Immunophenotypic analysis of peripheral blood stem cell harvests from patients with multiple myeloma

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Background and Objectives. Four-color multiparameter immunophenotyping has recently proven to be an attractive technique for evaluating the plasma cell (PC) compartment since it allows discrimination between myelomatous and normal PC. This study was designed to investigate: i) whether peripheral blood is less contaminated than bone marrow as a source for an autologous transplant; ii) the effect of growth factors on mobilizing myelomatous PC into peripheral blood; iii) the degree of contamination by myelomatous PC in apheresis samples; and iv) whether the number of PC increases during the last days of apheresis.

Design and Methods. Using 4-color antigen staining we investigated the composition of the PC compartment in 90 apheresis products from 40 patients with MM; in 17 cases bone marrow and peripheral blood samples were also simultaneously evaluated.

Results. (i) All pre-mobilization bone marrow samples analyzed were always contaminated with myelomatous PC whereas only 41% of the post-mobilization peripheral blood samples were contaminated. Moreover, the use of peripheral blood would lead to a reduction of $>5\times10^5$ infused myelomatous PC; (ii) mobilization with cytokines increased the number of circulating PC, generally because of an expansion of the normal PC population; (iii) forty-eight percent of all peripheral blood stem cell harvests were contaminated with myelomatous PC, although normal PC usually represented the predominant population; (iv) no significant changes were observed in the amount of contaminating myelomatous PC during the first three days of apheresis.

Interpretation and Conclusions. Multiparameter immunophenotyping is a useful approach for investigating the PC compartment in apheresis products.

Key words: multiple myeloma, plasma cells, peripheral blood, apheresis, immunophenotyping.

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During the last decade, high-dose chemotherapy followed by autologous stem cell support has become the treatment of choice for young patients with multiple myeloma (MM) because of the higher response rates and better survival after this strategy than those generally obtained with conventional therapy.[1-5] However, most transplanted patients ultimately relapse. This relapse is mainly caused by the residual malignant plasma cells (PC) surviving in the patient after the conditioning regimen, although the number of PC reinfused with the transplant may also have some influence on the relapse rate.

Peripheral blood is the preferred source for autologous stem cell transplants, since peripheral blood stem cells are easy to collect,[5-7] are associated with a faster hematopoietic recovery[8-11] and are less contaminated with PC than bone marrow (BM) harvests.[12,13] Nevertheless, it is well known that in most MM patients myelomatous PC are not only present in the bone marrow, but might also be circulating in peripheral blood.[14-16] Moreover, there are concerns that the number of circulating myelomatous PC[17] could increase in response to cytokine-based mobilization protocols,[18-21] although the mechanisms that could induce mobilization of bone marrow PC into peripheral blood as well as the time-course of PC mobilization after growth factor administration are still poorly understood.

Several techniques have been used to evaluate contamination of stem cell harvest products in MM. Reverse transcription polymerase chain reaction (RT-PCR) analysis of the CDRIII region of the immunoglobulin (Ig) heavy chain m-RNA is the preferred method since it is highly sensitive and allows simultaneous identification of both clonal PC and their B-cell precursors. However, RT-PCR is a semi-quantitative and time-consuming approach, since patient-specific oligonucleotide primers are required.[19,22-26]

Immunophenotypic analysis of cytoplasmic Ig light chain restriction is seen as an alternative method for evaluating tumor contamination of peripheral blood products in MM, given its speed, simplicity and quantitative nature. Several groups[27-29] have investigated the presence of clonal PC in peripheral blood samples by analysis of monotypic cytoplasmic Ig light chain expression specifically on PC, identified by their high CD38 expression of and/or reactivity for CD138, but the sensitivity of this approach is relatively low (10^{-3}-10^{-4}).[30] An alternative to this immunophenotypic approach is to use phenotypic aberrations as a tool for discriminating

between myelomatous and normal PC.[18,31-37] Based on this multiparameter flow cytometry approach, a sensitivity of up to 10^{-5} (identification of one aberrant PC among 10^5 normal cells) is reached,[38] and this technique could be applied to the majority of patients with MM, since phenotypic aberrations are found in more than 90% of the cases.[31,32,38]

Based on this, the aim of our study was to answer four questions concerning the quality of the samples obtained from patients with MM undergoing peripheral blood stem cell transplantation: (i) is the peripheral blood contaminated less with myelomatous PC than is bone marrow? (ii) do growth factors specifically mobilize myelomatous PC into peripheral blood? (iii) what is the frequency of myelomatous and normal PC in the peripheral blood-derived apheresis samples? (iv) does the number of myelomatous PC increase during the last days of collection?

Design and Methods

Patients' characteristics

Forty patients with MM scheduled to receive an autologous peripheral blood stem cell transplant were included in the study. Patients were diagnosed according to the Chronic Leukemia Myeloma Task Force criteria.[39] The patients' characteristics at the time of diagnosis are shown in Table 1. All patients had been uniformly treated according to the PETHEMA-94 trial which uses front-line therapy of four alternating cycles of VBCMP (vincristine, bischloro-ethylnitrosourea, cyclophosphamide, melphalan, prednisone) and VBAD (vincristine, bischloro-ethylnitrosourea, adriamycin, dexamethasone) followed by stem cell collection and autologous transplantation. At the time of mobilization, all patients had responded to front-line therapy (6 had a incomplete response, 31 a partial response and 3 were considered to have achieved minor responses.[40] Moreover, all patients were in morphologic remission (<5% PC in bone marrow) at the time of peripheral blood stem cell mobilization.

Peripheral blood stem cell mobilization

Mobilization of peripheral blood stem cells was performed by administering granulocyte colony-stimulating factor (G-CSF) at a dose of 10 μg/kg/day given over 5 consecutive days alone (n=35 cases) or in combination with cyclophosphamide (Cy) at doses of 4 g/m^2 (n= 5 cases). Peripheral blood stem cell harvest procedures were initiated when more than 3×10^6 CD34$^+$ cells/kg could be identified in the peripheral blood by flow cytometry; the stem cells were collected by apheresis using a *CS 3000 PLUS* (Baxter Healthcare Corporation, Deerfield, IL, USA) or a *Cobe Spectra* (Cobe BCT, Lakewood, CO, USA). PBSC collections were cryopreserved at -196°C in dimethyl-sulphoxide (DMSO); but prior to cryopreservation, an aliquot of each of the peripheral blood stem cell products obtained (0.5 mL) was used for immunophenotypic analysis.

Table 1. Characteristics of the patients enrolled in the study.

Characteristic	Patients n=40
Age (years)#	56 (31-70)
Sex	
Male	22
Female	18
Type of M-component	
IgG	22
IgA	14
Light chain only	4
Stage at diagnosis	
II	19
III	21
β2-microglobulin (mg/dL)#	4.8 (0.2-16.3)
Creatinine (mg/dL)#	1.2 (0.6-3.3)
C-reactive protein (mg/dL)#	3.4 (0.1-15)
Hemoglobin (g/dL)#	10.7 (5.4-15.7)

#Results expressed as median and range (in brackets).

Samples analyzed

A total of 90 apheresis products from the 40 patients undergoing a peripheral blood stem cell transplant were analyzed (median 2 aphereses/case; range: 1 to 4). In addition, just prior to the administration of the mobilization protocols, both peripheral blood and bone marrow samples were collected from 17 patients for immunophenotypic analysis. In 11 of these 17 cases, a post-mobilization peripheral blood sample from the first day of stem cell collection was also evaluated. The presence and number of myelomatous and normal PC were immunophenotypically investigated in all of these samples.

Immunophenotypic analysis

Briefly, a total of 2×10^6 cells from freshly obtained samples (<15 min) were incubated with the appropriate 4-color combinations of monoclonal antibodies (MoAb) using a direct immunofluorescence technique. The sources of the MoAb used were as follows: anti-CD38-FITC (IgG1 mouse; MHCD3801 clone) was obtained from Caltag Laboratories (Burlingame, CA, USA), anti-CD20-PE (IgG1 mouse; L27 clone), anti-CD28-PE (IgG1 mouse; L293 clone), anti-CD45-APC (IgG1 mouse; 2D1 clone), anti-CD56-PE (IgG1 mouse; clone NCAM16.2), and anti-CD117-PE

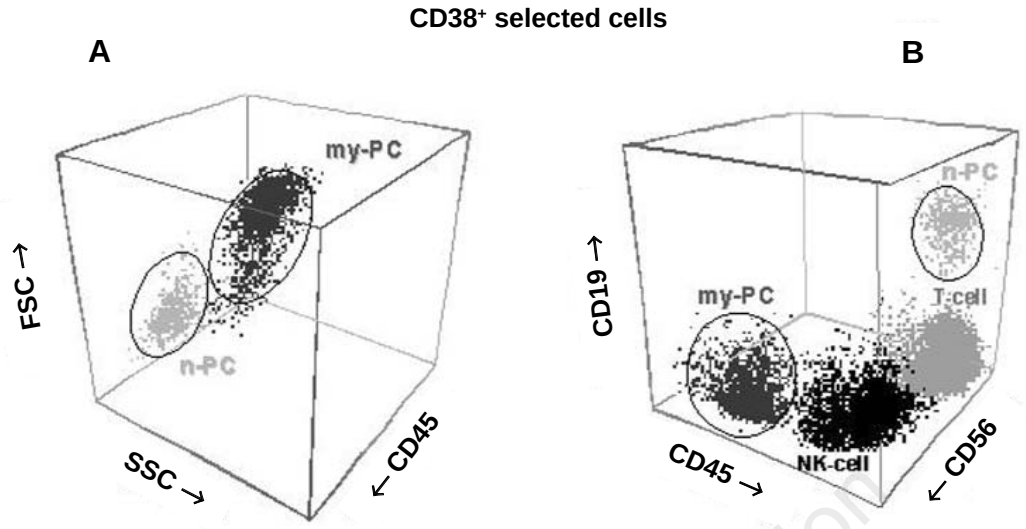

Figure 1. Representative immunophenotypic dot-plots showing the different laser-light-scatter (A) and antigenic characteristics (B) of the myelomatous PC (my-PC, colored black) and normal PC (n-PC, colored gray) in the CD38 positive cells. Note that the simultaneous expression of CD19, CD56 and CD45 antigens clearly distinguishes myelomatous from normal PC, and also allows the exclusion of other contaminating cells (NK and T-cells) from the analysis.

(IgG1 mouse; 104D2 clone) were from Becton/Dickinson Biosciences (BDB, San José, CA, USA); anti-CD19-PE/Cy5 (IgG_1 mouse; J4.119 clone) and anti-CD33-PE/Cy5 (IgG_1 mouse; D3HL60.251 clone) were from Immunotech (Marseille, France) and anti-CD138-PE/Cy5 (IgG_1 mouse; BB4 clone) was obtained from Cytognos S.L (Salamanca, Spain). In all cases a tube only stained for CD38-FITC was used to establish the autofluorescence level of the CD38-FITC-stained plasma cells present in the sample for the detectors used in the other monoclonal antibody stainings to measure PE-, PE/Cy5- and APC –associated fluorescence emissions. The fluorescence level of the plasma cells stained for CD38-FITC at the PE, PE/Cy5 and APC fluorescence detectors was recorded and used as a threshold for positivity. After incubation for 15 min at room temperature in darkness, the non-nucleated red cells were lysed (10 min at room temperature) using FACS lysing solution (BDB, San José, CA, USA). After a washing step, information on stained cells was acquired in a dual-laser FACSCalibur flow cytometer (BDB, San José, CA, USA). In order to increase the sensitivity of the technique, acquisition was performed in two consecutive steps. First, a total of 20,000 events/tube, corresponding to the total sample cellularity, were acquired; in the second step, information on at least 3×10^3 PC was acquired using an electronically activated *live-gate* drawn on a dot plot of intermediate/low side scatter versus CD38 strong positive cells (where PC are located). The Paint-A-Gate PRO software program (BDB, San José, CA, USA) was used for the data analysis. Cell debris was excluded from the analysis based on typical light-scatter characteristics, according to well-established methods. The distinction between myelomatous and normal PC was based on the specific phenotypic aberrations detected at diagnosis[31,38] as illustrated in Figure 1. This typically implies either expression on myelomatous PC of antigens present in the earlier B-cell maturation stages and constantly absent in normal PC (i.e. asynchronous expression of CD20 and/or CD117) and/or the expression of an antigen at very high intensity levels never reached in normal PC (i.e. over-expression of CD56, CD28 and/or CD33). In addition, we have previously reported on sorted-isolated PC in which the so-called normal PC (i.e. $CD38^{+++}CD138^{+}CD56^{-}CD19^{++}$) show a polyclonal nature based on the PCR-analysis of molecular markers of clonally rearranged segments of Ig genes, while the myelomatous PC population (i.e. $CD38^{+}CD138^{+}CD56^{+++}CD19^{-}$) had a clonotypic V(D)JH-rearranged product.[36] The threshold for positivity was established based on the baseline autofluorescence levels of the PC according to well-established methods.[41] Results obtained were expressed as absolute numbers of PC (both myelomatous and normal PC) per microliter of sample using the following calculation:

$$\text{absolute n. of PC}/\mu L = \frac{\text{WBC count } (10^3/\mu L) \times \text{percentage of PC}}{100}$$

The white blood cells (WBC) count was obtained in a CellDyN4000 hematology analyzer (Abott, Santa Clara, CA, USA).

Statistical analyses

Descriptive data are shown as median and range values. For continuous variables, the statistical significance of the differences observed between groups of patients was assessed using non-parametric tests (Mann-Whitney U and Wilcoxon tests for non-paired and match-paired variables, respectively). *p* values less than 0.05 were considered to be statistically significant. The SPSS 8.0 INC software (Chicago, IL, USA) was used for statistical analyses.

Results

Is peripheral blood less contaminated than bone marrow as a source for autologous stem cell transplants?

In order to assess the best source for hemopoietic progenitor cells — bone marrow versus peripheral blood — we simultaneously compared the number of myelomatous PC present in bone marrow samples obtained just prior to administration of the mobilizing therapy with those present in peripheral blood after mobilization. The pre-mobilization bone marrow samples were obtained because in bone marrow transplants stem-cell are harvested in a steady-state, while in peripheral blood transplants stem cells are collected after mobilization. All bone marrow samples from the 17 patients evaluated were contaminated with myelomatous PC (median of 39,920 myelomatous PC/μL; range: 198 to 2×10^6) whereas in the post-mobilization peripheral blood samples myelomatous PC were detected in only 7 of these cases (41%; median of 5 myelomatous PC/mL; range: 2 to 79). As shown in Table 2, patients in whom circulating myelomatous PC were immunophenotypically identified (n=7) showed a higher residual tumor load in their corresponding bone marrow sample obtained prior to mobilization than did those cases (n=10) in which circulating myelomatous PC were undetectable (*p*=0.001). In this latter group of patients, only PC with normal phenotype were observed in the post-mobilization peripheral blood samples. The levels of these phenotypically normal PC were significantly higher than those found in cases with contaminated peripheral blood: median of 33 normal PC/μL (range: 0.5 to 309 normal PC/μL) versus 1 normal PC/μL (range: 0 to 11 normal PC/μL), respectively (*p*=0.018).

Do growth factors mobilize myelomatous plasma cells into peripheral blood?

A comparison between the number of circulating PC present in peripheral blood samples obtained before and after mobilization was carried out in 11 patients. In all peripheral blood samples evaluated, the absolute numbers of circulating PC increased after mobilization: median of 1.1 PC/μL (range: 0 to 19 PC/μL) versus 18 PC/μL (range: 0.48 to 309 PC/μL), respectively. However, in a high proportion of cases (64%) this increase was due to an expansion of the normal PC compartment: median of 0.3 normal PC/μL (range: 0 to 1.5 normal PC/μL) versus 31 normal PC/μL (range: 0.48 to 309 normal PC/μL), in pre- and post-mobilization peripheral blood samples, respectively (*p*=0.018) (mean fold increase 100×). Higher levels of circulating myelomatous PC were found after mobilization therapy in only 4 out of the 11 cases analyzed (36%): median of 0.75 myelomatous PC/μL (range: 0 to 1.5 myelomatous PC/μL) versus 9.5 myelomatous PC/μL (range: 2 to 15 PC/μL), in pre- and post-mobilization peripheral blood samples, respectively (*p*=0.06) (mean fold increase 13×). It should be noted that in three of these cases, circulating myelomatous PC were already detected in peripheral blood prior to mobilization while in the fourth case the aberrant PC population was only detected in the post-mobilization peripheral blood sample. No correlation between the number of $CD34^+$ cells and PC was observed (*data not shown*).

Table 2. Correlation between the level of plasma cells (PC) in the bone marrow samples obtained prior to mobilization and the presence of circulating myelomatous PC in the peripheral blood after mobilization.

		Patients with circulating myelomatous PC post-mobilization (n=7 patients)	*Patients without circulating myelomatous PC post-mobilization (n=10 patients)*	*p value*
PC in bone marrow pre-mobilization	Myelomatous PC/μL	2.77 (0.12-20)	0.29 (0.002-5.55)	0.001
	Normal PC/μL	0 (0-0.16)	9.250 (0-1.14)	<0.001

What is the number of plasma cells present in apheresis samples?

In 43 out of the 90 apheresis products analyzed (48%), immunophenotypically aberrant PC were detected (median of 245 myelomatous PC/μL; range: 10 to 3,716 myelomatous PC/μL). These aphereses with myelomatous PC came from 19 patients (48% out of the 40 patients studied). It should be noted that when the first apheresis product contained myelomatous PC, the same occurred in the consecutive samples obtained from the same patient. Interestingly, in all but two of these 19 patients, myelomatous PC coexisted alongside nor-

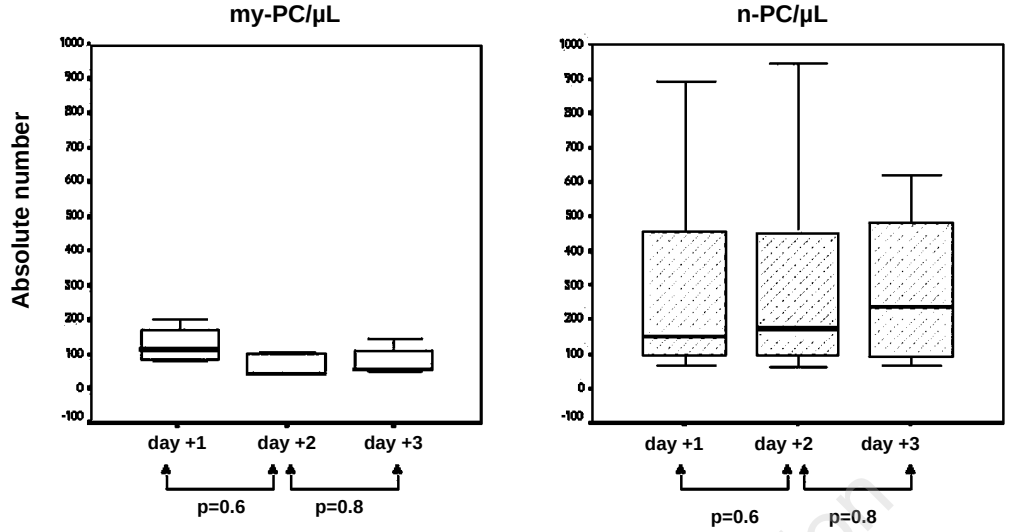

Figure 2. Overall changes observed in the absolute number of myelomatous (my-PC) and normal PC (n-PC) present in peripheral blood stem cell harvests from patients with MM according to the day of peripheral blood stem cell collection. Median and range values are represented by box-plot graphs and the statistical significance (*p* value) estimated in the comparison between groups is shown.

mal PC. The normal PC usually predominated over myelomatous PC and represented around 3/4 of the total PC present in the sample (median of 76%; range: 3% to 93%). In the remaining 47 apheresis products (52%), from a total of 21 patients, only PC displaying a normal phenotype were identified (median of 378 normal PC/µL; range: 57 to 5064 normal PC/µL).

Does the number of plasma cells increase during the period of daily peripheral blood stem cell collections?

The final goal of this study was to investigate whether or not the kinetics of mobilization of PC varies over the period of daily apheresis collection. We analyzed the sequential changes in the absolute numbers of myelomatous PC and normal PC in the first two harvest procedures in 31 patients and throughout the three consecutive days of stem cell collection in 13 patients. Overall, no significant changes (increase or decrease ≥25% in the number of PC over two consecutive days) were observed in the absolute numbers of either myelomatous PC or normal PC from day +1 to day +2 of harvest or from day +2 to day +3 (Figure 2). Regarding individual cases, a significant increase (≥25%) in the absolute number of myelomatous PC on day +2 was observed in 6 out of the 31 cases (19%). These patients showed a median number of 142 myelomatous PC/µL on day +1 versus 172 myelomatous PC/µL on day +2 (p=0.028). In contrast, another 6 cases showed a reduction of more than 25% in the absolute number of myelomatous PC between day +1 (median of 100 myelomatous PC/µl) and day +2 (median of 20 myelomatous PC/µL) (p= 0.03)(Figures 3 A, 3B). In the remaining 19 patients no differences in the absolute number of myelomatous PC were found between day +1 and day +2 of stem cell collection.

We also investigated whether the increase or decrease of myelomatous PC for each individual patient correlated with the corresponding normal PC, and vice versa. No correlation was found (*data not shown*).

When the peripheral blood stem cell harvest was extended for one more day (from day +2 to day +3), no significant change in the level of contamination was observed (Figure 2). In fact, only two of the 13 cases analyzed for 3 consecutive days showed significant changes in the number of myelomatous PC: in one of them, a higher level of myelomatous PC was detected on the third day of collection than on the previous day (median on day +2: 23 myelomatous PC/µL versus median on day +3: 93 myelomatous PC/µL) while, in the other, the opposite pattern occurred (median on day +2: 224 myelomatous PC/µL versus median on day +3: 56 myelomatous PC/µL).

A similar picture was observed in the sequential analysis of normal PC, which remained fairly stable overall during the daily peripheral blood stem cell collections (Figure 2), although increases or decreases ≥25% were found in the absolute number of normal PC once when individual cases were

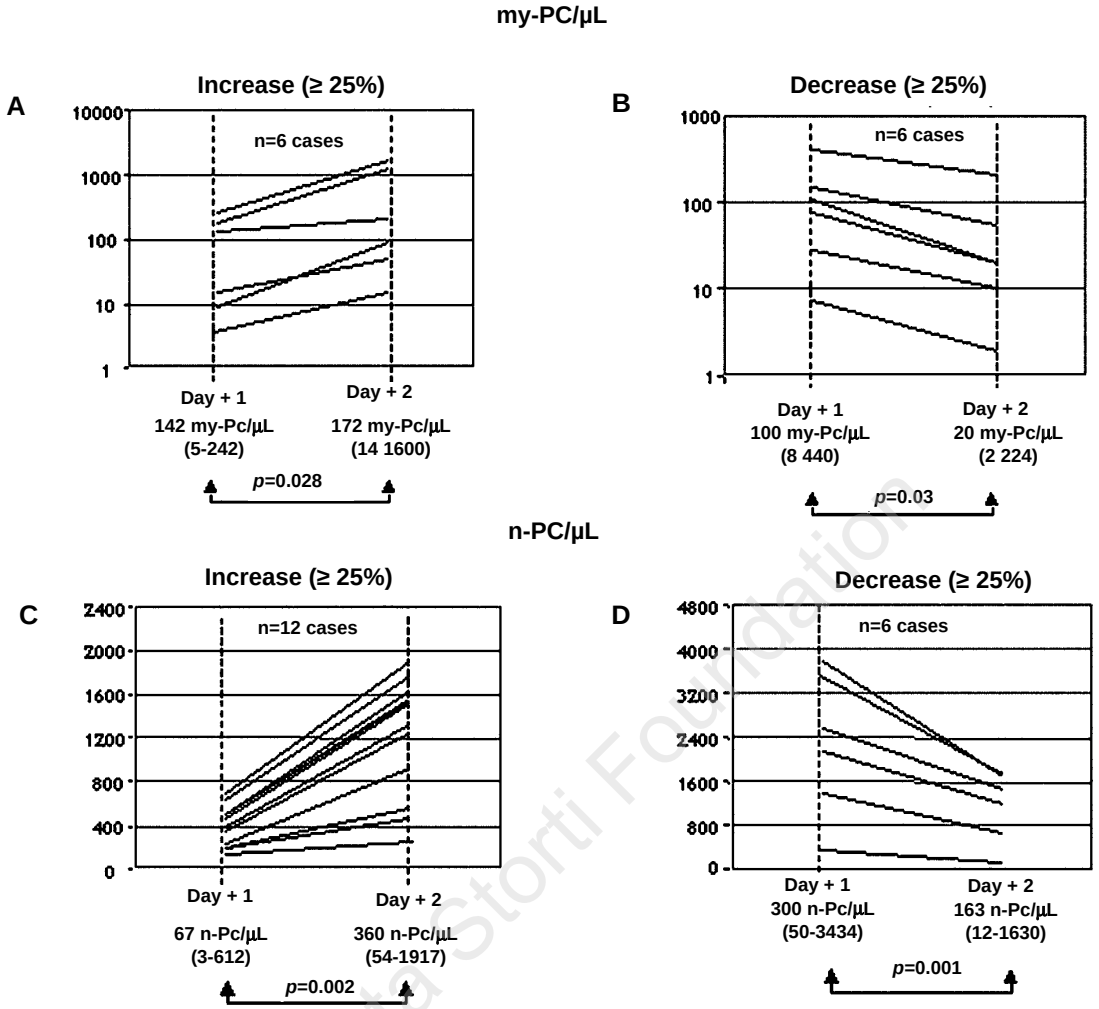

Figure 3. Individual changes found in the absolute number of myelomatous PC (my-PC) and normal PC (n-PC) during the first two days of peripheral blood stem cell harvest collections. The lines represent the increases (A,C) or decreases (B,D) exceeding 25% from day +1 to day +2 for both myelomatous-PC (top of the figure).

analyzed separately. In fact, the normal PC remained stable in only 13 out of the 31 cases analyzed. In the other cases, the number of normal PC either increased (n=12, 39%) or decreased (n=6, 19%) by ≥25%: from 67 normal PC/µ L to 360 normal PC/µL (p=0.002) and from 300 normal PC/µL to 163 normal PC/µL (p=0.01), respectively (Figures 3C, 3D).

Discussion

Conflicting results have been reported regarding the levels of residual PC contamination in apheresis products from patients with MM undergoing an autologous peripheral blood stem cell transplantation. Such discrepancies are probably related to the diversity of the methods used to detect the malignant PC. Most reported results are based on the detection of clonally rearranged VDJ segments of the immunoglobulin heavy chain gene[13,22-24,42-45] while information derived from immunophenotypic analyses is still scanty. Moreover, most immunophenotypic studies have used two- or three-color flow cytometric techniques to assess the cytoplasmic immunoglobulin light-chain restriction together with a surface antigen for identification of the PC.[27,29] In the present study, a four-color multiparameter immunophenotyping technique was used. This approach increases the specificity and sensitivity of the immunophenotypic analysis since it simultaneously combines information on six different parameters: laser-light scatter properties of the

cells – FSC (*forward light scatter*) and SSC (*sideward light scatter*) – and the antigenic expression of up to four different PC-associated antigens. The use of an amplified six-dimensional space facilitates this localization and identification of the PC population among the other cells present in the sample. In our experience, the simultaneous use of CD38 and CD45 is particularly useful for detecting PC in apheresis products, especially in those cases in which there is an increase in debris and signals corresponding to non-nucleated cells due to manipulation procedures. The use of CD45 also helps to distinguish between myelomatous PC (myelomatous PC are frequently CD45 negative or dimly positive) and $CD45^{high}$ T-lymphocytes or NK cells that might be contaminating the selected CD38 positive PC population. In line with these observations Rawstron *et al.*[32] have shown that the combination of CD38 and CD138 together with CD19, CD56 and CD45 is valuable for discriminating between normal and neoplastic plasma cells.

Peripheral blood stem cells are the preferred source for autologous transplants since their use is associated with more rapid hematopoietic reconstitution,[6,8] and at the same time might be contaminated by fewer PC than are bone marrow harvests.[13,22,42,46,47] Nevertheless, for an appropriate comparison between bone marrow and peripheral blood, the former sample should be obtained before mobilization (since steady state bone marrow is usually used for bone marrow stem cell transplants), while the peripheral blood sample should be obtained after mobilization (since peripheral blood stem cell products are always harvested after stimulation). In our study, this factor was taken into account, and in addition, all patients studied were in morphologic remission (<5% of PC). Despite this, immunophenotypic analyses showed that the bone marrow from all patients was contaminated by myelomatous PC whereas only 41% of the post-mobilization peripheral blood samples were contaminated. Interestingly, patients with circulating PC corresponded to those with the highest residual bone marrow tumor load. These results confirm and expand on previous observations[13,21] which indicate that tumor cell contamination in peripheral blood stem cell harvests is significantly lower than in bone marrow (differences in absolute numbers were ≥1 logarithm of myelomatous PC/μL), which would correspond to $>5\times10^5$ PC infused, if we had used bone marrow instead of peripheral blood for the transplant. Boccadoro *et al.* found that all aphereses from 64 patients analyzed contained $CD38^{++}/CD138^{+}$ cells, but they did not distinguish between normal and aberrant PC in their analysis,[27] while Pope *et al.* detected contamination ($CD38^{++}$ PC with monotypic cytoplasmic-κ^+ or λ^+ expression) in 16% of 25 patients.[29] Using four-color combinations, aimed at the detection of aberrant PC phenotypes identical to those observed at diagnosis, we found that 48% of the apheresis samples were contaminated by myelomatous PC. Phenotypically aberrant PC usually co-existed with normal PC, and in fact, in most cases, normal PC represented the predominant PC population (75% of the total of PC detected in the sample had a normal phenotype). These findings concur with the observations of Pope *et al.* who have reported that the majority of PC present in peripheral blood stem cell harvests have a polyclonal κ/λ light-chain distribution.

Regarding the possible influence of growth factors on mobilizing malignant PC into the peripheral blood, we observed an increase in the absolute number of circulating PC in all cases, but this increase was mainly due to expansion of the normal PC compartment. In fact, the malignant PC increased in only one third of cases, and in all except one of these, myelomatous PC were already present in the pre-mobilization peripheral blood sample.

Controversial results have been reported about the kinetics of PC mobilization after administration of growth factors. Several groups[19,48] have observed that higher proportions of myeloma cells could be collected during the last days of apheresis,[42] while others found minimal variations.[43,49] We found that the pattern of distribution of both myelomatous and normal PC observed in the first day of apheresis collection remained constant over successive days: when myelomatous and/or normal PC were detected in the first sample they were also identified in the subsequent samples. Regarding quantitative changes, no significant variations were observed in the absolute number of PC present in the apheresis products during the three days of collection. The absence of marked differences during the days of apheresis could be due to the fact that in most patients only two aphereses were required and it may well be that if four or more aphereses had been performed, the number of circulating myelomatous PC would have increased. Moreover, due to the presence of some inter-individual variations (in 19% of patients myelomatous PC increased ≥25% on the second day of aphereses) a safe practice recommendation would be not to perform more than three consecutive apheresis in order to avoid further stress and bone marrow exhaustion. Once again, our results indicate that it is important to discriminate between myelomatous and normal PC since the latter cell subset is frequently mobilized into peripheral blood. Previous reports[50] suggest that the pattern of expression of surface adhesion molecules in myelomatous PC may correlate with the mobilization of these cells into peripheral blood. Further studies are necessary to elucidate the potential role of the mobilization agents on the expression of surface integrins by neoplastic PC and their migration from bone marrow into peripheral blood.

In summary, in the present study, based on the use of a highly sensitive multiparameter immunophenotypic approach, we have shown that in patients with MM in morphologic remission who are being prepared for an autologous transplant: (i) the frequency of contaminating residual PC is lower in mobilized peripheral blood than in steady state bone marrow; (ii) mobilization increases the number of circulating PC but these are predominantly phenotypically normal PC; (iii) half of all harvests used for autologous transplantation in MM carry myelomatous PC; and, finally, (iv) the degree of contamination remains relatively stable throughout the first three days of apheresis collections.

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Contributions

GM: conception and design, analysis and interpretation of data, drafting the article and its revision, final approval of the version to be published; AO, JFSM: analysis and interpretation of data, drafting the article and its revision, final approval of the version to be published; the remaining authors; conception and design, analysis and interpretation of data. GM also created all the figures and tables included in this paper.

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In the following paragraphs, Dr. Kindler summarizes the peer-review process and its outcomes.

What is already known on this topic

Autologous hematopoietic stem cell grafting has been used for many years as a therapeutic tool for patients suffering from multiple myeloma. Growth factor-mobilized peripheral cell suspensions are favored over bone marrow harvests because they are easier to collect, allow a faster recovery of hematopoiesis, and contain fewer numbers of contaminating myelomatous plasma cells. However it is not clear whether such contamination is clinically important because purging myelomatous cells out of autografts does not alter patient survival rate, suggesting that malignant cells remaining in the patient are the source of relapse.

What this study adds

The authors have developed a flow cytometry-based immunophenotyping method to detect myelomatous plasma cells that is more sensitive and more discriminative that previously published methods. This may allow clarification of the role of contaminating myelomatous plasma cells in multiple myeloma relapse.