NEW CRITERIA TO IDENTIFY RISK OF PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE AND SMOLDERING MULTIPLE MYELOMA BASED ON MULTIPARAMETER FLOW CYTOMETRY ANALYSIS OF BONE MARROW PLASMA CELLS

RUNNING HEAD: PROGRESSION CRITERIA IN MGUS and SMOLDERING MULTIPLE MYELOMA

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ABSTRACT

Monoclonal gammopathy of uncertain significance (MGUS) and smoldering Multiple Myeloma (SMM) are plasma cell disorders with a risk of progression around 1% and 10% per year, respectively. We have previously shown that the proportion of BM aberrant plasma cells within the BMPC (bone marrow plasma cell) compartment (aPC/BMPC) assessed by FlowCytometry contribute to differential diagnosis between MGUS and MM. The goal of the present study was to investigate this parameter as a marker for risk of progression in MGUS (n=407) and SMM (n=93). Patients with a marked predominance of aPC/BMPC (≥95%) at diagnosis displayed a significantly higher risk of progression both in MGUS and SMM (p< 0.001). Multivariate analysis for progression free survival (PFS) selected the % aPC/BMPC (≥95%) as the most important independent variable in both entities, together with DNA aneuploidy and immunoparesis for MGUS and SMM, respectively. Using these independent variables, we have identified three risk categories in MGUS (PFS at 5 years of 2%, 10% and 46%, respectively (p<0.001)) and SMM patients (PFS at 5 years of 4%, 46%, and 72%, respectively (p<0.001)). Our results show that multiparameter FC evaluation of BMPC at diagnosis is a valuable tool that could help to individualize the follow-up strategy for MGUS and SMM patients.
INTRODUCTION

Monoclonal gammopathy of uncertain significance (MGUS) and Smoldering multiple myeloma (SMM) represent two forms of asymptomatic plasma cell disorder that share in common a variable period of stable disease but which may eventually progress to symptomatic Multiple Myeloma (MM). MGUS is the most common plasma cell disorder, whose incidence increases with age, affecting approximately 3% of population over 50 years of age and up to 10% in those over 70 years. Diagnosis of MGUS is characterized by the presence of a monoclonal immunoglobulin in serum $\leq$ 3 g/dl, and a $< 10\%$ of plasma cells in bone marrow, in the absence of end-organ damage, related to the proliferation of monoclonal plasma cell. Smoldering multiple myeloma (SMM) is also an asymptomatic plasma cell disorder that fulfills the diagnostic criteria of multiple myeloma (MM), although it is characterized by the absence of lytic lesions, anemia, renal insufficiency or hypercalcemia (CRAB symptoms). Its estimated prevalence ranges from 15% to 44% of newly diagnosed MM.

When progression occurs, the majority of cases evolve to overt MM. The rate of progression is significantly lower for MGUS cases (around 1% per year) than for SMM (10% per year). Therefore, a different follow-up strategy is needed in these two groups of monoclonal plasma cell disorders, with a closer follow-up in SMM than in MGUS. However, it would be most valuable to have individualized information on factors associated with the risk of progression at diagnosis in order to determine patient follow-up strategy according to their individual risk of progression. For both entities these factors include the amount of monoclonal component, the presence of Bence-Jones proteinuria, the isotype of the monoclonal immunoglobulin (IgA), and an abnormal serum free light-chain ratio. Particularly in the case of SMM, the current standard of care is close follow-up...
without treatment until MM symptoms develop. Nevertheless, this policy may change with the availability of novel non-cytotoxic agents. The obvious cohort of SMM patients in which these new agents should be tested are those at high risk of progression. Therefore, it is most important to identify this patients group in order to define the final value of “preventive treatment approaches”.

In recent years, multiparameter flow cytometry (FC) immunophenotyping has been increasingly used in the setting of different haematological malignancies\(^{12-17}\). Concerning plasma cell dyscrasias, it has been shown that, based on the expression of several markers, normal and myelomatous plasma cells (PC) can be easily differentiated \(^{12,18,19}\). We have previously shown that phenotypically aberrant PC (aPC) correspond to clonal PC, while PC with a normal phenotype are polyclonal\(^{12,20}\). Moreover, the proportion of aPC/BMPC was the most important criteria for differential diagnosis between MGUS and MM patients\(^{12}\). Thus, in the vast majority of MM patients, almost all PC (>95%) display an aberrant phenotype, while in MGUS patients normal and malignant plasma cells coexist\(^{12}\). Based on this background, we wanted to investigate whether or not this valuable parameter (aPC/BMPC) for differential diagnosis between MGUS and MM could also help to predict the risk of transformation of MGUS and SMM into symptomatic disease.

**MATERIAL & METHODS**

**Patients**

A total of 500 consecutive patients diagnosed between January 1996 and September 2003 who fulfilled criteria of monoclonal gammopathy of uncertain significance (MGUS) (n=407) or smoldering multiple myeloma (SMM) were included in this study. Informed written consent was obtained from all the patients included according with the Human Investigations Committee at the University Hospital of Salamanca.
Both entities were defined according to the International Myeloma Working Group. MGUS was characterized by the evidence of monoclonal component (MC) of less than 3g/dL, absence or less than 1g/24 hours Bence-Jones proteinuria, and bone marrow plasma cell (BMPC) infiltration less than 10%. SMM was defined by MC ≥3g/dL, and/or BMPC infiltration ≥10% and/or the presence of Bence-Jones proteinuria greater than 1g/dL, and in all cases absence end-organ damage (lytic bone lesions, hypercalcemia, renal insufficiency or anemia) was required. With respect to the criteria used for the definition of SMM, 14 out of 93 cases (15%) had both more than 10% of PC in BM and high MC (≥3 g/dL), 64 patients (68%) had ≥10% PC in the BM but with lower MC (<3 g/dL). The remaining 15 patients (16%) had an infiltration of BMPC lower than 10%, and diagnosis of SMM was performed in these cases on the basis of “reconfirmed” MC levels greater than 3.0. Immunoparesis was defined as a reduction (under the lower normal limit) in the levels of one or two immunoglobulin (Ig), with respect to the values of the corresponding uninvolved Ig. Progression to multiple myeloma was established according to the criteria defined by the International Working Group.

With a minimum follow-up of 24 months, at the time of closing this study, the median follow-up was 56 months (range: 24 to 187 months). Thirty three MGUS cases (8%) and 47 SMM patients (43%) have already progressed. Progression was defined as transformation from MGUS or SMM into symptomatic myeloma, amyloidosis or chronic lymphoproliferative disease. The median time to progression in MGUS patients was 44 months (range 4.4-120), and the majority of them progressed to symptomatic MM (n=26; 79%), 4 cases progressed to chronic lymphoproliferative disease (12%), 2 to amyloidosis (6%), and one to plasmatic cell leukaemia (3%). The median time to progression for SMM
patients was 18 months (range: 2-92 months), and all of them evolved into symptomatic MM.

The following variables collected at diagnosis were included in the analysis: ECOG status, hemoglobin (Hb), platelet count, erythrocyte sedimentation rate (ESR), serum creatinine, serum liver function tests, β2-microglobulin, C reactive protein (CRP), total protein, albumin and MC serum levels, presence of urine MC, serum concentration of polyclonal Ig, percentage of PC in the BM aspirate evaluated by optical microscopy, and skeleton X-ray (Table 1). The percentage of BMPC was calculated after counting 200-cells. Quantification of the MC was carried out using serum protein electrophoresis on cellulose acetate. Serum polyclonal Ig levels were measured by means of nephelometry.

**Immunophenotypic Studies**

Immunophenotypic analyses were performed on erythrocyte-lysed K3-EDTA anticoagulated whole BM samples. A total of 2×10^6 cells/tube were stained-lysed-and-then-washed using a direct immunofluorescence technique and multicolour staining, aimed at the specific identification and immunophenotypic characterization of PC: CD38-FITC/CD56-PE/CD19-PerCP-Cy5/CD45-APC). In specific cases additional staining for cytoplasmatic Ig light chains were used to clarify the polyclonal vs. monoclonal nature of PC. The cIgKappa/ cIgLambda staining was performed in a four colour tube including simultaneous surface staining for CD38 plus either CD45 or CD56 or CD19 depending on the type of antigenic aberrancy. All monoclonal antibodies used were purchased from Becton/Dickinson, Biosciences, BDB, San José, CA, except for CD38-FITC (Caltag Laboratories, San Francisco CA), and CD19-PE-Cy5 (Immunotech, Marseille, France). Acquisition was performed in a FACSCalibur flow cytometer (BDB) using double-step
procedure using with the CellQUEST software (BDB). In the first step, a total of $20 \times 10^3$ events from the total BM cellularity were measured; in the second step, information on a minimum of $3 \times 10^5$ cells were acquired and stored through an electronic "live-gate" drawn on the SSC/CD38++ events where PCs are included (Figure 1). In cases with very low numbers of PC the acquisition of cells was increased in order to reach the minimum target of PC events ($\geq 1000$). We first identified PC based on the expression of CD138/high expression of CD38 with and interm SCC. Once PC were identified, we focused our analysis on the PC compartment, and within it, we discriminated between PC with a normal and aberrant phenotype (polyclonal and clonal PC, respectively)\textsuperscript{21-23}. For this purpose, we used a validated immunophenotypic approach\textsuperscript{23,24} where the absence of CD19 and/or CD45, the decreased expression of CD38 and over expression of CD56 were used for the identification of aberrant phenotypes on PC (Table 2). For each case analysed data recorded were: i) total percentage of PCs from the whole nucleated BM celullarity and; ii) percentage of abnormal plasma cells (aPC) within the BM PC compartment. The latter parameter will be referred to in the text as: aPC/BMPC.

DNA index assessed by flow cytometry was performed using a double-staining procedure for nuclear DNA (with propidium iodide) and surface antigens (with anti-CD38 plus anti-CD138 monoclonal antibodies) as previously described\textsuperscript{25}, and the analysis was performed specifically on the PC (CD38 plus CD138+) cells. Aneuploidy was defined as hypo and hyperdiploidy bases on flow cytometry assessment.

The analysis by FC and the evaluation of the disease status, including the decision that therapy was needed, were performed by independent observers.

**Statistical analyses**
The Chi square and the Mann-Whitney U tests were used to estimate the statistical significance of differences observed between groups. Survival curves were plotted according to the Kaplan and Meier method, using log-rank and Breslow tests for comparison. In the univariate analysis for progression free survival (PFS), the following variables were tested: age, sex, hemoglobin, heavy chain, light chain, amount of MC, renal function, % of BMPC by optical microscope, % of BMPC by multiparameter FC, % of aPC/BMPC, presence of immunoparesis, DNA ploidy, and presence of Bence-Jones proteinuria. Multivariate analysis was performed to explore the independent effect of variables that showed a significant influence on progression free survival in the univariate analysis. The Cox regression model that was used employed the stepwise methods “forward conditional” as the variable selection model. Quantitative parameters were considered both as continuous as well as dichotomic variables.

All statistical analyses were performed using SPSS software - 12.5 versions - (SPSS Inc. Chicago, IL.).

RESULTS:

The clinical and biological characteristics of MGUS and SMM patients analyzed are summarized in Table 1. Median age (70 years) at diagnosis and sex distribution was similar in both groups. The frequency of IgG paraprotein was slightly higher in MGUS than in SMM (75% vs. 64%) while the opposite figure was observed for IgA (21% vs. 34%). The median value of the serum MC was 1.6 g/dL (range: 0.3 to 3.0 g/dL) and 2.37 g/dL (range: 0.8 to 6.5) for MGUS and SMM, respectively. The light chain was kappa in 73% (n=298) of the cases of MGUS patients and in 65% of SMM cases. Immunoparesis was present in 25% of MGUS patients (18% had decreased levels of only one Ig and 7% had low levels of two Igs) and in 52% of SMM patients (22% of one Ig and 30% of both chains). Bence
Jones proteinuria was detected in 40 (17%) MGUS and 14 (22%) SMM patients. The median percentage of bone marrow plasma cell PC (BMPC) by conventional morphology was 4% and 14% in MGUS and SMM, respectively.

As expected, multiparameter FC showed lower percentages of BM PC than morphology, probably due to a dilutional effect. The median value of PC infiltration by FC was significantly higher in patients with SMM than in MGUS (median 2.9% vs. 1.0%, \( p < 0.001 \)). When the analysis was restricted to the PC compartment and we discriminated between normal and phenotypically aberrant PC (aPC) (clonal PC), we observed that, in SMM, aberrant PC markedly predominate (median 97% aPC from the total BMPC cellularity) while in MGUS the distribution of abnormal/normal PC is more balanced, with 73% of PC displaying an aberrant phenotype. Moreover, if we consider the cut-off value of \( > 95\% \text{aPC/BMPC} \) which was previously used for differential diagnosis between MGUS and MM (it should be remembered that in symptomatic MM almost all PC (\( \geq 95\% \)) display an aberrant phenotype), we observed that while 60% of SMM were above this threshold, only 18% of MGUS had \( \geq 95\% \text{aPC} \) (\( p < 0.001 \)).

Interestingly, the percentage of phenotypically aPC/BMPC at diagnosis allowed the discrimination of two groups of patients with significantly different risks of progression to overt MM, both in MGUS and in SMM patients. Thus, in the MGUS group, 24% of patients (18/73) with more than 95% aPC/BMPC progressed with a median time to progression of 107 months, while only 4% (15/330) patients progressed in the group of cases with less than 95% of aPC/BMPC (\( p < 0.001 \)), with a cumulative probability of progression at 5 years of 25% vs. 5% respectively (Figure 2a). Similarly, within SMM patients the rate of progression to symptomatic MM for cases with \( > \) or \( < \) of 95% aPC/BMPC was 63% (36/56) vs. 10% (4/37) (\( p < 0.001 \)) (Figure 2b), with a cumulative
probability of progression at 5 years of 64% vs. 8%, respectively.

Other factors associated with a significant impact on progression free survival (PFS) in both groups of patients were: bone marrow plasma cells infiltration by conventional morphology (p<0.002), overall percentage of PC evaluated by flow cytometry (p<0.01), and immunoparesis (p<0.001) (Table 3). In addition, in MGUS patients, a Bence-Jones proteinuria higher than 0.2g /24 hours (p< 0.001), DNA aneuploidy (p= 0.01) and MC levels ≥2gr/dl (p<0.001) also had significant impact on PFS (Table 3). Other variables such as gender, isotype of the MC Ig, β2-microglobulin serum levels, ESR, haemoglobin, C reactive–protein were not associated with a different incidence of disease progression.

Interestingly, when we focused on patients with low risk of progression based on conventional criteria, such as low amounts of MC (<2 g/dl for MGUS and < 3g/dl for SMM) or the absence of immunoparesis, the evaluation of the percentage of aPC/BMPC still allowed the discrimination of two groups of patients at different risks of progression. Thus, among cases with low MC, those patients with ≥95% aPC/BMPC showed lower PFS than those with <95% aPC/BMPC, with significant impact on MGUS (p< 0.002) as well as SMM (p<0.001) (Figure 3). In the same way, upon restricting analysis to patients with no immunoparesis, cases with ≥95% of aPC/BMPC had poor prognosis in both diagnostic categories (SMM and MGUS) (p<0.02) (Figure 3). Similarly, the presence of ≥ or < 95% aPC/BMPC was able to discriminate two prognostic subgroups within patients with high BMPC infiltration or high MC in MGUS as well as SMM patients (data not shown).

In order to explore whether or not the percentage of immunophenotypically aPC/BMPC evaluated by FC was an independent prognostic factor for PFS among MGUS and SMM patients, a multivariate analysis was performed. Interestingly, in both diagnostic groups the sole common variable with independent prognostic value for PFS was the aPC/BMPC
(p<0.001 for MGUS and p=0.003 for SMM patients). In the MGUS group the DNA index also had independent prognostic value (p=0.001), with a risk of progression increased by a factor 8.2 in cases with ≥95% of aPC/BMPC, and by 4.6 in patients with DNA aneuploidy. In contrast, for the SMM patients, presence of immunoparesis was selected as also having independent prognostic value for PFS (p=0.02), with a risk of progression to symptomatic MM increased by a factor of 5.4 in cases with ≥95% of aPC/BMPC, and by 2.5 in patients with low levels of one or more uninvolved immunoglobulin at diagnosis.

Based on variables with independent prognostic value for MGUS transformation (≥95% aPC/BMPC and DNA aneuploidy) we established a prognostic index, by assigning one point for each adverse factor. Accordingly, three risk groups of MGUS patients were defined: cases with no risk factors (PFS at 5 years of 2%), cases with one risk factor (10% PFS at 5 years) and patients with both risk factors (46%PFS at 5 years). The same approach was used for SMM (but now based on ≥95% aPC/BMPC and immunoparesis). The PFS at 5 years was 4%, 46% and 72% for patients with none, one, or two risk factors, respectively (p<0.001) (Figure 4b).

DISCUSSION

The term MGUS, introduced by Kyle more than 25 years ago26, denotes the presence of a monoclonal protein without evidence of multiple myeloma, amyloidosis, macroglobulinaemia or other related plasma cell lymphoproliferative disorders26. Since SMM was first described by Kyle and Greipp and Alexanian in 198027,28, several series of asymptomatic MM have been reported9,29-33. These patients do not display evidence of end-organ damage and are biologically similar to patients with MGUS, although the risk of developing a symptomatic myeloma is much higher8. Patients should not be treated unless progression occurs, and the monitoring follow-up has to be closer for SMM than it is for
MGUS. Several efforts have been made to identify parameters that will probably predict progression from MGUS or SMM to active MM. For MGUS, the size of serum M protein\(^4,9,9\), as well as the IgA isotype\(^9\), an abnormal serum free light chain ratio\(^11\), detectable BJ protein excretion, >5% of PC in BM and presence of immunoparesis\(^9\) have been identified as a predictor of progression. Moreover, Rajkumar et al\(^11\) have recently show that the combination of a high M component (>1.5g/dl), a monoclonal protein other than IgG and an abnormal serum free light chain ratio is associated with a high risk of progression (58% at 20 years as compared to 5% when none of these risk factors were present). As far as SMM is concerned, it has been shown that the risk of progression is increased in cases with MC levels of >3 g/dl\(^5,8,9,32\), IgA isotype\(^8,9\), BJ protein excretion greater than 50 mg/24 h\(^9\), evolving SMM type\(^29\), >10% of PC in BM\(^9\) and occult bone lesions on magnetic resonance imaging (MRI)\(^8,33\). It should be noted that the presence of immunoparesis has been associated with a high risk of progression\(^9\) in MGUS, but not in SMM\(^8\).

Multiparameter FC is an increasingly widely used technique that can be applied to the identification of PC among all BM cells, as well as the discrimination of phenotypically abnormal plasma cells from their normal counterpart. The antigens most frequently used for the identification of aberrant PC phenotype include: CD19, CD45 and CD56 in combination with CD38/CD138\(^12,18,23-25\). Thus, the over expression of CD56 together with the absence of reactivity for CD19 and for CD45 and/or decreased amounts of CD38 have been found to be common characteristics of MM plasma cells\(^12,18,34\). Such aberrant phenotypes of BM plasma cells have been used for the study of minimal residual disease in MM, and it has been suggested that the persistence of immunophenotypically abnormal PC in the BM after treatment is associated with a worse clinical outcome\(^13,21-23,35\). In addition,
we have shown that the proportion of aPC/BMPC was the most important criteria for
differential diagnosis between MGUS and MM patients. Nevertheless, up until now the
potential value of PC immunophenotypic investigation for predicting the risk of
progression of MGUS and SMM patients has not been explored. Based on this background,
we wanted to investigate the potential impact of the detection of aPC/BMPC on the risk of
transformation of MGUS and SMM into symptomatic disease. In our study, at five years,
the risk of progression was 25% and 64% respectively for MGUS and SMM cases in which
the vast majority of PC have an aberrant phenotype (≥95% aPC/BMPC). These figures are
clearly higher than those of patients with <95% aPC/BMPC, in which the risk of
progression at five years is 5% and 8% for the MGUS and SMM group, respectively. It
should be emphasized that the immunophenotypic strategy used here is very simple and
cost-effective, since it only requires a tube sample with four monoclonal antibodies which
results in low cost.

Based on the two parameters with independent value in the multivariate analysis (≥95%
aPC/BMPC, and DNA aneuploidy for MGUS, and ≥95% aPC/BMPC, and presence of
immunoparesis for SMM), we propose a simple scoring system, assigning 1 point to each
adverse variable, which allows prognostic stratification of MGUS and SMM patients at
diagnosis into the three risk categories. The cumulative probability of progression from
MGUS to MM at five years was significantly different for the 3 subgroups: 2%, 10% and
46%, respectively. A similar pattern was observed for SMM with a risk of progression to
symptomatic disease at 5 years of 4%, 46% and 72%, respectively, for patients with none,
one or two risk factors. As mentioned above, the Mayo Clinic group has recently proposed
a new risk classification based on free light chain measurements. Of particular interest
would be to prospectively explore whether free light chain and % aPC represent
independent prognostic factors.

The current standard of care for MGUS and SMM is follow-up without treatment until symptomatic disease develops, but this policy is associated with a significant emotional burden and high cost. Inclusion of multiparameter FC analyses of the BMPC population in the diagnostic evaluation of MGUS and SMM patients could be of great help for establishing an individualized follow-up strategy according to their risk of progression. Moreover, the availability of novel, non-cytotoxic drugs, represent an attractive opportunity to investigate their efficacy in SMM (and even MGUS) patients with high risk of progression.

In summary, in the present study we show that multiparameter FC evaluation of BMPC at diagnosis of MGUS and SMM is a valuable tool for predicting the risk of progression to overt MM, since it allows the identification of patients at high risk of early progression (those in which the vast majority of PC display an aberrant phenotype), which may benefit from early treatment interventions, as opposed to a group of patients (in which both, aberrant and normal PC coexist) who will likely be free of progression for a long period of time and could be safely monitored without treatment over many years.
Reference List


Table 1. Clinical and biological characteristics of MGUS and SMM patients according with the type of Immunoglobulin

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MGUS</th>
<th>SMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender* (male/female)</td>
<td>205 / 202</td>
<td>46 / 47</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71 (26-95)</td>
<td>69 (39-88)</td>
</tr>
<tr>
<td>Serum MC (mg/dl)</td>
<td>1.60 (0.3-3.0)</td>
<td>2.37 (0.8-6.5)</td>
</tr>
<tr>
<td>Immunoparesis</td>
<td>90 (25%)</td>
<td>47 (52%)</td>
</tr>
<tr>
<td>% Of BM PC by morphology</td>
<td>4 (1-10)</td>
<td>14 (4-55)</td>
</tr>
<tr>
<td>% Of BM PC by FC</td>
<td>1.0 (0.1-37)</td>
<td>2.9 (0.9-27)</td>
</tr>
<tr>
<td>% Of abnormal PC</td>
<td>73 (0-100)</td>
<td>97 (17-100)</td>
</tr>
<tr>
<td>% of patients with &gt;95% aPC</td>
<td>73 (18%)</td>
<td>56 (60%)</td>
</tr>
<tr>
<td>Presence of BJ proteinuria*</td>
<td>40 (17%)</td>
<td>14 (22%)</td>
</tr>
<tr>
<td>Aneuploidity (%)</td>
<td>229 (58%)</td>
<td>62 (67%)</td>
</tr>
<tr>
<td>Progression (%)</td>
<td>33 (8%)</td>
<td>40 (43%)</td>
</tr>
</tbody>
</table>

Results expressed as median and (range) or as * number of cases (percentage).

Table 2. Aberrant phenotypic profile in SMM & MGUS patients.

<table>
<thead>
<tr>
<th>CD38</th>
<th>CD45</th>
<th>CD19</th>
<th>CD56</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>42 (50%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>+</td>
<td>-/dim</td>
<td>-</td>
<td>+</td>
<td>9 (11%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>+</td>
<td>Dim</td>
<td>-</td>
<td>-</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>dim</td>
<td>++</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>
Table 3. Clinical and biological parameters associated with risk of progression of MGUS and SMM in the univariate analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MGUS</th>
<th>SMM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median PFS (Months) Progression (%) p value (Long-rank)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoparesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12/266 (4.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>One or two Ig</td>
<td>17/91 (18%)</td>
<td></td>
</tr>
<tr>
<td>% of BMPC (optical microscope)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5% or 15%</td>
<td>Nr 11/251 (4%)</td>
<td>0.003</td>
</tr>
<tr>
<td>≥ 5% or 15%</td>
<td>Nr 21/144 (15%)</td>
<td></td>
</tr>
<tr>
<td>% of BMPC (flow cytometry)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1% or 5%</td>
<td>Nr 7/196 (3%)</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>≥ 1% or 5%</td>
<td>Nr 25/210 (12%)</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 g/dl MGUS and &lt;3 g/dl SMM</td>
<td>Nr 11/279 (4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥2 g/dl MGUS and ≥3 g/dl SMM</td>
<td>Nr 20/111 (18%)</td>
<td></td>
</tr>
<tr>
<td>Ligh chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>23/216 (10%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lambda</td>
<td>6/165 (3%)</td>
<td></td>
</tr>
<tr>
<td>DNA Ploidy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>11/172 (6%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Aneuploid</td>
<td>18/108 (17%)</td>
<td></td>
</tr>
<tr>
<td>Bence-Jones proteinuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.2 g/24 hours</td>
<td>10/216 (5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥0.2 g/24 hours</td>
<td>7/26 (26%)</td>
<td></td>
</tr>
</tbody>
</table>

* cut-off point of bone marrow plasma cell infiltration of 5% for MGUS and 15% for SMM.

** cut-off point of bone marrow plasma cell infiltration by flow cytometry of 1% for MGUS and 5% for SMM.
Figure 1: Gating strategy for PC analysis by multiparametric flow cytometry
Figure 2: Time To Progression (TTP) in MGUS (1a) and SMM (1b) according to the percentage of immunophenotypically aberrant plasma cell. For MGUS, median 107 months vs. not reached for patients with ≥95% or <95% aberrant PC, respectively, (p<0.001)(Figure 1A). For SMM median 34 months vs. not reached for patients with ≥95% or <95% aberrant PC, respectively (p<0.001)(Figure 1B).
Figure 3: Impact of percentage of abnormal PC in Time To Progression (TTP) for patients at low risk.

In panel A and B patients with low MC (<2 gr/dl for MGUS and <3gr/dl for SMM): TTP is longer in MGUS patients with <95% aberrant PC (p<0.002) (2A), in SMM the median TTP is 44 months vs. no reached for patients with ≥95% or <95% aberrant PC (p< 0.001) (2B).

In panel C and D patients without immunoparesis: median TTP not reached vs. not reached for patients with MGUS and <95% or ≥ 95% aberrant PC, respectively (p=0.02) (2C), and median not reached vs. 51 months for patients with SMM and <95% or ≥ 95% aberrant PC (p< 0.001) (2D).
Figure 4: Time to Progression (TTP) in MGUS (Panel A) and SMM (Panel B) according to the score system.

In panel A the score system for MGUS for was built on the basis of the percentage of immunophenotypically aberrant PC within the BMPC compartment (<95% aberrant PC score of 0; ≥95% score of 1), and DNA index: aneuploid (score 1) or diploid (score 0): In patients with Score 1 the median TTP has not been reached, in those with Score 2 the median TTP is 120 months, while in patients with Score 3 the median TTP is 61 months. (p<0.001).

In panel B the score system for SMM was built on the basis of the percentage of immunophenotypically aberrant PC within the BMPC compartment (<95% aberrant PC score of 0; ≥95% score of 1), and the presence (score 1) or absence (score 0) of immunoparesis: In patients with Score 1 the median TTP has not been reached, in those with Score 2 the median TTP is 73 months, while in patients with Score 3 the median TTP is 23 months. (p<0.001).
New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells

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