Original Article

Blood-based risk stratification for pre-malignant and symptomatic plasma cell neoplasms to improve patient management

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Abstract: Standard risk stratification (sRisk) guides clinical management in monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM) and multiple myeloma (MM). Nonetheless, clinical results are considerably heterogeneous among patients with similar risk status. Blood and bone marrow samples from 276 MGUS, 56 SMM and 242 MM in regular clinical practice were analyzed at diagnosis by flow cytometry. Higher levels of aberrant circulating plasma cells (cPC) (> 0.0035% of leukocytes), combined with albumin, beta2-microglobuline and lactate-dehydrogenase levels, offered minimally-invasive risk stratification (RcPC) with results comparable to sRisk. RcPC and sRisk 10-year progression-free-survival (10y-PFS) rates were: 93.8% vs. 95.1% for low-risk, 78.4% vs. 81.7% for intermediate-risk and 50.0% vs. 47.8% for high-risk MGUS; 58.3% vs. 57.8% low-risk, 44.4% vs. 45.8% intermediate-risk and 8.9% vs. 15.0% high-risk SMM; and 44.4% vs. 44.4% low-risk, 36.1% vs. 36.8% intermediate-risk, and 13.3% vs. 16.2% high-risk MM. Circulating-PC > 0.0035% vs. cPC<0.0035% was an independent prognostic factor for PFS (HR=4.389, P=1.2×10⁻¹⁵, Harrell C-statistic =0.7705±0.0190) and over-all survival (OS, HR=4.286, 2.3×10⁻⁹, Harrell C-statistic =0.8225±0.0197) that complemented sRisk in patients with low-sRisk (10y-PFS rates 48.1% vs. 87.3%, P=1.2×10⁻⁸) and intermediate-sRisk (10y-PFS rates 28.9% vs. 74.1%, P=8.6×10⁻¹²). Patients with high cPCs values are associated with higher proliferation and lower apoptosis rates of PC. Circulating-PC > 0.0035% identified MGUS, SMM and MM patients at higher risk of progression or death and predicted a cohort of patients that after relapse from stringent complete response showed shorter OS. These patients could benefit from early consolidation therapy, tandem ASCT or intensive maintenance.

Keywords: Plasma cell neoplasms, MGUS, SMM, MM, circulating plasma cells, risk stratification

Introduction

Novel treatments for multiple myeloma (MM) combining immunomodulatory drugs (lenalidomide, thalidomide, or pomalidomide), proteasome inhibitors (bortezomib, carfilzomib, or ixazomib) and tandem autologous stem cell transplantation (ASCT) have increased the rate of complete response (CR) and prolonged treatment-free and survival periods [1]. Nevertheless, the disease is considered incurable and displays substantial clinical heterogeneity in presentation and course, underlining the need for new biomarkers that allow us to adapt the therapy not only to the patient’s biological and clinical conditions, but also to the real risk of the disease. Currently there are several risk stratification systems depending on the type of plasma cell neoplasms (PCN), ranging from premalignant monoclonal gammapathy of undetermined significance (MGUS) [2-4] and smoldering MM (SMM) [2, 3, 5, 6] to the symptomatic
MM [7]. MGUS is the most common PCN, with a prevalence of 3.2% in the general population older than 50, and increasing with age [8]. Although the progression rates to malignant PCN in asymptomatic MGUS and SMM are about 1% and 10% per year, respectively [8, 9], prognostic biomarkers are also needed in MGUS and SMM for counseling, clinical care and follow-up, and for the design of clinical studies in patients at high risk [10].

Since different parameters are used for the risk stratification of pre-malignant and malignant PCNs, currently, a large group of biochemical (Albumin, beta2-microglobulin -b2m-, lactate dehydrogenase -LDH-), immunological (serum Monoclonal-protein, IgA, IgG, IgM and light-chains), histological (total and aberrant BM-PC infiltration), cytogenetic (Fluorescence In situ Hybridization, FISH, for del(17p) and t(4;14)), cytometric (BM-PC immunophenotype and labeling index), and/or imaging (MRI and/or PET/CT) parameters should be accessible [6, 7, 11-13]. Unfortunately, the unavailability of some of these parameters in less developed countries could hinder the worldwide expansion and effectiveness of the newest therapeutic protocols.

Multiple myeloma is a complex disease and several factors come into play in its prognosis. Genetic subtypes of MM have different underlying biological features that define the proliferative, apoptotic and dissemination properties of the myelomatous cell and contribute to the clinical heterogeneity of the disease [14]. Although, the precise mechanisms underlying the dissemination of myeloma tumor cells remain largely unknown [15], several studies have recurrently shown that the presence of aberrant circulating PCs (cPC) in peripheral blood (PB) is a marker for disease activity in patients with MM [16], with an adverse independent prognostic significance in MGUS [17], SMM [18-20], MM [21-23], and light chain amyloidosis [24]. Furthermore, the presence of cPCs can predict early relapse after autologous stem cell transplantation (ASCT) [25, 26], and appears to be useful as a predictive factor in relapsed MM [27].

In recent years, detection of cPC has gained interest for MM mainly because of the minimal invasive nature of blood vs. bone marrow analyses. High-sensitivity next-generation flow (NGF) is able to detect cPCs systematically in the blood of MM patients at diagnosis, with higher cPC counts having an adverse prognostic impact, thus suggesting that disease dissemination via blood confers a malignant behavior to MM [28]. Besides, NGF provides additional insight in the monitoring of treatment effectiveness; NGF is able to detect cPC in 26% of patients after therapy, pointing out patients with a higher tumor regrowth and/or dissemination capacity [29], which ultimately determine disease progression, most probably due the more immature and prominent stem cell-like features of cPCs [28].

This study of a large cohort of newly diagnosed MGUS, SMM and MM patients in real-world medicine was conducted to explore the prognostic utility of cPC monitoring by using flow cytometry at diagnosis. The predictive capacity of cPC was evaluated in combination with easily evaluable serum biochemical parameters or in combination with the standard risk stratification for each PCN stages. Likewise, its predictive capacity was evaluated in relation to the treatments currently used in ASCT eligible and ineligible patients. Finally, the presence of cPC was evaluated in relation to the proliferative and apoptotic capacity of bone marrow PCs.

Materials and methods

Patients

EDTA anti-coagulated PB and BM samples were obtained at diagnosis from 570 consecutive patients with PCNs in regular clinical practice from 7 hospitals in the Region of Murcia, Spain, between 2010 and 2017. This study was approved by the Research Ethics Committee. Institutional review board (IRB-00005712). Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Following the IMWG criteria [7], patients were classified in 276 MGUS, 56 SSM, and 242 MM. Mean follow-up for each stage were 57.3±30.5, 69.0±34.5, and 51.8±35.4 months for MGUS, SMM and MM, respectively. Standard risk stratification (sRisk) in MGUS (0 vs. 1-2 vs. 3 factors) [11], SMM (0 vs. 1 vs. > 1 factors) [30] and MM (RISS-I vs. RISS-II vs. RISS-III) [7] was done following updated criteria and patients grouped as low (Risk-I), intermediate (Risk-II), and high
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(Risk-III) risk. In MGUS disease progression was computed when progressed to SMM or MM; in SMM progression was computed when progressed to symptomatic MM. MGUS and SMM patients progressing to symptomatic MM were treated as MM patients. In MM, progression, complete response and relapse was estimated following Uniform Response Criteria for Multiple Myeloma of the IMWG [31, 32]. Treatments and management were at the discretion of the hematologists based on patient condition and tumor risk. Briefly, conventional first-line therapy for patients not eligible for ASCT included bortezomib, melphalan, and prednisone (VMP), bortezomib and dexamethasone (Vd) or more recently lenalidomide and dexamethasone (Rd). In ASCT-eligible patients, first-line therapy included bortezomib, cyclophosphamide, dexamethasone (VCD) or bortezomib, doxorubicin, and dexamethasone (PAD) or more recently bortezomib, thalidomide, and dexamethasone (VTd) or bortezomib, lenalidomide, and dexamethasone (VRd), and ASCT conditioning with melphalan 200 mg/m² (dose ranging from 200 to 100-140 mg/m² if renal impairment).

Plasma cell immunophenotyping

Plasma cell immunophenotype and MRD studies in BM samples and detection of peripheral blood cPC were performed in a minimum of 1×10⁶ white cells with FACSCanto-II and DIVA-Software (Becton Dickinson; BD; San Jose, CA, USA) following consensus criteria [4, 33, 34], previously validated [35], and are described in more detail in Figure 1. Briefly, total PCs were identified as CD38⁺CD138⁺ cells in the Synthesis + G2/M phases of the cell cycle.

Fluorescent in situ hybridization (FISH)

Cytogenetic abnormalities were evaluated in interphase nucleus from BM-PCs purified using RosetteSep® Human Multiple-Myeloma-Cell Enrichment Cocktail (Stemcell Technologies, Grenoble, France). The following FISH probes from Metasystems (Altlassheim, Germany) were used to evaluate: translocations of the immunoglobulin heavy chain gene region (IGH) with break-apart IGH probe (catalog n°: D-5061-100-0G, cut-off: 3%) and dual fusion probes to determine the most common IGH partners CCND1 (catalog n°: D-5111-100-0G, cut-off: 2%), FGFR3 (catalog n°: D-5108-100-0G, cut-off: 2%), MAF (catalog n°: D-5112-100-0G, cut-off: 2%) and MAFB (catalog n°: D-5105-100-0G, cut-off: 2%); copy number of chromosomes 5, 9 and 15 with 5p15/9q22/15q22 hyperdiploidy probes (catalog n°: D-5095-100-TC, cut-off: 10%); amplification/deletion of 17p13 (TP53) and 17q22 (LPO/MPO) with locus-specific probes (catalog n°: D-5048-100-0G, cut-off: 10%); amplification/deletion of 1q21-22 (CKS1B) and 1p32.3 (CDKN2C) with locus-specific probes (catalog n°: D-5099-100-0G, cut-off: 10%), and monosomy-13/deletion 13q14.2 (DLEU1) and 13q34 (LAMP1) with locus-specific probes (D-5054-100-0G, cut-off: 10%). For each probe 300 plasma cells were analyzed with Metafer (Metasystems).

Statistical analysis

Statistical analyses were performed using the SPSS version 15.0 (SPSS Inc, Chicago, IL). ANOVA and DMS post-hoc tests were used to analyze continuous variables. Receiver operating characteristic (ROC) was used to explore patient PFS and to determine the optimal cutoff values for cPCs. PFS was estimated as months from the diagnosis date to disease progression or death. Survival curves were plotted according to the Kaplan-Meier method. The log-rank test was used to estimate significant differences. Multivariate analysis of prognostic factors for PFS and OS was performed using the Cox proportional hazards model (stepwise regression). Hazard ratio (HR) and 95% confidence interval were estimated. Harrell C-statistic was obtained using STATA-14 (Somersd package).
Figure 1. Plasma cell (PC) immunophenotyping. A. Flow cytometry analysis for peripheral blood and bone marrow samples were performed with FACSCanto-II and DIVA Software (BD). Photomultiplier (PMT) voltages were adjusted daily using CS&T beads (BD). Fluorescence compensation was finely adjusted using negative events for each fluorochrome as reference. A total of three millions white cells were stained for both tube-1: CD3+CD20 FITC, CD19 PE, CD38 PerCP-Cy5.5, CD56 PE-Cy7, CD27 APC, CD45 APC-Cy7, Annexin-V V450, and CD138 BV510 (BD); and tube-2: cyIgG, cyIgA, cyIgD, or cyIgM FITC, cyLambda PE, and Lambda cit PE-A.

B

C

Multiple myeloma

Multiple myeloma

Apoptosis: Low

High

Proliferation: High

Low

1.1% 3.5% 49.3% 2.1% 13.5% 8.2% 12.6% 3.8%
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Cy7, cyKappa APC, CD45 APC-Cy7, CD19 BV421, and CD138 BV510 (BD). One million cells were recorded for each tube. After doublet discrimination (in a FSC-H/FAC-A dotplot), total PCs (Blue) were identified as CD38++CD138+ events. Aberrant PC (cyan) were identified as CD45++/negative, CD19++/negative, CD20+, CD56+, CD27++/negative, and/or monoclonal restriction for the heavy and/or light immunoglobulin chains (specific gating strategy was followed for each patient based on their phenotype). Mature B cells (red) were identified as lymphocytes (FSC/SSC blue and red events for PCs and B lymphocytes, respectively) and proliferative (Synthesis + G2/M phases of the cell cycle analysis) rates, respectively.

P<0.05 was considered statistically significant.

Results

Patient characteristics

Table 1 presents biological, clinical and therapeutical characteristics of patient. Ten-year PFS (10y-PFS) and OS (10y-OS) rates were 86.4% and 90.8% for MGUS, 50.0% and 75.0% for SMM, and 40.6% and 54.5% for MM. According to the type of treatment, 10y-PFS and 10y-OS rates were 26.8% and 40.2% for No-ASCT (P<0.018), 35.5% and 66.1% for ASCT with PAD and VCD, and 60.7% and 85.5% for ASCT with VTd or VRd (Figure 2).

Risk stratification of PCN based on aberrant cPCs (RcPC)

Increasing percentages of aberrant cPC were observed for MGUS, SMM and MM (0.009±0.006, 0.029±0.017, and 0.87±0.25; P=0.009) (Figure 3A). The ROC analysis showed that the cutoff value with the highest prognostic capacity for PFS was 0.0035% cPC (area under the curve, AUC=0.753, sensitivity =72.0% and specificity =81.0%) (Figure 3B). As expected, increasing percentages of patients with cPCs > 0.0035% were observed for MGUS, SMM and MM (8.3%, 36.4% and 64.55%; P=1x10^-28) (Figure 3C). Next, we explored the prognostic capacity of cPCs > 0.0035% by itself for PFS and OS. The 10y-PFS and 10y-OS rates for patients with cPC below/over 0.0035% were 91.7%/50.0% (P=1.5x10^-7) and 94.9%/75.0% (P=0.013) for MGUS, 62.0%/38.5% (P=0.061) and 90.5%/69.3% (P=0.07) for SMM, and 48.0%/28.3% (P=3.0x10^-4) and 72.0%/44.6% (P=1.1x10^-4) for MM, respectively (Figure 3D).

Cox regression analysis of total PCN showed that cPC > 0.0035% was an independent prognostic factor for PFS (HR=4.389, P=1.2x10^-15, Harrell C-statistic =0.7705±0.0190) and OS (HR=4.286, 2.3×10^-9, Harrell C-statistic =0.8225±0.0197) when analyzed together with sex (shorter PFS and OS for men than women), age (shorter PFS and OS for elders) and standard risk stratification (HR=1.770, P=2.5×10^-5 for PFS and HR=2.689, 1.4×10^-7 for OS) (Figure 3E). Comparable HR results were observed when MGUS, SMM and MM were analyzed separately (see Figure 3F).

To evaluate the predictive capacity of the cPC analysis, risk stratification was calculated following similar criteria to those of the RISS [7], but substituting the presence of high-risk cytogenetics for the presence of cPCs > 0.0035% (“RcPC” stratification). In the case of MGUS and SMM, high risk (RcPC-III) was assigned when cPCs > 0.0035% even if b2m<.5 mg/dL (see Figure 4A for RcPC stratification criteria). Standard risk and RcPC stratifications showed comparable PFS and OS curves (Figure 4B) and similar distribution of patients within the risk groups for MGUS, low (46.7% vs. 44.2%), intermediate (50.4% vs. 47.4%) and high (2.9% vs. 8.3%) risk; for SMM, low (42.8% vs. 33.9%), intermediate (48.2% vs. 42.8%) and high (8.9% vs. 23.2%) risk; and for MM, low (22.3% vs. 22.3%), intermediate (65.3% vs. 59.9%), and high (12.4% vs. 17.7%) risk (Figure 4C). It is noteworthy that high-risk patients were slightly more frequent in all NPCs with the RcPC than with sRisk. Besides, similar 10y-PFS and 10y-OS rates were observed within the sRisk and RcPC groups for patients with MGUS, low (93.8% vs. 95.1% and 95.3% vs. 96.7%), intermediate (78.4% vs. 81.7% and 85.6% vs. 86.3%) and high (50.0% vs. 47.8% and 62.5% vs. 69.6%) risk; SMM, low (58.3% vs. 57.8% and 87.5% vs. 84.2%), intermediate (44.4% vs. 45.8% and 70.4% vs. 66.7%) and high (8.9% vs. 15.0% and 30.0% vs. 40.1%) risk; and MM, low (44.4% vs. 44.4% and 79.6% vs. 79.6%), intermediate (36.1% vs. 36.8% and 47.5% vs. 49.3%) and high (13.3% vs. 16.2% and 16.7% vs. 18.6%) risk (Figure 4D). Survival curves for MGUS, SMM and MM patients according to the sRisk and RcPC stratifications are shown in Figure 5.
Higher tumor burden and proliferation and lower apoptosis rates of BM-PC are associated with higher RcPC

Next, we evaluated the biological characteristics of BM-PCs in the RcPC groups, which allowed us to understand the differences in patient survival. Decreasing PFS (77.4%, 57.2%, and 27.8%, \( P=2\times10^{-21} \)) and OS (90.8%, 66.9%, and 41.8%, \( P=2\times10^{-18} \)) rates observed in the low, intermediate and high RcPC risk groups for total NPCs (Figure 6A) were inversely associat-
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Kaplan-Meier and Log-rank tests for Progression-Free (PFS) and Overall Survival (OS) according to the type of PCN: monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), or multiple myeloma (MM); and according to the type of first-line treatment: No-ASCT with VMP, Vd or Rd; ASCT with PAD or VCD; or ASCT with VTd or VRd. Ten-year PFS and OS rates are shown for each group of patients.

ed with increasing percentages (7.9%, 20.7%, and 40.7%, P=9×10^{-15}, Figure 6B), absolute numbers (0.58±0.1, 2.4±0.5 and 15.1±4.2×10^3/µl, P=1×10^{-16}, Figure 6C) and proliferation rates (1.5%, 2.4% and 3.9%, P=3×10^{-4}, Figure 6D) of BM-PCs. However, decreasing survivals were directly associated with decreasing apoptosis rates of BM-PCs (8.5%, 6.2%, and 2.9%, P=6×10^{-9}, Figure 6D).

cPCs complements predictive capacity of standard risk stratification

The study of cPCs not only allowed non-invasive risk stratification in pre-malignant and symptomatic PCNs, but also complemented the standard risk stratification estimated by studying bone marrow samples. In fact, according to the absence/presence of cPCs > 0.0035%, we observed 10y-PFS and 10y-OS rates of 87.3%/47.1% (P=1.2×10^{-8}) and 95.8%/81.5% (P=0.004) for low-risk patients; 74.1%/29.0% (P=8.6×10^{-12}) and 83.0%/47.8% (P=2.2×10^{-7}) for intermediate-risk patients; and 33.3%/17.4% (P=0.58) and 66.7%/21.7% (P=0.08) for high-risk patients (Figure 7A). Noteworthy is that 18.6% of standard low-risk and particularly 38.9% of standard intermediate-risk patients who had cPC > 0.0035% showed PFS and OS rates close to those seen in standard high-risk patients.

Similar results were observed for each type of NPC according to the absence/presence of cPC > 0.0035%. MGUS, SMM and MM patients showed 10y-PFS rates of 96.6%/71.4%, 66.6%/60.0% and 55.0%/33.3% for sRisk-I and 86.3%/25.0%, 70.0%/25.0% and 44.8%/29.3% for sRisk-II, respectively; and 10y-OS rates of 97.8%/71.4%, 88.9%/80.0% and 90.0%/80.0% for sRisk-I and 89.0%/75.0%, 95.0%/75.0% and 62.0%/42.1% for sRisk-II, respectively (Figure 7B). The smaller number of sRisk-III patients did not allow for the evalua-
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<th>95% CI</th>
<th>P</th>
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<td>2.610-5.111</td>
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HR: hazard ratio; 95% CI: 95% confidence interval
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Figure 3. Aberrant circulating plasma cells (cPC) numbers and prognostic capacity. A. Percentage of aberrant cPCs (in total leukocytes) in peripheral blood of patients with monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), or multiple myeloma (MM). P values estimated by ANOVA. B. ROC curve analysis of cPC for Progression-Free survival (PFS). C. Frequency of patients with cPC > 0.0035% in MGUS, SMM and MM patients. P values estimated by contingency tables and chi-squared test. D. Kaplan-Meier and Log-rank tests for PFS and Overall Survival (OS) according to the absence or presence of cPC > 0.0035% in MGUS, SMM and MM patients. E. Cox regression analysis for PFS and OS for sex, age, standard risk stratification (sRisk), and cPC > 0.0035% in total leukocytes. F. Hazard ratio (HR) and 95% confidence interval for progression or death in Total, MGUS, SMM, and MM patients observed in the Cox regression analysis.

Figure 4. Prognostic utility of aberrant circulating plasma cells (cPC) on progression-free (PFS) and overall survival (OS). A. Stratification criteria for the standard risk stratification (sRisk) and cPC-based risk stratification (RcPC). B. Kaplan-Meier and Log-rank tests for PFS and OS according to sRisk and RcPC stratifications. C. Distribution of patients with risk-I, -II and -III for sRisk and RcPC stratifications in MGUS, SMM and MM. Number of patients for each PCN stage and risk is indicated. D. Ten-year PFS and OS for sRisk and RcPC groups for MGUS, SMM and MM patients.

The newest therapies for malignant PCNs accessible in routine clinical practice are increasing the rate of CR and prolonging survival periods, but are also demanding adequate biomarkers to guide risk-adapted therapies. Certainly, the risk stratification provided by the cPCs analysis is predictive in all types of therapies including tandem ASCT with VTd and VRd. Thus, RcPC stratification was able to predict 89.0% and 100% 10y-OS rates in patients with low RcPC risk treated with ASCT either with PAD/VCD or VTd/VRd, compared to 44.5%, 45.5% and 45.5% 10y-OS rates in patients with high RcPC risk treated with No-ASCT and ASCT either with PAD/VCD or VTd/VRd, respectively (Figure 8A).

Stringent complete response (sCR), with negative MRD 3 to 6 months after therapy, was achieved in 39.6%, 68.4% and 64.5% of
Figure 5. Comparative prognostic utility of sRisk and RcPC stratifications in MGUS, SMM and MM. Kaplan-Meier and Log-rank tests for progression-free and overall survival according to the sRisk and the RcPC stratifications.
**Figure 6.** Biological characteristics of bone marrow plasma cells (BM-PC) in RcPC groups. A. Ten-year PFS and OS rates in total PCN patients. B. Percentage of BM-PC in the histology study. C. Number per μl of BM-PC. D. Proliferation (Synthesis + G2/M phases of the cell cycle) and apoptosis (Anexine-V⁺) rates of BM-PC.

**Figure 7.** Circulating plasma cell (cPCs) analysis complements standard risk stratification. A. Kaplan-Meier and Log-rank tests for progression-free (PFS) and overall survival (OS) according to the absence/presence of cPC > 0.0035% in the standard risk groups (sRisk). Ten-year PFS and OS rates are indicated for each risk group. B. Ten-year PFS and OS rates for sRisk-I and sRisk-II MGUS, SMM and MM patients according to the absence/presence of cPC > 0.0035%.

Patients from our series treated with Non-ASCT and ASCT with PAD/VCD or VTd/VRd, respectively. Relapse occurred in 73.5%, 57.1% and 37.0% of patients in these treatment groups, respectively (Figure 8B). Patients who relapsed after sCR showed lower 10y-OS rates com-
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Figure 8. Risk stratification estimated with cPCs maintains its prognostic capacity in different types of treatments and clinical responses. A. Kaplan-Meier and Log-rank tests for Overall Survival (OS) according to the RcPC stratification for the three types of treatments: No-ASCT with VMP, Vd or Rd; ASCT with PAD or VCD; or ASCT with VTd or VRd. B. Percentage of patients with stringent complete response (sCR) with negative minimal residual disease (MRD) and percentage of patients who relapsed after sCR according to the type of treatment. C. Kaplan-Meier and Log-rank tests for OS according to disease relapse after sCR and according to the RcPC stratification for no-relapsed and relapsed patients.

pared to those who did not relapse (51.6% vs. 86.0%, P=1.6×10^{-4}) (Figure 8C). Nonetheless, low RcPC risk was able to predict 78.6% and 100% 10y-OS rates in relapsed and non-relapsed patients, respectively, compared to intermediate RcPC risk (46.7% and 70.0%) and high RcPC risk (26.7% and 80.0%) (Figure 8C). Therefore, high RcPC risk helped to identify patients that after relapse will show much reduced PFS.

Discussion

In the era of personalized cancer therapies, the availability of clinically relevant and biologically meaningful biomarkers is imperative. In multiple myeloma, great effort has been made to find molecular alterations that allow for adequate risk stratification [14, 36-38] and targeted therapies [39-41]. However, very few of these biomarkers have been translated into generalized clinical practice, except for the high-risk alterations detected by FISH such as del(17p) and t(4;14) [7], which, in combination with albumin, b2m, and LDH, lead to a risk stratification that overestimates intermediate risk patients. Treatment approaches for intermediate-risk patients are currently between regimens used for low and high-risk patients.
Although the outcome of patients with MM have drastically improved over the past decade, considerable heterogeneity in clinical course and survival is observed among patients with similar risk status, suggesting that additional factors govern the sensitivity and resistance of myeloma therapies. In cancer, the array of genetic alterations imprint specific proliferation and apoptosis profiles on transformed cells, which ultimately govern its dissemination and the fate of the disease [42-44]. The results of this study demonstrate that cPCs is an adverse independent prognostic factor in MGUS, SMM and MM, which is directly related to the proliferation rate and inversely related to the apoptosis rate of the myelomatous cells. The presence of cPCs at diagnosis establishes an unfavorable prognosis, regardless of the type of PCN, which identifies patients at the highest risk of progression and death. Such prognosis was maintained throughout the clinical course of the disease, even when patients were treated with the most effective drugs currently used in regular clinical practice, and even when the therapy reached its maximum effectiveness, CR with negative MRD.

Currently, different risk stratification systems apply for each PCN stages; however, blood cPC-based risk stratification is able to offer valid risk estimation across the entire PCN spectrum (MGUS, SMM and MM) based on the combination of parameters such as: 1) serum albumin, b2m and LDH levels, indicative of patient clinical status, tumor burden and disease activity, respectively, and 2) cPCs, indicative of higher tumor growth/dissemination and disease progression based on their stem cell-like features [29]. Although, the precise biological significance of cPCs in PCN remains largely unknown, compared to BM-PC, cPCs display more immature phenotype, features of quiescent cells with greater resistance to chemotherapeutic agents and higher self-renewal potential [revised in 15]; suggesting that cPCs might constitute MM stem cells [45] with higher resistance to current therapeutic approaches both in ASCT eligible and ineligible patients, as suggested by the results observed in our series.

Although the presence of cPCs was described for the first time in 1962 [46] and ever since its prognostic value in pre-malignant and malignant PCN has been undoubtedly established [18, 19, 22, 47, 48], the truth is that its implementation in routine clinical practice is marginal, which is surprising for a fast and cheap technology accessible in most centers treating MM. It is unquestionable that the inclusion of del(17p) and t(4;14) cytogenetic alterations has notably improved risk stratification in MM. However, this methodology, more laborious and less accessible, underestimates high-risk patients, even after the inclusion of patients with high LDH in this group [7]. It is known that the frequency of high-risk cytogenetics rarely exceeds 25% [49] (13.9% in our series of MM) and that concurrent elevated levels of LDH and b2m are rarely observed at the onset of the disease (7% in our series of MM) [50]. In contrast, RISS overestimates the intermediate-risk group and, as a consequence, these patients show notable clinical variability, indicating that some of these patients could have benefited from more effective first-line treatments [51]. The truth is that in our series the presence of cPC > 0.0035% identified 18.6% of standard low-risk and particularly 38.9% of standard intermediate-risk patients who showed rates of PFS and OS close to those seen in standard high-risk patients. Therefore, the benefits of risk stratification based on cPCs are multiple, since it makes this technology accessible to more centers worldwide and contribute to improving current risk stratification systems, in order to offer first-line therapies better adapted to the biological risk of patients.

The frequency of PB involvement depends on the type of PCN and the sensitivity of the method used to detect cPCs, ranging from immunocytochemistry [52, 53] to conventional 4/8 color flow cytometry [18, 19, 22, 47, 48] or the new generation flow (NGF) [28]. NGF can detect cPCs up to 100% in active MM and SMM and in 59% of MGUS. In contrast, the flow cytometry method used in our study was 10 times less sensitive than NGF and detected cPCs in 64.5%, 36.7% and 8.8% of MM, SMM and MGUS, respectively. Although at first glance our method might appear to have insufficient sensitivity, the truth is that a cutoff of 0.0035% (35 cells in a million) was sensitive enough to offer a good prognostic capacity both on PFS and OS in all PCNs. In fact, in the work of Sanoja-Flores et cols [28] a cutoff of 0.058 cPCs/µl was established to differentiate MGUS from myeloma and to confer MGUS patients a shorter pro-
progression time to SMM or MM. Besides, a cutoff of 0.1 cPCs/µl was set as the optimal cutoff with a prognostic capacity for PFS and OS in MM [28]. These results are equivalent to those described by us, since 0.0035% in our series was equivalent to 0.22 cPCs/µl. This small difference could be due to differences in sample processing: in our study 200 µl of PB were directly labeled, while in NGF the bulk-lysis processing required 3 additional lysis/wash steps, which could have led to selective loss of cell populations. Other studies have established cutoffs of 0.1% (28 time higher than ours) [13] or 0.02% (6 time higher than ours) [54, 55] cPCs as high-risk factor for PFS and/or OS in MM. Therefore, and although NGF can offer great advantages for conducting non-invasive MRD studies [29], our method offers sufficient sensitivity to establish at diagnosis a useful risk stratification for all types of PCNs, and can be accessible to most centers working with conventional flow cytometry. However, the International Myeloma Working Group (IMWG) should promote the use of standardized methods to analyze cPCs, which would make inter-laboratory results comparable, as well as multicenter clinical trials to set consensual cPCs cutoffs with the highest prognostic value, so that this marker can be translated into clinical practice soon.

Analysis of cPC could also facilitate risk-adapted follow-up and clinical management in MGUS and SMM. In MGUS progression to malignant PCNs occurs at a rate of 1% per year [8]. However, in our series, low-risk patients with cPCs<0.0035% had progression rates 3 times lower and high-risk patients with cPCs > 0.0035% 10 times higher, supporting a direct negative effect of the MGUS clone inducing severe organ damage [56]; therefore, it would be reasonable to assess the possibility of early treatment in these high-risk cases. In SMM, excluding ultra-high-risk cases (BM-PC≥60% or FLC-ratio≥100) who should be diagnosed and treated as symptomatic MM, the optimal time for treatment remains controversial [57-59]. However, and although our data should be confirmed in larger series of SMM, cPC<0.0035% identified patients with long 10-year PFS and OS and therefore those for whom a watchful waiting would be justified.

In general, low cPCs values at diagnosis together with normal levels of albumin and b2m identify patients with long OS, close to 100%, regardless of the type of PCN, the treatment or the response achieved. In line with previous reports [28], our results show that these patients will show a good long-term outcome, even when they do not reach MRD-negativity or they relapse after sCR. Therefore, the analysis of cPCs seems to have prognostic value even with the most effective drugs currently used in clinical practice, although it should be evaluated for the new upcoming therapies such as CART-BCMA and BiTE.

These results show that risk stratification estimated at diagnosis by combining blood cPC analysis and usual biochemical parameters provides a rapid and accessible prognosis useful to identify MGUS, SMM and MM patients at higher risk of progression and dead. The prognostic capacity of cPC complemented standard risk stratification systems for different types of PCNs and remained valid even in MM patients who relapsed after sCR, and therefore it would be equally useful for the most effective drugs used in current real-world clinical practice. Thus, RcPC predicted a cohort of patients who showed much shorter OS after relapse and could benefit from early consolidation therapy, tandem ASCT or intensive maintenance. Our results also show that patients with high cPCs values are associated with higher proliferation and lower apoptosis rates which ultimately would explain the worse clinical course of these patients.

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None.

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Risk stratification for plasma cell neoplasms

References


Risk stratification for plasma cell neoplasms


