Abstract

**Introduction:** The serum free light chain (sFLC) has been widely used in the assessment of response in patients with multiple myeloma and other plasma cell dyscrasias. However, its use in Waldenstrom macroglobulinemia (WM) has not been previously assessed. We sought to examine the role of sFLC in response and progression of patients with WM.

**Methods:** This study was conducted in a cohort of 48 patients with a diagnosis of WM, untreated (n = 20) or relapsed/refractory (n = 28), prospectively treated on a bortezomib and rituximab trial.

**Results:** Involved FLC (iFLC) response occurred in 79% patients versus 60% by M-spike protocol criteria. The median time to response was shorter with iFLC than per protocol (2.1 and 3.7 months; P = 0.05). Progression defined using iFLC also correlated well to progression in the protocol (κ = 0.63). However, the median time to progression (TTP) was more rapid by iFLC than per protocol (13.7 and 18.9 months). We also confirmed that a flare in iFLC in post–rituximab therapy did not correlate with lack of response or shorter TTP.

**Conclusion:** Involved sFLC may be a useful marker of tumor measurement, showing earlier response and progression compared with IgM or M-spike measurements. *Clin Cancer Res;* 17(9); 1–6. ©2011 AACR.

Introduction

Waldenstrom macroglobulinemia (WM) is a low-grade B-cell lymphoma characterized by bone marrow infiltration of lymphoplasmacytic cells that secrete immunoglobulin M (IgM) in the serum (1–3). The current consensus recommendations use the serum monoclonal protein to determine response and progression (4, 5). However, the IgM level lacks sensitivity because of its prolonged half-life (3). In addition, in about 50% of patients receiving rituximab therapy, the IgM increases temporarily, a term called IgM flare. This leads to discontinuation of therapy in some patients. Therefore, there is a need to identify new markers reflective of tumor burden in WM. The serum free light chain (sFLC) assay is a nephelometric measurement of κ and λ light chains. An abnormal κ/λ FLC ratio indicates an excess of one light chain type versus the other and is interpreted as a surrogate for clonal expansion on the basis of extensive testing in healthy volunteers and patients with myeloma, amyloidosis, and renal dysfunction (6–9). The assay is conducted on automated chemistry analyzers, is widely available, and is commonly used to monitor patients with multiple myeloma (MM), oligosecretory myeloma, and primary amyloidosis, as well as patients with the light chain–only form of myeloma. The sFLC assay has shown significant application in response, progression, and prognosis of plasma cell dyscrasias, particularly in MM (6–12). However, the role of sFLC in the measurement of tumor response in WM has not been previously examined. We have previously shown that the use of involved sFLC values accurately diagnosed patients with WM and differentiated them from patients with IgM-MGUS (monoclonal gammopathy of undetermined significance).

In this study, we hypothesized that sFLC can be used in the assessment of response and progression of patients with WM. This study represents the first prospective assessment of the role of sFLC in patients with WM.
Translational Relevance

The serum free light chain (sFLC) has been widely used in the assessment of response in patients with multiple myeloma and other plasma cell dyscrasias. However, its use in Waldenstrom macroglobulinemia (WM) has not been previously assessed. In this study, we show that sFLC can be a useful marker of tumor measurement, showing early assessment of tumor response and early progression compared with IgM or M-spike measurements. The use of sFLC in the measurement of tumor response in WM is warranted.

Previously untreated (N = 26) or relapsed and/or refractory (N = 37). The inclusion criteria included a measured creatinine 2.5 times or less the upper limit of the normal (ULN) range. Approval of this protocol was obtained from Dana-Farber Cancer Institute and was in accordance with the Declaration of Helsinki.

Eligible patients must have had measurable sFLC levels at baseline. Patients were excluded for normal involved FLC (iFLC) at baseline (N = 12), no baseline or cycle 1 sFLC data (N = 1), or no follow-up data available after therapy initiation (N = 2). A total of 48 patients were included in this study.

Serum FLC assay

The serum free κ and λ light chain levels were measured using the sFLC assay (Freelite; The Binding Site Ltd.; ref. 13). The clonal FLC (either κ or λ depending on the type of light chain involvement) was considered the involved immunoglobulin FLC (9). The DFCI laboratory normal ranges are as follows: κ: 3.3–19.4 mg/L; λ: 5.7–26.3 mg/L; ratio, κ/λ: 0.26–1.65. IgM normal range (nephelometry): 40–230 mg/dL. Abnormal serum iFLC value is defined as greater than the ULN of κ or λ value depending on the type of clonal serum light chain. Abnormal FLC ratio is defined as outside the normal range of ratio (<0.26 or >1.65).

Response

The serum iFLC test was used for response and progression evaluation. Response per protocol was defined according to consensus recommendations (10). Serum iFLC and IgM response (nephelometry) was defined as achievement of partial response (PR) and better (at least 50% decreases from baseline in the iFLC level and in the IgM value or normalization of the level).

Progression

Progressive disease was defined according to consensus recommendations (10), for example, at least 25% increase in serum monoclonal M-spike, using serum protein electrophoresis (SPEP), or progression of symptoms attributable to WM, and confirmed by a second measurement at least 2 weeks apart. Various definitions of progression have been described by the use of serum iFLC. Therefore, we have examined 2 iFLC definitions, a 25% or a 50% increase in the level of serum iFLC from nadir with an increase to a value greater than the ULN. IgM progression was defined using similar terms.

Statistical analysis

Response rate was summarized as percentage of patients with the 90% confidence interval (CI). Concordance between serum iFLC, IgM, and protocol (M-spike) response rate was evaluated using k-statistics. The level of agreement for the k-statistic was as follows: ≤0, poor; 0.0–0.2, slight; 0.2–0.4, fair; 0.4–0.6, moderate; 0.6–0.8, substantial; and 0.8–1.0, almost perfect. We also calculated the maximum percentage decrease in the iFLC and IgM levels; their correlation was evaluated using Spearman correlation coefficient. For responders by both iFLC and IgM criteria or responders by both iFLC and M-spike criteria, we calculated time to response (defined as months from therapy initiation to the date of response); comparisons were conducted using the Wilcoxon signed-rank test. Time to progression (TTP; defined as months from therapy initiation to the date of progression, censored at last known to be in remission or date of initiation of new therapy) was estimated using Kaplan–Meier methodology. We also did landmark analysis to compare TTP by FLC, IgM or protocol response status at 2, 4 and 6 months after therapy initiation; log-rank test was used. The statistical analysis was carried out using SAS version 9.2 (SAS Institute Inc.). All P values are 2 sided.

Results

Patient characteristics

Baseline characteristics of these patients (N = 48) are described in Table 1. There was no difference in the baseline serum FLC levels between patients on the upfront arm of the study or those with relapsed/refractory disease (data not shown).

Serum iFLC response

Twenty-nine (60%, 90% CI: 48%–72%) patients responded as per protocol response criteria, evaluated using the SPEP. The iFLC response occurred in 38 (79%, 90% CI: 67%–88%) patients with 21 (44%) having 50% reduction, 2 (4%) having normalization of iFLC values, and 15 (31%) meeting both criteria. According to serum IgM protein measurement by nephelometry, 35 (73%, 90% CI: 60%–83%) patients responded with a PR or better.

Previous studies examined other FLC response definitions by using FLC κ/λ ratio or the difference between involved FLC and uninvolved FLC (dFLC). Here, 12 of 47 (26%, 90% CI: 15%, 38%) patients achieved normal FLC ratio. Of these, 11 (92%) achieved response by iFLC response criteria. According to dFLC criteria, 36 (75%) patients showed a 50% decrease in dFLC, of which 35 (97%) also had iFLC response (κ = 0.89, 95% CI: 0.74–0.99). We therefore considered serum iFLC measurement
Correlation of iFLC with IgM and M-spike response

Table 2 A compared iFLC response with IgM and protocol response criteria (M-spike by SPEP). Concordance of iFLC response and protocol response was fair ($\kappa = 0.38$, 95% CI: 0.13–0.64). Of note, iFLC response rate was 3 of 3 (100%) in complete response (CR), 24 of 26 (92%) in PR, 9 of 13 (69%) in minor response (MR), and 2 of 5 (40%) in stable disease (SD) per protocol. Lack of concordance occurred, as 11 patients had iFLC response but did not reach response as per SPEP in the protocol. We believe this relates to the high sensitivity of serum iFLC test and might accentuate lack of sensitivity of the SPEP technique. Interestingly, an agreement between serum iFLC and IgM response occurred in 41 (85%) patients, 33 responders, and 8 nonresponders ($\kappa = 0.60$, 95% CI: 0.34–0.86). Of the 5 patients who achieved iFLC response but not IgM response, 3 had IgM reduction of more than 45% but less than 50% compared with baseline. There was a high concordance between IgM response and protocol M-spike response ($\kappa = 0.63$, 95% CI: 0.41–0.85, data not shown). There was a significant correlation between maximum percentage reduction in iFLC and IgM (Spearman correlation coefficient = 0.64, $P < 0.0001$). Maximum reduction estimated using iFLC and IgM by protocol response obtained using SPEP M-spike is shown in Figure 1A and B, respectively.

Time to iFLC response

Time to iFLC response was compared with IgM ($N = 33$, Fig. 1C) and SPEP response ($N = 27$, Fig. 1D) among patients who achieved response by both criteria. Median time to iFLC response was 1 month earlier than either IgM (nephelometry) or SPEP, 2.1 months (range: 0.9–28.7) versus 3.0 months (0.9–14.7; $P = 0.07$) and 3.7 months ($P = 0.05$), respectively. Sixteen (48%) patients achieved iFLC response earlier in comparison with IgM. Only 5 (21%) patients achieved earlier IgM response, and 10 (30%) patients achieved IgM and iFLC response at the same time.

Serum iFLC progression

On the basis of protocol criteria of progression, 23 (48%) patients showed progression. We examined 2 iFLC definitions that were previously described, 50% and 25% increase in the level of iFLC from nadir with increase to a value greater than the ULN. Twenty-eight (58%) patients and 23 (48%) showed progression defined using iFLC 25% and 50% increase criteria [Table 2 (B)]. There was significant correlation between progression by iFLC more than 25% increase and M-spike, having concordance with both markers in 39 (81%) patients ($\kappa = 0.63$, 95% CI: 0.41–0.84). The 25% iFLC increase criteria showed better concordance than 50% definition ($\kappa = 0.58$, 95% CI: 0.35–0.81). Similar trends were observed using IgM progression criteria. We therefore propose the use of 25% increase in iFLC from nadir as the progression definition in WM.

Median follow-up was 19.1 months (range: 5.2–43.5 months). Median TTP per the protocol was 18.9 months (95% CI: 10.5–NR), and it was 13.7 months (95% CI: 9.5–19.1) obtained using iFLC of more than 25% and IgM of more than 25% criteria, respectively. These results show more rapid detection of progression by iFLC than M-spike and IgM measurements. Therefore, iFLC seems a sensitive marker to determine progression earlier in WM.

Early response by iFLC

We examined using iFLC whether response 2 months after therapy initiation (early response) can predict overall response to therapy. Seventeen (35%) patients achieved early iFLC response, which was not associated with baseline iFLC levels. Interestingly, patients with early iFLC response had intermediate/high International Staging System (ISS)-
WM stage, elevated β2-microglobulin or low hemoglobin levels of less than 11.5 g/dL (P < 0.05). Early iFLC response was related to overall IgM response (P = 0.02). We could not detect significant association between early iFLC response and overall response by M-spike.

Serum iFLC flare
We examined correlation between IgM flare and iFLC flare. We defined flare as 15% increase from the prior value. Flare occurred during cycles 2 and 3 as well as during cycles 5 and 6, as rituximab was given in cycles 1 and 4. On the basis of this, 11 (23%) patients had iFLC flare only, 8 (17%) had IgM flare only, and 6 (13%) had both iFLC and IgM flare. There were 23 (48%) patients who did not flare by either iFLC or IgM. An early flare occurred in only 35% of patients following the first cycle of rituximab. These results confirm that iFLC can show flare in post–rituximab therapy, with a lack of correlation with lack of response or shorter TTP and increased occurrence of relapse.

Correlation of response with time to progression
At the time of analysis, there were only 2 of 48 patients died in this study population. Therefore, there is a limited power to evaluate correlations of variables, including response, with overall survival or progression-free survival (PFS). In the context of this limited power, we compared TTP by FLC/IgM/M-spike response at 2, 4 and 6 months after initiation of therapy using the landmark methodology. Overall, we did not detect any significant association between FLC/IgM/M-spike response and TTP defined by protocol (Supplementary Table S1), no matter which landmark time point was chosen. Results were similar if TTP was defined by FLC or IgM criteria (25% or 50% increase, data not shown).

Given the good concordance between the FLC response and the IgM or M-spike response, there were very few patients who had response by just 1 criterion (Table 2A). Therefore, we compared TTP between patients who had both FLC and IgM response versus all others (FLC

<p>| Table 2. Comparing iFLC response (A) and progression (B) with IgM and protocol criteria |
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Abbreviation: PD, progressive disease.

*The progression of iFLC and IgM was defined as 25% increase from nadir with value greater than ULN.
response only, IgM response only, nonresponse by both criteria), and no significant association was detected (Supplementary Table S1). Results were similar if we compared TTP between patients who had both FLC and protocol (M-spike) response versus all others. However, we acknowledge the sample size is limited to draw definite conclusions in this study.

In this study, 12 patients with normal iFLC level were excluded from analysis. When comparing response and TTP (by protocol criteria), we did not find any difference between patients with normal or abnormal iFLC level at baseline (data not shown).

Discussion

The sFLC assay is a nephelometric measurement of κ and λ light chains that circulate as light chain monomers or dimers and that are not bound to Ig heavy chain. The sFLC assay has shown significant clinical application in plasma cell dyscrasias, particularly in MM, primary systemic amyloidosis, and MGUS. In MM, sFLC is used to monitor response to therapy and is included in the response criteria (14) on the basis of its sensitivity to assess lower tumor burden compared with SPEP. However, empiric definition of sFLC response was proposed for patients with MM in the international uniform response criteria, but it was not validated. Another possible advantage of the measurement of clonal light chain over IgM M-spike is that light chain has a significantly shorter half-life (10).

This study described the role of sFLC in WM. Here, we analyzed the sFLC in a prospective study of patients treated uniformly in a clinical trial of bortezomib and rituximab. The median iFLC values were higher in our study than in previously published studies of MM (5, 6). This may be because we have included in our study only patients with abnormal sFLC values at baseline. Interestingly, involved sFLC value range was not as wide in WM as in myeloma (1), although the overall number of patients studied is too small to draw definite conclusions. iFLC response was comparable with the response obtained using SPEP or IgM measurement by nephelometry, indicating that it can be used in the future as a reliable measurement of disease response. Seventy-nine percent of patients achieved an iFLC response compared with 60% of patients achieving response obtained using SPEP. Previous studies have examined other FLC response definitions by using the FLC κ/λ ratio or the difference between involved and uninvolved FLC. In this study, we found that serum iFLC measurement is not inferior to the FLC κ/λ ratio and to the dFLC value for this study.

The median time to iFLC response was 1 month earlier than either IgM (nephelometry) or SPEP, indicating that this test could be used to assess response earlier than current measurements of disease. Similarly, iFLC greater than 25% showed a more rapid detection of progression compared with M-spike and IgM measurements. Therefore, iFLC seems a sensitive marker to determine response and progression earlier in WM. These results are comparable with studies carried out in MM.
Interestingly, patients with early iFLC response were more likely to have intermediate/high ISS-WM stage, elevated β2-microglobulin or low hemoglobin levels less than 11.5 g/dL. These results may be similar to those observed by Van Rhee and colleagues (15), indicating that high sFLC and rapid reductions after therapy could reflect a more aggressive disease. Further studies are warranted to examine the role of sFLC and early sFLC response and its correlation with TTP and PFS.

Finally, we examined whether sFLC measurement can be used in patients with an IgM flare post–rituximab therapy to predict whether those patients will respond after the flare. In this study, we observed that iFLC could show a flare in post–rituximab therapy, with a lack of correlation between IgM and iFLC in the post–rituximab flare. Therefore, serum iFLC cannot replace IgM to differentiate progression from flare when patients are treated with rituximab therapy. In addition, the iFLC flare status prior to cycle 3 did not correlate with a lack of response or a shorter TTP or an increased occurrence of relapse.

In summary, iFLC may be a useful and sensitive marker of tumor measurement in WM that correlates well with IgM and M-spike measurements. The serum iFLC marker showed a more rapid time to response and time to progression than those observed using IgM or M-spike measurements. We propose to study sFLC measurement in future, large, prospective trials to further confirm whether early determination of sFLC will help in making decision of treatment modification during the course of therapy.

Disclosure of Potential Conflicts of Interest

I.M. Ghobrial is a consultant/advisory board member of Millennium, Celgene, Novartis, and Onyx. X. Leleu is a consultant/advisory board member of Janssen Cilag, Celgene, Amgen, Novartis, Roche, MedPharma.

Authors’ Contribution

X. Leleu, W. Xie, and I. M. Ghobrial wrote the manuscript; W. Xie and E. Weller conducted statistical analysis; and M. Bagshaw, R. Leduc, R. Banwait, and N. Roper collected data. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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