Monitoring Multiple Myeloma

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Abstract: Multiple myeloma (MM) is a B-cell malignancy characterized by the accumulation in bone marrow of terminally differentiated plasma cells. MM is a slowly growing, heterogeneous disease with no known cure. Patients with MM have a median survival of approximately 5 years, during which they may experience significant morbidity. More reliable and rapid determination of changes in the clinical status of patients with MM is becoming increasingly important because of the increasing number of available treatments for these patients. Currently available tests for monitoring patients with MM most often include assessments of monoclonal paraprotein and serum free light chain levels, but the tests have several limitations. Measurement of serum B-cell maturation antigen level may overcome these limitations and improve outcomes for patients with MM. Newer radiologic procedures such as positron emission tomography/computed tomography are superior to plain radiographs, but are costly and inconvenient. Bone marrow examination directly identifies malignant cells, but the heterogeneous nature of the disease makes it problematic to use routinely to follow patients with MM. The development of new markers and approaches to more accurately and quickly assess changes in tumor burden in patients with MM should result in better outcomes for these patients.

Introduction

Multiple myeloma (MM) is a B-cell malignancy characterized by the accumulation in bone marrow of terminally differentiated plasma cells, all of which produce the same monoclonal immunoglobulin. MM is a slowly growing, heterogeneous disease with no known cure. Patients with MM have a median survival of approximately 5 years, during which time they may experience significant morbidity.[1] Until recently, MM was defined by the presence of end-organ damage, manifesting as hypercalcemia, renal failure, anemia, and bone lesions (CRAB).[1] After clinicians diagnose MM, they must stage the disease, stratify it by risk, and estimate the prognosis.[2] These steps guide patient counseling, clinical decision making, and treatment selection. In addition, the assessment of treatment response is crucial for ongoing management of disease.

When the number of therapies for MM grew, it became increasingly important to define a uniform prognostic index that would

allow physicians to individualize treatment decisions and monitor patients' responses to specific therapies quickly and accurately. In 2005, the International Staging System (ISS)[3] was established on the basis of the outcomes of 10,750 patients with MM from North America, Europe, and Asia who were treated with standard therapy or autologous stem cell transplant.[3] Serum levels of albumin and β_2-microglobulin were used to evaluate outcomes.

Traditional prognostic factors, such as blood cell counts, renal function, hypercalcemia, M-protein levels, percentage of bone marrow involved, and immunoglobulin isotypes, were not found to have prognostic significance on multivariate analysis.[4,5] Genetic abnormalities were not included when the ISS was formulated until Avet-Loiseau and colleagues presented data demonstrating that high-risk cytogenetic features and lactate dehydrogenase (LDH) levels had prognostic significance that was independent of the ISS.[4] This finding led the International Myeloma Working Group (IMWG) to modify its staging criteria in 2016 to include the ISS stage, cytogenetic features, and LDH levels.[6] The new revised system is known as the ISS-R.[6-8]

With the ISS-R, patients are segregated into 3 risk groups on the basis of cytogenetic features and LDH data. High-risk disease is characterized by an ISS stage of II or III and the presence of t(4;14) and/or del(17p13), detected by fluorescence in situ hybridization (FISH), whereas low-risk disease is characterized by an ISS stage of I or II and the absence of these high-risk genetic features. Disease between these 2 categories is considered intermediate risk.[7,8] Even though evidence supports the superiority of ISS-R staging compared with Durie-Salmon staging, established in 2006, in identifying a truly high-risk subset of patients,[9-11] the system has been controversial. The study establishing ISS-R included only patients enrolled in experimental trials and 65% of them were younger than 65 years, whereas the average age of patients at diagnosis of MM is 70 years.[12] Additionally, information about chromosome 1 abnormalities was not collected in all trials, so that this prognostic parameter was omitted from the ISS-R staging.[8] Lastly, lack of interlaboratory standardization of FISH analysis and inconsistent cutoffs for LDH levels are additional limitations of the modified staging system.[8] Some studies, including our own, have challenged the validity of ISS staging within various subpopulations of patients with MM.[13-15] Other studies have shown that additional prognostic factors could improve the prediction of survival[16,17] and that acquired chromosomal transformations can change the predictive capabilities of the ISS.[18,19] As mentioned earlier, the IMWG guidelines also consider circulating protein markers, measured by protein electrophoresis and immunofixation, and serum free light chain (sFLC) analysis with derived κ/λ ratios for monitoring MM.

Monoclonal Paraprotein

Measuring monoclonal immunoglobulins in the serum of patients with MM remains the gold standard for monitoring the disease. Because the monoclonal immunoglobulin can be identified and quantified in most cases as an M spike on serum protein electrophoresis (SPEP),[20,21] tracking the monoclonal paraprotein (M-protein) concentration is considered one of the best ways to monitor a patient's response to treatment. Each M protein consists of 2 heavy chains (immunoglobulin G [IgG], IgA, IgM, IgD, or IgE) and 2 light chains (κ or λ). In most patients with newly diagnosed MM, the M-protein concentration is higher than 3 g/dL; however, after effective treatment, the M-protein concentration is reduced as monoclonal antibody–producing malignant clonal plasma cells are eliminated.[22] These changes in the M protein are routinely visualized by SPEP and/or immunofixation electrophoresis, the latter of which can determine whether the M protein disappears entirely. Although these techniques are adequate for many patients with MM, monitoring those in whom light-chain-only, oligosecretory, nonsecretory, or specific for IgA/IgM heavy chain MM is diagnosed can be challenging.[23] The slow rate of change in serum M-protein levels can also be problematic when the effects of new therapies for MM need to be assessed quickly.[23] Thus, these tests are generally performed monthly for patients on active therapy. One of the problems of using SPEP to assess changes in the clinical status of patients with MM is that in those with monoclonal IgA or IgM paraproteins, the M protein often overlaps with non-immunoglobulin proteins on the SPEP pattern, which makes accurate quantification of the M-protein levels both challenging and subjective. In addition, measuring M-protein levels is not applicable in patients with oligosecretory or nonsecretory disease because in these cases the levels of the paraprotein are low or nondetectable, respectively. Currently, the only way to assess myeloma tumor burden in patients with nonsecretory MM is with frequent bone marrow examination, a procedure that is both highly invasive and costly (see later section, "Bone Marrow Examination") and positron emission tomography/computed tomography (PET/CT), which is also costly and inconvenient. For patients with oligosecretory or light chain–only MM, the relatively new sFLC assay has been used. However, the test produces inconsistent results and so is not very reliable (see later section, "Serum Free Light Chain"). Another limitation of using serum M protein to monitor patients with MM is that it has a long half-life (approximately 21 days[24]), so the levels change very slowly, limiting the usefulness of M-protein measurement to detect rapid changes in disease status. More recently, the use of antibody-based therapeutics for patients with MM, such as daratumumab (Darzalex, Janssen Biotech)

and elotuzumab (Empliciti, Bristol-Myers Squibb), has been shown to complicate the interpretation of results generated by protein electrophoresis in patients receiving these treatments. Specifically, the use of humanized IgG κ monoclonal antibodies to treat patients with IgG κ MM may result in detectable M protein, which can be misinterpreted as evidence of residual disease, a false-positive test result.[25] Thus, tests that provide a more accurate assessment of changes in disease status, and more rapidly in real time, are needed to monitor patients with MM.

Serum Free Light Chain

Measurement of immunoglobulin sFLCs in patient serum became a practical option with the commercial availability of polyclonal nephelometric assays in 2001.[26] For more than a decade, it has been shown to be a superior alternative to urine studies owing to its convenience and better sensitivity. Previously, clinicians had to rely on urine samples to monitor patients with light chain MM, typically over a 24-hour period—an approach that often yields inaccurate results.[27,28] Studies have also shown that sensitivity is significantly improved by sFLC analysis when patients with MM who have residual disease are being monitored.[29] FLCs have a short half-life (2-6 hours) and therefore have been identified as having the potential to provide early monitoring of disease.[30,31] The IMWG guidelines currently recommend that sFLC assays be used in diagnostic screening panels and that the sFLC be used as a "biomarker of malignancy" (difference between involved and uninvolved sFLCs ≥100 mg/L) to define the presence of MM in the absence of other indications.[32] When response is evaluated, however, the IMWG recommends sFLC measurement only for patients in whom the levels of other markers are below what can be reliably measured—that is, a serum M-protein level of 10 g/L or less and a urine M-protein level of 200 mg or less per 24 hours.[33,34]

Many have argued that sFLC measurement is more sensitive and accurate than urine electrophoresis for patients with MM, and they now advocate that urine studies be replaced with sFLC evaluation.[35,36] In addition, Mori and colleagues have argued that although they are not a better biomarker for monitoring disease in patients with an intact immunoglobulin, relative changes in M-protein levels assessed with SPEP and sFLC levels improved the detection of residual disease after induction therapy with the proteasome inhibitor bortezomib (Velcade, Millennium/Takeda Oncology) and the immunomodulatory agent thalidomide (Thalomid, Celgene) or lenalidomide (Revlimid, Celgene).[37] Moustafa and colleagues have noted that normalization of the sFLC ratio, regardless of the depth of response, adds significant prognostic value to the monitoring of progression-free survival (PFS) and overall survival (OS) in patients with MM.[38] Additionally, the newer class of anti-MM drugs, the monoclonal antibodies, has brought about its own unique set of challenges for monitoring MM.[24] In a preliminary in vitro study, Rosenberg and colleagues showed that although treatment with monoclonal antibodies can confound the interpretation of SPEP and immunofixation electrophoresis results (especially in patients with IgG κ MM), treatment with daratumumab showed no effect on sFLC levels.[39]

Although there seems to be no shortage of publications supporting the use of sFLCs to monitor MM, the clinical utility of sFLC monitoring remains limited. The limitations are broad and by no means insignificant. The percentage of patients with MM who have sufficiently elevated levels of sFLCs is low, and therefore a significant number of subjects in whom this test can be used for a clinical evaluation of MM are excluded. Khoriaty and colleagues noted that although sFLC was a sensitive and specific biomarker for MM, its levels were elevated to a level that could be assessed in fewer than half (48%) of patients with MM.[40] In another study, Dejoie and colleagues reported that 98 of 157 patients (62%) with MM who had intact immunoglobulin had measurable disease with sFLC testing.[35] Among patients with renal failure, serum levels of FLC increase as much as 20- to 30-fold, so that the sFLC assay is often unreliable owing to antigenic excess.[41] Given that myeloma-related renal impairment occurs frequently in patients with MM, a significant portion of the MM population cannot receive an accurate assessment with the sFLC assay.

In 2016, the IMWG released a consensus statement updating the internationally accepted guidelines for monitoring patients with MM.[34] This most recent update continues to suggest that the sFLC assay can be used to assess response only when both the serum and urine M-component levels are deemed unmeasurable. The panel suggests that response measured with the sFLC assay can be either partial (indicated by a ≥50% reduction in the difference between involved and uninvolved sFLC levels) or complete; complete response (CR) is defined by the absence of evidence of disease on bone marrow biopsy and on serum and urine immunofixation electrophoresis, and by normalization of the sFLC ratio. Recently, it has been shown that unconventional immunologic recovery may abrogate normalization of the sFLC ratio even in the absence of any evidence of disease. In the study of Abbi and colleagues, in a cohort of 142 patients with MM who had achieved a CR, 17 (12%) were found to have an abnormal sFLC ratio without any other evidence of residual disease, including normal results of flow cytometry and FISH on highly selected plasma cells.[42] Interestingly, all of the abnormal FLC ratios were found among the patients with a κ-restricted type of MM.

Perhaps the most important limitation of evaluating sFLCs is the lack of a universal assay standard, which results in significant assay imprecision.[43,44] Tate and colleagues reported that in different reagent lots, sFLC results varied by as much as 45%, with median lot-to-lot variations of 19% and 20% for κ and λ light chains, respectively.[43] Additionally, discrepancies of between 17% and 32% were found in the sFLC ratios, suggesting that extreme caution is needed when the results of this test are interpreted. Furthermore, since its development in 2011, the N Latex FLC monoclonal antibody assay (Siemens) has introduced another significant complication: nonequivalence. It has been shown by 2 independent groups that the results of 2 different sFLC tests (N Latex FLC and Freelite [Binding Site]) vary with the testing platform, and that the 2 techniques are comparable but not equivalent.[45,46] They could not recommend an inter-platform comparison of results. It is because of this wide variation that Tate and colleagues, along with others, have called for a universal international standard to guarantee harmonization of values among different laboratories. Problems with the sFLC assay also include false-negative interpretation,[45,46] polymerization leading to as much as a 10-fold overestimation,[43,47] and nonlinearity upon serial dilution.[43]

An alternative method for monitoring M proteins has recently become available; the automated serum heavy/light chain (HLC) immunoassay (Hevylite, Binding Site) separately measures the intact immunoglobulin of each light chain type, and κ/λ (HLC) ratios can be derived to provide clonality.[48] Michallet and colleagues demonstrated that the HLC immunoassays were more sensitive than the conventional electrophoretic method for stratifying patients who had achieved a response that was partial or better: partial response (PR), very good PR, or CR. The drawback, however, is that patients with light chain myeloma and oligosecretory disease were not included in the analysis.[48]

Taken together, the benefits and drawbacks of the serum FLC and HLC assays suggest that the search for more reliable biomarkers must continue if a rapid and accurate method of evaluating a patient's response to MM therapy is to be developed.

Positron Emission Tomography/Computed Tomography and Magnetic Resonance Imaging

Medical imaging has played an important role in the diagnosis and initial staging of MM, and in differentiating it from other monoclonal plasma cell dyscrasias. Although the modified Durie-Salmon staging system, which was established in 2006, eliminated the detection of focal lesions as a criterion for the diagnosis of MM, this is still widely used as a functional parameter relevant to MM. A characteristic feature of the osseous (bone) manifestations of MM is that the lesions regress only slowly or not at all, even among patients with a CR.[49,50] Therefore, conventional radiography and computed tomography (CT) are not adequate for treatment monitoring. CT to assess bone lesions is supplemented by positron emission tomography (PET), which is highly sensitive for the detection of isolated focal medullary lesions. Additionally, PET/CT has been used for the initial staging and treatment monitoring of nonsecretory MM.[51,52] Several studies have demonstrated the higher sensitivity of PET/CT for the detection of focal bone lesions in comparison with conventional radiography.[53,54] PET/CT testing was improved with the introduction of radioactive tracers, such as ^{18}F-fluorodeoxyglucose; this makes it possible to differentiate between active and inactive lesions, which is especially useful during monitoring for possible relapse after the initiation of treatment for MM.[55] Although PET/CT-based methods have been shown to have a high degree of sensitivity and specificity and to be of key prognostic value in MM, PET/CT is still an expensive and somewhat inconvenient test.

Another imagining technique used to detect bone lesions and bone involvement in patients with MM is magnetic resonance imaging (MRI).[56] It is currently accepted that MRI and PET/CT are equally effective for detecting focal lesions, but MRI is better for detecting diffuse disease.[56-58] A recent study by Moreau and colleagues directly compared MRI and PET/CT with respect to the detection of bone lesions at diagnosis and prognostic value.[59] They demonstrated that at diagnosis, MRI results were positive in 95% of patients and PET/CT results were positive in 91% of patients.[59] These findings suggest that the 2 techniques are of comparable effectiveness in detecting bone lesions at diagnosis and may be complementary for defining response, as recently proposed by the IMWG.[10,32] Owing to the small sample size in this study, however, the results should be interpreted with caution, and it should be kept in mind that normalization of MRI findings after 3 cycles of therapy and before maintenance therapy was not predictive of PFS or OS.[59]

Bone Marrow Examination

Bone marrow examination continues to be the cornerstone for establishing the diagnosis of MM.[50] In association with other clinical and laboratory parameters, bone marrow findings are used to differentiate patients with indolent precursors of active MM, such as monoclonal gammopathy of undetermined significance (MGUS), from those with smoldering MM. Bone marrow examination is also the currently accepted way to evaluate the

course of disease in patients with nonsecretory myeloma, and it is required for the definitive categorization of a CR to anti-MM therapy.[60] The biopsy procedure has proved safe and is only rarely associated with any serious adverse events, the most common of which is hemorrhage.[51] Traditionally, core biopsy samples are collected and evaluated for morphologic characteristics indicative of MM, and plasma cell infiltration is measured with immunophenotypic methods.[52] The most recent consensus statement from the IMWG regarding the monitoring of MM states that bone marrow plasma cell estimation for diagnosis is based on either conventional bone marrow aspiration or biopsy; however, the measurement is preferably derived from a core biopsy specimen.[32]

Bone marrow biopsy, which has long been considered the most accurate method of measuring plasma cell infiltration, is not without limitations. Although many institutions continue to rely solely on bone marrow aspiration, this is unwise. The typical aspirate smear tends to underestimate the degree of plasmacytosis owing to differences in the level of blood contamination and sampling variability resulting from focal disease distribution. As has been demonstrated on multiple occasions,[53-55,60] immunohistochemically stained trephine biopsy samples consistently show more malignant plasma cell infiltration than do the corresponding aspirate samples. In one study, Wei and colleagues[61] reported that 25% of the patients sampled would have been misclassified as having less extensive disease if results from the examination of aspirate smears had been used alone rather than concurrently with results from the examination of CD138-stained trephine samples. This finding indicates the need for trephine biopsy and CD138 immunohistochemistry to ensure that the most accurate estimate of bone marrow plasma cell infiltration is obtained. In the past decade, CD138 immunohistochemical staining of a trephine biopsy section has been repeatedly validated as a superior method of identifying malignant myeloma cells,[62-64] and it has been widely adapted to measure bone marrow plasmacytosis. Although CD138 immunohistochemical staining requires more extensive training than traditional hematoxylin and eosin staining, the accuracy of the former technique makes the additional investment well worthwhile.

Minimal Residual Disease

Recent advances, including the introduction of novel therapies to treat MM, have brought about unprecedented improvements in the PFS and OS of patients with MM.[62,65,66] The number of newly approved MM therapies continues to grow, and they are expected to improve further the outcomes of patients with MM. Because of the changes, clinicians must now ask themselves an important question: should the aim of future MM therapy be palliative or curative? Novel, more sensitive primary endpoints have become a focus of myeloma research so that the potentially increasingly curative profile of MM therapies can be better evaluated.[67] Reservoirs of residual disease—so-called minimal residual disease (MRD)—are thought to be the cause of eventual relapse in patients with MM. The ideal test for MRD should have several relevant characteristics, including a high degree of applicability, sensitivity, specificity, feasibility, accessibility, and reproducibility. It should also be of proven clinical value. To date, none of the currently available approaches has achieved all these goals. However, novel testing methodologies continue to be developed and improved to measure MRD more sensitively. The most commonly used modalities for monitoring MRD are allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), next-generation sequencing (NGS), and multiparametric flow cytometry (MFC).

Allele-Specific Oligonucleotide Polymerase Chain Reaction

ASO-PCR of diverse heavy chain rearrangements is used to assess the MRD status of patients with MM.[68,69] ASO real-time quantitative PCR (ASO-qPCR) has replaced the less sensitive ASO-PCR. However, its future clinical utility as a test for MRD status remains underwhelming. One significant shortcoming of ASO-qPCR is its failure rate. ASO-qPCR is feasible only in up to 80% of patients, owing to the lack of known clonal targets for amplification in the IgH locus in the remaining 20% of individuals.[70] Additionally, ASO-qPCR requires patient-specific reagents, which greatly increases the cost of the test and the expertise needed to run the test.[71] Like other methods for determining MRD status, PCR relies on bone marrow biopsy sampling. This type of sampling is invasive, and results can be inaccurate owing to the heterogeneous nature of bone marrow involvement. When the El Programa Español de Tratamientos en Hematología (PETHEMA) group used ASO-qPCR to determine MRD status, only 42% of samples were evaluable because of lack of clonality, unsuccessful sequencing, and/or suboptimal ASO performance.[69] However, the correlation between ASO-qPCR and multicolor flow cytometry was found to be strong when the tests were evaluated in the patients for whom MRD testing was feasible (correlation coefficient for bivariate analysis [r]=0.881, P<.0001).[35] In another study,[71] when the 2 techniques were compared with the results of NGS to measure MRD status in 378 samples from 55 patients, both routinely reached the same sensitivity level of 1×10^{-5} (≥1 myeloma cell in 10^5 bone marrow cells), and excellent concordance was observed. Moreover, several studies in which ASO-qPCR was used have shown that it determines outcomes effectively in the transplant setting.[72-74] Although MRD evaluation by

ASO-qPCR is sensitive and specific, it has been found to have serious shortcomings and has been supplanted by NGS methods.

Next-Generation Sequencing

The development and refinement of NGS techniques, such as massive parallel sequencing, will undoubtedly reshape the MM landscape over the years to come with profound revelations regarding prognostication, therapeutic choice, and disease monitoring. This sequencing-based method uses consensus primers to amplify and sequence all rearranged immunoglobulin gene segments present in a myeloma cell. Specifically, NGS has the potential to redefine CR and identify reservoirs of residual disease. A high degree of analytic reproducibility with the use of a specific, in-house technique has been demonstrated,[65] and it appears this same fully automated technique can be implemented in any laboratory with NGS capability, minimizing variation among laboratories.

Preliminary studies suggest that NGS is a widely applicable test that measures positivity in 91% of cases of MM.[70] Additionally, NGS-based assessments have been found to measure residual disease with a sensitivity of 0.0001% (<1 in 10^6 cells) and are at least as sensitive as[70] and possibly more specific than[72,74,75] ASO-PCR assessments. NGS has demonstrated the ability to monitor subclonal evolution, which is a known mode of disease progression owing to the Darwinian branching nature of MM.[71] In the future, it may be possible to use NGS methods to monitor MRD with peripheral blood samples instead of bone marrow samples. Pilot studies have already begun, with relatively promising results.[76] In fact, recent studies suggest that peripheral blood may be a more accurate indicator of MRD than bone marrow–derived cells.[77]

Despite the early promising results with blood testing, repeated bone marrow aspiration, which is painful and difficult for patients, is currently required for NGS.[76] The quality of the specimen tested is an important factor in assessing MRD.[71] Additionally, relapse still occurs in many patients who achieve MRD-negative status, suggesting that the threshold for the number of detectable cells with which MRD has traditionally been evaluated is too low and requires further improvement.[78] One other important drawback of NGS is its reliance on a substantially infiltrated baseline sample for effective clonotypic identification.[74] Finally, as the disease progresses, somatic hypermutation[71] can confound the results of the test. Somatic hypermutation is a characteristic of MM,[79] suggesting that a substantial number of false-negative results are caused by clonal evolution.

Multiparametric Flow Cytometry

The MRD measurement obtained with MFC has been identified as a possible early prognostic biomarker.[80] Patients with MM who achieve MRD-negative status have been shown to have a longer PFS and a longer OS.[80-82] Originally, MFC methods were validated for use only among patients receiving a stem cell transplant[83]; however, their utility has expanded to include virtually all patients with MM.[84-87] Additionally, the methods used by MFC to detect MRD among patients who have achieved a CR in response to MM therapy have improved dramatically. Most recently, next-generation flow cytometry has been shown to improve the sensitivity and prognostic capability of both the first-generation 4- or 6-color MFC assays and the newer 8-color MFC assays.[87] The greater sensitivity of next-generation flow cytometry than of conventional flow cytometry was attributed to an approach that optimized a combination of fluorochromes and antibody reagents to increase specificity at very low MRD levels, allowing a greater number of cells to be evaluated. These new methods were shown to provide a sensitive, more standard approach for MRD detection in MM, which purportedly overcomes some of the major limitations of conventional flow cytometry methods, such as lack of precision and standardization.[87]

However, many barriers must still be overcome before MFC can be implemented as a practical means of monitoring MM. Although improvements have been made, there remains no standard MFC method. Additionally, although the IMWG has incorporated MRD status in its response criteria,[34] no consensus exists on the role MRD status should play in patients who do not achieve a CR. A high level of expertise is required to perform the assay properly, and great care must be taken to avoid the pitfalls of the test. During their validation, Flores-Montero and colleagues[87] noted that blood contamination, which has the potential to confound the assay results, was present in 17 of 110 bone marrow samples from patients with a very good PR or better, including the only 2 samples from patients with disease progression. In these cases, the authors suggested that a second bone marrow sample might be needed—an unwelcome procedure for frail or elderly patients with MM. Additionally, given the heterogeneous immunophenotypic presentation of normal plasma cells,[87] the authors of the study indicated that the optimization techniques required to improve the sensitivity and specificity of their assay required 5 separate combinations of fluorochrome-conjugated antibody clones to be tested, and that the composition of the optimal panel could not be predicted even by someone with pre-existing expertise. Although the costs of a successful assay have been touted as manageable,[80] they increase significantly if a multitude of next-generation flow cytometry reagents are necessary.

The increasingly sensitive assays continue to outperform their predecessors, suggesting that survival benefits are improving with each technologic advance.[86-89] Further improvements are sure to be made in the future, which paradoxically means that the absolute definition of MRD remains open. Finally, the use of antibody therapies has already been shown to have the potential to interfere with the markers used in the flow assay.[90] Flores-Montero and colleagues[87] noted that only 2 of many CD38 antibody clones proved effective for detecting CD38 on malignant plasma cells from patients who had MM treated with daratumumab. As more emphasis is placed on developing a greater number of monoclonal antibody therapies, a greater number of malignant plasma cell markers may be affected, and MRD test results from patients receiving these treatments may be difficult to interpret.

Other Biomarkers

Although a panoply of potential biomarkers have been suggested,[91] few appear to provide accessible, accurate, and consistent clinical information. Serum interleukin 6 (IL-6) is a major growth factor in MM.[92] This pleiotropic cytokine[93] activates many pro-survival and anti-apoptotic pathways simultaneously, affecting myeloma cells primarily through paracrine signaling from bone marrow stromal cells, and to a lesser extent through autocrine signaling in a subset of patients.[94-96] However, IL-6 remains a questionable indicator of disease.[97] Although some groups found that survival times were significantly shorter in patients whose IL-6 levels remained above a threshold of 7 pg/mL,[98] others were unable to find a correlation between plasma IL-6 levels and disease activity.[99]

Sclerostin, a potent antagonist of Wnt signaling secreted by mature osteocytes[100] in normal bone tissue, is overexpressed in patients with MM.[101,102] Additionally, elevated serum sclerostin levels have been found to correlate with reduced osteoblast function and poor survival.[103] Unfortunately, these results have been difficult to replicate, and other groups have been unable to detect significant differences between the sclerostin levels of patients with MM and those with MGUS or healthy donors.[104] Age[105] and proximity to bone marrow[106] may both play a role in explaining these discordant results. Treatment with corticosteroids, specifically dexamethasone, is known to elevate sclerostin expression.[107] Elevated levels of sclerostin among patients with MM therefore may be the result of superficial upregulation of sclerostin via a glucocorticoid-mediated pathway. This suggests that the marker may play a role only in patients not taking dexamethasone as part of their myeloma therapy, which is uncommon with current treatment regimens for MM.

Syndecan-1, also known as CD138, is a heparin sulfate proteoglycan that is abundant on the surface of malignant and healthy plasma cells.[108] Levels of soluble syndecan-1 are higher in patients with MM than in healthy donors.[109-111] Many promising studies have suggested that syndecan-1 is a significant prognostic marker for MM[109-116]; however, its utility for monitoring MM remains an open question. Evaluation of syndecan-1 levels in patients with MM showed much higher levels in bone marrow than in peripheral blood samples,[115] suggesting that results would be more accurate if the more invasive procedure were performed to assess syndecan-1 levels. Baseline serum levels of syndecan-1 have been found to be lower in patients who responded to chemotherapy than in nonresponders, but they do not predict therapeutic response or survival.[109] In one study, syndecan-1 expression was shown not to correlate with the degree of bone marrow or peripheral blood involvement.[116] Taken together, the results of various studies evaluating soluble syndecan-1 are mixed, and further investigation is required before syndecan-1 measurement can be included in the repertoire of MM tests.

Measurement of the serum levels of B-cell maturation antigen (BCMA), one of the new protein markers used to follow patients with MM, may be without the limitations of the tests previously discussed. BCMA (or TNFSFR17) is an extracellular protein present on the surface of mature B cells and terminally differentiated plasma cells.[117] It has been shown to be shed from the surface of plasma cells through the action of the enzyme complex gamma secretase.[118] Shed BCMA is found in both the serum and plasma of patients with MM, and levels are higher than those in age- and sex-matched healthy subjects.[119] Specifically, serum BCMA levels are higher in patients with active MM than in those with smoldering disease, whereas levels are lower in patients with MGUS than in those with smoldering MM.[119] Notably, BCMA levels can be measured in very small amounts of serum with a routine enzyme-linked immunosorbent assay (ELISA). Evaluation of the serum BCMA level in patients with MM can indicate changes in clinical status because the levels trend with the traditional MM markers, including M-protein elevation and bone marrow plasmacytosis. BCMA is also the first circulating marker that can be used to follow patients with nonsecretory myeloma.[119] An important advantage of this new biomarker is that changes in clinical status can be assessed more rapidly than with conventional serum M protein in patients with secretory disease because the turnover of BCMA in serum (24-36 hours)[17] is much more rapid than that of M protein (3-4 weeks).[120] Recently, our group showed that monitoring of serum BCMA levels at least weekly during the first cycle of a new therapy was consistently

more effective than monitoring of serum M protein because BCMA levels changed more rapidly than those of the conventional marker.[117] Moreover, BCMA levels are independent of renal function,[120] so that results are more reliable than those obtained with sFLC levels in patients with MM; this finding was confirmed when levels of the 2 biomarkers were compared in patients with MM assessed at multiple time points during their disease course.[17] BCMA also predicts both PFS and OS in patients with MM.[120] Thus, this single protein marker, serum BCMA, has the potential to be a better monitoring tool for following patients with MM and predicting their outcomes.

Conclusion

The more reliable and more rapid determination of changes in the clinical status of patients with MM is becoming increasingly important as they and their treating physicians consider an expanding number of therapies during the course of disease. A goal will be to use these markers to help determine optimal therapeutic choices for individual patients. Currently available tests for monitoring most often include periodic assessments of M-protein and sFLC levels, but the tests have several limitations. M-protein assessment is not applicable in many patients with MM, and the slow changes in M-protein levels delay the detection of changes in clinical status that often become clinically significant. The sFLC levels also cannot be assessed in many patients undergoing treatment. Levels of sFLC may be unreliable, especially in patients with renal impairment, which is common in MM. The development of tests that can more quickly and reliably assess changes in clinical status, such as measurement of the serum BCMA level, may overcome these limitations and improve outcomes for patients with MM. Newer radiologic procedures, such as PET/CT, have identified lesions that are not seen on plain radiographs, but these are costly and inconvenient. Bone marrow examination directly identifies malignant cells, but the heterogeneous nature of myelomatous involvement within the marrow and the use of different techniques to obtain bone marrow, aspiration vs biopsy, makes it problematic to use bone marrow examination to follow patients with MM routinely. These problems also are relevant when bone marrow is used as a tissue source to determine MRD. Patients with MM are being more frequently assessed for MRD as treatments become ever more effective, with the result that the lower levels of tumor cells remaining in patients cannot be identified with conventional protein-based tests. The multiple approaches used to assess MRD—ASO-PCR, NGS, and MFC—are becoming more reliable, but they are expensive, and their importance in clinical decision making has not been demonstrated.

The development of new markers and approaches to assess changes in tumor burden and level of involvement in patients with MM more accurately and quickly will provide several clinical benefits. First, patients will learn sooner whether therapies are working, so that they can avoid the unnecessary side effects of ineffective treatment and move on to another therapeutic option before their disease further affects them adversely. Second, it may be possible to use fewer drugs and lower doses if the markers can detect changes in clinical status within days rather than months. Physicians will then add more drugs and use higher doses only if the less aggressive treatment is ineffective, and unnecessary side effects and the costs of additional agents and higher doses will be avoided. Third, the new biomarkers may detect changes in tumor burden quickly enough to help guide the dosing and scheduling of new drugs. For example, measurement of serum BCMA levels has indicated that during monthly treatment with immunomodulatory agents, the tumor burden increases during the standard 1-week-off period,[17] suggesting that continuous treatment with these agents may be optimal. Fourth, the techniques may help determine whether patients can discontinue therapy without compromising their outcomes, and also detect patients with early disease progression who might benefit from early therapeutic intervention. It will be important to generate carefully designed studies that can provide clear data to support the utility of these markers and techniques in improving the outcomes of patients with MM.

Disclosures

Dr Berenson is a director of OncoTracker, Inc, a company that is involved in the development of BCMA as a disease marker. Dr Spektor and Mr Udd have no disclosures or conflicts of interest to report.

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