C-Reactive Protein and β-2 Microglobulin Produce a Simple and Powerful Myeloma Staging System

By Regis Bataille, Mario Boccadoro, Bernard Klein, Brian Durie, and Alessandro Pileri

Multiple myeloma (MM) staging procedures are still inadequate for detection of the optimal therapeutic procedure for an individual patient. The Durie & Salmon staging system and serum β2-microglobulin (β2M) are used worldwide because of their easy clinical application. Other prognostic parameters, such as myeloma cell proliferative activity, are of exceeding importance, but are not as simple as standard methods. Recently, interleukin-6 (IL-6) has been shown to be a major growth factor for MM. IL-6 is a pleiotropic cytokine acting on several cell lineages, and, at the hepatocyte level, stimulates the synthesis of acute phase proteins, such as the well known C-Reactive Protein (CRP). Serum CRP concentration actually reflects the IL-6 activity. A survival analysis carried out in 162 MM patients at diagnosis showed that serum CRP level is a highly significant prognostic factor. Moreover, serum CRP was independent of serum β2M. This feature allowed stratification of MM patients into 3 groups according to CRP and β2M serum levels: (1) low risk group, CRP and β2M < 6 mg/L (50% of patients); (2) intermediate risk group, CRP or β2M ≥ 6 mg/L (35% of patients); (3) high risk group, CRP and β2M ≥ 6 mg/L (15% of patients). Survival was 54, 27, and 6 months, respectively (P < .0001). We thus propose a new and powerful myeloma staging system based on simple and reliable laboratory evaluations.

SURVIVAL in multiple myeloma (MM) varies from a few months to many years. Currently available staging systems are still inadequate for precise assessment of an individual prognosis. Nevertheless, accurate prognostic evaluation is mandatory for selection of the optimal therapeutic strategy: new aggressive chemotherapy regimens with or without bone marrow transplant or growth factor support have been recently proposed, but they can be most strongly considered only in patients with aggressive disease.

The Durie & Salmon staging system and serum β2-microglobulin (β2M), the two worldwide methods used for prognostic classification, mainly reflect tumor burden. This is not an absolute criterion. It has been shown, for instance, that proliferative activity of the bone marrow myeloma cells (ie, labeling index, LI) is not related to the tumor burden, but is a powerful independent prognostic factor. Other parameters, such as the immunologic phenotype of circulating lymphocytes, also give useful prognostic information. However, these tests are more complex and time-consuming than the Durie & Salmon staging system or serum β2M determination, which are at present widely used in large cooperative groups.

Recently, interleukin-6 (IL-6) has been shown to be a major growth factor for human myeloma cells. IL-6 stimulates in vitro and in vivo plasma cell growth. Moreover, in MM patients high serum IL-6 levels are related to myeloma cell proliferation and disease severity. Anti-IL-6 monoclonal antibodies (MoAbs) block myeloma cell proliferation in vivo, this being one type of evidence of the in vivo role of IL-6 in myeloma pathogenesis. IL-6 is a pleiotropic cytokine active on B cells and several other cell lineages. For example, it is active on hepatocytes and regulates the major acute phase proteins in liver cells. In particular, it has recently been shown that only IL-6 induces synthesis of C-Reactive Protein (CRP) by human hepatocytes in primary cultures. In vivo, a significant correlation between serum IL-6 and CRP levels has been found in rheumatoid arthritis. After infusion of anti-IL-6 MoAbs in MM, serum CRP level decreases to undetectable levels within 10 days, and rapidly increases again at the end of treatment. Taken together, all these features show that CRP production is totally dependent on IL-6, and that serum CRP level actually reflects IL-6 activity.

These observations prompted us to evaluate the serum CRP level in monoclonal gammopathy of undetermined significance (MGUS) subjects and in MM patients. Moreover, serum CRP level was analyzed in MM at diagnosis, during the remission phase, and at relapse. A survival analysis was performed to assess the prognostic value of serum CRP level. Finally, serum CRP was related to other prognostic factors such as the proliferation of bone marrow myeloma cells and serum β2M levels. Its strong prognostic value, associated with that of serum β2M level, allowed development of a simple and powerful myeloma staging system.

PATIENTS AND METHODS

This study was performed on all available frozen sera from MM patients at diagnosis in two hematologic institutions (Department of Immunology and Rheumatology, Montpellier, France, and Department of Medicine and Experimental Oncology, Section of Hematology, University of Torino, Italy) from January 1985 to November 1990. This series almost represents the consecutive patients referred to these institutions. Serum CRP level was measured in 162 MM patients at diagnosis, at relapse, and during remission. The follow-up was from diagnosis of MM to death or end of study in all patients.

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Submitted September 26, 1991; accepted April 2, 1992.

Supported by Association pour la Recherche sur le Cancer (Paris, France), Ligue Nationale contre le Cancer (Paris, France), Ligue Regionale contre le Cancer (Montpellier, France), and by CNR, Progetto Finalizzato Applicazioni Cliniche Ricerca Oncologica Associazione Italiana per la Ricerca sul Cancro (Milano, Italy).

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Blood, Vol 80, No 3 (August 1), 1992: pp 733-737
measured in 52 subjects with MGUS and 162 MM patients at diagnosis. Moreover, in the MM patient group, 49 determinations were performed during remission phase, and 33 at relapse. MGUS and MM were defined as previously reported.\(^2\) In MM at diagnosis, mean age was 63.8 ± 10.5 years; 88:74 was the male/female ratio; 101 patients showed IgG isotype, 42 IgA, and 17 Bence Jones; one was IgD and one nonproducing. Ninety-four MM patients were Durie & Salmon stage III, 43 stage II, and 25 stage I. Patients were treated with alternating VMCP/VBAP (vincristine, melphalan, cyclophosphamide, prednisone/vincristine, BCNU, Adriamycin, prednisone) or melphalan and prednisone. Because these treatments showed no statistically significant difference in terms of survival,\(^3\) no distinction was made in the survival analysis. Median survival for MM patients was 36 months; median follow-up for censored patients was 28.7 months (range 2.5 to 120 months); 55% of patients were dead at the time of analysis.

Serum CRP concentrations were evaluated by commercially available enzyme-linked immunsorbent assay (ELISA) kits (Eurogenetics, Tessenderlo, Belgium), or nephelometry (Behring, Mannheim, Germany). These two methods gave very similar results on a series of 48 patient sera (Wilcoxon 2 paried test, \(P = .41\)). Serum \(\beta2M\) levels were detected using radio immunoassay (RIA) kits (Pharmacia, France). All sera were stored at -20°C before measurement.

Plasma cell proliferation was evaluated on fresh samples: isolated bone marrow cells were incubated in vitro with bromodeoxyuridine. The percentage of myeloma cells in S-phase (LI) was evaluated by antibromodeoxyuridine MoAb (Dakopatts, Glostrup, Denmark) and fluorescein-labeled goat antimouse Ig (Becton & Dickinson, Mountain View, CA) as previously described.\(^8\)

CRP values are expressed in terms of the median plus the distribution range, because they were not normally distributed in all groups. Comparison between groups was made by Wilcoxon’s test; the Spearman rank sum test was used for correlation analysis. The results of clinical follow-up are expressed in a life-table format according to the Kaplan-Meier method; the log rank test was used to compare survival curves. Multivariate analysis was performed using the Cox model. All data were processed with the SAS statistical software package (SAS Institute Inc, Cary, NC).

RESULTS

Median serum CRP levels were 0.7 mg/L (range 0 to 89.5) in MGUS, and 3.75 mg/L (range 0 to 121) in MM patients at diagnosis (normal CRP range: 0 to 6 mg/L). However, only 4 of 52 subjects displayed above-normal values in the MGUS group, compared with 60 of 162 in MM patients at diagnosis (Chi square = 15.23; \(P < .0001\)). Moreover, low values were observed in MM during remission phase (0.9 mg/L, range 0 to 100), and high values again at relapse (3.9 mg/L, range 0 to 79.7) (diagnosis versus remission, \(P < .0001\); remission versus relapse, \(P < .008\)) (Fig 1).

To assess the prognostic value of serum CRP level, a survival analysis was performed in 162 MM patients evaluated at diagnosis. A CRP cutoff of 6 mg/L was chosen because it represents a widely used upper limit for normal subjects. Moreover, using this cutoff, patients were still separated in two well-balanced risk groups: 60 and 102 patients, respectively. Patients with values ≥6 mg/L showed a reduced survival in comparison with patients with low values (21 v 48 months, \(P < .0001\)) (Fig 2, Table 1).

The significance of several recognized prognostic factors has been tested by univariate analysis in this patient series: serum \(\beta2M\), Durie & Salmon stage, serum albumin, LI, and age (Table 1). Serum albumin, using a previously defined cutoff of 30 g/L,\(^4,5\) identified a very poor survival patient subgroup. However, this subgroup represented only 14.1% of the whole series. Serum \(\beta2M\) level (cutoff of 6 mg/L) separated patients into two groups showing a significantly different survival (14 v 48 months, \(P < .0001\); Table 1).

The correlation between serum CRP and other prognostic factors was evaluated. No direct relationship was detected with serum creatinine, serum \(\beta2M\), or tumor stage. However, a trend for an inverse relationship between CRP and albumin was observed (\(r = -.44; \ P < .0001\)). The distribution of clinical parameters according to serum CRP level was evaluated. The 6 mg/L cutoff divided the MM population in two groups showing different stage (\(P < .002\), LI \(P < .008\), and albumin \(P < .0004\)). \(\beta2M\) also reached statistical significance \(P < .01\) (Table 2). Other clinical parameters, such as isotype, creatinine, age, and gender were equally distributed in these two groups.

A multivariate analysis was performed using parameters statistically significant in the univariate analysis: CRP,
Table 1. Correlates of Survival: Univariate Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cutoff Value</th>
<th>Median Survival Mo.</th>
<th>No. of Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>&lt;6 mg/L</td>
<td>48.0</td>
<td>102</td>
<td>.0001</td>
</tr>
<tr>
<td>β2M</td>
<td>≥6 mg/L</td>
<td>21.0</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Li</td>
<td>&lt;2%</td>
<td>48.4</td>
<td>48</td>
<td>.01</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;60</td>
<td>57.3</td>
<td>63</td>
<td>.007</td>
</tr>
<tr>
<td>Albumin</td>
<td>&lt;30 g/L</td>
<td>7.0</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>NR*</td>
<td>25</td>
<td>99</td>
<td>.001</td>
</tr>
</tbody>
</table>

*NR, not reached.

β2M, stage, albumin, age were used as continuous variables (Table 3). Li was available in 66 patients only and excluded from this analysis. The stepwise Cox proportional hazard model contained, first, albumin, followed by β2M (Table 3). CRP did not enter this model. However, if albumin was excluded, CRP came first because of its mutually exclusive correlation with albumin. CRP was followed by β2M, and no other parameters entered this model (Table 3).

MM patients were stratified into three groups according to CRP and β2M serum levels: (1) low-risk group, CRP and β2M <6 mg/L; (2) intermediate-risk group, CRP or β2M ≥6 mg/L; and (3) high-risk group, CRP and β2M ≥6 mg/L (Fig 3). Eighty-one patients (50%) entered group 1, 56 entered group 2 (34.6%), and 25 entered group 3 (15.4%). Survival was 54, 27, and 6 months, respectively (P < .0001) (Table 4).

DISCUSSION

IL-6 is a central in vitro growth factor for the malignant plasma cells in MM.12,13 This is now proved by the ability to reproducibly obtain human myeloma cell lines whose proliferation is totally dependent on the addition of IL-6.14 Several arguments support the concept that IL-6 is also involved in myeloma cell growth in vivo. (1) A positive correlation exists between the degree of the in vitro myeloma-cell response to IL-6, and the in vivo myeloma cell proliferative activity.14,16 (2) Serum IL-6 levels are increased in MM patients and are a reflection of disease severity.18,19 (3) IL-6 is directly detectable (in terms of mRNA and protein) in the bone marrow from MM patients.20 (4) Anti-IL-6 murine monoclonal antibodies, given to patients with advanced MM, resulted in a strong inhibition of myeloma cell proliferation.20,21 Of major interest, serum CRP levels were highly increased in these patients with active MM, and became undetectable during the anti-IL-6 therapy.20 Thus, our data with anti-IL-6 therapy in MM confirmed that CRP synthesis by hepatocytes is totally dependent on IL-6 and serum CRP level actually reflects IL-6 activity in vivo. Taken together, these data prompted us to investigate CRP as a putative prognostic parameter in MM. Incidentally, few studies have ever been devoted to acute phase proteins (not including CRP) in MM.29

Our present findings showed that serum CRP level (1) was significantly increased in MM compared with MGUS, (2) reflected MM disease activity, (3) strongly correlated with patient survival, and (4) was a powerful prognostic indicator in MM. Of major interest, the prognostic value of CRP was found to be independent of β2M, another strong prognostic indicator. Finally, we have shown that simultaneous use of both these parameters constitutes a simple and powerful myeloma staging system.
Table 4. Stratification of Myeloma Patients According to CRP and β2M

<table>
<thead>
<tr>
<th>Risk Categories</th>
<th>Risk Parameters</th>
<th>No. of Patients (%)</th>
<th>No. Alive (%)</th>
<th>Median Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low CRP and &lt; 6 mg/L</td>
<td>81 (50.0)</td>
<td>45 (55.5)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Intermediate CRP or ≥ 6 mg/L  β2M</td>
<td>56 (34.6)</td>
<td>24 (42.8)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>High CRP and ≥ 6 mg/L  β2M</td>
<td>25 (15.4)</td>
<td>2 (8)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Serum CRP levels were significantly higher in patients with active MM (diagnosis, relapse) than inactive MM severity. Finally, serum CRP levels were directly related to survival in 162 previously untreated MM patients. These data clearly showed that serum CRP level, by directly reflecting IL-6 production in vivo, is a strong indicator of disease activity in MM. This concept is further supported byous data showing that serum IL-6 levels reflect disease prognosis and survival in MM patients, with a return of remission associated with increased serum CRP levels. Moreover, no direct correlation between CRP and LI was found, and this relation is a strong argument in favour of CRP as a reflection of the effect of IL-6 on plasma cell proliferation.

Increased serum CRP levels delineated a subset of patients with high cell mass, low serum albumin, but high LI. Actually, a link between CRP and LI was found, and this relation is a strong argument in favour of CRP as a reflection of the effect of IL-6 on plasma cell proliferation.

In conclusion, serum CRP level is shown to be a new and powerful prognostic factor in MM, and, like β2M, its determination is rapid, simple, reliable, and inexpensive.


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