cytometry, molecular studies, conventional cytogenetics, and fluorescence in situ hybridization (FISH). The morphologic determination of residual disease often is difficult, complicated by the fact that normal plasma cells also may be present in the bone marrow, especially after treatment. An optimal assay for monitoring residual disease in multiple myeloma should be robust, universally applicable, and able to quantitate the number of neoplastic plasma cells. Although a molecular study, Southern blotting, is considered the “gold standard” for demonstrating clonality at a molecular level, the technique has several major drawbacks, with lack of sensitivity being most pronounced when applied to the study of myeloma. In the present study, we compared the detection rates of residual disease in plasma cell myeloma using different ancillary tests on bone marrow specimens in addition to morphologic examination.
Materials and Methods

Cases
Cases were identified by a computer search of consecutive specimens in the pathology files of the City of Hope National Medical Center, Duarte, CA, from July 2002 to June 2003. The diagnosis of myeloma was established in each case in house or at outside facilities (referral cases). Bone marrow aspirate and trephine biopsy samples were obtained for each patient at initial examination for a follow-up examination after treatment. Morphologic assessment was performed in all cases, and ancillary studies, including immunohistochemical analysis, flow cytometry, conventional cytogenetics, FISH, and molecular study for B-cell gene rearrangements, were used in a variable number of cases when they were needed. The results of the morphologic review of bone marrow and ancillary testing for each patient were obtained by review of original pathology reports.

Immunohistochemical Analysis
The cases with no bone marrow aspirate available or the cases with negative or nondiagnostic flow cytometric results were studied by immunohistochemical analysis. The immunophenotype of the plasma cells was characterized through immunostaining of paraffin-embedded bone marrow biopsy and clot sections using heat-induced epitope retrieval and the streptavidin-biotin complex method with automated staining equipment (DAKO Autostainer, DAKO, Carpinteria, CA) according to the manufacturer’s instructions. The antibodies used for the studies were CD138, κ, and λ (BioGenex, San Ramon, CA). Appropriate positive and negative control samples were included.

Flow Cytometry
The cases with aspirate available were studied routinely by flow cytometry. Flow cytometric studies were performed on separate cell suspensions of bone marrow aspirates. Samples were analyzed by 3-color analysis, using permeabilized techniques with monoclonal antibodies in triple combinations as follows: CD38 phycoerythrin (PE) (Coulter, Miami, FL)/CD45 PE–Texas red (ECD; Coulter)/κ fluorescein isothiocyanate (DAKO); CD38/CD45/λ FITC (DAKO); CD138 PE (Coulter)/CD45/κ; and CD138/CD45/λ. Briefly, cells were incubated with CD45, CD38, and CD138 at room temperature for 15 minutes in the dark and then fixed with Fixation Medium (Fix & Perm Kit, Caltag, Burlingame, CA). After washing and centrifugation, the cellular part was incubated with Permeabilization Medium (Fix & Perm Kit, Caltag) and κ or λ antibody. Plasma cells were analyzed for κ or λ expression by setting the gate to collect CD38+/CD45 dim (CD45–) or CD138+/CD45 dim (CD45–) populations. The number of events acquired a minimum of 2,500 events in the gate. Analysis was performed on a Beckman Coulter Epics XL MCL flow cytometer (Coulter) using XL system II software (Coulter).

Conventional Cytogenetics
Cytogenetic analysis was performed on bone marrow aspirates based on treatment protocol requirement and using standard techniques on metaphase cells derived from 24-, 48-, or 72-hour unstimulated cell cultures.5 GTG banding was used to identify the individual chromosomes. Chromosome abnormalities were described according to the International System for Human Cytogenetic Nomenclature.6 When available, a minimum of 20 metaphase cells per sample was analyzed.

Fluorescence In Situ Hybridization
FISH analysis was performed based on treatment protocol requirement. Dual-color FISH studies were performed to determine the following: (1) the number of interphase nuclei containing the CCND1/immunoglobulin heavy chain (IgH) (LSI CCND1/IgH probe, Vysis, Downers Grove, IL) fusion gene, resulting from the (t(11;14)(q13;q32) characteristically associated with multiple myeloma (probe sensitivity, 100%; 95% confidence interval [CI], 76.8%-100%; specificity, 100%; 95% CI, 69.2%-100%); (2) the presence or absence of chromosome 13 long arm sequences using a locus-specific DNA probe, BAC 480p3, which maps to 13q14.3, and a control identifier probe for chromosome 13 telomeric DNA sequences (BAC 19015 [13qtel]) (probe sensitivity, 100%; 95% CI, 89.1%-100%; specificity, 100%; 95% CI, 89.1%-100%); and (3) the presence or absence of the TP53 gene and chromosome 17 copy number using a locus-specific DNA probe, BAC 199f11, which maps to 17p13.1 in association with a reference probe for chromosome 17 (17q21.1, BAC 62n23, erb-b2 locus; probe sensitivity, 100%; 95% CI, 78.2%-100%; specificity, 100%; 95% CI, 78.2%-100%).

Chromosome spreads were prepared by standard methods, and cutoff values for positivity and negativity were calculated as described previously.5 Slides were incubated in 2× saline sodium citrate (SSC) at 37°C for 30 minutes and denatured in 70% formamide/2× SSC (pH 7.0) at 72°C for 2 minutes. Slides were hybridized using the aforementioned probes (Vysis). A volume of 5 µL of probe was heat denatured at 72°C for 5 minutes, applied to denatured slides, covered with an 18-mm round coverslip, and sealed with rubber cement. Slides were incubated in a humidified chamber at 37°C overnight. Nonspecific probe binding was removed by a 0.4× SSC/0.3% Nonidet P40 (NP40; Vysis) (pH 7.0) posthybridization wash at 72°C for 2 minutes, followed by 2× SSC/0.1% NP40 (pH 7.0) at room temperature for 1 minute. Cells were counterstained with 4′,6-diamino-2-phenyl indole dihydrochloride (Vysis). Lymphocytes from a
phenotypically normal female were used as a negative control sample. In all, a minimum of 200 interphase nuclei was scored for each probe and each case.

**Detection of IgH and κ Light Chain Gene Rearrangements by Polymerase Chain Reaction Analysis**

Detection of IgH and κ light chain gene rearrangements was performed based on treatment protocol requirement. Genomic DNA was isolated from bone marrow aspirates. Polymerase chain reaction (PCR) and Southern blot procedures were performed for analysis of IgH and κ light chain gene rearrangements, respectively. The PCR primers and conditions used to detect IgH and κ light chain gene rearrangements have been described previously.7 PCR products were analyzed by using the ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA) for IgH rearrangements using consensus FR3A/V_{L\text{H}}, FR2A/V_{L\text{H}}, and FR2B/V_{L\text{H}} primers.8 Southern blot procedures were performed for analysis of κ light chain genes when needed.

**Statistics**

The Pearson \(\chi^2\) was used to compare detection rates among the studies.

**Results**

**Clinical Findings**

A total of 121 cases of multiple myeloma were analyzed. The study population consisted of 80 men and 41 women. The median age of the men at initial examination was 59 years (range, 39-72 years) and of the women, 54 years (range, 35-68 years). Of the samples, 72 were obtained after transplantation (38 patients had undergone autologous stem cell transplantation) and 49 after chemotherapy. The time to bone marrow transplantation varied from 3 weeks to 6 years (median, 14 weeks; mean, 54 weeks). Of the 121 patients, 70 were found to have residual myeloma by one or more of the pathology assays.

**Morphologic Assessment**

All 121 cases were evaluated by morphologic assessment of bone marrow specimens, which is based on the examination of bone marrow aspirate smears or biopsy imprints and trephine biopsy or clot sections. Image 1A shows the absence of residual disease in the bone marrow.9 Among the 121 cases, 58 cases were positive for residual disease by morphologic assessment (residual disease in 4 cases was detected by morphologic assessment alone), and 12 cases showed a false-negative result compared with the final diagnosis. Figure 1A illustrates the detection of IgH rearrangements using consensus FR3A/V_{L\text{H}}, FR2A/V_{L\text{H}}, and FR2B/V_{L\text{H}} primers.8 Southern blot procedures were performed for analysis of κ light chain genes when needed.

**Immunohistochemical Findings**

Of the 121 cases, 50 were evaluated by immunohistochemical analysis using CD138, κ, and λ stains. Light chain restriction was used as a surrogate marker for clonality. A κ clone was defined as a κ/λ ratio of 5.0 or more and a λ clone as a κ/λ ratio of 0.5 or less.10 Among the 50 cases tested, 26 cases (52%) were diagnosed as containing residual disease. Of these 26 cases, 25 (96%) cases showed residual disease by immunohistochemical studies (Table 3). A single false-negative case was detected by flow cytometry and molecular studies.

As described in the “Materials and Methods” section, immunohistochemical analysis was performed on cases with no available bone marrow aspirate and cases with negative or nondiagnostic flow cytometry. Table 3 shows the number of cases with residual disease detected by combined ancillary studies, mainly immunohistochemical analysis, flow cytometry, and molecular studies. Of 8 cases with negative flow cytometric results, 8 showed residual disease by immunohistochemical analysis (100% detection rate), whereas 11 of 12 cases detected by flow cytometry were detected by immunohistochemical analysis as well. The 8 cases negative by PCR were positive by immunohistochemical analysis (100% detection rate). Of 5 cases with positive results of molecular studies, 3 were detected by immunohistochemical analysis. Of the 2 negative immunohistochemical cases, 1 was not detected by flow cytometry (Table 3). All samples with residual disease showed CD138 positivity of the plasma cells. Of 25 cases, 14 revealed κ light chain restriction (56%) and 11 cases showed λ light chain restriction (44%).

**Flow Cytometric Findings**

The criteria used for light chain restriction were as described in “Immunohistochemical Findings.”10 Of 121 cases, 86 were studied by flow cytometry, and 54 cases had a final diagnosis of residual disease. Of these 54 cases, 39 (72%) were positive for residual disease by flow cytometric studies (Figure 1 and Table 1). In addition, 7 of 10 cases with negative results of molecular studies were positive by flow cytometry.

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Cytochemistry, and 16 of 21 cases with positive molecular study results were positive flow cytometrically as well (Table 3). CD138 and CD38 antibodies usually showed strong binding, clearly separating the majority of plasma cells from the other cellular components. In the present study, all cases with residual disease were positive for CD138 and CD38. Cytoplasmic κ monotypic expression was seen in 27 (69%) of 39 cases, and cytoplasmic λ monotypic expression was found in 12 (31%) of 39 cases.

Conventional Cytogenetics

Conventional cytogenetic studies were performed in 49 cases, of which 40 eventually were diagnosed with residual disease. However, only 6 of these cases (15%) showed cytogenetic abnormalities (Figure 1 and Table 1). The findings of chromosomal abnormalities in these 6 cases were variable but consistent with a diagnosis of multiple myeloma.

Fluorescence In Situ Hybridization

Only 12 cases with residual disease were tested by standard FISH analysis. Six cases were positive for residual disease by FISH by 1 or more of 3 probes, for a detection rate of 50% (Table 1). Five cases revealed a 14q32 translocation, with 1 case showing monosomy 13 and 1 showing a TP53 deletion. Two cases with abnormal FISH results showed chromosomal abnormalities by conventional cytogenetics.
However, 3 cases with abnormal FISH results had normal karyotypes by conventional cytogenetics.

Molecular Studies of Immunoglobulin Gene Rearrangements

Molecular analyses were performed in 72 cases, of which 40 cases eventually showed residual disease. Of the 40 cases, 24 showed heavy chain and/or κ light chain gene rearrangements (60% detection rate) (Table 1). Four cases with positive heavy chain rearrangements by molecular studies were negative for neoplastic plasma cells by either immunohistochemical analysis or flow cytometry. One case with positive heavy chain rearrangement was negative for residual disease by morphologic examination and flow cytometry.

Discussion

The complete remission rate in multiple myeloma has been increased by high-dose chemotherapy followed by bone marrow or stem cell transplantation. However, many patients eventually still experience relapse as a consequence of residual disease. For the detection of residual disease, morphologic assessment alone usually does not distinguish

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Detection of Residual Disease in Multiple Myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Disease</td>
<td>Morphologic Assessment</td>
</tr>
<tr>
<td>Detected</td>
<td>58</td>
</tr>
<tr>
<td>Not detected</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
</tr>
<tr>
<td>Detection (%)</td>
<td>83</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

* P < .05 vs flow cytometry; † P < .01 vs FISH and molecular studies; † P < .001 vs conventional cytogenetics.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results of Ancillary Testing of False-Negative Cases by Morphologic Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case No.</td>
<td>Immunohistochemical Analysis</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
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<td>5</td>
<td>ND</td>
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<td>+</td>
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<tr>
<td>11</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>Detection (%)</td>
<td>100 *</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization; ND, not done.

* P < .01 compared with flow cytometry and molecular studies.
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normal from neoplastic plasma cells. Accordingly, at present, more sensitive techniques are needed to evaluate the effectiveness of new treatment strategies in multiple myeloma. Current approaches to detect residual disease are based on morphologic assessment of bone marrow specimens, analysis of the paraprotein levels, or PCR analysis of the immunoglobulin heavy chain variable-diversity-joining region. Additional methods of assessing residual disease in myeloma include immunophenotyping by immunohistochemical analysis and flow cytometry and conventional cytogenetics and FISH. Complete remission is defined as the absence of the original monoclonal paraprotein in serum and urine by immunofixation and fewer than 5% plasma cells in the bone marrow.

### Table 4

**Abnormal Cytogenetic Results in Cases With Residual Multiple Myeloma**

<table>
<thead>
<tr>
<th>Sex/Age (y)</th>
<th>History</th>
<th>Conventional Cytogenetic Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/45</td>
<td>Follow-up</td>
<td>46,XY,t(1;18)(p13;q24.1),der(5)t(5;?)(q15;?),der(14)t(11;14)(q13;q32.3)(17)(46,XY)</td>
</tr>
<tr>
<td>M/68</td>
<td>10 mo after BMT</td>
<td>40-46,XY,add(1)(q12),del(2)(2,7)(q21, q11.2),-3,del(5)(p13)(p15),add(6)(q21),del(7)(p13),+8,add(9)(p1,2),del(10)(q24q26),+11,add(1)(p15),del(11)t(3;11)(p13;q21),+12,del(12)(q13q15),del(13)(q15p43.3),del(13)(q12q14),-14,-15,del(16)(t;1;16)(q21, q13),-17-20,add(20)(q13.1)</td>
</tr>
<tr>
<td>M/58</td>
<td>Postchemotherapy</td>
<td>45,X,-Y,t(5;17)(q15;p13),t(11;14)(q13;q32)[4]/46,XY[46,XY]</td>
</tr>
<tr>
<td>M/63</td>
<td>2 y after BMT</td>
<td>46,XY,t(11;14)(q13;q32.3)[15]/46,XY[1]</td>
</tr>
<tr>
<td>M/64</td>
<td>130 d after BMT</td>
<td>45,X,-Y,t(5;17)(q15;p13),t(11;14)(q13;q32)[4]/46,XY[46,XY]</td>
</tr>
<tr>
<td>M/71</td>
<td>2 y after BMT</td>
<td>45,X,-Y,t(5;17)(q15;p13),t(11;14)(q13;q32)[4]/46,XY[46,XY]</td>
</tr>
<tr>
<td>M/71</td>
<td>2 y after BMT</td>
<td>45,X,-Y,t(5;17)(q15;p13),t(11;14)(q13;q32)[4]/46,XY[46,XY]</td>
</tr>
</tbody>
</table>

BMT, bone marrow transplantation.
Within the bone marrow of patients with myeloma, CD138 (syndecan-1) used for immunophenotyping was found to be a specific marker for plasma cells and was not expressed by other hematopoietic cells or endothelial cells. Syndecans are a family of cell surface proteoglycans, of which syndecan-1 is the prototypical member. Syndecan-1 is expressed on cells from human myeloma cell lines and mediates cell-cell adhesion, adhesion to type I collagen, and inhibition of invasion into type I collagen gels.

Immunohistochemical analysis by CD138 combined with κ and λ immunostains showed the highest detection rate in the present study compared with the other ancillary tests. In the present study, immunohistochemical analysis showed the highest detection rate in the cases in which residual disease was not detected by morphologic examination (Table 2). Although immunohistochemical study was not done on all the cases studied and basically was a backup study for flow cytometry, it showed a high detection rate in the cases with positive and negative flow cytometric results (Table 3). In addition, the assay is relatively cost-effective in comparison with the other methods. Immunohistochemical analysis

**Image 2I** Histogram of flow cytometry for a patient with residual disease of multiple myeloma. Approximately 3% κ monoclonal, CD38+, and CD138+ plasma cells are detected. These plasma cells are negative for CD45. ECD, PE–Texas red; FITC, fluorescein isothiocyanate; FS, forward scatter; LIN, linear; PE, phycoerythrin; SS, side scatter.

**Image 3I** Interphase nuclei showing deletion of 13q involving band 13q14.3 (one green signal). The red signals denote an internal control locus indicating the cell has 2 copies of chromosome 13 (fluorescence in situ hybridization).
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may have limited value in small biopsy specimens owing to the often patchy nature of disease in multiple myeloma. Regardless, it is the ancillary test of choice for detecting residual disease in multiple myeloma if an adequate bone marrow biopsy specimen is provided.

Plasma cells are terminally differentiated B cells and, hence, express a number of B-cell antigens and myeloma-specific antigens, such as CD138. Therefore, flow cytometry can offer a quick and efficient method not only for quantifying low levels of neoplastic plasma cells but also for assessing normal immune reconstitution.\textsuperscript{4,14} Plasma cells may be identified by using 3-color flow cytometry by high CD38, high CD138, and low CD45 expression. The neoplastic plasma cell population then can be confirmed by cytoplasmic κ or λ expression.

Initial flow cytometric methods for detection of intracellular (cytoplasmic/nuclear) antigens in lymphoplasmacytic cells were acknowledged as laborious, technically difficult, and not reliable.\textsuperscript{15} Recently, a variety of permeabilizing agents, such as the Fix & Perm Kit (Caltag), FACS lysing solution (Becton Dickinson Immunocytometry System, San Jose, CA), and IntraPrep (Beckman Coulter, Fullerton, CA) have enabled access to intracellular antigens without severely altering important light-scattering properties.\textsuperscript{16} These methods have improved the accuracy of cytoplasmic immunoglobulin light chain determination by flow cytometry.\textsuperscript{17}

The expression of CD19 and CD56 also can be used to distinguish between normal and neoplastic plasma cells. The former are consistently CD19\textsuperscript{+}CD56\textsuperscript{−}, whereas the latter are CD19\textsuperscript{−} or CD19\textsuperscript{+}CD56\textsuperscript{+}.\textsuperscript{2,4}

The flow cytometric assay has several advantages over current methods for the assessment of residual disease in patients with myeloma. The assay uses the same markers for each patient, is applicable to more than 98% of patients, and does not require knowledge of presentation characteristics to detect residual disease. The sensitivity is approximately 1 log greater than that of consensus-primer fluorescent IgH PCR. Although the sensitivity is lower than that of the patient-specific PCR approach, the assay can be performed rapidly, which means it can be used routinely to aid treatment decisions. In contrast with allele-specific PCR, which measures all B-lineage cells as the neoplastic cells, including clonally related B lymphocytes, the flow cytometric method measures neoplastic plasma cells directly.\textsuperscript{4,18,19} In addition, flow cytometric study is a better quantitative assay for residual neoplastic plasma cells than immunohistochemical analysis.\textsuperscript{4} In the present study, flow cytometry showed the second highest detection rate of residual disease using CD38, CD138, and CD45 in conjunction with cytoplasmic κ or λ expression. An adequate bone marrow aspirate and reliable permeabilization techniques, however, are required. In comparison with immunohistochemical analysis, the lower detection rate by flow cytometry may be due to the hypocellularity of some bone marrow aspirates commonly observed in posttreatment marrow specimens.
Conventional cytogenetic analysis of multiple myeloma is difficult owing to the nonproliferative nature of the malignant plasma cells. At disease presentation, abnormal metaphases have been reported in about 50% of cases, whereas residual detection, as observed in the present study, was near 15%. However, the introduction of FISH, which precludes the need for metaphase cells, has greatly improved our ability to detect nonrandom cytogenetic changes in the majority of patients with multiple myeloma. Specifically, translocations involving the immunoglobulin heavy chain gene at 14q32, monosomy or deletions of chromosome 13, and hyperdiploidy (trisomy of 5, 9, and 15) have been reported in a significant number of patients from both cytogenetic and interphase FISH studies, and distinct biologic subtypes of multiple myeloma are characterized by unique genetic abnormalities leading to differing clinical outcomes. As a result of these significant findings, interphase FISH has become the method of choice for the detection of nonrandom cytogenetic changes at disease presentation. Although cytogenetics and FISH studies showed a low detection rate for residual disease in the present study, they still might have significant roles in the assessment of the biologic behavior of the disease.

Such observations led to the development of a new technique, target FISH. This sequential 2-step technique identifies the plasma cells on a cytocentrifuged slide using May-Grünwald-Giemsa stain, mapping their location on the slide, followed by destaining and FISH analyses to detect IgH rearrangements, chromosome 13 or 17p deletions, or hyperdiploidy in the targeted plasma cells. However, a sequential immunocytochemical and FISH approach also would improve residual disease detection.

Southern blotting is considered to be the gold standard approach for the demonstration of clonality at a molecular level. Despite this, it has a number of major drawbacks in the study of myeloma. The most obvious is the relative lack of sensitivity. In recent years, PCR-based approaches for the assessment of residual disease of multiple myeloma have improved the sensitivity of detection. Unfortunately, this approach still is less optimal for residual disease analysis of post–germinal center–derived B-cell malignant neoplasms with a high level of somatic hypermutation in the variable region of IgH or κ light chain genes because of mismatches between the mutated DNA sequence and the used corresponding consensus primers. Our study by PCR analysis using consensus primers demonstrated a 50% detection rate for residual disease in patients with multiple myeloma, which is intermediate among the ancillary studies. The use of multiple sets of primers may increase the detection rate of minimal residual disease for myeloma, and by using real-time quantitative PCR, it is possible to see kinetics of bone marrow tumor load, which may be used to guide therapeutic decisions. PCR analysis with disease-specific primers would be ideal for residual disease detection in myeloma, but these are significantly more expensive and time-consuming.

In addition to morphologic examination, immunohistochemical analysis and flow cytometry are the most helpful ancillary studies for the detection of residual disease in patients with multiple myeloma, with immunohistochemical analysis the most effective single method. Immunohistochemical analysis and flow cytometry are sensitive, cost-effective, and fast and, therefore, are highly recommended for clinical practice. Conventional cytogenetics, an assay that requires mitotic cells, lacks high sensitivity and, therefore, is not useful for residual disease detection. However, cytogenetics and standard FISH studies are clinically useful for prognostic factors, and plasma cell–targeted FISH analysis shows promise for detection of residual disease in the future. Similarly, molecular detection of immunoglobulin gene rearrangements using consensus primers lacks high sensitivity. Nevertheless, if the patient has had previously positive molecular data, these methods for the detection of residual disease may be useful.

References

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