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

Running title: Flow cytometry in solitary plasmacytoma

Authors: Bruno Paiva *1, Mauricio Chandia *2, Maria-Belen Vidiriales 2, Enrique Colado 3, Teresa Caballero-Velázquez 4, Fernando Escalante 5, Alfonso Garcia de Coca 6, Maria-Carmen Montes 7, Ramon Garcia-Sanz 2, Enrique M. Ocio 2, Maria-Victoria Mateos 2, Jesus F. San Miguel 1

Authors affiliation: 1) Clinica Universidad de Navarra; Centro de Investigaciones Medicas Aplicadas (CIMA), Pamplona; (2) Hospital Universitario de Salamanca; IBSAL, IBMCC (USAL-CSIC), Salamanca; (3) Hospital Universitario Central de Asturias, Oviedo; (4) Hospital Universitario Virgen del Rocio/CSIC/Universidad de Sevilla, Instituto de Biomedicina de Sevilla (IBIS), Sevilla; (5) Complejo Hospitalario de Leon, Leon; (6) Hospital Clinico Universitario de Valladolid, Valladolid; (7) Hospital Virgen de la Concha, Zamora; Spain

Correspondence:

Jesus F. San Miguel, M.D; Ph.D.
Clinica Universidad de Navarra; Centro de Investigacion Medica Aplicada (CIMA)
Av. Pio XII 36, 31008 Pamplona, Spain
e-mail: sanmiguel@unav.es
Key point

- MFC is a valuable biomarker to discriminate "true" SBP patients from those with "occult" BM clonal PCs and high-risk of progression to MM

Abstract

Solitary plasmacytoma represents a heterogeneous group of patients; approximately half develop multiple myeloma (MM) in 2-3 years, while others remain disease-free at 10-years. By definition, these patients do not have morphological bone marrow (BM) plasma cell (PC) infiltration. Here, we investigated if 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 if BM clonality predicted higher risk of progression. BM clonal PCs were detected in 17/35 (49%) SBP and 11/29 (38%) EMP patients. 71% of Flow-positive vs. only 8% of Flow-negative SBP patients evolved to MM (median time-to-progression of 26 months vs. not reached; HR: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.
Introduction
Solitary plasmacytoma (SP) is a rare neoplasm defined by localized clonal plasma cell (PC) infiltration without systemic tumor dissemination. 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. Localized clonal PCs infiltrates may arise either in the bone (solitary bone plasmacytoma; SBP) or extraosseous (extramedullary plasmacytoma; EMP). Approximately 50% of patients with SBP and 15% with EMP will evolve into multiple myeloma (MM). 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 approximately 30% of patients with SBP, which resulted in higher risk of progression. In fact, a negative MRI is now commonly considered as a prerequisite for the diagnosis of SBP. Other potential biomarkers for risk of progression include the presence of M-component at diagnosis or its persistence after treatment, as well as clonal expansion of free light-chains detectable in urine or in serum, the latter using the free light-chain (FLC) assay.

Here, we hypothesized that sensitive BM evaluation of SP patients through MFC could have additional value since it may unravel the presence of clonal PCs, which in an otherwise presumed localized disease would translate into higher risk of developing MM.
Patients and methods

A total of 64 patients newly-diagnosed with histological 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, <3g/dL of M-component, and no additional lytic lesions on the conventional skeletal survey or any other imaging technique whenever available (MRI in 20 and PET/CT 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 (30Gy-50Gy) with (8%) or without surgery (84%), or surgery alone (8%).

Erythrocyte-lysed whole BM samples were stained in ≤24-hours using a direct immunofluorescence technique, as described elsewhere. Briefly, a backbone antigen combination including CD19, CD38, CD45, and CD56 was systematically evaluated, allowing to identify the BMPC compartment on the basis of strong CD38 expression and intermediate side-scatter signal, and to detect clonal PCs by the recognition of aberrant phenotypic expression profiles (Supplementary Table 1). Data acquisition was performed in a two-step procedure, as previously described. 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., Chicago, IL).
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 out of the 64 (44%) patients with SP (Flow-positive), and slightly more frequently in those cases with SBP (49%) compared to 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 infra-expression of CD19 and CD45 with or without over-expression of CD56 (73% of cases; Supplementary Table 1). Overall, these results suggest similar phenotypic profiles of BM clonal PCs from SP patients vs. MGUS or MM.10,11 However, (in contrast to MM) both clonal and normal PCs coexist within the BMPC compartment. In fact, while in SBP patients median percentage of clonal PCs exceed 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. Noteworthy, 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 clinic; 12 SP should be no exception to this paradigm. While 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 TTP to MM,9 though approximately 20% of cases with normal FLCs may still develop MM at 5-years.9 Disappearance of the M-component upon radiotherapy may also be of prognostic value,3 but it will be uninformative in the subset of patients with undetectable M-component at diagnosis.7,9 In the present series, those patients with detectable M-component at diagnosis (47%) had shorter median TTP to active MM (median 66
months vs. NR in patients with undetectable M-component), but differences did not reached significant (\(P= .15 \)). TTP was similar for patients with persistent M-component after radiotherapy, and immune paresis was a rare event (only one SBP patient). Altogether, these observations stress how challenging the identification of SP patients at risk of developing MM is.

At cellular level, Warsame et al has shown that the presence of >5% BMPCs by conventional microscopy is associated with significantly shorter TTP, 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. not reached; HR:17.4; \(P< .001 \)) (Figure 1A). Among patients with EMP, only 20% of Flow-positive cases evolved into MM as compared to 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 to Flow-positive cases without M-component (24 vs. 39 months; \(P= .81 \)). Noteworthy, 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). Notwithstanding, while Flow-positivity could be of significant importance, a negative result could still reflect a patchy BM involvement.

In summary, we show that MFC is a valuable biomarker for the management of patients with SBP, but 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 tailor 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.

Acknowledgments
This work was supported by the Cooperative Research Thematic Network (RTICC) grants RD12/0036/0058, RD12/0036/0069 and RD12/0036/0052 of the Red de Cancer (Cancer Network of Excellence), Instituto de Salud Carlos III, Spain, Instituto de Salud Carlos III/Subdirección General de Investigación Sanitaria (FIS: PI060339; 06/1354; 02/0905; 01/0089/01-02; PS09/01897/01370; G03/136; Sara Borrell: CD13/00340), Consejería de Educación, Junta de Castilla y León Valladolid, Spain (HUS396A12-1), Asociación Española Contra el Cáncer (AECC; GCB120981SAN), Spain.

Authorship and Conflict of Interest Statement
*BP and *MC equally contributed. JFSM and BP conceived the idea and designed the study. BP, MC, MBV, EC and TC-V performed immunophenotypic analysis. EC, TC-V, FE, AGdC, MCM, RGS, EMO, MVM and JFSM provided study material or patients. BP and MC performed statistical analysis. BP, MC, and JFSM analyzed and interpreted data. BP, MC and JFSM wrote the manuscript.

All authors reviewed and approved the manuscript, and declared no conflict of interests to disclose.
References


Table 1. Demographics and characteristics of patients with solitary bone plasmacytoma (SBP; n=35) and extramedullary plasmacytoma (EMP; n=29).

<table>
<thead>
<tr>
<th>Patient demographics and characteristics</th>
<th>SBP</th>
<th>EMP</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>57%</td>
<td>69%</td>
<td>NS</td>
</tr>
<tr>
<td>Median age, years</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>β2-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 LDH</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 free light-chain ratio (n=18)</td>
<td>69%</td>
<td>40%</td>
<td>-</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>-</td>
</tr>
<tr>
<td>* Urine M-component (g/dL)</td>
<td>0.5 (0.05 – 0.5)</td>
<td>-</td>
<td>-</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>-</td>
</tr>
<tr>
<td>Bone marrow plasma cells by conventional morphology, %</td>
<td>2 (0 – 5)</td>
<td>1 (0 – 5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Median values are given only for patients with detectable M-component in serum or urine
Figure legend

Figure 1. TTP to MM according to sensitive MFC immunophenotypic evaluation of the BM PC compartment. Time-to-progression (TTP) of patients with solitary plasmacytoma (SP) grouped according to the presence versus absence of bone marrow (BM) phenotypically aberrant clonal plasma cells (PCs) by multiparameter flow cytometry (MFC) immunophenotyping is shown in Figures 1A and 1B for patients with solitary bone plasmacytoma (SBP) and extramedullary plasmacytoma (EMP), respectively.
A) TTP (solitary bone plasmacytoma)

Flow-positive: 26m; 63% at 3-years
Flow-negative: NR; 6% at 3-years
HR: 17.4; $P < .001$
B) TTP (extramedullary plasmacytoma)

Flow-positive: NR; 20% at 3-years
Flow-negative: NR; 6% at 3-years
HR: 10.9; $P = .35$
Multiparameter flow cytometry for staging of solitary bone plasmacytoma: new criteria for risk of progression to myeloma

Bruno Paiva, Mauricio Chandia, Maria-Belen Vidriales, Enrique Colado, Teresa Caballero-Velázquez, Fernando Escalante, Alfonso Garcia de Coca, Maria-Carmen Montes, Ramon Garcia-Sanz, Enrique M. Ocío, María-Victoria Mateos and Jesus F. San Miguel