**Immunophenotypic evaluation of the plasma cell compartment in multiple myeloma: a tool for comparing the efficacy of different treatment strategies and predicting outcome**

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Multiparametric immunophenotyping can be a sensitive method for analyzing the plasma cell (PC) compartment in patients with multiple myeloma because it allows discrimination between myelomatous and normal PCs. Using this approach, we compared the efficacy of high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) with that of conventional chemotherapy.

**Introduction**

Immunophenotyping is an attractive technique for evaluating changes in the plasma cell (PC) compartment in patients with multiple myeloma (MM) because it allows discrimination between myelomatous PCs (MM-PCs) and normal PCs (N-PCs). The distinction between the 2 is based on the presence of phenotypic aberrations in MM-PCs that are absent in N-PCs. Using immunoglobulin (Ig)-gene rearrangement and sorting experiments, we previously demonstrated the clonal and polyclonal nature of MM-PCs and N-PCs, respectively. Moreover, dilutional experiments showed that multiparameter flow cytometry immunophenotyping has a sensitivity of $10^{-4}$ to $10^{-3}$. Although the myeloma clone includes not only PCs but also less mature B cells, PCs can proliferate and propagate MM and constitute the hallmark of the disease; therefore, investigation of changes in the composition of the PC compartment should be clinically informative. The aims of the current study were to compare the efficacy of autologous stem cell transplantation (ASCT) and chemotherapy by means of analysis of the PC compartment and to determine whether changes in this tumor cell compartment correlated with disease outcome.

**Study design**

A total of 87 patients with MM were included in the study. Criteria for entering the study were the presence of MM-PCs with an aberrant phenotype at diagnosis (in a series of 120 patients, 110 patients [92%] had such aberrancies); response to initial chemotherapy (thus, 23 patients [21%] with refractory disease were excluded); and inclusion in the 94 Spanish Program for the Study and Treatment of Hematological Malignancies (PETHEMA) trial. In this protocol, after 4 cycles of vincristine, bis-chloroethyl-nitrosourea, melphalan, cyclophosphamide, and prednisone–vincristine, bis-chloroethyl-nitrosourea, doxorubicin, and dexamethasone, patients with a response were randomly assigned to receive either ASCT (with melphalan [200 mg/m² of body-surface area], 47 patients) or 8 additional cycles of chemotherapy (40 patients). Subsequently, all patients received maintenance therapy with interferon and dexamethasone. The 2 arms were well balanced according to prognostic features, including the percentage of bone marrow PCs (BMPCs) at diagnosis (Table 1). The criteria used for response and relapse were those employed in previous PETHEMA trials. In 29 of the 87 patients (33%), disease progressed or relapse occurred, and in 11 of them, progression occurred in the first 2 years after diagnosis. The median follow-up and progression-free survival (PFS) in the overall series were 65 months and 53 months, respectively.

Immunophenotypic evaluation of the BMPC compartment was done at diagnosis and after treatment (3 months after ASCT and 1 month after the total 12 cycles of chemotherapy). The methods used for analyses of PCs were described previously. We used a panel of monoclonal antibodies in quadruple combinations (CD38/56/19/45, CD138/28/33/38, and CD20/117/138/38). The PC phenotypic aberrancies identified at diagnosis were used as patient-specific probes for follow-up analyses. To increase the sensitivity level of the technique, we used a 2-step acquisition procedure in which up to $2 \times 10^6$ cells were acquired through a specific “live gate” drawn on SSC/CD38+/CD138- cells. In all cases, an FL1/FL2/FL3 isotype-matched negative control CD38 for
antigen-positive cells was used to evaluate specifically the PC autofluorescence level.

For data analysis, Paint-A-Gate PRO software (Becton Dickinson, San Jose, CA) was used according to well-established methods. Three variables were evaluated. Whole bone marrow (BM) cellularity was assessed and the percentages of phenotypically aberrant MM-PCs and N-PCs in the BM were determined. We also measured MM-PCs and N-PCs in purified PCs selected through a specific electronic gate and assessed the percentage of N-PCs among the total PCs (ie, the percentage of N-PCs among total purified/gated BMPCs [N-PCs/TPCs]). PCs were considered normal if they met the following criteria:

- Percentage of N-PCs among total purified/gated BMPCs (% N-PCs/TPCs).
- Percentage of normal PCs cells among total BM cells (% N-PCs).
- Clinical disease stage III/IV (%).
- Serum creatinine at least 178 μmol/L (%).
- Hemoglobin less than 100 g/L (%).
- Calcium greater than 2.9 mmol/L (%).
- Albumin less than 30 g/L (%).
- LDH greater than 460 U (%).
- β2-microglobulin at least 60 mg/L (%).
- C-reactive protein at least 60 g/L (%).
- ECOG grade at least 2 (%).
- Diploid DNA content (%).
- S-phase plasma cells at least 3% (%).
- BMPCs, % (range).
- Median follow-up (mo).

ASCT indicates autologous stem cell transplantation; LDH, lactic dehydrogenase; ECOG, Eastern Cooperative Oncology Group; and BMPCs, bone marrow plasma cells.

Results and discussion

Changes in the PC compartment in patients treated with ASCT compared with patients given chemotherapy

As shown in Table 2, the percentage of MM-PCs was significantly lower after transplantation than after chemotherapy. In fact, ASCT induced a reduction in MM-PCs of more than 2 logs, whereas chemotherapy resulted in a reduction of only 1 log. In parallel, there was a greater increase in the percentage of N-PCs in the transplantation group than in the chemotherapy group, although this difference was not significant. When the analysis was restricted to purified/gated PCs, recovery of N-PCs was significantly greater in the transplantation group (percentage of N-PCs/TPCs, 86% versus 35%; P = .01; Table 2). Moreover, the proportion of patients considered to have no minimal residual disease (MRD)—ie, MM-PCs were not detected at a sensitivity limit of 10−4 and only N-PCs were present—was significantly higher in the transplantation group than in the chemotherapy group (36% versus 15%; P = .04).

In patients with MM, ASCT is considered to provide higher response rates and longer survival than chemotherapy, but in most studies of this issue, response to treatment was based on standard criteria. Thus, it would be useful to have additional indications of the superiority of ASCT. Because MM results from expansion of malignant PCs, we hypothesized that use of sensitive techniques to assess changes occurring in this tumor cell compartment could provide additional insights into the differences between these 2 treatment methods. We found that, in comparison with chemotherapy, ASCT produced a significantly higher reduction in the percentage of residual MM-PCs together with a greater recovery of normal BMPCs. This PC profile resembled that observed in patients with monoclonal gammopathy of undetermined significance, in whom N-PCs and MM-PCs coexist, rather than that in patients with MM at diagnosis, in whom almost all BMPCs (>95%) are phenotypically aberrant.

We also correlated the immunophenotypic response to treatment (no MRD) with the electrophoretic response at the same time point. Almost all the patients who had only an objective response (on electrophoresis) had MRD (44 of 48 patients [92%]). Among the 39 patients who had complete remission (on electrophoresis), about half (20 patients) still had MRD. When immunofixation was used to define complete remission, 74% of patients had results consistent with those of immunophenotypic studies (MRD, 60%; and no MRD, 14%), but 14% of patients were found to have MRD on phenotyping though immunofixation assessment detected no MRD, whereas the remaining 12% had no MRD on phenotyping but did have MRD by immunofixation.

Effect of changes in the PC compartment on PFS

In all 87 patients, we evaluated the effect on PFS of changes observed in the PC compartment induced by treatment by analyzing BM samples obtained 3 months after ASCT or after 12 cycles of chemotherapy. As shown in Figure 1, patients with low levels of residual disease after treatment (<0.12% MM-PCs) had a trend toward an improved PFS (P = .1). Patients with a high percentage of N-PCs (≥0.14%) had a slightly longer
median PFS (P = .1). However, analysis of purified/gated PCs was the most powerful variable for discriminating between different risk categories. Thus, patients in whom at least 30% of gated PCs had a normal phenotype had a significantly longer PFS than patients with less than 30% N-PCs (median PFS, 60 ± 6 months versus 34 ± 12 months; P = .02).

It is known that the percentage of BMPCs assessed by conventional morphologic analysis has limited prognostic value, probably because of the patchy pattern of BM infiltration and possible dilution of the BM sample with blood, which can produce bias. These 2 factors might also affect the precision of flow cytometry–based immunophenotypic approaches for measuring MM-PCs and N-PCs with respect to overall BM cellularity. The new variable we used here (analysis of PC subsets in purified/gated BMPCs) is likely to be less influenced by sampling errors and could represent a more objective indicator for disease monitoring in patients with MM. Although the cut-off level of at least 30% for N-PCs/TPCs showed the highest predictive value in discriminating among MM patients at different risks of relapse, higher cut-off levels might be more accurate for specific assessment of patients undergoing ASCT, since the percentage of N-PCs/TPCs was significantly higher in this group of patients.

Recovery of N-PCs after treatment may lead to recovery of noninvolved Igs. As shown in Table 3, patients with an increase of more than 50% in the level of noninvolved Igs had a significantly higher percentage of N-PCs and N-PCs/TPCs, together with a lower percentage of MM-PCs, than patients with an increase in polyclonal Igs of less than 50% (P = .05). However, the level of Ig recovery did not distinguish 2 different prognostic groups (P = .8).

It is possible that recovery of Ig requires more time than recovery of PCs, and perhaps the prognostic importance of Ig recovery must be evaluated at later time points.

In summary, our results show that multiparameter flow cytometry immunophenotyping is a rapid, easily standardized, sensitive method for evaluating the PC compartment in patients with MM and that it may offer valuable prognostic information.

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References


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