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ORIGINAL ARTICLE

Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia

D Behl[1], LF Porrata[2], SN Markovic[2,3], L Letendre[2,3], RK Pruthi[2], CC Hook[2], A Tefferi[2], MA Elliot[2], SH Kaufmann[2,3], RA Mesa[2] and MR Litzow[2]

[1]*Department of Medicine, Mayo College of Medicine, Rochester, MN, USA;* [2]*Division of Hematology/Department of Medicine, Mayo College of Medicine, Rochester, MN, USA and* [3]*Department of Oncology, Mayo College of Medicine, Rochester, MN, USA*

Absolute lymphocyte count (ALC) recovery postautologous stem cell transplantation is an independent predictor for survival in acute myelogenous leukemia (AML). The role of ALC recovery after induction chemotherapy (IC) in AML is unknown. We hypothesize that ALC recovery after IC has a direct impact on survival. We have now evaluated the impact of ALC recovery after IC on overall survival (OS) and leukemia-free survival (LFS) in 103 consecutive, newly diagnosed AML patients treated with standard IC and consolidation chemotherapy (CC) from 1998 to 2002. ALC recovery was studied at days 15 (ALC-15), 21 (ALC-21), 28 (ALC-28) after IC and before the first CC (ALC-CC). Superior OS and LFS at each time point were observed with an ALC-15, ALC-21, ALC-28, and ALC-CC $\geqslant$500 cells/μl. Patients with an ALC $\geqslant$500 cells/μl at all time points vs those who did not have superior OS and LFS (not reached vs 13 months, $P<0.0001$; and not reached vs 11 months, $P<0.0001$, respectively). Multivariate analysis demonstrated ALC $\geqslant$500 cells/μl at all time points to be an independent prognostic factor for survival. Our data suggest a critical role of lymphocyte (immune) recovery on survival after IC in AML.
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Introduction

In the setting of autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML), it has been shown that peripheral blood absolute lymphocyte count (ALC) on day 15 is an independent prognostic factor of clinical outcome.[1] The same observation in ASCT has been made in multiple myeloma,[2] Hodgkin's lymphoma,[3] breast cancer,[4] primary systemic amyloidosis,[5] and non-Hodgkin's lymphoma. [2]It has been suggested by some authors that ALC recovery in children with acute lymphoblastic leukemia (ALL) (Hudson G *et al. Blood* 2003;**102**: Abstract #1391) and AML (Lomas C *et al. Blood* 2003;**102**: Abstract #3250) after chemotherapy is a favorable prognostic factor. However, the role of ALC recovery on survival after standard induction chemotherapy (IC) in AML in adults has not been well described.

To assess whether ALC recovery after IC had the same prognostic significance in adult AML, we analyzed the ALC recovery in a group of newly diagnosed, previously untreated AML patients at different time points after IC.

Correspondence: Dr LF Porrata, Mayo Clinic College of Medicine Mayo Clinic College of Medicine, 200 First St SW, Rochester, MN 55905, USA.
E-mail: porrata.luis@mayo.edu, Dr MR Litzow, litzow.mark@mayo.edu
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Materials and methods

Patient population

Between 1998 and 2002, 188 new, previously untreated adult AML patients were seen at the Mayo Clinic. A total of 103 (54.7%) of these 188 patients were eligible for the study, and received IC described below. Patients who could not receive IC because of other comorbidities, or received only palliative chemotherapy such as Hydroxyurea, or refused chemotherapy (43 patients) were excluded, as also were the patients who opted for chemotherapy at centers closer to their homes (21 patients). In all, 19 patients underwent standard IC but their day 14 bone marrow revealed persistent leukemia, and they needed a second course of induction. They were excluded from the study to keep statistical analysis of the study parameters simple. This subset was fairly small and thus, we decided not to analyze it separately. Two patients were lost to follow-up.

The 103 AML patients could be classified as follows according to the French–American–British (FAB) classification: 12 patients with M0, 15 patients with M1, 21 patients with M2, 13 with M3, 17 with M4, two with M4eo, three with M5a, onewith M5b, two with M6, two with M7, one patient with NK/myelocytic, one with granulocytic sarcoma, and 10 unclassified.

All patients gave written, informed consent allowing utilization of their medical records for medical research. Approval for the retrospective review of their records was obtained from the Mayo Clinic Institutional Review Board and was in accordance with the Declaration of Helsinki.

Induction chemotherapy

In all, 90 patients underwent IC with Idarubicin (12 mg/m^2/day) on days 1, 2 ,3 and continuous infusion cytarabine (100 mg/m^2/day) on days 1–7; 13 patients with acute promyelocytic leukemia (APL) underwent IC with Idarubicin (12 mg/m^2) on days 2, 4, 6, 8 and all-*trans*-retinoic acid (45 mg/m^2) daily until complete remission (CR); and one patient with M4 received mitoxantrone (6 mg/m^2), etoposide (80 mg/m^2), and cytarabine (1000 mg/m^2) on days 1–6.

End point

The primary end point of the study was to assess the impact of early ALC recovery after IC for AML on overall survival (OS) and leukemia-free survival (LFS.)

Prognostic factors

The prognostic factors used in the study were age (>60 vs $\leqslant 60$ years), FAB classification (M3 vs other), cytogenetics (unfavorable vs other) secondary AML vs *de novo* disease, lactate dehydrogenase (LDH) > normal for age/sex, white blood cell count (WBC) $\geqslant 35 \times 10^9$/l at diagnosis, and absolute neutrophil count (ANC) recovery and platelets (Plts) recovery at days 15, 21, 28 after IC and before first consolidation chemotherapy (CC).

Response and survival

CR was defined as normal bone marrow morphology with 20% cellularity and fewer than 5% blasts, resolution of previously abnormal cytogenetics, no evidence of extramedullary leukemia, ANC $\geqslant 1500$ cells/μl, and Plts $\geqslant 100\,000$ cells/μl for at least 4 weeks. Patients who recovered their peripheral blood counts, and had more than 5% and less than 25% myeloblast were considered to be in partial remission (PR), as were patients fulfilling the bone marrow criteria of CR but without full recovery of peripheral blood platelet and/or white blood cell counts. OS was measured from the date of initiation of treatment to the date of death or last follow-up. LFS was defined as the time from initiation of treatment to disease progression, relapse, death, or last follow-up. Those who died were considered to have disease progression unless there was clear documentation in the records that no progression of AML had occurred.

Statistical analysis

OS and LFS were analyzed using the method described by Kaplan and Meier.[6] The differences between survival curves were tested for statistical significance using the two-tailed log-rank test. The Cox proportional hazards model was used to perform a univariate analysis on continuous and dichotomized variables. Each continuous variable was dichotomized from finding the optimal cut point on the basis of log-rank statistic. The Cox proportional hazard model was used to assess ALC $\geqslant 500$ cells/μl at all time points as prognostic factor for OS and LFS rates as well as to adjust for other known prognostic factors. The prognostic factors included in the analysis are outlined under the prognostic factors in the Materials and methods section. A logistic regression model was used to estimate and test the association of variables with CR while simultaneously adjusting for variables included in the model. The χ^2 analyses and Fischer Exact tests were used to determine relations between categorical variables; the Wilcoxon rank-sum tests and Spearman correlation coefficient were used for continuous variables. All *P*-values represented were two-sided, and statistical significance was declared at $P<0.05$.

Results

The median age for the study group was 59 years (range: 18–81 years) at the time of diagnosis. The median follow-up was 18 months (range: 1–78 months). Distributions of baseline characteristics for these patients are presented in Table 1 and are summarized on the basis of whether the patients achieved an upper limit ALC recovery $\geqslant 500$ cells/μl at all time points vs those who did not. There was more secondary AML in the latter group, but otherwise there were no statistically significant differences between the groups at baseline. To rule out the possibility that hematopoietic recovery affects ALC recovery after IC, we analyzed the association between ANC/Plts and ALC recovery. The distribution between ANC and Plts recovery at days 15, 21, 28 after IC and at CC was similar between the groups. We identified no association between ANC or Plts recovery and ALC-15 (ANC-15: $r_s=0.014$, $P=0.9$; Plts-15: $r_s=0.13$, $P=0.2$); ALC-21 (ANC-21: $r_s=0.06$, $P=0.55$; Plts-21: $r_s=0.073$, $P=0.47$); ALC-28 (ANC-28: $r_s=0.03$, $P=0.8$; Plts-28: $r_s=0.11$, $P=0.29$); and ALC-CC (ANC-CC: $r_s=0.19$, $P=0.13$; Plts-CC: $r_s=0.04$, $P=0.74$). The distribution of the numbers and type of consolidation therapies and stem cell transplantation (SCT) was similar between both groups.

Survival

By July 2005, 63% (65/103) of the patients had died. The median OS and LFS for the cohort group were 20 months and 13 months, respectively. Recurrence or progression of disease was the cause of death in 60 patients. Five patients died of other complications: two patients of diffuse alveolar hemorrhage, one patient of chronic myelogenous leukemia, one patient of cerebral hemorrhage, and one patient of acute respiratory distress syndrome. A recent report[7] suggests that hematopoietic recovery (i.e., platelet recovery) in acute leukemia predicts clinical outcome after IC. Thus, we studied ANC and Plts recovery in association with clinical outcome in our cohort of patients. As a continuous variable we did not identify a correlation between survival and ANC or Plts recovery at any time point (see Table 2). Categorically, we did not identify a correlation between ANC or Plts recovery at any time point looking at different cut-points between the 25% and the 75% quartiles. Of the 60 patients who died due to disease relapse or progression, 28% (17/60) achieved an ALC $\geqslant 500$ cells/μl at all time points, and 72% (43/60) did not. When ALC-15, ALC-21, ALC-28, and ALC-CC as continuous variables were analyzed independently by univariate analysis, all were found to be predictors for OS and LFS. Using the ALC $\geqslant 500$ cells/μl as the cutoff value, ALC-15 $\geqslant 500$ cells/μl, ALC-21 $\geqslant 500$ cells/μl, ALC-28 $\geqslant 500$ cells/μl, and ALC-CC $\geqslant 500$ cells/μl were all found to predict OS and LFS. ALC-15 $\geqslant 500$ cells/μl (OS: RR $=0.551$, $P<0.0026$; LFS: RR $=0.510$, $P<0.0002$); ALC-21 $\geqslant 500$ cells/μl (OS: RR $=0.674$, $P<0.0064$; LFS: RR $=0.510$, $P<0.0094$); ALC-28 $\geqslant 500$ cells/μl (OS: RR $=0.591$, $P<0.0001$; LFS: RR $=0.624$, $P<0.0001$); and ALC-CC $\geqslant 500$ cells/μl (OS: RR $=0.591$, $P<0.0377$; LFS: RR $=0.669$, $P<0.05$). Thus, to be able to compare ALC recovery with the other prognostic factors, we dichotomized patients into patients achieving an ALC $\geqslant 500$ cells/μl at all time points vs patients that did not.

Superior median OS (Figure 1) and LFS (Figure 2) estimates were observed in the 40 patients achieving an ALC $\geqslant 500$ cells/μl at all time points vs the 63 patients that did not (not reached vs 13 months, $P<0.0001$ and not reached vs 11 months, $P<0.0001$, respectively). Of the patients, 24 did not receive CC after IC: five patients died of treatment-related complications, 15 patients died of progressive leukemia, and four patients went directly to SCT. Of the patients, 28% (29/103) underwent SCT. In the ALC $\geqslant 500$ cells/μl at all time points, two patients underwent ASCT and five patients underwent allogeneic SCT. In the group with ALC <500 cells/μl, nine patients underwent ASCT and 13 patients underwent allogeneic SCT. Even though SCT was not a factor for OS ($P=0.9$) and LFS ($P=0.50$) when compared to patients treated with IC plus CC, the number of such patients in our study was too small to draw any relevant statistical conclusions. Of the 85 patients who did not undergo allogeneic SCT, we studied predictive factors for CR. Univariately, the following factors were associated with CR: age <60 years (19/36 (53%)) vs $\geqslant 60$ year (12/49 (24%), ($P<0.012$); FAB-M3 (11/12 (92%) vs other (20/73 (27%)), ($P<0.0001$); cytogenetics (only 77 cases in this subgroup) – others (24/46 (52%)) vs unfavorable (5/31 (16%)), ($P<0.0017$); *de novo* AML (30/61

Table 1 Baseline characteristics based on patients with an ALC $\geqslant$500 cells/μl at all time points vs patients who did not

Characteristics	*ALC recovery $\geqslant 0.5 \times 10^9$/l at all time points (N = 40)*	*ALC recovery $< 0.5 \times 10^9$/l at one or more time points (N = 63)*	P-*value*
Age (years)			0.74
>60	21	31	
$\leqslant$60	19	32	
Sex			0.55
Female	16	29	
Male	24	34	
FAB classification			0.24
M3	7	6	
Other	33	57	
Cytogenetics			0.33
Favorable	6	10	
Intermediate	20	23	
Unfavorable	10	26	
No information	4	4	
Induction chemotherapy			0.18
Idarubicin+cytarabine (7+3)	32	57	
Idarubicin+*trans*-retinoic acid	7	6	
MEC	1	0	
LDH*			0.66
>Normal for age/sex	24	33	
Normal	12	21	
Secondary AML			0.09
Yes	7	22	
No	33	41	
WBC at diagnosis			0.64
$\geqslant$35	7	14	
<35	32	50	
ANC-15, median (range)	0.3 (0.1–0.68)	0.5 (0.1–3.85)	0.27
ANC-21, median (range)	0.9 (0.1–5.0)	0.8 (0.1–3.7)	0.68
ANC-28, median (range)	0.64 (0.1–4.5)	0.79 (0.1–19.83)	0.88
ANC-CC, median (range)	3.84 (0.64–6.61)	3.69 (0.3–9.73)	0.88
Plts-15, median (range)	16 (5–95)	14 (2–109)	0.48
Plts-21, median (range)	21 (2–396)	19 (2–503)	0.20
Plts-28, median (range)	162.5 (12–723)	106.5 (3–611)	0.38
Plts-CC, median (range)	188 (33–375)	186.5 (14–430)	0.84
Type of consolidation therapies			0.54
Ida +ARA-C followed by HD-ARA-C	11	13	
Ida+ARA-C	13	20	
HD-ARA-C	4	9	
Ida+ATRA	4	2	
Maintenance ATRA	1	1	
MEC	1	0	
Number of consolidation therapies, median (range)	2 (1–4)	2 (1–5)	0.27
Stem cell transplantation			0.68
Autologous	2	9	
Allogeneic	5	13	

ANC-15, ANC-21, ANC-28, and ANC-CC = absolute neutrophil count $\times 10^9$/l recovery after induction chemotherapy at days 15, 21, 28, and at consolidation chemotherapy; ARA-C = cytarabine; ATRA = *trans*-retinoic acid; Ida = idarubicin; FAB = French–American–British (FAB) classification; LDH = lactate dehydrogenase; Plts-15, Plts-21, Plts-28, and Plts-CC = platelet count $\times 10^9$/l recovery after induction chemotherapy at days 15, 21, 28, and at consolidation chemotherapy; WBC = white blood cell count $\times 10^9$/l; cytogenetics = favorable (t(8;12), t(15;17) and inv(16)/t(16;16)), intermediate (normal karyotype and missing Y chromosome, all others as well as complex chromosomal changes were classified as unfavorable; MEC = mitoxantrone, etoposide, cytarabine.
*Data available in only 90 patients.

(49%)) vs secondary AML (1/24 (4%)), ($P<0.004$); and ALC $\geqslant$500 cells/μl at all time points (19/32 (59%)) vs ALC <500 cells/μl (12/53 (23%)), ($P<0.001$). When these factors were accounted for in the multivariate logistic regression model, ALC $\geqslant$500 cells/μl at all time points remained a significant, independent predictive factor for CR ($P<0.007$). An ALC $\geqslant$500 cells/μl at all time points was associated with an adjusted odds ratio for CR of 7.3 (95% CI, 1.9–35.4).

Table 2 Univariate analysis for overall and leukemia-free survival

Prognostic factors	*Overall survival*			*Leukemia-free survival*		
	RR	*95% CI*	P-*value*	*RR*	*95% CI*	P-*value*
Age >60 years	1.518	1.195–1.942	<0.0006	1.257	1.010–1.569	<0.0405
ALC recovery ⩾500 cells/μl at all time points	0.474	0.294–0.692	<0.0001	0.554	0.363–0.736	<0.0001
FAB (M3 vs other)	0.257	0.061–0.547	<0.0001	0.462	0.255–0.719	<0.0001
LDH >normal for age/sex	1.249	0.961–1.612	0.0954	1.033	0.803–1.314	0.7960
Secondary AML	1.571	1.227–2.000	<0.0004	1.333	1.053–1.671	<0.0179
Cytogenetics (Unfavorable vs other)	1.631	1.256–2.105	<0.0002	1.442	1.137–1.822	<0.0027
WBC $\geqslant 35 \times 10^9$/l	1.088	0.803–1.429	0.5670	1.187	0.900–1.526	0.2160
ANC-15	1.076	0.462–1.844	0.83	0.884	0.379–1.522	0.71
ANC-21	1.675	0.769–2.720	0.16	1.388	0.630–2.229	0.35
ANC-28	1.055	0.946–1.135	0.29	1.079	0.975–1.159	0.12
ANC-CC	0.899	0.734–1.091	0.28	0.994	0.785–1.123	0.52
Plts-15	0.994	0.976–1.008	0.44	0.988	0.970–1.003	0.12
Plts-21	0.999	0.995–1.003	0.77	0.999	0.994–1.002	0.51
Plts-28	0.998	0.996–1.009	0.85	0.997	0.996–1.003	0.30
Plts-CC	0.999	0.996–1.002	0.28	0.996	0.993–1.001	0.13

ALC recovery ⩾500 cells/μl at all time points = patients that achieve a higher ALC cut-off values of $\geqslant 0.5 \times 10^9$ at all time points (15, 21, or 28 days after induction chemotherapy, and at first consolidation chemotherapy); AML = acute myelogenous leukemia; ANC-15, 21, 28,CC = absolute neutrophil count at days 15, 21, 28 after induction chemotherapy and at first consolidation chemotherapy; FAB = French–American–British (FAB) classification; LDH = lactate dehydrogenase; cytogenetics = favorable (t(8;12), t(15;17) and inv(16)/t(16;16)), intermediate (normal karyotype and missing Y chromosome, all others as well as complex chromosomal changes were classified as unfavorable; Plts-15, 21, 28, CC = platelet count at days 15, 21, 28 after induction chemotherapy and at first consolidation chemotherapy; WBC = white blood cell count $\times 10^9$/l.

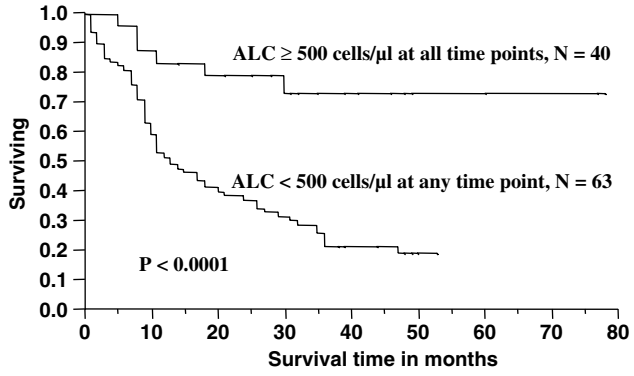

Figure 1 Kaplan–Meier estimates of overall survival (OS) of patients achieving an ALC recovery ⩾500 cells/μl at all time points vs patients achieving ALC recovery <500 cells/μl at any time point. The median OS was not reached in the group with an ALC recovery ⩾500 cells/μl at all time points and 13 months in the group with an ALC recovery <500 cells/μl at any time point. The OS rates at 4 years were 73 vs 19%, respectively, $P<0.0001$.

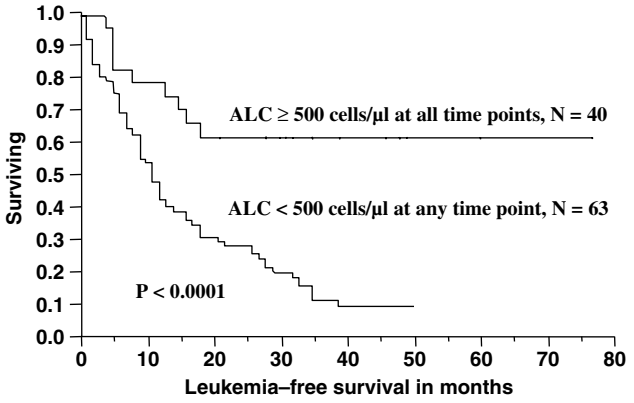

Figure 2 Kaplan–Meier estimates of leukemia-free survival (LFS) of patients achieving an ALC recovery ⩾500 cells/μl at all time points vs patients achieving ALC recovery <500 cells/μl at any time point. The median LFS was not reached in the group with an ALC recovery ⩾500 cells/μl at all time points and 11 months in the group with an ALC recovery <500 cells/μl at any time point. The OS rates at 4 years were 62 vs 9%, respectively, $P<0.0001$.

We also analyzed the subgroup of patients who did not undergo SCT separately. Of the 74 patients who did not undergo SCT, patients achieving an ALC ⩾500 cells/μl at all time points ($N=27$) experienced improved OS and LFS compared with patients with an ALC <500 at one or more time points ($N=47$) (OS not reached vs 11 months, $P<0.0001$; LFS not reached vs 9 months, $P<0.0001$, respectively). ALC recovery was also identified as an independent predictor for OS and LFS in this subgroup analysis when compared to age, FAB, cytogenetics, and secondary AML (OS: RR = 0.516, $P<0.0038$; LFS: RR = 0.485, $P<0.0013$). Also, analyzing the 16 patients with favorable cytogenetics (seven patients achieved ALC ⩾500 cells/μl at all time points and nine patients did not), a better OS and LFS was observed in the patients with ALC ⩾500 cells/μl at all time points than those with ALC <500 cells/μl. (OS not reached vs 36 months, $P<0.028$; and LFS not reached vs 29 months, $P<0.046$, respectively). However, this is again too small a subgroup of patients from the cohort to draw any significant conclusions.

Univariate analysis

Additional prognostic factors were examined in univariate analysis as indicated in Table 2. Age (>60 vs ⩽60 years), FAB classification (M3 vs other), cytogenetics (unfavorable vs other), and secondary AML vs *de novo* disease were significant predictors for improved OS and LFS in the univariate analysis.

Multivariate analysis

When the same factors were subjected to multivariate analysis (Table 3) ALC ⩾500 cells/μl at all time points was an independent predictor of OS (RR = 0.536, $P<0.0008$) and LFS (RR = 0.591, $P<0.0011$).

Table 3 Multivariate analysis for overall and leukemia-free survival

Prognostic factors	*Overall survival*			*Leukemia-free survival*		
	RR	*95% CI*	P-*value*	*RR*	*95% CI*	P-*value*
Age >60 years	1.725	1.300–2.318	<0.0002	1.333	1.038–1.752	<0.0241
ALC recovery ⩾500 cells/μl at all time points	0.536	0.331–0.788	<0.0008	0.591	0.401–0.821	<0.0011
FAB (M3 vs other)	0.361	0.085–0.787	<0.0056	0.557	0.238–0.825	<0.0108
Secondary AML	1.142	0.865–1.500	0.3434	1.027	0.787–1.327	0.8392
Cytogenetics (unfavorable vs other)	1.750	1.316–2.335	<0.0001	1.481	1.139–1.923	<0.0036

ALC recovery ⩾500 cells/μl at all time points = patients that achieve a higher ALC cutoff values of $\geqslant 0.5 \times 10^9$ at all time points (15, 21, or 28 days after induction chemotherapy, and at first consolidation chemotherapy); AML = acute myelogenous leukemia; FAB = French–American–British (FAB) classification; cytogenetics = favorable (t(8;12), t(15;17) and inv(16)/t(16;16)), intermediate (normal karyotype and missing Y chromosome, all others as well as complex chromosomal changes were classified as unfavorable.

Discussion

In the present study, we have shown that adult patients with higher ALC following IC have enhanced OS and LFS. Indeed, multivariate analysis identifies postinduction ALC as strong independent predictor of treatment outcome in this patient population. To our knowledge, this is the first report linking early ALC regeneration to prognosis after IC in adult AML. Previous studies have shown that patients achieving a higher ALC recovery after ASCT for AML experienced superior survival.[1] Moreover, early ALC regeneration after IC has been reported to correlate with improved survival in pediatric AML. Lomas and coworkers studied 30 consecutive patients with AML, and found that day 28 ALC postchemotherapy was significantly higher in the OS and event-free survival groups (Lomas C *et al*. *Blood* 2003;**102**: Abstract). The ALC seemed to have no relation to cytogenetics, FAB type or treatment stratification. Similarly, Hudson and coworkers studied 100 consecutive children with ALL, and reported that postchemotherapy recovery of ALC correlated at all time points with OS (Hudson G *et al*. *Blood* 2003;**102**: Abstract).

However, the impact of early ALC recovery on survival after standard IC has not been studied in a systematic fashion in adults before. The time point selection (days, 15, 12, 28, and before CC) to analyze ALC recovery after IC was based on our previous studies showing that day 15 after SCT is a prognostic factor for survival, and the reports by Lomas C *et al*. (*Blood* 2003;**102**: Abstract) and Hudson G *et al*. (*Blood* 2003;**102**: Abstract) who examined ALC on days 21 and days 28 after IC. To simplify and be able to compare ALC against other prognostic factors, we dichotomized patients into those who achieved an ALC ⩾500 cells/μl at all time points vs those who did not, as ALC ⩾500 cells/μl was identified as the optimal cutoff point value at each time interval. Using this new category, we observed superior survival in AML patients who demonstrated early ALC after IC and demonstrated that early ALC is a prognostic factor independent of other known prognostic factors. Despite a higher number of secondary AML and poor risk cytogenetics in the ALC <500 cells/μl group that could have affected the clinical outcome analysis, ALC recovery after IC remained an independent predictor for CR when compared to age at presentation, cytogenetics, FAB classification, and secondary AML.

Faderl *et al*.[7] recently reported in *de novo* ALL that the time to platelet (TPR) recovery predicted outcome in ALL patients achieving a CR. The authors suggested that TPR could be a surrogate marker of the host's ability to contain and fight minimal residual disease. Thus, a concern of the survival advantage observed with early ALC recovery may be a reflection of a faster bone marrow recovery leading to less adverse consequences of prolonged cytopenias and the ability to proceed with further courses of chemotherapy without delay. Our study showed no association between ANC/Plts and ALC recovery suggesting a different recovery kinetics between hematopoietic (i.e., ANC/Plts) and immunologic recovery (i.e., ALC). Our study did not show an association between hematopoietic recovery (i.e., ANC/Plts) and survival in AML patients after IC. This discordance could be explained from the statistical standpoint by the small sample (103 patients) in our study compared to 249 patients in Faderl's study. Our study analyzed a heterogeneous AML groups of patients (*de novo* and secondary AML) compared to a homogeneous *de novo* ALL patients in Faderl's study. As a result of the different biology between *de novo* and secondary AML, a study looking at TPR in homogenous *de novo* AML group is warranted to assess if TPR is also a surrogate marker for clinical outcome in AML after IC.

As this was a retrospective study, further investigation is required to determine whether a specific subset of lymphocytes is responsible for producing the superior clinical outcome. Mackall *et al*.[8] reported normalization of natural killer (NK) cell numbers after repeated cycles of chemotherapy, while T- and B-cell numbers remained persistently low. Recently, Lowdell *et al*.[9] reported that prolonged remission after treatment in AML patients depends on the autologous cytolytic activity of NK cells, and this activity can be measured as 'leukemia cytolytic activity (LCA)'. Patients who ultimately relapsed had significantly lower LCA than those who remained in remission ($P<0.001$). Based on these observations, Lowdell *et al*.[9] proposed that this immune response, rather than the chemotherapy patients receive, is responsible for continued remission. NK cells are known to have definite antileukemia activity.[10,11] Moreover, NK cells can mature without the presence of a functioning thymus, possibly explaining why they recover so promptly.[12,13] Collectively, these prior observations and the present results suggest that NK cells have a role in the antileukemia immunosurveillance of AML patients after standard IC. Thus, we are opening a prospective study to analyze the quantitative and qualitative immune recovery after IC in AML patients, specifically focussing on observing the time course in which T cells, B cells, and NK cells recover, and their relationship to clinical outcome.

A variety of immunological strategies are currently undergoing preclinical and clinical testing for AML, including monoclonal antibodies,[14] gene therapy,[15] dendritic cell vaccinations,[16] and systemic treatment with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), the cytotoxic cytokine utilized by NK cells.[17] Our observation showing the

effect of ALC recovery on survival after IC highlights not only the importance of further studies of immunological therapies for AML, but also suggests that status of the host immune system should be carefully monitored as a potential variable influencing outcome as these immunological therapies are tested in the clinic.

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