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Flow cytometric detection of circulating myeloma cells before transplantation in patients with multiple myeloma: a simple risk stratification system

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Detection of circulating myeloma cells (CMCs) by flow cytometry in patients with multiple myeloma (MM) indicates active disease. We hypothesized that detection of CMCs at the time of stem-cell collection prior to autologous stem-cell transplantation (ASCT) identifies patients at high risk of rapid progression. A cohort of patients undergoing ASCT was identified. CMCs were determined by gating on CD38⁺/CD45⁻ cells using flow cytometry. The impact of CMCs on overall survival (OS) and time to progression (TTP) was

evaluated in univariate and multivariate analyses. Of 246 patients undergoing ASCT, 95 had CMCs. Complete response (CR) rates after transplantation were 32% and 36% for patients with and without CMCs, respectively ($P = .50$). OSs were 33.2 and 58.6 months ($P = .01$) whereas TTPs were 14.1 and 22 months, respectively ($P = .001$). On multivariate analysis, CMCs remained independent of cytogenetics and disease status at time of transplantation ($P = .03$). CMCs and cytogenetics were combined in a new scoring

system. Patients with neither, one, or both parameters had a median OS of 55, 48, and 21.5 months and a median TTP of 22, 15.4, and 6.5 months, respectively. CMCs at the time of ASCT is an independent prognostic factor and in combination with cytogenetics provides a powerful scoring system that stratifies patients and guides management. (Blood. 2006; 107:3384-3388)

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Introduction

Multiple myeloma is a tumor resulting from expansion of a monoclonal population of plasma cells that constitutes 10% of all hematologic malignancies.¹ Therapy for this disease has improved significantly over the last few years although it remains incurable.² Patients with a good performance status are usually offered high-dose therapy (HDT) followed by autologous stem-cell transplantation (ASCT). HDT/ASCT is associated with complete response (CR) rates of up to 40% and responses are possibly higher with tandem transplantation.³⁻⁵ While a significant proportion of patients have a durable response after HDT/ASCT, others relapse relatively quickly and do not appear to benefit from the procedure. Although HDT/ASCT is a relatively safe procedure with a mortality risk of approximately 1% to 2%, morbidity is significant due to the toxicity of the conditioning regimen.⁴⁻⁶ Therefore, proper pretransplantation identification of patients who may or may not benefit from standard conditioning regimens is important, since such patients may be enrolled in experimental conditioning regimens. In addition, accurate risk stratification may allow meaningful comparisons between different studies and help generate new hypotheses for therapeutic interventions. Moreover, patients who may not achieve long-term control of the disease after HDT/ASCT might be candidates for maintenance therapy after transplantation.

Various groups have evaluated the impact of a number of parameters that are associated with a poor response to HDT with rapid relapse of the disease. Thus, high β_2 -microglobulin level, an elevated lactate dehydrogenase (LDH) level, age, response to therapy, a high bone marrow plasma-cell labeling index (PCLI),

and cytogenetic abnormalities such as del13q, t(4,14)(p16.3;q32), and -17p13 are all associated with rapid relapse of the disease.^{3,4,7-9} However, some of these prognostic parameters are abnormal in only a small subset of patients or are only available in a limited number of institutions treating patients with HDT/ASCT. Thus, there is a need for a simple and widely available pretransplantation risk stratification system.

The presence of circulating myeloma cells (CMCs) detected by a slide-based immunofluorescence method has been shown to predict early relapse of the disease after HDT/ASCT.^{10,11} However, this test is time-consuming, technically difficult, and not widely available. Flow cytometry is widely available and can detect CMCs.¹² Detection of circulating myeloma cells at the time of diagnosis of multiple myeloma is associated with a shortened survival.^{13,14} The presence of CMCs is not a consequence of disease burden but suggests biologically aggressive disease.¹⁴ Thus, we hypothesized that detection of CMCs by flow cytometry prior to hematopoietic stem-cell collection would be a predictor of biologically aggressive disease leading to early relapse regardless of initial response to HDT/ASCT therapy.

Patients and methods

Patients

Patients with multiple myeloma seen at Mayo Clinic Rochester are prospectively enrolled in a database that has relevant demographic, clinical,

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Table 1. Demographic and clinical characteristics of cohort, (N = 246)

Characteristic	n	Median	Range
Sex, male	155	—	—
Age, y	246	57.2	30-74.9
β_2 -microglobulin level, $\mu\text{g/mL}$	242	2.28	1.0-15.2
PCLI, %	246	0.3	0.0-11.2
BMPC, %	246	13.0	0.13-82.0
LDH level, U/L	246	172.5	73.0-1331

— indicates not applicable; PCLI, bone marrow plasma-cell labeling index; BMPC, percentage of bone marrow plasma cells; and LDH, lactate dehydrogenase.

and laboratory characteristics and is continuously updated. All patients undergoing HDT/ASCT for multiple myeloma between January 1, 1999 and December 31, 2002 had flow cytometric analysis of their peripheral blood performed within 2 weeks before hematopoietic stem-cell collection and are included in this analysis. Bone marrow cytogenetics, analyzed immediately before stem-cell collection for ASCT, were scored as “abnormal” if there was any cytogenetic abnormality except loss of the Y chromosome in males, which is considered to be a normal variant. This study was approved by the Mayo Foundation Institutional Review Board in compliance with the Declaration of Helsinki and federal regulations.

Flow cytometry

Peripheral-blood mononuclear cells were isolated by density gradient centrifugation on Ficoll at 300g for 15 minutes, washed in phosphate-buffered saline, and incubated with monoclonal antibodies against CD38 and CD45. Monoclonal myeloma cells were identified by gating on a population of cells that express high levels of CD38 but stain only weakly with CD45 (CD38⁺/CD45⁻).^{12,14,15} In this analysis, 50 000 events are evaluated and the number of myeloma cells counted using the described gating procedure.

Response criteria

Response to HDT/ASCT was as defined by Blade et al.¹⁶

Statistical analysis

Differences between nominal variables were studied using the chi-square statistic, and differences between continuous variables were evaluated using the Mann-Whitney test. Overall survival (OS) was calculated from the date of ASCT to the time of death or last contact. Time to progression (TTP) was defined as the interval between ASCT and evidence of progressive disease. Survival analysis was performed using the method of Kaplan and Meier.¹⁷ Differences between survival curves were evaluated using the log-rank test. Univariate and multivariate analysis was performed using the Cox proportional hazards model.¹⁸ For the multivariate analysis, parameters with the highest χ^2 in the univariate analysis were included first and additional parameters were added in a step-wise fashion. Any parameter that remained independent was retained whereas parameters that lost their independence were discarded from subsequent analyses.

Results

Patient characteristics

Between January 1, 1999 and December 31, 2002, a total of 246 patients underwent HDT/ASCT for multiple myeloma. The relevant characteristics of these patients are shown in Table 1. The majority of patients had responded to induction therapy, with almost half of the patients (122) receiving transplants in plateau phase. The remaining patients received transplants for either relapsed disease (57 had relapsed off therapy and 19 patients relapsed while on therapy) or primary refractory disease (48 patients). All of the patients were mobilized with a combination of cyclophosphamide (3 g/m²) followed by hematopoietic growth factor (HGF). No patient was given maintenance therapy after HDT/ASCT.

Circulating plasma cells

Flow cytometry identified CMCs in 95 patients (38.6%; Figure 1A-C). The median number of circulating myeloma cells was 9 (range, 1-1533) per 50 000 events. In a study of 10 healthy adults, none had detectable CMCs in the CD38⁺/CD45⁻ gate.¹⁹ Thus, detection of cells in this gate by flow cytometry is almost certainly due to myeloma.¹² In order to evaluate the relevance of CMCs at the time of ASCT, patients were divided into 2 groups: one group with and another without CMCs prior to hematopoietic stem-cell collection. Patients with CMCs had higher levels of β_2 -microglobulin, PCLIs, LDH, and bone marrow plasmacytosis; and abnormal karyotypes. Patients with CMCs also had a higher incidence of relapsed disease at the time of transplantation (Table 2). There was no difference in the time between diagnosis of myeloma and transplantation between the 2 cohorts ($P = .334$). The response to transplantation was similar in both groups at 95%, with complete response rates of 33% and 36% for patients with and without CMCs, respectively ($P = .644$).

Survival analysis

With a median follow-up of 34 months (range, 0-74.7 months), median OS from the time of HDT/ASCT for the whole cohort was 50 months, although this was 58.6 months for patients without and 33.2 months for patients with CMCs ($P = .005$, log-rank test; Figure 2A). The median TTP was 18 months for the whole cohort. Patients with CMCs had a median TTP of 14.1 months whereas for those without CMCs the median TTP was 20.4 months ($P < .001$; Figure 2B). On univariate analysis, Durie Salmon stage, the PCLI, percentage of bone marrow plasma cells (BMPC), abnormal cytogenetics, disease status at the time of transplantation, and failure to achieve a CR after HDT/ASCT also had a similar negative impact on OS and TTP,

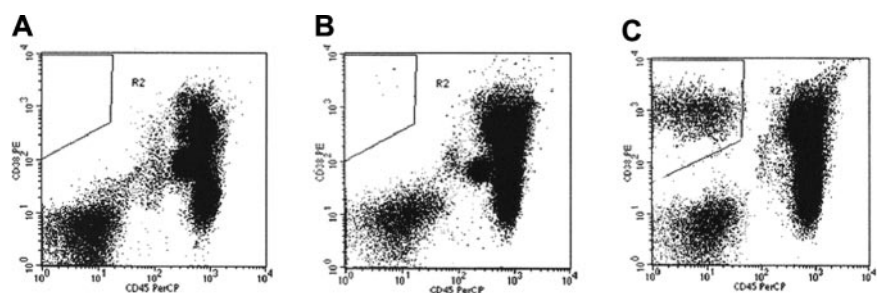


Figure 1. Detection of circulating myeloma cells (CMCs) by flow cytometry. CMCs are detected by gating for a population of CD38⁺/CD45⁻ cells. Healthy individuals do not have any cells in the CD38⁺/CD45⁻ gate (A). Panels B and C represent patients with a few (5) or many (1080) CMCs, respectively. Each analysis evaluates 50 000 events.

Table 2. Demographic, clinical, and laboratory findings in patients with (n = 95) or without circulating myeloma cells (n = 151)

Characteristic	Circulating myeloma cells		P
	Present	Absent	
Sex, male, %	65	62	.561
Age, median, y	58.3	56.7	.306
β ₂ -microglobulin level, median μg/mL	2.85	2.025	< .001
PCLI, median, %	0.80	0.0	< .001
BMPC, median, %	27.0	7.0	< .001
Cytogenetics			< .001
Normal	64	83	—
Abnormal	36	17	—
LDH level, median U/L	180	169	.041
Durie-Salmon stage, %			.634
II	28	30	—
III	72	70	—
Disease status at transplantation, %			.001
Plateau	36	58	—
Relapse off therapy	24	23	—
Relapse on therapy	14	4	—
Primary refractory	26	15	—
Conditioning regimen, %			.237
MEL 200	84	87	—
Other MEL	7	3	—
MEL/TBI	9	10	—
Complete response, %	33	36	.644
Time to transplantation, months			.334
Median	7.6	6.7	—
Range	3.6-66.0	3.8-60.8	—

PCLI indicates bone marrow plasma-cell labeling index; BMPC, percentage of bone marrow plasma cells; LDH, lactate dehydrogenase; MEL 200, melphalan at a dose of 200 mg/m²; MEL/TBI refers to melphalan and total body irradiation; and —, not applicable.

as shown in Table 3. These observations are in agreement with previously published series.^{3,4,6-8} The impact of CMCs on both OS and TTP was statistically significant as both a continuous and a categoric variable.

We performed multivariate analysis for both OS and TTP on the cohort of patients. In both analyses, the PCLI and cytogenetics were included first due to their strong and independent effect on prognosis. All the parameters identified as predictors of an inferior OS or TTP (Table 3) were included in the analyses. Disease status ($P = .033$), PCLI ($P = .033$), abnormal bone marrow cytogenetics ($P = .008$), and CMCs ($P = .014$) at the time of transplantation remained independent predictors of an inferior OS. A similar analysis for TTP identified achieving a CR after transplantation ($P < .001$), disease status ($P < .001$), CMCs ($P < .001$), and cytogenetic abnormalities ($P = .002$) as being independent predic-

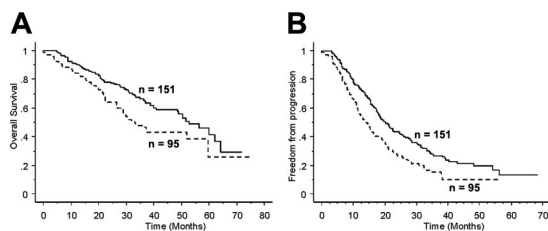


Figure 2. Kaplan-Meier plots based on the presence or absence of circulating myeloma cells detected by flow cytometry. (A) Overall survival (OS). (B) Time to progression (TTP). The presence of circulating myeloma cells is associated with an adverse outcome with respect to both OS and TTP ($P = .005$ and $P < .001$, respectively).

Table 3. Univariate analysis of the impact of various parameters on overall survival and time to progression for the whole cohort

Variable	Overall survival		Time to progression	
	HR	P	HR	P
Sex	0.77	.18	0.78	.10
β ₂ -microglobulin level, μg/mL	1.112	.003	1.052	.11
LDH level, U/L	1.003	< .001	1.001	.39
PCLI, %	1.197	< .001	1.133	< .001
BMPC, %	1.016	< .001	1.019	< .001
Durie-Salmon stage	1.685	.017	1.512	.012
Abnormal cytogenetics	2.275	< .001	2.394	< .001
Disease status at transplantation, relapse vs plateau	1.238	< .001	1.27	< .001
Response, other vs CR	1.372	.14	2.05	< .001
Circulating myeloma cells				
Continuous	1.001	.008	1.001	.031
Nominal	1.62	.009	1.60	.001

HR indicates hazard ratio; LDH, lactate dehydrogenase; PCLI bone marrow plasma-cell labeling index; and BMPC refers to the percentage of bone marrow plasma cells.

tors of a shorter TTP. In this analysis the PCLI did not have an impact on TTP ($P = .12$).

Given the results of the multivariate analysis, we wanted to combine various parameters to develop a simple scoring system that can be used to risk stratify patients for whom HDT/ASCT is being considered. Patients who undergo transplantation may be in plateau phase after responding to induction therapy or may have chemotherapy-sensitive/resistant relapsed disease or primary refractory disease. Thus, the status of the disease at the time of transplantation comprises a heterogeneous group of patients. Moreover, given that the PCLI is not widely available and response to HDT/ASCT is a post hoc event, we developed a prognostic scoring system based on bone marrow cytogenetics and CMCs. Patients were divided into 3 groups: low risk (neither abnormality), intermediate risk (either one), or high risk (both). As can be seen from Figure 3 and Table 4, this stratification strategy identifies patients at extremely high risk of progression shortly after HDT/ASCT (median time to progression of 6.2 months). The low-risk group had a median OS of 55 months and a TTP of 22 months after a single ASCT.

We also evaluated our scoring strategy for patients who received transplants in plateau phase or with chemotherapy-sensitive disease. This combined cohort had 178 patients: 108 patients in the low-risk group, 56 in the intermediate-risk group, and 14 in the high-risk group. The OS for patients with a score of 0 was 59 months (Figure 4A). Patients with a score of 1 had a median survival of 48 months and patients with a score of 2 survived for a median of 15.5 months after HDT/ASCT. The TTPs were 21.6

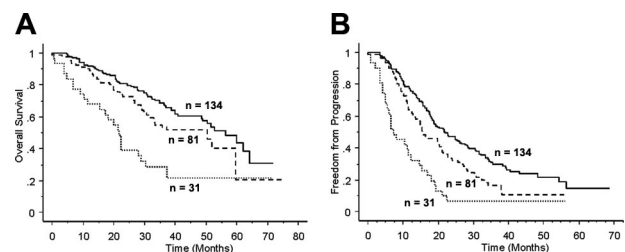


Figure 3. Kaplan-Meier plots based on the risk stratification combining cytogenetics and presence of circulating myeloma cells. (A) OS. (B) TTP. Patients with normal cytogenetics and no circulating myeloma cells have a superior OS and TTP compared with patients with one or both of these parameters.

Table 4. Overall survival and time to progression in the 3 risk groups based on cytogenetics and circulating myeloma cells

Risk group	n	OS, mo	TTP, mo
Low	134	55	21.8
Intermediate	81	48	15.4
High	31	21.5	6.5

months, 15.2 months, and 6 months for patients with a score of 0, 1, and 2, respectively (Figure 4B).

Discussion

HDT/ASCT is not curative for myeloma but patients who achieve a CR appear to do better and may not require therapy for a variable amount of time. Time off therapy is important since the agents used for the disease have significant toxicity, adversely affect the quality of life of patients, and require very frequent monitoring.²⁰ HDT/ASCT is also associated with significant morbidity, although mortality has decreased to below 5%, especially in centers experienced with the procedure.^{4,5} Despite the relative safety of HDT/ASCT, optimal patient management dictates that this therapeutic modality should only be offered to patients who are expected to benefit. Thus, identification of patients who may not benefit is important, since patients with high-risk disease may be better served by enrolling them in experimental conditioning regimens or can be considered for maintenance therapy after HDT/ASCT or recruited to other therapies with potentially less toxicity.

Various groups have reported on an increasing number of variables that predict the outcome of therapy after HDT/ASCT. However, often these prognostic parameters are only abnormal in a small proportion of patients and therefore have limited utility. Other tests such as the PCLI are only available in a select number of centers. Thus, there is a need for widely available and reliable tests that can be combined to provide proper risk stratification of patients being considered for HDT. Here we report on a simple system that identified patients at low and high risk of progression after HDT, and the technology required for this risk stratification strategy is widely available and therefore applicable in most centers. Flow cytometric detection of circulating myeloma cells identifies a group of patients at increased risk of progressive disease and, when combined with abnormal cytogenetics, it provides a powerful yet simple scoring system.

The presence of CMCs does not predict failure to respond to HDT/ASCT. Indeed, in our series response rates to HDT were

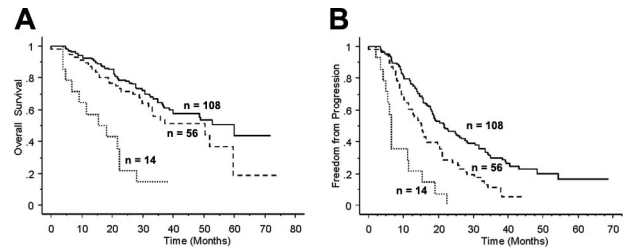


Figure 4. Kaplan-Meier plots for patients who received transplants, either in plateau phase or with chemotherapy-sensitive disease, stratified on the scoring system developed. (A) OS. (B) TTP. In this group of patients, both OS and TTP are inferior for patients with either or both risk factors.

similar in both cohorts of patients. However, the TTP for patients with CMCs was significantly shorter and they seem to benefit less from the procedure compared with patients without CMCs. Achieving a CR is considered an important goal of HDT/ASCT, since various studies have shown that achieving a CR is associated with an improved TTP and OS.^{3,4,20,21} However, achieving a CR is not the only factor associated with an improved survival, since CR rates with single and tandem transplantations were not significantly different in the IFM 94 trial, yet patients who underwent tandem transplantation had an improved survival.⁵ The lack of correlation between CR and OS and TTP has already been reported by our group in a smaller cohort of patients.²² Our current observations suggest that the picture is more complicated since a subset of patients may achieve a CR and yet relapse relatively quickly. Therefore, the presence of CMCs does not predict for chemosensitivity but rather for short time to progression perhaps in part due to a higher proliferation rate as reflected by the higher PCLI (Table 2). The poor outcome with CMCs should not be interpreted as justification for purging of the apheresis product since there is no evidence that purging of the hematopoietic stem-cell prior to transplantation confers any benefit.²³ CMCs may not be involved in posttransplantation relapse but may reflect loss of adhesion indicative of unfavorable biology.

The presence of circulating myeloma cells at the time of transplantation seems to be an excellent surrogate for high-risk disease. The presence of cytogenetic abnormalities combined with myeloma cells detected by flow cytometry identified a group of patients who will relapse soon after HDT/ASCT. It is not clear whether these patients benefit from HDT/ASCT, and this issue can only be addressed in a randomized trial. In contrast, patients without any of these prognostic parameters can expect almost 2 years without the need for therapy after a single transplantation.

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