CLINICAL TRIALS AND OBSERVATIONS

Brief report

The relationship between the serum free light chain assay and serum immunofixation electrophoresis, and the definition of concordant and discordant free light chain ratios

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"Stringent" complete remission in myeloma has been defined by a normal serum free light chain ratio (SFLCR) in addition to the standard criteria for CR. 2648 serial samples from 122 IgG or IgA myeloma patients were studied to explore the relationship between SFLCR and serum immunofixation electrophoresis (SIFE). SFLCR was normal in 34% of cases with positive SIFE and abnormal in 66%. SFLCR was normal in 69% of cases with negative SIFE and abnormal in 31%. When evaluated with SIFE as the benchmark, the sensitivity of SFLCR was 66% and specificity was 69%. These findings were unchanged when abnormal SFLCR values were classified as concordant (< 0.26 for λ disease and > 1.65 for κ) or discordant (< 0.26 for κ disease and > 1.65 for λ). Additional studies are required to determine the temporal relationship between SFLCR normalization and paraprotein clearance. Until then, the role of SFLCR in defining response remains controversial. (Blood. 2009;114:38-39)

Introduction

The serum free light chain (SFLC) assay enables detection of an abnormal protein in patients with plasma cell dyscrasias who secrete no or small quantities of monoclonal protein in the serum.[1,2] Thus, the SFLC ratio (κ:λ; SFLCR) may be abnormal (normal range, 0.26-1.65) in patients who have a negative serum immunofixation electrophoresis (SIFE). However, the relationship between SFLCR and SIFE in patients who do secrete detectable paraprotein has not been studied.

Moesbauer et al reported 5 patients in whom normalization of SFLC levels and SFLCR preceded SIFE negativity by approximately 5 weeks.[3] They also reported 4 patients in whom SFLC levels increased a median of 3 months before SIFE became positive. It was not specified if the change in SFLC levels was associated with an abnormal SFLCR. Presumably partly based on this, the newly proposed international response criteria for myeloma define an entity called "stringent" complete remission (CR) based on negative SIFE and normal SFLCR (with absent clonal plasmacytosis).[4] The definition of "CR" is similar to that in the old criteria,[5] and it is distinguished from "stringent CR" by lack of requirement of normal SFLCR. The new criteria aim to replace the old, which did not specify SFLC-based assessment at all. The new criteria have yet to be validated prospectively in clinical practice, whereas the old criteria have been used in multiple clinical trials.

The obvious implication of using normalization of SFLCR to define "stringent CR" is that SFLCR is the most sensitive indicator of residual disease. Thus, one would expect to see abnormal SFLCR when SIFE is normal but not vice versa. We wanted to explore the relationship between SIFE and SFLCR in patients with secretory IgG or IgA myeloma.

Methods

From among patients with myeloma investigated and treated at Northwestern University between January 2004 and June 2008, 2648 samples from 122 patients were identified that satisfied the following criteria: known IgG or IgA paraprotein heavy-chain isotype, and the availability of concomitant SFLC assay and SIFE. Patients with biphenotypic disease were excluded. Patients known to have either IgG or IgA disease but with more than one identifiable band in any of the samples on SIFE, usually oligoclonal bands during immune reconstitution during or after therapy, had those samples excluded. These inclusion and exclusion criteria ensured lack of any ambiguity in interpretation of either SIFE or SFLC.

Samples were collected from patients serially at various stages of the disease and therapy, ranging from initial presentation to remission after therapy to relapse. This retrospective review was approved by the Institutional Review Board of Northwestern University, and patients' informed consent was obtained in accordance with the Declaration of Helsinki.

SFLC levels were measured using standard techniques (Freelite, The Binding Site) performed on a Dade-Behring Nephelometer.[1] The χ^2 test was used to assess the significance of the relationship between SFLCR and SIFE.

The usual practice is to simply classify SFLCR as normal (0.26-1.65) or abnormal (< 0.26 or > 1.65). In this analysis, we also explored the concept and significance of concordance or discordance of an abnormal SFLCR.[6] An abnormal SFLCR was considered concordant if less than 0.26 for λ disease and greater than 1.65 for κ disease, and discordant if less than 0.26 for κ disease and greater than 1.65 for λ disease. Our hypothesis was that a discordant SFLCR should be physiologically analogous to normal because it does not reflect an excess of the abnormal light chain associated with the original monoclonal protein.

Results and discussion

SIFE showed the original M protein in 2342 samples (88%). SFLCR was abnormal in 1636 (62%). The abnormal ratio was concordant in 1536 and discordant in 100.

Table 1. The relationship between SFLC and SIFE

SIFE	SFLC ratio				
	Normal	Abnormal	Discordant abnormal	Concordant abnormal	Normal or discordant abnormal
Positive	802 (79%)	1540 (94%)	72 (72%)	1468 (96%)	874 (79%)
Negative	210 (21%)	96 (6%)	28 (28%)	68 (4%)	238 (21%)
P		<.001 vs normal	.092 (vs normal)	<.001 vs discordant abnormal	<.001 vs concordant abnormal

SFLC indicates serum free light chain; and SIFE, serum immunofixation electrophoresis.

As Table 1 shows, the proportion of patients with normal SFLCR, but a positive SIFE was much greater than those with an abnormal SFLCR and a negative SIFE. The pattern of SIFE results with discordant abnormal SFLCR was similar to normal SFLCR and significantly different from concordant normal SFLCR, suggesting that abnormal SFLCR should be classified into discordant and concordant and only the latter considered indicative of potentially active disease. It is possible that a discordant abnormal SFLCR is analogous to oligoclonal bands being detected on SIFE.

SIFE is currently the standard indicator of the presence of a monoclonal protein. When evaluated against that standard, the sensitivity of SFLCR in detecting the presence of a monoclonal protein was 66% and specificity 69%. The sensitivity dropped to 63%, but specificity improved to 77% if discordant abnormal SFLCR were considered normal. What if either positive SIFE or concordant abnormal SFLCR were considered to signify residual disease (2410; 91%), and a negative SIFE as well as a normal or discordant abnormal SFLC ratio were required to rule out residual monoclonal protein (238; 9%)? In this case, the sensitivity of SIFE was 97% and that of an abnormal concordant SFLCR 62%. These assumptions do not allow calculation of specificity.

Although the presence of an abnormal SFLCR in the setting of a negative SIFE may be expected because of the greater sensitivity of the SFLC assay in some clinical situations (hyposecretory disease and some cases of nonsecretory disease), approximately one-third of samples showed a normal SFLCR despite positive SIFE, an unexpected finding. This unusual finding can be explained satisfactorily only if serial data show that normalization of SFLCR on therapy is invariably followed by SIFE negativity (ie, normalization of SFLCR heralds impending paraprotein clearance) and SFLCR invariably becomes abnormal before SIFE becoming positive on relapse. However, no such serial observations are available, including in this particular analysis, to answer this important question. We are in the process of studying serial correlation between the SFLCR and SIFE.

SFLC estimation allows measurement of tumor burden in situations in which more conventional assays are not feasible, such as selected nonsecretory and hyposecretory patients and light chain disease with anuric renal failure. However, it is clear that prospective validation of this assay is required in other clinical settings. For example, we have shown recently that SFLCR does not correlate well with total proteinuria in patients with myeloma and therefore cannot replace 24-hour urine protein estimation.[7]

Our data indicate that normal SFLCR cannot rule out the presence of residual disease as conventionally defined by a positive SIFE and that a normal SFLCR is much more likely to be seen in the presence of a positive SIFE than is a negative SIFE in the presence of abnormal SFLCR. Additional studies are required to determine the temporal relationship between SFLCR normalization and paraprotein clearance and to determine whether the "stringent CR" entity truly defines a more robust disease response than "nonstringent CR." Until then, the role of SFLC estimation and incorporation of SFLCR into assessment of response in myeloma remains controversial.

Authorship

Contribution: S.S. and J.M. treated the patients, designed the study, analyzed and interpreted the data, and wrote the paper; E.V. and S.A. helped with patient management and data interpretation; and J.K. and V.S. helped with data collection, verification, and interpretation.

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