Multiparameter flow cytometry for staging of solitary bone plasmacytoma: new criteria for risk of progression to myeloma

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Solitary plasmacytoma represents a heterogeneous group of patients; approximately half develop multiple myeloma (MM) in 2 or 3 years, whereas others remain disease-free at 10 years. By definition, these patients do not have morphologic bone marrow (BM) plasma cell (PC) infiltration. Here, we investigated whether sensitive BM evaluation of patients with solitary bone plasmacytoma (SBP; n = 35) and extramedullary plasmacytoma (EMP; n = 29) through multiparameter flow cytometry (MFC) would unravel the presence of clonal PCs in otherwise disease-free BM, and whether BM clonality predicted higher risk of progression. BM clonal PCs were detected in 17 of 35 SBP (49%) and 11 of 29 EMP (38%) patients. Seventy-one percent of flow-positive vs only 8% of flow-negative SBP patients evolved to MM (median time to progression of 26 months vs not reached; hazard ratio, 17.4; P < .001). No significant differences were observed among EMP cases. Our results highlight the importance of MFC for sensitive BM evaluation of SBP patients, to predict risk of developing treatment-requiring MM and to plan disease monitoring. (Blood. 2014;124(8):1300-1303)

Introduction

Solitary plasmacytoma (SP) is a rare neoplasm defined by localized clonal plasma cell (PC) infiltration without systemic tumor dissemination.1 Consequently, an M-component is typically absent or present in low amounts; both the skeletal survey and the bone marrow (BM) are normal, and no related organ or tissue impairment is observed.2 Localized clonal PC infiltrates may arise either in the bone (solitary bone plasmacytoma [SBP]) or extraceous (extramedullary plasmacytoma [EMP]). Approximately 50% of patients with SBP and 15% with EMP will evolve into multiple myeloma (MM).3,4 Thus, identifying those patients more likely to progress could allow tailored follow-up, and probably reduce the anxiety of patients with low risk of progression.

Because both entities account for <5% of PC dyscrasias, investigational studies are uncommon, and the identification of biomarkers to predict risk of transformation to MM is needed. Magnetic resonance imaging (MRI) has shown the ability to detect occult BM disease in ~30% of patients with SBP,5 which resulted in higher risk of progression.6 In fact, a negative MRI is now commonly considered as a prerequisite for the diagnosis of SBP.8

Other potential biomarkers for risk of progression include the presence of M-component at diagnosis or its persistence after treatment,7 as well as clonal expansion of free light chains (FLCs) detectable in urine or in serum, the latter using the FLC assay.9

Here, we hypothesized that sensitive BM evaluation of SP patients through multiparameter flow cytometry (MFC) could have additional value because it may unravel the presence of clonal PCs, which in an otherwise presumed localized disease would translate into higher risk of developing MM.

Study design

A total of 64 patients newly diagnosed with histologic confirmation of SBP (n = 35) or EMP (n = 29) over the last 15 years are the focus of this study. All patients had <5% BMPCs by microscopy, <3 g/dL M-component, and no additional lytic lesions on the conventional skeletal survey or any other imaging technique whenever available (MRI in 20 and positron emission...
Table 1. Demographics and characteristics of patients with SBP (n = 35) and EMP (n = 29)

<table>
<thead>
<tr>
<th>Patient demographics and characteristics</th>
<th>SBP</th>
<th>EMP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>57%</td>
<td>69%</td>
<td>NS</td>
</tr>
<tr>
<td>Median age, y</td>
<td>65 (39-81)</td>
<td>59 (34-83)</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>135 (102-172)</td>
<td>149 (126-167)</td>
<td>.01</td>
</tr>
<tr>
<td>Serum albumin, g/dL</td>
<td>3.8 (2.5-5.0)</td>
<td>4.3 (3.7-5.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.8 (0.5-1.4)</td>
<td>0.8 (0.6-1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum beta-2-microglobulin, mg/L</td>
<td>2.1 (0.8-7.4)</td>
<td>1.7 (0.8-2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.5 (2.4-10.9)</td>
<td>9.8 (8.9-10.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Elevated lactate dehydrogenase</td>
<td>16%</td>
<td>6%</td>
<td>NS</td>
</tr>
<tr>
<td>Positive immunofixation and/or electrophoresis</td>
<td>59%</td>
<td>29%</td>
<td>.03</td>
</tr>
<tr>
<td>Abnormal serum FLC ratio, 2</td>
<td>69%</td>
<td>40%</td>
<td>NS</td>
</tr>
<tr>
<td>Serum M-component, g/dL,*</td>
<td>0.8 (0.05-1.8)</td>
<td>1.1 (0.5-1.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Urine M-component, g/dL,*</td>
<td>0.5 (0.05-0.5)</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>Immune paresis</td>
<td>2%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Disappearance of the M-component upon radiotherapy, n = 16</td>
<td>54%</td>
<td>100%</td>
<td>NS</td>
</tr>
<tr>
<td>BMPCs by conventional morphology, %</td>
<td>2 (0-5)</td>
<td>1 (0-5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

*Median values are given only for patients with detectable M-component in serum or urine. All EMP patients had undetectable Urine M-component. The number of patients with available information on serum FLC and M-component precludes statistical analysis.

tomography/computed tomography scanning in 14). Table 1 shows the demographics and disease characteristics of SBP and EMP patients. BM samples were collected after informed consent was given, in accordance with local ethical committee guidelines and the Declaration of Helsinki. Patients were treated with radiotherapy (30-50 Gy) with (8%) or without surgery (84%), or by surgery alone (8%).

Erythrocyte-lysed whole BM samples were stained in ≥24 hours using a direct immunofluorescence technique, as described elsewhere.10,11 Briefly, a backbone antigen combination including CD19, CD38, CD45, and CD56 was systematically evaluated, allowing for identification of the BMPC compartment on the basis of strong CD38 expression and intermediate side-scatter signal, and detection of clonal PCs by the recognition of aberrant phenotypic expression profiles (supplemental Table 1, see supplemental Data available at the Blood Web site). Data acquisition was performed in a 2-step procedure, as previously described.10,11 Patients were defined as flow-positive when ≥20 PCs were detectable by MFC, at a sensitivity level of 10-4.

Patients were followed until progression to MM, death, or last follow-up. Curves were plotted by the Kaplan-Meier method and the log-rank test used to estimate statistical significant differences. Statistical analyses were conducted using the SPSS software (version 15.0; SPSS Inc).

Results and discussion

Median follow-up for the whole series was 3 years. Overall, 38% of SBP patients and 14% of EMP cases evolved into treatment-requiring MM, and 82% of total progressions were observed during the first 3 years; these numbers illustrate the typical clinical course of patients with SBP and EMP.1

BM clonal PCs were detected in 28 of the 64 patients with SP (44%; flow-positive), and slightly more frequently in those cases with SBP (49%) compared with EMP (38%). Median percentage of BM clonal PCs was 0.20% (0.01%-5%) and 0.096% (0.02%-0.35%) for patients with SBP and EMP, respectively. Clonal PCs were mainly detected by simultaneous infraexpression of CD19 and CD45 with or without overexpression of CD56 (73% of cases; supplemental Table 1). Overall, these results suggest similar phenotypic profiles of BM clonal PCs from SP patients vs monoclonal gammapathy of undetermined significance or MM.10,11 However, (in contrast to MM) both clonal and normal PCs coexist within the BMPC compartment. In fact, although in SBP patients median percentage of clonal PCs exceeds that of normal PCs (66% vs 34%), the opposite pattern was found among EMP cases (74% median normal PCs).

Thus, conventional microscopic BM evaluation may fail to detect such low levels of occult disease. It is noteworthy that in 12 of the 28 flow-positive cases an MRI was also performed, and proved to be negative.

Diagnosis and monitoring of MM still relies mostly on conventional “gold standard” techniques, but a comprehensive set of novel assays is paving their way into the clinic12; SP should be no exception to this paradigm. Although contradictory results have been reported regarding the prognostic value of diagnostic levels of M-component,1 Dingli et al showed that patients with SBP with an abnormal serum FLC ratio have shorter time to progression (TTP) to MM,2 though ~20% of cases with normal FLCs may still develop MM at 5 years.3 Disappearance of the M-component upon radiotherapy may also be of prognostic value,4 but it will be uninformative in the subset of patients with undetectable M-component at diagnosis.5,6 In the present series, those patients with detectable M-component at diagnosis (47%) had shorter median TTP to active MM (median 66 months vs not reached [NR] in patients with undetectable M-component), but differences did not reach significance (P = .15). TTP was similar for patients with persistent M-component after radiotherapy, and immune paresis was a rare event (only 1 SBP patient). Altogether, these observations stress how challenging it is to identify SP patients at risk of developing MM.

At the cellular level, Warsame et al have shown that the presence of >5% BMPCs by conventional microscopy is associated with significantly shorter TTP,7 but in-depth evaluation of true clonality within the PC compartment has not been investigated. Herein, 71% of SBP patients displaying BM clonal PCs progressed into MM, in contrast to only 6% among flow-negative patients (P < .001); median TTP was significantly shorter if BM clonality was present (26 months vs NR; hazard ratio, 17.4; P < .001) (Figure 1A). Among patients with EMP, only 20% of flow-positive cases evolved into MM as compared with 6% of flow-negative patients, and TTP was not significantly different (Figure 1B). Baseline M-component was observed in 62% of flow-positive patients (no correlation being observed between serum M-component concentration and percentage of BM clonal PCs), but these showed similar TTP compared with flow-positive cases without M-component (24 vs 39 months; P = .81). It is noteworthy that the risk of transformation was virtually absent among flow-negative cases without M-component, with only 1 patient (4%) evolving into MM (12.5 years after diagnosis and with multiple skin plasmacytomas but no evidence of BM disease). Nonetheless, although flow positivity could be of significant importance, a negative result could still reflect patchy BM involvement.

In summary, we show that MFC is a valuable biomarker for the management of patients with SBP, but is less informative among EMP cases. One possible explanation could rely on more preserved numbers of normal PCs in EMP, and thus larger series of patients with longer follow-up are needed. From a clinical standpoint, our results highlight the high risk of progression for SBP patients with “minimal occult” BM disease which could contribute to the tailoring of patients’ follow-up. Conversely, flow diagnostic criteria may also allow the accurate identification of “true” SP, characterized by
flow-negative BM and absence of M-component which would represent a signature for curability.

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Authorship

Contribution: J.F.S.M. and B.P. conceived the idea and designed the study; B.P., M.C., M.-B.V., E.C., and T.C.-V. performed immunophenotypic analysis; E.C., T.C.-V., F.E., A.G.d.C., M.-C.M., R.G.-S., E.M.O., M.-V.M., and J.F.S.M. provided study material or patients; B.P. and M.C. performed statistical analysis; B.P., M.C., and J.F.S.M. analyzed and interpreted data and wrote the manuscript; and all authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Figure 1. TTP to MM according to sensitive MFC immunophenotypic evaluation of the BMPC compartment. (A-B) TTP of patients with SP grouped according to the presence vs absence of BM phenotypically aberrant clonal PCs by MFC immunophenotyping for patients with SBP and EMP, respectively.
References


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