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## Flow cytometric detection of circulating myeloma cells pretransplant in patients with multiple myeloma: a simple risk stratification system.

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## **Abstract**

**Background:** Detection of circulating myeloma cells (CMC) by flow cytometry in patients with multiple myeloma (MM) indicates active disease. We hypothesized that detection of CMC at the time of stem cell collection prior autologous stem cell transplantation (ASCT) identifies patients at high risk of rapid progression.

**Methods:** A cohort of patients undergoing ASCT was identified. CMC were determined by gating on CD38<sup>+</sup>/CD45<sup>-</sup> cells using flow cytometry. The impact of CMC on overall survival (OS) and time to progression (TTP) were evaluated in univariate and multivariate analyses.

**Results:** Of 246 patients undergoing ASCT, 95 had CMC. Complete response (CR) rates post transplant were 32% and 36% for patients with and without CMC ( $p=0.50$ ). OS were 33.2 and 58.6 months ( $p=0.01$ ) while TTP were 14.1 and 22 months respectively ( $p=0.001$ ). On multivariate analysis, CMC remained independent of cytogenetics and disease status at time of transplant ( $p=0.03$ ). CMC and cytogenetics were combined in a new scoring system. Patients with neither, one or both parameters had median, OS of 55, 48 and 21.5 months and median TTP of 22, 15.4 and 6.5 months respectively.

**Conclusion:** CMC 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.

**Key words:** multiple myeloma, stem cell transplant, flow cytometry, plasma cells, prognosis

## Introduction

Multiple myeloma is a tumor resulting from expansion of a monoclonal population of plasma cells that constitutes 10% of all hematologic malignancies.<sup>1</sup> Therapy for this disease has improved significantly over the last few years although it remains incurable.<sup>2</sup> 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 rates of up to 40% and responses are possibly higher with tandem transplantation.<sup>3-5</sup> 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 1-2%, morbidity is significant due to the toxicity of the conditioning regimen.<sup>4-6</sup> Therefore, proper pretransplant 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 transplant.

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  $\beta_2$ -microglobulin, an elevated lactate dehydrogenase (LDH), 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.<sup>3,4,7-9</sup> 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 pre-transplant risk stratification system.

The presence of circulating myeloma cells (CMC) detected by a slide based immunofluorescence method has been shown to predict early relapse of the disease after HDT/ASCT.<sup>10,11</sup> However, this test is time consuming, technically difficult and not widely available. Flow cytometry is widely available and can detect CMC.<sup>12</sup> Detection of circulating myeloma cells at the time of diagnosis of multiple myeloma is associated with a shortened survival.<sup>13,14</sup> The presence of CMC is not a consequence of disease burden but suggests biologically aggressive disease.<sup>14</sup> Thus, we hypothesized that detection of CMC 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.

## **Methods**

*Patients:* Patients with multiple myeloma seen at Mayo Clinic Rochester are prospectively enrolled in a database that has relevant demographic, clinical 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 as 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 and all patients had given informed consent to participate in this study.

*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<sup>+</sup>/CD45<sup>-</sup>).<sup>12,14,15</sup> In this analysis, 50,000 events are evaluated and the number of myeloma cells counted using the above gating procedure.

*Response criteria:* Response to HDT/ASCT was as defined by Blade et al.<sup>16</sup>

*Statistical analysis:* Differences between nominal variables were studied using the chi square statistic and differences between continuous variables 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.<sup>17</sup> Differences between survival curves were evaluated using the log-rank test . Univariate and multivariate analysis was performed using Cox's proportional hazards model.<sup>18</sup> For the multivariate analysis, parameters with the highest  $\chi^2$  in the univariate analysis were included first and additional parameters added in a step-wise fashion. Any parameter than remained independent was retained while 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) being transplanted in plateau phase. The remaining patients were transplanted either for relapsed disease (57 had relapsed off therapy and 19 patients relapsed while on therapy) or for primary refractory disease (48 patients). All the patients were mobilized with a combination of cyclophosphamide ( $3\text{g}/\text{m}^2$ ) followed by hematopoietic growth factor (HGF). No patient was given maintenance therapy post HDT/ASCT.

### *Circulating plasma cells*

Flow cytometry identified CMC 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 CMC in the  $\text{CD}38^+/\text{CD}45^-$  gate.<sup>19</sup> Thus, detection of cells in this gate by flow cytometry is almost certainly due to myeloma.<sup>12</sup> In order to evaluate the relevance of CMC at the time of ASCT, patients were divided into 2 groups: one group with and another without CMC prior to hematopoietic stem cell collection. Patients with CMC had higher levels of  $\beta_2$ -microglobulin, PCLI, LDH, bone marrow plasmacytosis and abnormal karyotypes. Patients with CMC also had a higher incidence of relapsed disease at the time of transplant (Table 2). There was no difference in the time between diagnosis of myeloma and transplantation between the 2 cohorts ( $p=0.3335$ ). The response to transplantation was similar in both groups at 95%

with complete response rates of 33% and 36% for patients with and without CMC (p=0.6436).

### *Survival analysis*

With a median follow-up of 34 months (range 0 – 74.7months), 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 CMC (p=0.0052, log-rank test) (Figure 2A). The median TTP was 18 months for the whole cohort. Patients with CMC had a median TTP of 14.1 months while for those without CMC, the median TTP was 20.4 months (p=0.0005) (Figure 2B). On univariate analysis, Durie Salmon stage, the PCLI, BMPC, abnormal cytogenetics, disease status at the time of transplant and failure to achieve a CR after HDT/ASCT also had a similar negative impact on OS and TTP as shown in Table 3. These observations are in agreement with previously published series.<sup>3,4,6-8</sup> The impact of CMC on both OS and TTP was statistically significant both as a continuous as well as a categorical 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 = 0.0334), PCLI (p = 0.0334), abnormal bone marrow cytogenetics (p = 0.008) and CMC (p = 0.014) at the time of transplant, remained independent predictors of an inferior OS. A similar analysis for TTP identified achieving a CR after transplant (p <0.0001), disease status (p = 0.0004), CMC (p <0.0001) and cytogenetic abnormalities (p = 0.0022) as

being independent predictors of a shorter TTP. In this analysis the PCLI did not have an impact on TTP ( $p=0.12$ ).

Given the results of the multivariate analysis, we wanted to combine various parameters to develop a simple scoring system that can be utilized to risk stratify patients for whom HDT/ASCT is being considered. Patients who undergo transplant may be in plateau phase after responding to induction therapy, may have chemotherapy-sensitive/resistant relapsed disease or primary refractory disease. Thus the status of the disease at the time of transplant 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 CMC. 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 transplanted in plateau phase or with chemotherapy sensitive disease. This combined cohort had 178 patients: 108 patients in the low risk group, 56 with intermediate risk and 14 with high-risk disease. 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 TTP were 21.6 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.<sup>20</sup> HDT/ASCT is also associated with significant morbidity although mortality has decreased to below 5% especially in centers experienced with the procedure.<sup>4,5</sup> Despite the relative safety of HDT/ASCT, optimal patient management dictates that this therapeutic modality should only be offered to patients that 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 post 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 CMC does not predict failure to respond to HDT/ASCT. Indeed, in our series response rates to HDT were similar in both cohorts of patients. However, the TTP for patients with CMC was significantly shorter and they seem to benefit less from the procedure compared to patients without CMC. 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.<sup>3,4,20,21</sup> However, achieving a CR is not the only factor associated with an improved survival since CR rates with single and tandem transplants were not significantly different in the IFM 94 trial, yet patients who underwent tandem transplantation had an improved survival.<sup>5</sup> The lack of correlation between CR and OS and TTP has already been reported by our group in a smaller cohort of patients.<sup>22</sup> 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 CMC 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 CMC 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 transplant confers any benefit.<sup>23</sup> CMC may not be involved in post transplant relapse but may reflect loss of adhesion indicative of unfavorable biology.

The presence of circulating myeloma cells at the time of transplant 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 transplant.

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### **Figure legends**

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

Figure 2. Kaplan-Meier plots for overall survival (A) and time to progression (B) based on the presence or absence of circulating myeloma cells detected by flow cytometry. The presence of circulating myeloma cells is associated with an adverse outcome with respect to both OS and TTP ( $p=0.0052$  and  $0.0005$  respectively).

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

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

**Table 1 Demographic and clinical characteristics of cohort, N=246**

<b>Characteristic</b>	<b>N</b>	<b>Median</b>	<b>Range</b>
Gender			
Male	155		
Age	246	57.2	30 – 74.9
$\beta_2$ -microglobulin	242	2.28	1.0 – 15.2
PCLI	246	0.3	0.0 – 11.2
BMPC (%)	246	13.0	0.13 – 82.0
LDH	246	172.5	73.0 – 1331

PCLI refers to bone marrow plasma cell labeling index

BMPC refers to the percentage of bone marrow plasma cells

LDH is lactate dehydrogenase

**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	
Gender			
Male (%)	65	62	0.5605
Age (median)	58.3	56.7	0.3062
$\beta_2$ -microglobulin (median)	2.85	2.025	<0.0001
PCLI (median)	0.80	0.0	<0.0001
BMPC (median)	27.0	7.0	<0.0001
Cytogenetics			
Normal	64	83	0.0005
Abnormal	36	17	
LDH (median)	180	169	0.0410
Durie-Salmon Stage			
II (%)	28	30	0.6339
III (%)	72	70	
Disease status at transplant			
Plateau (%)	36	58	0.0008
Relapse off therapy	24	23	
Relapse on therapy	14	4	
Primary refractory	26	15	
Conditioning Regimen			
MEL 200 (%)	84	87	0.2366
Other MEL	7	3	
MEL/TBI	9	10	
Complete Response			
Yes (%)	29	33	0.6436
Time to transplant			
Median (months)	7.6	6.7	0.3335
Range	(3.6-66)	(3.8-60.8)	

PCLI = bone marrow plasma cell labeling index, BMPC = bone marrow plasma cells, LDH = lactate dehydrogenase

**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
Gender	0.77	0.18	0.78	0.10
$\beta_2$ -microglobulin	1.112	0.003	1.052	0.11
LDH	1.003	0.0003	1.001	0.39
PCLI	1.197	<0.0001	1.133	<0.0001
BMPC	1.016	0.0005	1.019	<0.0001
Durie Salmon Stage	1.685	0.017	1.512	0.012
Cytogenetics Abnormal	2.275	<0.0001	2.394	<0.0001
Disease status Relapse vs Plateau	1.238	0.0005	1.27	<0.0001
Response Other vs CR	1.372	0.14	2.05	<0.0001
Circulating myeloma cells				
Continuous	1.001	0.008	1.001	0.031
Nominal	1.62	0.009	1.60	0.001

HR = estimated hazard ratio, LDH = lactate dehydrogenase, PCLI = bone marrow plasma cell labeling index, BMPC = bone marrow plasma cells

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

<b>Risk Group</b>	<b>N</b>	<b>OS (months)</b>	<b>TTP (months)</b>
Low	134	55	21.8
Intermediate	81	48	15.4
High	31	21.5	6.5

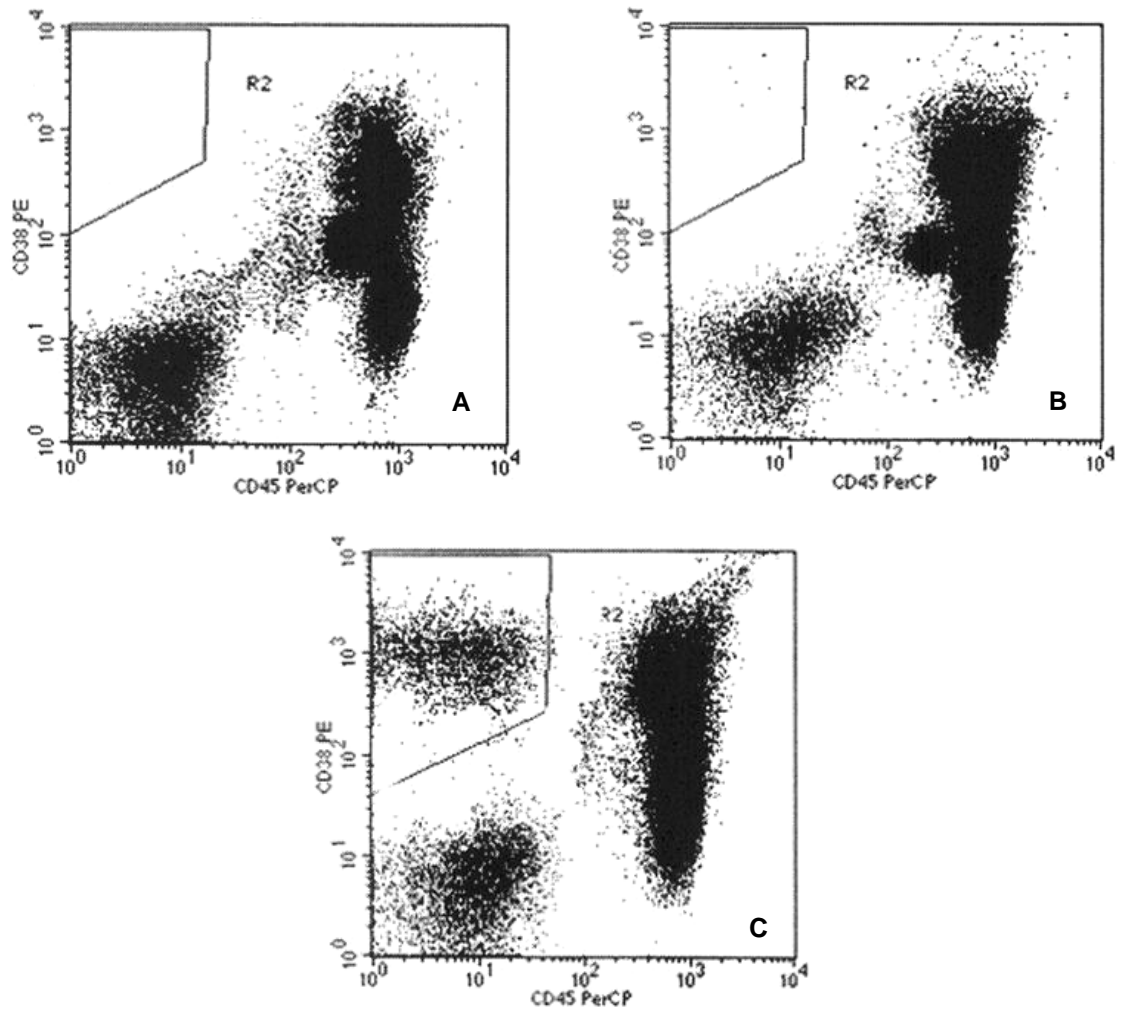


Figure 1.

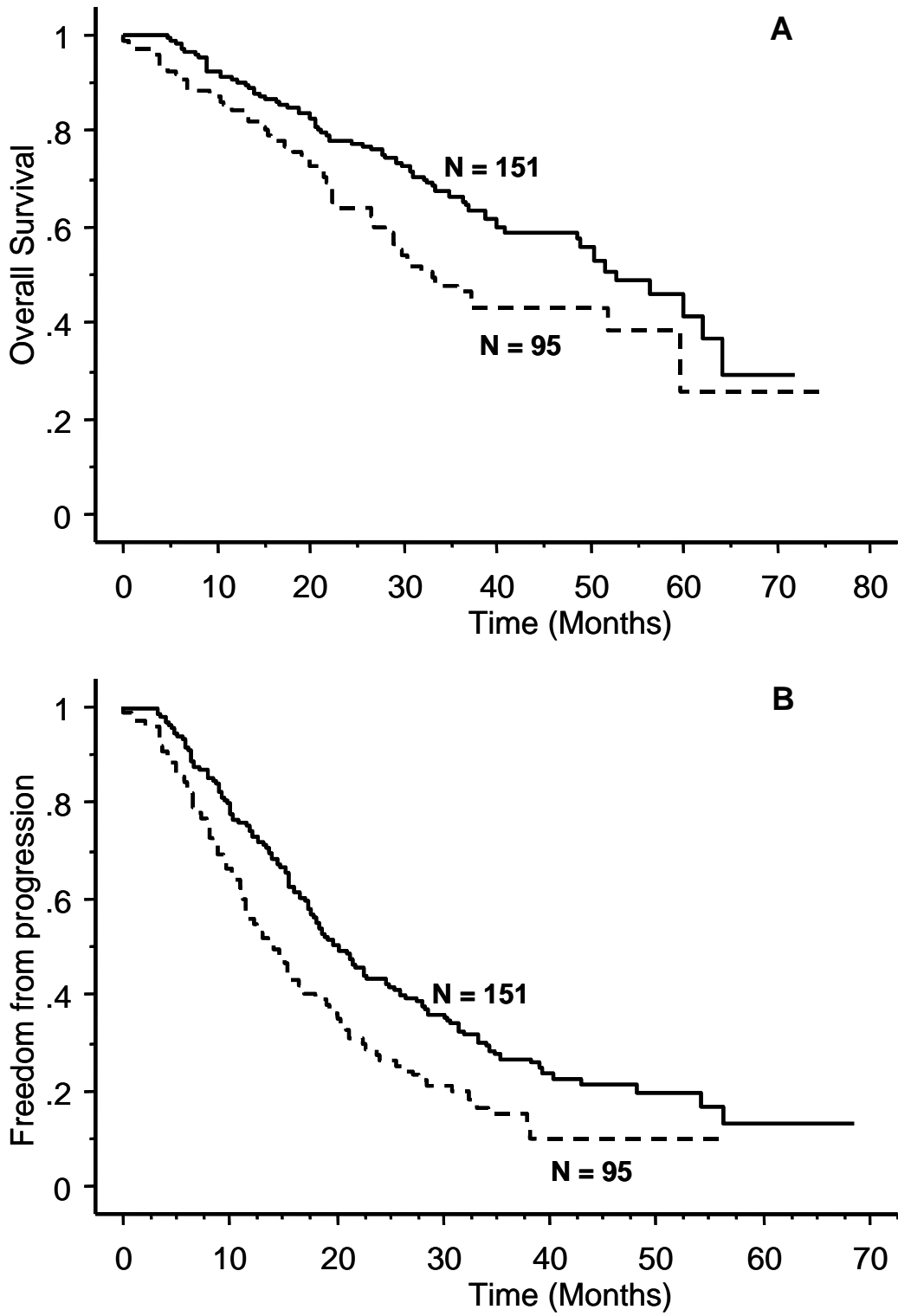


Figure 2

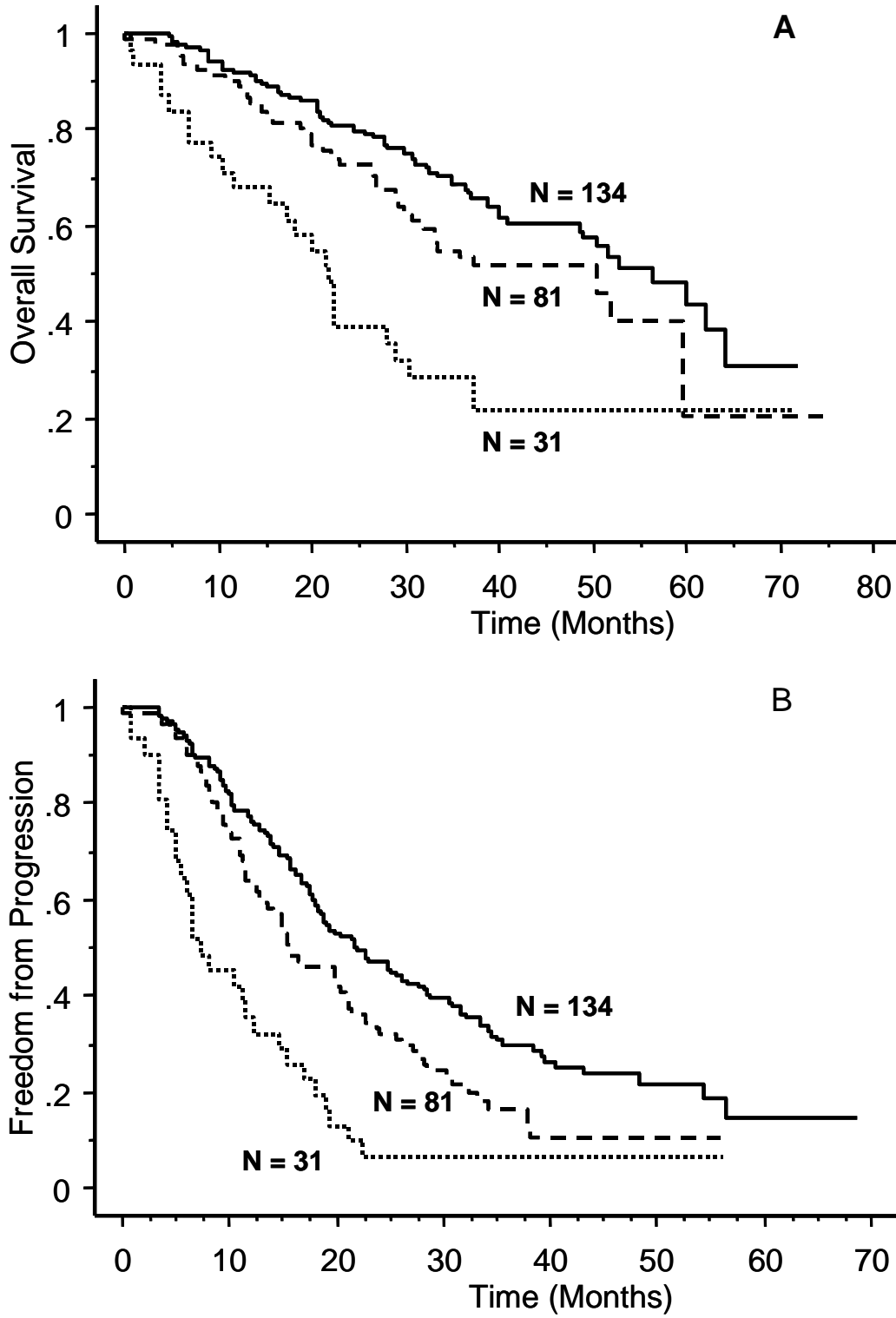


Figure 3

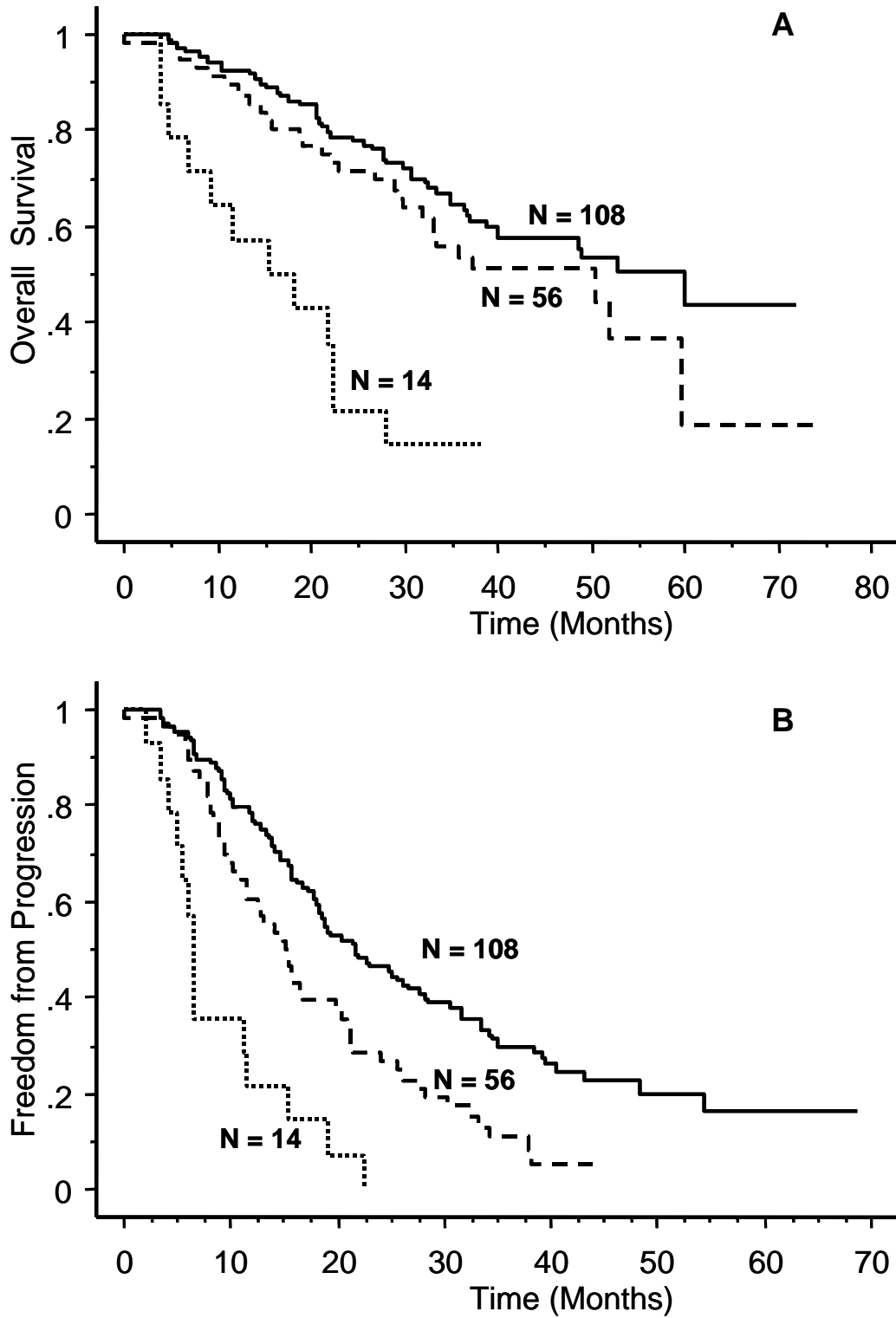


Figure 4

