Early Lymphocyte Recovery Predicts Superior Survival after Autologous Hematopoietic Stem Cell Transplantation for Patients with Primary Systemic Amyloidosis

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ABSTRACT

Purpose: **Absolute lymphocyte count recovery at day 15 (ALC-15) post-autologous stem cell transplantation (ASCT) is a powerful prognostic indicator for survival for multiple hematologic malignancies and metastatic breast cancer. The relationship of ALC-15 with clinical outcomes in primary systemic amyloidosis is unknown.**

Experimental Design: **We evaluated 145 consecutive patients with primary systemic amyloidosis who underwent ASCT at the Mayo Clinic from 1996 to 2003. The ALC-15 threshold was set at 500 cells/μL based on our previous observations.**

Results: **The median patient follow-up was 22 months (range, 3-87 months). Higher hematologic complete response was observed in patients with an ALC-15 ≥ 500 cells/μL compared with patients with an ALC-15 < 500 cells/μL (41% versus 21%, $P < 0.0008$, respectively). The median overall survival and progression-free survival times were significantly better for the 59 patients that achieved an ALC-15 ≥ 500 cells/μL compared with 86 patients with ALC-15 < 500 cells/μL (not reached versus 53 months, $P < 0.0003$ and not reached versus 27 months, $P < 0.0001$, respectively). Multivariate analysis showed ALC-15 to be an independent prognostic factor for overall survival and progression-free survival.**

Conclusions: **ALC-15 ≥ 500 cells/μL is associated with significantly improved clinical outcomes following ASCT in patients with primary systemic amyloidosis.**

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INTRODUCTION

Day 15 absolute lymphocyte count (ALC-15) after autologous stem cell transplantation (ASCT) has been reported as a powerful independent prognostic indicator of clinical outcomes for metastatic breast cancer (1, 2) and multiple malignant hematologic (3–8) conditions including multiple myeloma (6). Because of the superior survival observed in multiple myeloma (MM) patients achieving higher ALC-15 (≥500 cells/μL) post-ASCT, we hypothesized that ALC-15 may also affect survival in another plasma cell dyscrasia treated with ASCT, primary systemic amyloidosis. Herein we present the results of our study evaluating the role of ALC-15 following ASCT in patients with amyloidosis.

MATERIALS AND METHODS

Patient Population. Between 1996 and 2003, a total of 173 ASCT have been done at the Mayo Clinic for patients with amyloidosis. The diagnosis of amyloidosis was made as previously described (9). Clinical MM was defined as the presence of an M protein in serum or urine associated with lytic bone disease or ≥30% monoclonal plasma cells in the bone marrow (10). A total of 145 of 173 (84%) consecutive patients were eligible for the study. Twelve patients were excluded because they had not achieved their >100 days follow-up visit due to recent transplant; six patients died before day 15 post-ASCT and 10 patients had a concurrent diagnosis of another malignancy (8 patients with multiple myeloma, 1 patient with Waldrestrom macroglobulinemia, and 1 patient with lymphoplasmacytic lymphoma). Data for this retrospective study were prospectively collected over time and entered into a computerized database. Response to therapy, relapse, and survival data are updated continuously. No patients were lost to follow-up. All patients gave written, informed consent allowing the use of their medical records for medical research. Approval of the study was obtained from the Mayo Clinic Institutional Review Board and was in accordance with U.S. federal regulations and the Declaration of Helsinki.

End Points. The primary end point of the study was to assess the impact of ALC-15 on overall survival (OS) and progression-free survival (PFS) from the time of transplant. Secondary end point was to determine the agreement, as well as the correlation between the autograft absolute lymphocyte count (A-ALC) and ALC-15 in amyloidosis. The ALC-15 was obtained from the standard complete blood cell count, and the infused A-ALC for each apheresed unit collection was calculated as follows: A-ALC = [(% collection lymphocytes) × (absolute WBC)]/kg.

Prognostic Factors. Prognostic factors for post-transplant OS and PFS evaluated in this study included age, albumin, alkaline phosphatase, β-2 microglobulin, bone marrow plasma

cells, circulating plasma cells, conditioning regimen, C-reactive protein, creatinine, ejection fraction, interventricular septal thickness, lactate dehydrogenase (LDH), number of organs involved by amyloidosis, number of prior therapies to ASCT, plasma cell labeling index (11), serum M spike, stem cell mobilization regimes, time to autotransplantation, troponin T level, 24 hours urine protein, and urine M protein.

Peripheral Blood Stem Cell Collection. Thirty patients were mobilized by cyclophosphamide 3 g/m^2 and granulocyte macrophage colony-stimulating factor (GM-CSF) and 115 patients by granulocyte-colony stimulating-factor alone (G-CSF). Apheresis was done by processing 11 to 14 L of blood. Patient underwent daily apheresis sessions to achieve a target of 5×10^6 CD34 cells/kg. A minimum target of 2×10^6 CD34 cells/kg was required for the patient to be considered for transplantation.

Conditioning Regimens. The conditioning regimen was determined based on previously published risk-adapted approach (12). Fifteen patients received melphalan (140 mg/m^2) and total body irradiation (12 Gy), 84 patients received melphalan (200 mg/m^2), 37 patients received melphalan (140 mg/m^2), eight patients received melphalan (100 mg/m^2), and one patient received melphalan (160 mg/m^2).

Response and Survival. The hematologic response criteria conformed to those established for multiple myeloma (13). All patients who had measurable serum or urine M protein were monitored for changes in the M peak. A response required a 50% reduction in the size of the serum or urine M peak after transplantation. Two response criteria were applied when the light chain or the immunoglobulin protein was detectable but not quantifiable by electrophoresis. The first required complete eradication of the immunoglobulin light chain as seen by immunofixation and <5% marrow plasma cells. Beginning in June 2002, the immunoglobulin free light chain assay was applied to quantify the free light chains. When this second criterion was applied, the free light chain value had to diminish by >50% as long as the pretransplant level was >0.026 g/L for λ free light chain and >0.019 g/L for κ free light chain (14, 15). Light chain response was assessed on day >100. The organ-based response criteria varied depending on the dominant organ involved (16). For patients with renal amyloidosis, organ response required a 50% decrease in 24-hour albumin excretion. In patients with hepatic involvement, the response required a 50% reduction in the serum alkaline phosphatase level. Echocardiographic regression of cardiac amyloidosis required a 2-mm decrease in the thickness of the interventricular septum or an increase of 20% in the ejection fraction. A neurologic response required clinical regression of peripheral neuropathy confirmed by electromyography. Organ response was assessed on day >100 and every 6 months.

OS was measured from the date of transplant to the date of death or last follow-up. PFS was defined as the time from transplant to the time of progression. Those who died were considered to have had disease progression unless documented evidence clearly indicated no progression had occurred.

Statistical Analysis. OS and PFS were analyzed using the approach of Kaplan and Meier (17). Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. The Cox proportional hazards model (18) was used to evaluate ALC-15 as a prognostic factor for post-transplant OS and PFS as well as to assess and adjust for other known prognostic factors. The cutoff and definition of ALC-15 $\geq$ 500 cells/μL recovery after ASCT was used based on our previous work (1, 3, 4, 6). Risk ratios reported are for risk associated with patients having high ($\geq$500 cells/μL) versus low (<500 cells/μL) ALC-15 values. Other prognostic factors tested included age ($\geq$50 years), albumin (<3.5g), alkaline phosphatase (>375 UL, 1.5 x normal), β-2 microglobulin (>2.7 mg/L), bone marrow plasma cells (>20%), circulating plasma cells (>1%), conditioning regimen (melphalan/total body irradiation versus MEL alone), C-reactive protein (>0.8 mg/d), creatinine (>2.0 mg/L), ejection fraction (<60%), interventricular septal thickness ($\geq$15 mm), LDH (elevated for age/sex), number of organs involved by AL (>2), number of prior therapies to ASCT ($\geq$1), 24-hour urine protein (>3 g/d), plasma cell labeling index (>1%), serum M spike ($\geq$1.0 mg/dl), stem cell mobilization regimen (G-CSF versus Cytoxan/GM-CSF), urine M protein (>0.25 g/d), time to autotransplantation (as a continuous variable), and troponin T ($\geq$0.035 μg/dl). Multivariate analysis done using Cox regression models tested all variables with a $P <$ 0.2 in the univariate analysis. The choice of optimal cutoff of infused A-ALC was based on its utility as a marker for ALC-15 using box plot, ROC curves, and area under the curve analyses. Prediction of ALC-15 recovery was explored further in logistic regression models, univariately assessing continuously and dichotomized values of A-ALC as well as the other potential prognostic factors described above. χ^2 tests were used to determine relationships between categorical variables; two-sample t tests (and Wilcoxon rank-sum tests as appropriate) and Spearman correlation were used to evaluate associations for continuous variables. All Ps represented were two sided, and statistical significance was declared at $P < 0.05$.

RESULTS

Patient Characteristics. The median age at the time of transplantation for this cohort of 145 patients was 55 years (range, 31-71 years). Distributions of additional baseline characteristics for these patients are presented in Table 1 and summarized based on whether patients achieved ALC-15 $\geq$ 500 cells/μL versus ALC-15 $\geq$ 500 cells/μL. The median time from diagnosis to transplantation was 4.4 months (range, 1.3-74.7 months). There was no difference between the groups for the baseline characteristics in Table 1, as well as for the type of serum M protein (P = 0.70), the type of urine M protein (P = 0.08), kidney involvement (P = 0.32), heart involvement (P = 0.49), liver involvement (P = 0.84), peripheral nerve involvement (P = 0.36), and autonomic nerve involvement (P = 0.47). The only statistical differences observed between the groups were LDH and conditioning regimens. None of the patients received purged or CD34-selected stem cells and none of the patients developed clinically evident autologous graft versus host disease. Supportive care was standard for prospective transplanted patients including prophylactic fluoroquinolone antibiotics and fluconazole.

Post-Transplant Overall Survival and Time to Progression. By February 2004, 34 deaths had occurred among the 145 patients in the study. By day 100 post-ASCT, of the three

Table 1 Baseline characteristics of patients according to the absolute lymphocyte count at day 15 (ALC-15) after ASCT

Characteristics	ALC-15 < 500 cells/μL (n = 86)	ALC-15 ≥ 500 cells/μL (n = 59)	P
Age (y)			
Median (range)	55 (35-69)	55 (31-71)	0.88
Sex			
Females	32	27	0.50
Males	52	32	
Prognostic factors for amyloidosis			
Age (y)			
≥50	63	40	0.58
<50	23	19	
Albumin (g/dl)			
≥3.5	20	10	0.41
<3.5	66	49	
Alkaline (units/L) phosphatase			
>375	17	7	0.26
≤375	69	52	
β-2 microglobulin (mg/L)			
≥2.7	29	15	0.36
<2.7	57	44	
Bone marrow plasma cells (%)			
>20	15	5	0.15
≤20	71	54	
Circulating plasma cells (%)			
>1	3	2	0.97
≤1	83	57	
C-reactive protein (mg/L)			
>0.8	12	8	0.95
≤0.8	74	51	
Creatinine (mg/L)			
>2.0	9	1	0.05
≤2.0	77	58	
Ejection fraction (%)			
≥60	66	48	0.54
<60	26	11	
Interventricular septal thickness (mm)			
≥15	26	14	0.45
<15	60	45	
LDH			
Elevated	23	6	0.02
Normal for age/sex	63	53	
No. organs involved			
>2	15	11	0.85
≤2	71	48	
Plasma cell labeling index (%)			
≥1	5	2	0.70
<1	81	57	
Serum light chain by immunofixation			
λ	46	31	0.79
κ	13	11	
None	27	16	
Serum M spike (g/dl)			
>1.0	18	6	0.11
≤1.0	68	53	
24 h urine protein (g)			
>3.0	48	34	0.87
≤3.0	38	25	
Troponin T* (μg/L)			
≥0.035	12	4	0.20
<0.035	50	36	
Urine light chain by immunofixation			
λ	57	41	0.79
κ	20	11	
None	9	7	
Urine M protein (g/d)			
>0.25	31	23	0.73
≤0.25	55	36	

Table 1 Continued

Characteristics	ALC-15 < 500 cells/μL (n = 86)	ALC-15 ≥ 500 cells/μL (n = 59)	P
Free κ light chain before ASCT† (g/L)			
>0.026	24	18	0.80
≤0.026	15	9	
>0.019	14	7	0.43
≤0.019	25	20	
No. prior therapies			
0	58	38	0.59
1	23	16	
2	5	4	
3	0	1	
Stem cell mobilization regimens			
Cytoxan/GM-CSF	16	14	0.46
G-CSF	70	45	
Conditioning regimens			
Melphalan/TBI	6	9	0.05
Melphalan 200	46	38	
Melphalan 160	0	1	
Melphalan 140	28	9	
Melphalan 100	6	2	

*Only available data in 102 patients.
†Only available data in 66 patients.

patients with an ALC-15 ≥ 500 cells/μL, one died of pneumonia, one of pulmonary embolus, and one of sepsis. Of the 10 patients with an ALC-15 < 500 cells/μL, three died of multiorgan failure, two of septic shock, one of pneumonia, one of ARDS, one of disseminated aspergillosis, one of sudden cardiac death, and one of brain stem hemorrhage. All CR patients in the cohort are true CR (CR + negative immunofixation). Of the 66 patients with available free light chain data, only four patients were classified as very good partial response. These four very good partial response patients were combined with patients that achieved a PR. The median follow-up on living patients in this cohort was 22 months (range, 3-87 months). The median post-transplant OS (Fig. 1) and PFS (Fig. 2) times were significantly better for patients with ALC-15 ≥ 500 cells/μL compared with patient with ALC-15 < 500 cells/μL (not reached versus 53 months, $P < 0.0003$ and not reached versus 27 months, $P < 0.0001$, respectively). By the time of this analysis, there was no statistically difference between the groups for organ response [67% (39 of 58 patients) in the ALC-15 ≥ 500 cells/μL group versus 58% (50 of 86 patients) in the ALC-15 < 500 cells/μL group, $P = 0.30$]. However, by day >100 visit, we found a higher hematologic response rate in the ALC-15 ≥ 500 cells/μL group of 85% (50 of 59 patients) compared with 58% (50 of 86 patients) in the ALC-15 < 500 cells/μL group ($P < 0.0009$). The ALC-15 ≥ 500 cells/μL group achieved a higher hematologic complete response rate of 41% (24 of 59 patients) compared with only 15% (13 of 86 patients) in the ALC-15 < 500 cells/μL group ($P < 0.0008$).

Univariate Analysis. ALC-15, β-2 microglobulin, circulating plasma cells, conditioning regimen, C-reactive protein, interventricular septal thickness, LDH, number of organs involved by amyloidosis, stem cell mobilization regimen, troponin T level (analysis done on the 102 patients with available data), and urine M protein were significant predictors for OS. ALC-15, β-2 microglobulin, circulating plasma cells, conditioning regimen, creatinine, interventricular septal thickness, LDH, number of organs involved by amyloidosis, number of prior therapies to ASCT, stem cell mobilization regimen, troponin T level (analysis done on the 102 patients with available data), and urine M protein were significant predictors for PFS (Table 2).

Multivariate Analysis. ALC-15 was an independent predictor for OS [relative risk (RR), 0.388; $P < 0.0001$] and PFS (RR, 0.417; $P < 0.0001$) when compared with the predictors identified in the univariate analysis (Table 3).

In the subset of 102 patients with troponin T levels available, ALC-15 was also found to be an independent prognostic factor for OS (RR, 0.278; $P < 0.0005$) and PFS (RR, 0.427; $P < 0.0014$) when compared with the significant factors including troponin T (19, 20) in the univariate analysis.

Role of Infused Autograft Lymphocytes and ALC-15. We have previously published a strong correlation between the A-ALC and ALC-15 (21, 22). We set out to assess if a strong correlation also exist between A-ALC and ALC-15 post-ASCT in amyloidosis. Box plots showed that patients achieving an ALC-15 ≥ 500 cells/μL received higher numbers of A-ALC compared with patients achieving ALC-15 < 500 cells/μL [median A-ALC infused: 0.89×10^9 lymphocytes/kg (range, $0.57\text{-}2.8 \times 10^9$ lymphocytes/kg) versus 0.43×10^9 lymphocytes/kg (range, $0.02\text{-}0.95 \times 10^9$ lymphocytes/kg)]. ROC and area under the curve analysis showed that A-ALC was a significant marker for ALC-15 recovery (area under the curve = 0.97, $P < 0.0001$). We identified A-ALC ≥ 0.5×10^9 lymphocytes/kg to be an optimal cutoff value to predict an ALC-15 ≥ 500 cells/μL. In regards to the association between ALC-15 recovery and the A-ALC cutoff, these dichotomized variables were found to be significant correlated with each other ($P < 0.0001$) as were their continuous counterparts ($r_s = 0.83$, $P < 0.0001$). We identified no association between ALC-15 and CD34 ($P = 0.42$), number of prior therapies ($P = 0.40$), conditioning regimens ($P = 0.29$), and other patient characteristics/prognostic factors.

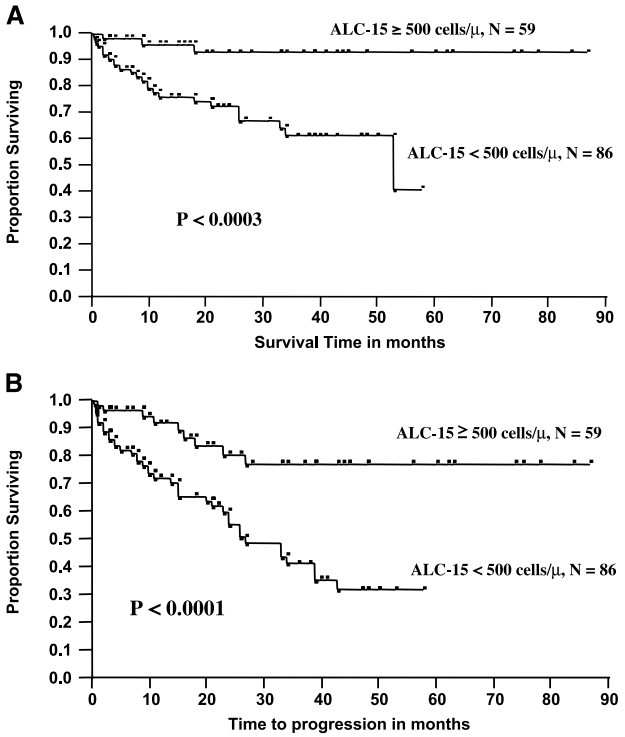

Fig. 1 *A,* Kaplan-Meier estimates of OS of patients achieving an ALC-15 ≥ 500 cells/μL versus patients achieving an ALC-15 < 500 cells/μL post-ASCT in amyloidosis. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were only three events in the ALC-15 ≥ 500 cells/μL group versus 25 events in the ALC-15 < 500 cells/μL group. The median OS was not reached in the group of patients with an ALC-15 ≥ 500 cells/μL and 53 months in the group of patients with an ALC- 15 < 500 cells/μL. The OS rates at 5 years were 93% and 59%, respectively ($\chi^2 = 13.06$, $P < 0.0003$). *B,* Kaplan-Meier estimates of PFS of patients achieving an ALC-15 ≥ 500 cells/μL versus patients achieving an ALC-15 < 500 cells/μl post-ASCT in amyloidosis. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were nine events in the ALC-15 ≥ 500 cells/μL group versus 40 events in the ALC-15 < 500 cells/μL group. The median PFS was not reached in the group of patients with an ALC-15 ≥ 500 cells/μL and 27 months in the group of patients with an ALC- 15 < 500 cells/μL. The PFS rates at 5 years were 77% and 32%, respectively ($\chi^2 = 14.83$, $P < 0.0001$).

Using the cutoff value of A-ALC ≥ 0.5 × 10^9 lymphocytes/kg, the median post-transplant OS (Fig. 2*A*) and PFS (Fig. 2*B*) times were significantly better for patients infused with an A-ALC ≥ 0.5 × 10^9 lymphocytes/kg compared with patients infused with an A-ALC < 0.5 × 10^9 lymphocytes/kg (not reached versus 53 months, $P < 0.0004$ and not reached versus 26 months, $P < 0.0001$), respectively). In the same cohort of patients, A-ALC was also an independent predictor by multivariate analysis for OS (RR, 0.405; $P < 0.0001$) and PFS (RR, 0.475; $P < 0.0001$) when compared with the significant predictors identified in the univariate analysis. When comparing RR for OS and PFS, A-ALC has similar prognostic significance to ALC-15, as well as, the OS and PFS survival for both variables are almost identical. The identification of A-ALC as prognostic factor for survival post-ASCT in amyloidosis and its correlation with ALC-15 suggests that the manipulation of A-ALC collection could be used as an immunotherapeutic strategy to enhance immune recovery post-ASCT. We identified that the stem cell mobilization regimen affects A-ALC collection and therefore ALC-15 recovery. We identified patients mobilized with G-CSF alone achieved a higher A-ALC (median of 0.50 × 10^9 lymphocytes/kg) compared with patients mobilized with Cytoxan/GM-CSF (median, 0.42 × 10^9 lymphocytes/kg; $P < 0.05$). Thus, patients mobilized with G-CSF achieved a higher ALC-15 (median, 0.500 cells/μL) compared with patients mobilized with Cytoxan/GM-CSF (median, 0.290 cells/μL; $P < 0.030$).

The number of organs involved by amyloidosis was another strong prognostic factor for survival. We combined A-ALC and number of organs involved to assess outcome post-ASCT in amyloidosis. Patients infused with an

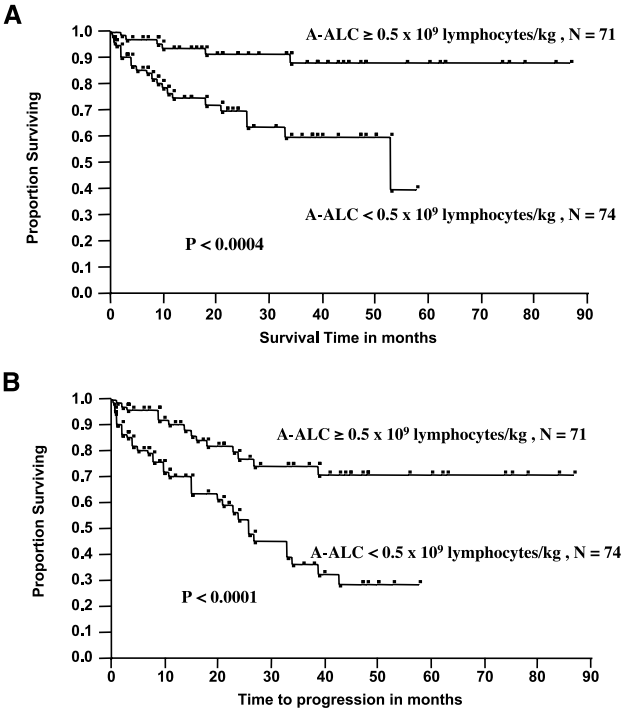

Fig. 2 *A,* Kaplan-Meier estimates of OS of patients infused with an A-ALC ≥ 0.5 × 10^9 lymphocytes/kg versus patients infused with an A-ALC < 0.5 × 10^9 lymphocytes/kg post-ASCT in AL. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were six events in the A-ALC ≥ 0.5 × 10^9 lymphocytes/kg group versus 22 events in the A-ALC < 0.5 × 10^9 lymphocytes/kg. The median OS was not reached in the group of patients infused with an A-ALC ≥ 0.5 × 10^9 lymphocytes/kg and 53 months in the group of patients infused with an A-ALC < 0.5 × 10^9 lymphocytes/kg. The OS rates at 5 years were 88% and 40%, respectively ($\chi^2 = 12.59$, $P < 0.0004$). *B,* Kaplan-Meier estimates of PFS of patients infused with an A-ALC ≥ 0.5 × 10^9 lymphocytes/kg versus patients infused with an A-ALC < 0.5 × 10^9 lymphocytes/kg post-ASCT in amyloidosis. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were 14 events in the A-ALC ≥ 0.5 × 10^9 lymphocytes/kg group versus 35 events in the A-ALC < 0.5 × 10^9 lymphocytes/kg. The median PFS was not reached in the group of patients infused with an A-ALC ≥ 0.5 × 10^9 lymphocytes/kg and 26 months in the group of patients infused with an A-ALC < 0.5 10^9 lymphocytes/kg. The PFS rates at 5 years were 77% and 32%, respectively ($\chi^2 = 15.47$, $P < 0.0001$).

Table 2 Univariate analysis for OS and PFS for all patients in the cohort

Prognostic factors at transplantation	OS		PFS	
	RR (95% confidence interval)	*P*	RR (95% confidence interval)	*P*
ALC-15 $\geq 0.5 \times 10^9$ lymphocyte/kg	0.459 (0.267-0.710)	0.0002	0.527 (0.355-0.741)	0.0001
β-2 Microglobulin ≥ 2.7 mg/L	1.482 (1.039-2.085)	0.031	1.475 (1.093-1.967)	0.012
Circulating plasma cells >1%	2.601 (1.255-4.501)	0.014	2.491 (1.351-4.000)	0.006
Conditioning regimen: melphalan/total body irradiation versus melphalan alone	0.638 (0.407-1.106)	0.102	0.716 (0.494-1.119)	0.132
C-reactive protein ≥ 0.8 mg/L	1.375 (0.870-2.030)	0.160		
Creatinine > 2.0 mg/L			1.475 (0.910-2.177)	0.107
Interventricular septal thickness ≥ 15 mm	1.523 (1.069-2.141)	0.021	1.424 (1.045-1.908)	0.026
LDH > normal	1.477 (0.933-2.183)	0.091	1.526 (1.045-2.127)	0.030
No. organs involved > 2	1.876 (1.289-2.671)	0.0015	1.796 (1.300-2.429)	0.0007
No. prior therapies to ASCT ≥ 1			1.316 (0.984-1.743)	0.06
Stem cell mobilization regimen:				
G-CSF versus Cytoxan/GM-CSF	0.666 (0.456-1.002)	0.051	0.766 (0.570-1.054)	0.100
Troponin T ≥ 0.035*	2.278 (1.413-3.593)	0.0012	2.177 (1.492-3.111)	0.0001
Urine M protein > 0.25 g/d	1.319 (0.907-1.919)	0.146	1.412 (1.066-1.876)	0.016

*Only available data in 102 patients.

A-ALC $\geq 0.5 \times 10^9$ lymphocyte/kg and <3 organs involved had a superior survival compared with patients infused with an A-ALC $< 0.5 \times 10^9$ lymphocyte/kg and ≥3 organs involved (Fig. 3*A* and *B*). The RR for OS (RR, 0.311; $P < 0.0001$) and PFS (RR, 0.356; $P < 0.0001$) for A-ALC $\geq 0.5 \times 10^9$ lymphocyte/kg and <3 organs involved was superior when compared with the RR of either ALC-15 or A-ALC. The combination of these two variables should identify patients with good outcome in amyloidosis post-ASCT.

Discussion

ALC-15 ≥ 500 cells/μL has been reported as a significant prognostic factor for survival post-ASCT. Because of the association with better survival between MM and ALC-15 (6), we set out to investigate if the same exist in another plasma cell dyscrasia, amyloidosis. A small proportion of the cohort of patients in this study presented with high quantitative values of markers most commonly seen in MM, such as bone marrow plasma cells > 20%, plasma cell labeling index >1%, circulating plasma cells, and serum M protein > 1.0 g/dl. Our group has previously reported amyloidosis patients with these high value markers with low risk to transform to MM and the evolution of MM from amyloidosis is rare, occurring in 6 of 1,600 patients over 20 years (9, 10, 23, 24). None of the patients in the study has transformed into MM. We identified that patients achieving an ALC-15 ≥ 500 cells/μL had superior OS and PFS compared with patients achieving an ALC-15 < 500 cells/μL, suggesting for the first time that the patients own immune system may have a direct impact in survival in patients with amyloidosis post-ASCT.

A possible explanation for the survival advantage associate with ALC-15 ≥ 500 cells/μL post-ASCT in amyloidosis patients may be containment of the underlying malignant plasma cell clone by early reconstitution of immune surveillance. In patients with ALC-15 ≥ 500 cells/μL, we observed not only a higher hematologic response, but also a higher hematologic complete response compared with patients with an ALC-15 < 500 cells/μL. Although, we did not identified any difference in organ response between the ALC-15 ≥ 500 cells/μL group and the ALC-15 < 500 cells/μL, further follow-up is needed, as organ response requires a long follow-up interval.

Due to the retrospective nature of this study, we do not know the lymphocyte subsets involved in the ALC-15 recovery and their relationship to clinical outcome post-ASCT in amyloidosis. However, the most likely lymphocyte subsets involved by ALC-15 post-ASCT are natural killer (NK) cells. The immune reconstitution post-ASCT in amyloidosis follows the same pattern reported in ASCT (25, 26). Akpek et al. (27) reported early recovery of NK cells, followed by CD8 count and B cells at 3 months, and delayed CD4 count recovery, producing the reversed CD4/CD8 ratio observed post-ASCT. We have shown that by day 15 post-ASCT NK, cells are normal in numbers and function in relationship to T and B cells (28). A possible explanation for the rapid NK cell recovery post-transplant compared with T cells is that NK cells differentiation from bone marrow progenitor cells occurs rapidly without restriction of thymic involvement (29). Recently, we have shown in non-Hodgkin's lymphoma and multiple myeloma patients that if NK cell recovery does take place by day 15 post-ASCT, their estimated progression free survival at 2 years is 89% versus 0%

Table 3 Multivariate analysis for OS and PFS

Prognostic factors at transplantation	OS		PFS	
	RR (95% confidence interval)	*P*	RR (95% confidence interval)	*P*
ALC- 15 $\geq 0.5 \times 10^9$ lymphocyte/kg	0.388 (0.189-0.660)	<0.0001	0.417 (0.252-0.653)	<0.0001
Circulating plasma cells > 1%	3.066 (1.271-6.895)	<0.0151	2.806 (1.328-5.505)	<0.0088
No. organs involved > 2	2.191 (1.410-3.381)	<0.0007	1.750 (1.202-2.503)	<0.0042
Likelihood ratio, $P < 0.0001$				

NOTE. Statistical significance declared at $P < 0.0026$.

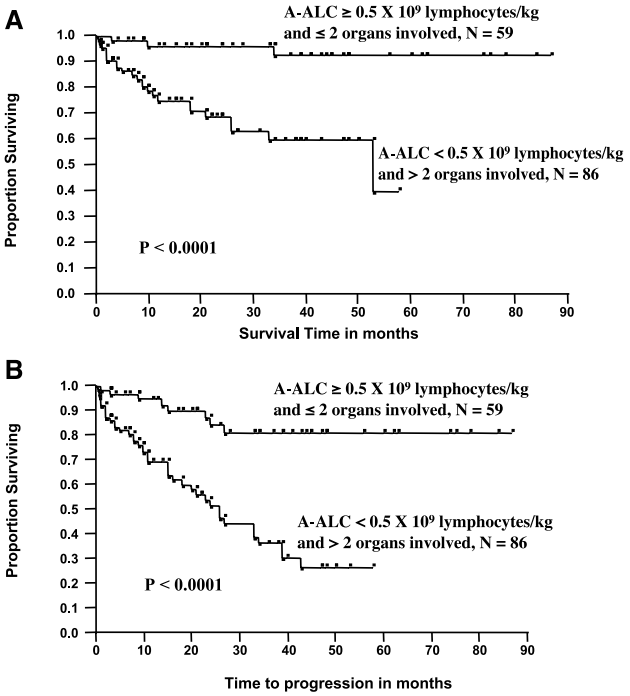

Fig. 3 *A,* Kaplan-Meier estimates of OS of patients with the variables of A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by amyloidosis versus patients with an A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by amyloidosis. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were three events in the A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by AL group versus 25 events inpatients with an A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by amyloidosis group. The median OS was not reached in the group of patients infused with A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by amyloidosis and 53 months in the group of patients with A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by amyloidosis. The OS rates at 5 years were 92% and 40%, respectively ($\chi^2 = 16.14$, $P < 0.0001$). *B,* Kaplan-Meier estimates of PFS of patients with the variables of A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by AL versus patients with an A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by amyloidosis. Dots above horizontal line, censored data; dots below horizontal line, uncensored data. There were eight events in the A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by amyloidosis group versus 45 events inpatients with an A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by amyloidosis group. The median PFS was not reached in the group of patients infused with A-ALC $\geq 0.5 \times 10^9$ lymphocytes/kg and <3 organs involved by amyloidosis and 26 months in the group of patients with A-ALC $< 0.5 \times 10^9$ lymphocytes/kg and ≥3 organs involved by AL. The PFS rates at 5 years were 81% and 26%, respectively ($\chi^2 = 22.98$, $P < 0.0001$).

for patients that have not achieved NK reconstitution (30). In our data, early T- or B-cell recovery did not affect clinical outcomes.

In comparison with allogeneic stem cell transplantation, where the source of engrafted lymphocytes can be differentiated (donor or host), in the ASCT setting, the possible sources of reconstituting lymphocytes are as follows: (a) lymphocyte surviving the high-dose chemotherapy, (b) lymphocytes differentiating from the transplanted autologous stem cells, or (c) infused lymphocytes form the autologous stem cell graft (31). We have recently reported that the infused A-ALC not only influences ALC-15 recovery but also affects survival post-ASCT in patients with myeloma or non-Hodgkin's lymphoma. Our current data confirms the same observation in amyloidosis. The two main lymphocyte subsets harvested during peripheral blood stem cell collections are T cells and NK cells (32). We have reported that the number of NK cells infused from the stem cell grafts, and not T or B cells, correlates with ALC-15 (33). These results argue in favor of the A-ALC (i.e., the number of NK cells in the autograft) as one of the main sources affecting ALC-15 recovery post-ASCT. The correlation between A-ALC and ALC-15 argues in favor of considering the autologous stem cell graft not only the source of $CD34^+$ stem cell driven hematologic recovery but also as the source of immunologic recovery with direct effect on survival post-ASCT. Similar *in vivo* autologous stem cell graft engineering, focused on NK cells, has been studied in breast cancer (34, 35). Several groups have shown enhanced numbers and function of NK cells in the mobilized autograft with the combination of G-CSF and interleukin 2 (IL-2).These studies also showed higher NK cell numbers by day 14 post-ASCT in patients mobilized with G-CSF + IL-2 compared with patients mobilized with G-CSF alone. Although theses studies had small numbers of patients, using an intent-to-treat analysis, Sosman et al. (35) found no statistical significance for either PFS ($P = 0.5$) or OS ($P = 0.7$) favoring the 23 patients mobilized with G-CSF + IL-2 compared with the nine patients mobilized with G-CSF alone. Other combinations of NK cell specific cytokines, such as IL-15 and IL-21, with G-CSF could be studied to assess their impact on autograft NK cell collection, NK cell recovery post-ASCT, and clinical outcomes post-ASCT. It seems that G-CSF is a better immunologic mobilization option than Cytoxan/GM-CSF because we observed decreased A-ALC collection in patients mobilized with Cytoxan/GM-CSF, most likely due to the myelosuppressive effects of Cytoxan. To enhance early lymphocyte recovery post-ASCT, other groups have used adoptive transfer of costimulated T cells (36–38). Before CD34 stem cell collections, patients underwent a steady-state single 20L apheresis procedure. This product served as the source of cells for *ex vivo* expansion of $CD3^+/CD28^+$ T cells. $CD3^+/CD28^+$ costimulated T cells were reinfused on day 14 post-ASCT. The authors observed a rapid lymphocytosis with improved T-cell recovery ($CD3^+$ and $CD4^+$ T cells) and reported responses to therapy in 16 patients with refractory/relapse non-Hodgkin's lymphoma, 32 patients with MM, and four patients with chronic myelogenous leukemia that were refractory to IFN-α and imatinib meslylate before ASCT. We are currently conducting a prospective study to analyze the target dose of A-ALC and autograft NK cells to achieve early NK cell recovery and to assess their impact on clinical outcomes post-ASCT.

In summary, the current report expands and continues to support our prior work underscoring the importance of lymphocyte recovery post-ASCT. This is the first study showing that ALC-15 is a significant predictor of survival post-ASCT in amyloidosis. The disadvantage of ALC-15, as a prognostic factor is that ALC is obtained after the ASCT has occurred. Thus, the identification of any factor before transplant that affects ALC-15 recovery post-ASCT could be used as an immunotherapeutic strategy to enhance immune recovery and improved survival post-ASCT. From a therapeutic standpoint, the correlation between A-ALC and ALC-15 suggests that the manipulation

of A-ALC, a marker obtained after stem cell mobilization and before ASCT, could be target to enhance ALC-15 recovery with direct impact on clinical outcomes post-ASCT. In a case control study, ASCT was found to achieve superior survival compared with standard therapy (39), an observation currently being examined in a phase 3 trial by a French myeloma intergroup (12). Further studies are under way attempting to improve the results of ASCT in amyloidosis, including the utilization of tandem transplantation (12). We hope that our report will provide insight into the relevance of immune reconstitution post ASCT in amyloidosis in support of ongoing efforts to extend the life expectancy of these patients.

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