Low platelet counts after induction therapy for childhood acute lymphoblastic leukemia are strongly associated with poor early response to treatment as measured by minimal residual disease and are prognostic for treatment outcome

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ABSTRACT

Background
Numerous reports have been published on the association between kinetics of leukemic cells during early treatment of childhood acute lymphoblastic leukemia and therapeutic outcome. In contrast, little is known about the prognostic relevance of normal blood counts in this setting.

Design and Methods
Normal hematopoiesis during and after induction treatment (days 8, 15 and 33) was correlated with therapeutic outcome in a cohort of 256 children with acute lymphoblastic leukemia treated in one of three consecutive ALL-BFM trials at a single institute. Replication analysis of positive findings was performed in an independent cohort of 475 patients from the ALL-BFM 2000 multicenter trial.

Results
A platelet count in the first quartile on treatment day 33 and a neutrophil count above the median on day 8 were significantly associated with treatment outcome, conferring multivariate risk ratios for an event of 3.27 (95% confidence interval 1.60-6.69) and 2.26 (95% confidence interval 1.23–4.29), respectively. Replication analysis confirmed the prognostic effect of platelet count on treatment day 33 and demonstrated a strong association with minimal residual disease-based risk group distribution ($P<0.00001$).

Conclusions
Platelet counts after induction treatment may improve treatment stratification for patients with childhood acute lymphoblastic leukemia and be of particular interest in non-minimal residual disease-based trials. *(ALL-BFM 2000 is registered at: ClinicalTrials.gov: NCT00430118. National Cancer Institute: Protocol ID 68529)*

Key words: leukemia, childhood, prognosis, normal hematopoiesis, blood count.

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The online version of this article has a Supplementary Appendix.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignant disease of childhood and can be cured in more than 80% of cases by the use of intensive multi-agent chemotherapeutic regimens.[1-3] In current protocols, this therapy is adapted according to the risk of treatment failure, which has become a common feature in present day clinical management of childhood ALL. The risk of treatment failure is assessed through the evaluation of prognostic factors which have been identified as a result of continuing research on the clinical and biological aspects of leukemias. These factors include clinical characteristics [e.g., gender, initial white blood cell count (WBC) and age at diagnosis], immunological features (e.g., leukemic immunophenotype), somatic features (e.g., non-random recurrent chromosomal aberrations such as the Philadelphia chromosome) as well as germline genetic characteristics (e.g., thiopurine methyltransferase genetics) which are assessable at diagnosis.[1-3] In addition, a variety of estimates of early response to treatment are used as prognostic factors for treatment allocation.[1-3] Overall, the risk assessment procedures applied by different study groups mainly lead to therapy stratification into two or three risk groups (e.g., standard/low, intermediate, high).

The Berlin-Frankfurt-Münster (BFM) Study Group started to assess the prognostic value of the so-called prednisone response as early as 1983.[4] The prednisone response is determined from the peripheral blood leukemic blast count on day 8 after starting treatment with 7 days of monotherapy with prednisone and one intrathecal dose of methotrexate on treatment day 1. A good response is defined as a peripheral blood blast count of less than 1000/μL while a poor response is characterized by a count of 1000 blasts/μL or more. Event-free survival rates between prednisone good and poor responders differ significantly (roughly 80% *versus* 30 to 40%).[4,5] In the early 1990s, the International BFM study group initiated clinical evaluation of a molecularly assessed early treatment response in childhood ALL.[6] This so-called minimal residual disease (MRD) analysis allows submicroscopic detection of leukemic clone-specific immunoglobulin and/or T-cell receptor gene rearrangements by polymerase chain reaction (PCR)-analysis and is approximately 1,000 to 10,000-fold more sensitive than methods based on morphological detection. Several independent studies have shown that MRD is a strong prognostic factor and is superior to morphologically assessed treatment response.[6-12] Of importance, over the last decade, MRD has become the most important predictor of outcome in ALL-BFM trials on treatment of childhood ALL.[13-15]

While numerous reports on the dynamics of leukemic cells during the early treatment phases of childhood ALL in association with prognosis have been published, little information is available on the potential importance of normal blood cells during this time period. We, therefore, evaluated the relationship of normal hematopoiesis during and after induction treatment with therapeutic outcome in a cohort of children with ALL treated in three consecutive ALL-BFM trials in a single institution.

Design and Methods

Patients

We identified 282 ALL patients who were treated at the Department of Pediatric Hematology and Oncology, Hannover Medical School, Germany, according to one of three consecutive ALL-BFM protocols from 1990 to 2004 (ALL-BFM 90, ALL-BFM 95, ALL-BFM 2000). The design, conduct, analysis and results of the multicenter trials ALL-BFM 90, 95 and 2000 have been described in detail elsewhere.[13-17] The institutional review boards of Hannover Medical School and all participating centers approved these studies. Informed consent was obtained in accordance with the Declaration of Helsinki. In all trials, patients were stratified into three branches (standard, intermediate, and high risk). In the ALL-BFM 90 study, a leukemic cell mass estimate was used, the so-called risk factor. This composite variable was calculated from the initial blast count in the peripheral blood and the sizes of liver and spleen below the costal margin in centimeters (risk factor = 0.2 × log (n. of blood blasts/μL + 1) + 0.06 × liver size + 0.04 × spleen size). Standard-risk patients had <1000/μL peripheral blood blasts on treatment day 8, a risk factor of less than 0.8, no central nervous system disease, no mediastinal mass, and no T-cell ALL. Intermediate risk was defined as ≤1000/μL peripheral blood blasts on treatment day 8, a risk factor of ≥0.8, or a risk factor of <0.8 and central nervous system disease and/or a mediastinal mass or T-cell ALL. High-risk patients had a prednisone poor-response, or ≥5% blasts in the bone marrow on treatment day 33, or were positive for a t(9;22) or *BCR/ABL* fusion RNA. In ALL-BFM 95, standard-risk patients had no high-risk criteria (see below), an initial WBC count <20×10^9/L, age at diagnosis of ≥1 and <6 years, and no T-ALL. Intermediate-risk patients had no high-risk criteria and an initial WBC ≥20×10^9/L, and/or age at diagnosis of <1 or ≥6 years, and/or T-ALL. High-risk patients had a prednisone poor-response, or ≥5% blasts in the bone marrow on treatment day 33, or were positive for a t(9;22) or t(4;11) or their molecular equivalents (*BCR/ABL* or *MLL/AF4* fusion RNA). In ALL-BFM 2000, risk group stratification included MRD analysis and required two MRD targets with sensitivities of ≤10^{-4}. Standard-risk patients were MRD-negative on treatment days 33 (TP1) and 78 (TP2) and had no high-risk criteria. High-risk patients had residual disease (≥10^{-3}) at TP2. MRD intermediate-risk patients had MRD detected at either one and or both time points but at a level of <10^{-3} at TP2. Although MRD-based stratification criteria were introduced in ALL-BFM 2000, established high-risk parameters were also retained: patients with a prednisone poor-response or ≥5% leukemic blasts in the bone marrow on day 33 or positivity for a t(9;22) or t(4;11) or their molecular equivalents (*BCR/ABL* or *MLL/AF4* fusion RNA) were stratified into the high-risk group independently of their MRD results.

In all three trials treatment consisted of standard drugs (eg, prednisone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, ifosfamide, cytarabine, 6-mercaptopurine, 6-thioguanine, and methotrexate) and, in some of the patients, cranial irradiation and/or hematopoietic stem cell transplantation (high-risk patients, only).

With the exception of high-risk patients in ALL-BFM 90 and ALL-BFM 95, all patients received induction, consolidation, and re-induction treatment, followed by maintenance therapy. High-risk patients in ALL-BFM 90 and ALL-BFM 95 were treated with a shorter induction regimen and continued on an intensive rotational consolidation schedule but did not receive the regular re-induction (protocol II). High-risk patients in ALL-BFM 2000 received the

regular induction regimen and were subsequently treated with a combination of intensive rotational consolidation and re-induction treatment.

Regarding cranial irradiation, in study ALL-BFM 90 the dose used was 12 Gy for all non-standard-risk patients. Standard-risk patients did not receive cranial irradiation. Patients with central nervous system involvement (mainly defined by >5 leukocytes/μL of cerebrospinal fluid with definable blasts) received a dose of 24 Gy (<2 years: 18 Gy, <1 year: no cranial irradiation). In the subsequent trials, ALL-BFM 95 and ALL-BFM 2000, preventive cranial irradiation, at a dose of 12 Gy, was only applied in high-risk patients and patients with T-cell ALL. All other cranial irradiation-related settings remained similar to those in ALL-BFM 90.

Patients were included in the present analysis if they had blood count data available for one of the following time-points: treatment days 8, 15 and 33. These time-points were chosen as they represent important response evaluation dates during and after induction treatment on ALL-BFM protocols. Consecutively enrolled patients from the ALL-BFM 2000 trial and data from the associated database were employed for the replication analysis.

Normal hematopoiesis

Blood counts were assessed in the clinical laboratory of the Department of Pediatric Hematology and Oncology at Hannover Medical School and retrieved through chart reviews. The findings of the treating institutions were used for the replication analysis. Patients' histories of platelet transfusions were made available through archived files from the Department of Transfusion Medicine at Hannover Medical School. Absolute neutrophil, absolute monocyte, and absolute lymphocyte counts were calculated from WBC counts and differential blood cell count percentages. A left-shifted blood count was defined by the presence of at least 1% metamyelocytes, myelocytes or promyelocytes in a differential count of 100 cells.

Statistical analysis

Differences in the distribution of individual parameters among subsets of patients were analyzed using Fisher's exact test or the χ^2 test for categorized variables and the Mann-Whitney-*U* test for continuous variables. The various blood count levels at the different time-points were examined as quartiles based on the frequency distribution in all patients with the respective information available. Event-free survival was defined as the time from diagnosis to the date of last follow-up in complete remission or first event. Events were resistance to therapy (non-response), relapse, secondary neoplasm or death from any cause. Failure to achieve remission due to early death or non-response was considered as an event at time 0. Patients lost to follow-up were censored at the time of their withdrawal. The Kaplan-Meier method was used to estimate survival rates, differences were compared with a two-sided log-rank test.[18,19] Cumulative incidence functions for competing events were constructed using the method of Kalbfleisch and Prentice, and were compared with Gray's test.[20,21] A Cox proportional hazards model was used to obtain the estimates and the 95%-confidence interval of the relative risk for prognostic factors.[22] Statistical analyses were conducted using the SAS statistical program (SAS-PC, Version 9.1; SAS Institute Inc., Cary, NC, USA).

Results

Of the 282 children with ALL treated from 1990 to 2005 in the Department of Pediatric Hematology and Oncology at Hannover Medical School, 256 had data on normal hematopoiesis available for treatment day 8, while 251 and 249 patients had additional data available for treatment days 15 and 33, respectively. Table 1 shows the characteristics of the entire cohort of 282 patients and separately for those patients included (n=256) and excluded due to unavailability of data (n=26). No significant differences were observed between included and excluded patients regarding important clinical and biological characteristics with potential impact on therapeutic outcome.

Table 2 gives a detailed overview of the prednisone prephase and the complete induction treatment in the three consecutive trials ALL-BFM 90, 95 and 2000 and allows exact comparison of the treatment phases during

Table 1. Characteristics of 282 pediatric patients with acute lymphoblastic leukemia treated at Hannover Medical School from 1990 to 2005.

	All patients n (%)	Included n (%)	Not included[f] n (%)	P[g]
Gender				
male	147 (52.1)	135 (52.7)	12 (46.2)	
female	135 (47.9)	121 (47.3)	14 (53.8)	0.54
Age at diagnosis (years)				
<1	5 (1.8)	5 (2.0)	-	
1-<6	177 (62.8)	162 (63.3)	15 (57.7)	
6-<10	58 (20.6)	54 (21.1)	4 (15.4)	
≥10	42 (14.9)	35 (13.7)	7 (26.9)	0.29
Presenting WBC[a] ($\times 10^9$/L)				
<20	185 (65.6)	172 (67.2)	13 (50.0)	
20-<100	64 (22.7)	56 (21.9)	8 (30.8)	
≥100	33 (11.7)	28 (10.9)	5 (19.2)	0.20
Immunophenotype				
B	241 (85.5)	223 (87.1)	18 (69.2)	
T	34 (12.1)	28 (10.9)	6 (23.1)	0.10
unknown	7 (2.5)	5 (2.0)	2 (7.7)	
DNA index[b]				
<1.16	179 (63.5)	164 (64.1)	15 (57.7)	
≥1.16	39 (13.8)	35 (13.7)	4 (15.4)	0.75
unknown	64 (22.7)	57 (22.3)	7 (26.9)	
TEL/AML1[c]				
positive	27 (9.6)	27 (10.5)	-	
negative	108 (38.3)	103 (40.2)	5 (19.2)	0.58
unknown	147 (52.1)	126 (49.2)	21 (80.8)	
BCR/ABL				
positive	4 (1.4)	3 (1.2)	1 (3.8)	
negative	222 (78.7)	208 (81.2)	14 (53.8)	0.24
unknown	56 (19.9)	45 (17.6)	11 (43.3)	
MLL/AF4[c]				
positive	1 (0.4)	1 (0.4)	-	
negative	118 (41.8)	109 (42.6)	9 (34.6)	0.99
unknown	163 (57.8)	146 (57.0)	17 (65.4)	
Prednisone response[d]				
good	248 (87.9)	227 (88.7)	21 (80.8)	
poor	28 (9.9)	23 (9.0)	5 (19.2)	0.16
unknown	6 (2.1)	6 (2.3)	-	
Risk group				
standard	88 (31.2)	80 (31.2)	8 (30.8)	
intermediate	156 (55.3)	144 (56.2)	12 (46.2)	
high	38 (13.5)	32 (12.5)	6 (23.1)	0.30

[a]WBC: white blood cell count; [b]ratio of DNA content of leukemic G_0/G_1 cells to normal diploid lymphocytes; [c]obligate screening for TEL/AML1 *fusion transcripts only in ALL-BFM 2000 and for* MLL/AF4 *only in ALL-BFM 95 and 2000; [d]good: <1000 leukemic blood blasts/μL on treatment day 8; poor: ≥1000/μL; [e]minimal residual disease, only performed in ALL-BFM 2000; [f]26 patients were excluded from the study; [g]P Fisher's exact test for all 2x2 comparisons, all others χ^2.*

Table 2. Prednisone prephase and induction treatment in the consecutive childhood leukemia trials ALL-BFM 90, 95 and 2000.

Drug	ALL-BFM 90 Dose	ALL-BFM 90 Administered on days	ALL-BFM 95 Dose	ALL-BFM 95 Administered on days	ALL-BFM 2000 Dose	ALL-BFM 2000 Administered on days
Prephase						
Prednisone (orally)	60 mg/m²/d	1-7	60 mg/m²/d	1-7	60 mg/m²/d	1-7
Induction						
Prednisone[1] (orally)	60 mg/m²/d	8-28	60 mg/m²/d	8-28	60 mg/m²/d	8-28
Dexamethasone[1] (orally)	-	-	-	-	10 mg/m2/d	8-28
Vincristine (IV)	1.5 mg/m²/d (max 2 mg)	8, 15, 22, 29	1.5 mg/m²/d (max 2 mg)	8, 15, 22, 29	1.5 mg/m²/d (max 2 mg)	8, 15, 22, 29
Daunorubicin[2] (IV)	30 mg/m²/d	8, 15, 22, 29	30 mg/m²/d	8, 15, 22, 29	30 mg/m²/d	8, 15, 22, 29
L-Asparaginase[3] (IV)	10,000 IU/m²/d	12, 15, 18, 21, 24, 27, 30, 33	5,000 IU/m²/d	12, 15, 18, 21, 24, 27, 30, 33	5,000 IU/m²/d	12, 15, 18, 21, 24, 27, 30, 33
Methotrexate[4] (IT)	12 mg/d	1, (8), 15, (22), 29	12 mg/d	1, 12, (18), (27), 33	12 mg/d	1, 12, (18), (27), 33

[1]After a common 7-day prednisone prephase in ALL-BFM 2000, patients were randomized to receive either prednisone or dexamethasone; [2]For standard-risk patients in ALL-BFM 95, the number of daunorubicin applications in induction was halved to two doses of 30 mg/m²; [3]CRASNITIN, an E. coli *asparaginase preparation from Bayer (Leverkusen, Germany) was given in ALL-BFM 90;* E. coli *asparaginase from Medac (Wedel, Germany) was used as the first-line asparaginase preparation in ALL-BFM 95 and 2000; the dose reduction from 10,000 IU/m² in ALL-BFM 90 to 5,000 IU/m² in subsequent trials was because of the higher activity and toxicity compared with the formerly used preparation (CRASNITIN); in case of allergic reactions,* Erwinia *L-asparaginase (ERWINASE; Speywood, London, UK) or PEG-asparaginase (ONCASPAR, Medac) was recommended as a substitute; high-risk patients in ALL-BFM 95 received only seven doses of asparaginase, sparing the dose on day 33, and continued on day 36 with intensive block-based treatment; [4]Doses were adjusted for children younger than 3 years; in ALL-BFM 95, the day 33 intrathecal (IT) methotrexate dose was scheduled on day 27 in high-risk patients; application days in parentheses indicate additional IT methotrexate doses for patients with CNS status CNS2, TLP+, or CNS3.*

which normal blood counts were evaluated in our study. It can be understood from this table that there were only slight differences regarding treatment between the three trials (detailed in the footnote to Table 2).

Table 3 presents the details of the blood cell counts observed at treatment days 8, 15 and 33 in our cohort of 256 patients with childhood ALL. Depending on the availability of data, hemoglobin levels, platelet count and WBC count on treatment day 8 were based on 256 individuals while the remaining values for day 8 were based on 246 individuals. On treatment day 15, hemoglobin levels and platelet and WBC counts were based on 251 individuals. The remaining values for day 15 were available for 229 individuals. After completion of induction, on treatment day 33, hemoglobin levels and platelet and WBC counts were based on 249 individuals and the remaining values on 227 individuals. Hemoglobin levels, platelet, WBC, absolute neutrophil, absolute monocyte, and absolute lymphocyte counts were analyzed in quartiles. Table 3 shows the cut-points for quartiles used for the different normal blood count values during induction treatment (treatment days 8 and 15) and after induction (treatment day 33). The numbers of patients per quartile of the different blood cell counts at the different time-points are shown in *Online Supplementary Table S1*. Left-shifted blood count was analyzed as a dichotomous variable (present or absent).

When analyzed regarding their effect on treatment outcome neither hemoglobin levels on treatment days 8, 15 and 33 nor monocyte levels at these same times showed significant associations (*data not shown*). Similarly, no associations were observed for presence or absence of a left-shifted blood count on treatment days 8, 15 and 33 (*data not shown*). While platelet counts on days 8 and 15 did not reveal significant associations with outcome, low platelet counts (in quartile 1) on treatment day 33 were related to a dismal 8-year event-free survival (Figure 1A) due to an increased 8-year cumulative incidence of recurrent disease (Figure 1B). Furthermore, patients with low neutrophil counts on treatment day 8 fared better compared to patients with higher counts. Patients in quartiles 1 and 2 had an 8-year event-free survival of 0.90±0.04 and 0.91±0.04, respectively, compared to 0.66 ± 0.07 and 0.70 ± 0.06 for patients in quartiles 3 and 4 (Figure 1C). As for platelet counts on treatment day 33, the dismal outcome for patients with high neutrophil counts on treatment day 8 was due to recurrent disease (Figure 1D). Neutrophil counts on treatment day 33 were not significantly associated with event-free survival. This was due to a relatively large number of events other than relapse in the quartiles 3 and 4 (*data not shown*). However, the cumulative incidence of relapse in quartiles 3 and 4 for neutrophil counts on day 33 was significantly lower than that in quartiles 1 and 2 (P=0.031; *data not shown*). With respect to lymphocyte counts, patients with counts on treatment day 8 in quartiles 2 and 3 did significantly better than those whose counts were in quartile 1 or 4 (8-year event-free survival rates of 0.91±0.04 and 0.86±0.04 *versus* 0.73±0.06 and 0.67±0.06, respectively; P=0.003). Besides this non-linear association of normal lymphocyte values on day 8 with outcome, the other lymphocyte counts evaluated in this study – those at treatment days 15 and 33 – were not associated with outcome (*data not shown*). None of the additional specific blood count variables investigated in this study showed significant associations with outcome. In multivariate Cox regression analyses including known prognostic variables (gender, immunophenotype, WBC count at diagnosis, trial, risk group criteria of ALL-BFM 95), platelet counts on treatment day 33 in quartile 1 and neutrophil counts on day 8 in quartiles 3 and 4 were independent predictors of outcome conferring risk ratios for an event of 3.27 (95% confidence interval 1.60–6.69) and 2.26 (95% confidence interval 1.23–4.29) when compared to the remaining three platelet quartiles or the first and sec-

ond neutrophil quartiles, respectively.

The sample analyzed in our single institution study consisted of patients being treated in three consecutive clinical trials, ALL-BFM 90 and 95, using classical risk factors and cytomorphological response to treatment for therapy stratification, and ALL-BFM 2000, mainly relying on sensitive molecular measurements of residual disease for risk group allocation. Thus, we next tried to replicate our findings by analyzing an independent sample of patients drawn from the entire population of the ALL-BFM 2000 multicenter trial. For this purpose, platelet counts on treatment day 33 as well as lymphocyte and neutrophil counts on day 8 were collected by chart review for an additional 475 patients. Besides a larger proportion of older patients in the replication sample and a larger standard risk group due to MRD-based stratification of all patients in the replication cohort, the characteristics of these 475 patients did not differ significantly from the initial single center study population (*Online Supplementary Table S2*). Applying the same cut-points as in the initial set of patients, we did not detect significant differences in outcome explained by the lymphocyte count on treatment day 8. When analyzing the prognostic cut-points for platelet counts on day 33 (quartile 1 *versus* quartiles 2-4) and neutrophil counts on day 8 (quartiles 1 and 2 *versus* 3 and 4) from the initial set of patients, we again observed a significantly worse outcome for patients whose platelet counts were in quartile 1 (Figure 2A) but not for those with neutrophil counts in quartiles 3 and 4 (Figure 2B). This latter finding was due to the fact that in the analysis of single quartiles, the negative prognostic impact of neutrophil quartile 3, which was detected in our initial analysis, could not be replicated in our second analysis. Nevertheless and even though disadvantageous, we maintained the initial cut-points as this second analysis aimed at validating the prognostic values of the initial analysis. In multivariate Cox regression analyses including known prognostic variables (gender, immunophenotype, WBC count at diagnosis, risk group criteria of ALL-BFM 95), the negative effect on outcome conferred by a platelet count in quartile 1 retained its significance (risk ratio for an event 1.81; 95% confidence interval 1.14 – 2.88, P=0.012; for univariate risk ratios see *Online Supplementary Table S3*). However, neutrophil count in quartiles 3 and 4 lost its negative prognostic value in comparison to counts in quartiles 1 and 2 (risk ratio for an event 1.34; 95% confidence interval 0.83–2.16, P=0.229). Similar results were obtained when neutrophil counts on day 8 were categorized according to the median (*data not shown*). Of interest, when we applied the same multivariate Cox analysis, but instead of using the ALL-BFM 95 risk group stratification criteria introduced MRD-based risk group as a covariate to the model, the negative prognostic impact of a platelet count in quartile 1 on day 33 lost its independent effect (risk ratio for an event 1.44; 95% confidence interval 0.90–2.31, P=0.133). The strong association of MRD levels with platelet counts on treatment day 33, responsible for this phenomenon, is shown in Table 4. Seventy-four percent of MRD high-risk patients had platelet counts in quartile 1 at treatment day 33 while this was the case for only 40% of intermediate-risk and 28% of standard-risk patients. The positive and negative predictive values for platelet counts in quartile 1 and having high-risk MRD levels were 18% and 96%, respectively.

Discussion

In our study on normal hematopoiesis during and after induction treatment for childhood ALL, we were able to demonstrate that platelet counts on treatment day 33 and neutrophil counts on day 8 were significantly associated with treatment outcome. We were able to replicate the effect of platelet count on treatment day 33 in an independent cohort of 475 patients and demonstrated its strong relationship with MRD measurements. Only very few other studies on normal hematopoiesis and treatment outcome of hematologic malignancies are available in the medical literature. The first study on this issue was published by Faderl *et al.*, who hypothesized that time to platelet recovery (defined by a count of >100×10^9/L) is an

Table 3. Blood cell counts during (treatment days 8 and 15) and after induction treatment (treatment day 33) of childhood acute lymphoblastic leukemia.

	Day 8[c]	Day 15[d]	Day 33[e]
Hemoglobin (g/L)			
Range	6.0-15.0	6.0-14.9	5.9-16.5
25th percentile	8.5	7.9	8.4
50th percentile	9.6	9.0	9.4
75th percentile	10.8	10.5	10.4
Platelet count (x10^9/L)			
Range	3.4-576.6	4.3-766.0	21.0-655.0
25th percentile	27.0	44.0	162.0
50th percentile	49.0	90.0	236.0
75th percentile	103.5	209.0	316.5
White blood cell count (x10^9/L)			
Range	0.5-229.6	0.1-15.7	0.3-25.2
25th percentile	1.5	1.4	1.6
50th percentile	2.5	2.0	2.7
75th percentile	4.4	3.4	4.4
Leukemic blast count (x10^9/L)			
Range	0-207.1	0-4.1	0-16.1
25th percentile	0	0	0
50th percentile	0.02	0	0
75th percentile	0.15	0	0
Absolute neutrophil count (x10^9/L)[a]			
Range	0-13.33	0-10.67	0-10.80
25th percentile	0.17	0.16	0.49
50th percentile	0.51	0.63	1.03
75th percentile	1.31	1.36	1.98
Left-shifted blood counts [n (%)][b]			
yes	73 (29.7)	13 (5.7)	87 (38.3)
no	173 (70.3)	216 (94.3)	140 (61.7)
Absolute monocyte count (x10^9/L)[a]			
Range	0-2.30	0-0.36	0-1.77
25th percentile	0	0	0.03
50th percentile	0.04	0	0.08
75th percentile	0.10	0.04	0.17
Absolute lymphocyte count (x10^9/L)[a]			
Range	0.02-11.79	0.02-10.52	0-7.80
25th percentile	1.03	0.70	0.71
50th percentile	1.52	1.16	1.26
75th percentile	2.60	1.93	2.20

[a]calculated from white blood cell counts and differential blood cell count percentages; [b]defined by the presence of at least 1% metamyelocytes, myelocytes or promyelocytes in a differential count of 100 cells; [c]hemoglobin, platelet count and white blood cell count based on 256 individuals, remaining values based on 246 individuals; [d]hemoglobin, platelet count and white blood cell count based on 251 individuals, remaining values based on 229 individuals; [e]hemoglobin, platelet count and white blood cell count based on 249 individuals, remaining values based on 227 individuals.

essential component of complete remission in acute leukemia.[23,24] They analyzed time to platelet recovery in 249 adults with ALL who entered remission after one course of induction chemotherapy and were able to show that time to platelet recovery was significantly and independently associated with both disease-free and overall survival if it occurred within a maximum of about 60 days after the start of therapy. These results are in accordance with our findings on the role of platelet counts on treatment day 33 and most likely reflect good treatment response, clearance of leukemic cells from the bone marrow and hematopoietic recovery.

A second study was published by Laughton *et al.*, who evaluated myelosuppression during induction and consolidation chemotherapy in 227 children uniformly treated for ALL on consecutive Australian and New Zealand Children's Cancer Study Group protocols.[25] They found that a slow rate of myeloid recovery at the end of induction chemotherapy, reflected by a low absolute neutrophil count, was highly predictive of relapse. Multivariate analysis confirmed the independent prognostic significance of MRD and absolute neutrophil count at the end of induction chemotherapy in this study. On the basis of the latter results, the authors concluded that the responses of normal myeloid and leukemic cells to chemotherapy predict outcome by distinct mechanisms. In contrast to our study, Laughton *et al.* did not investigate neutrophil counts during induction treatment. We cannot, therefore, compare our findings on a prognostic role of neutrophil counts on treatment day 8 to results from other studies on child-

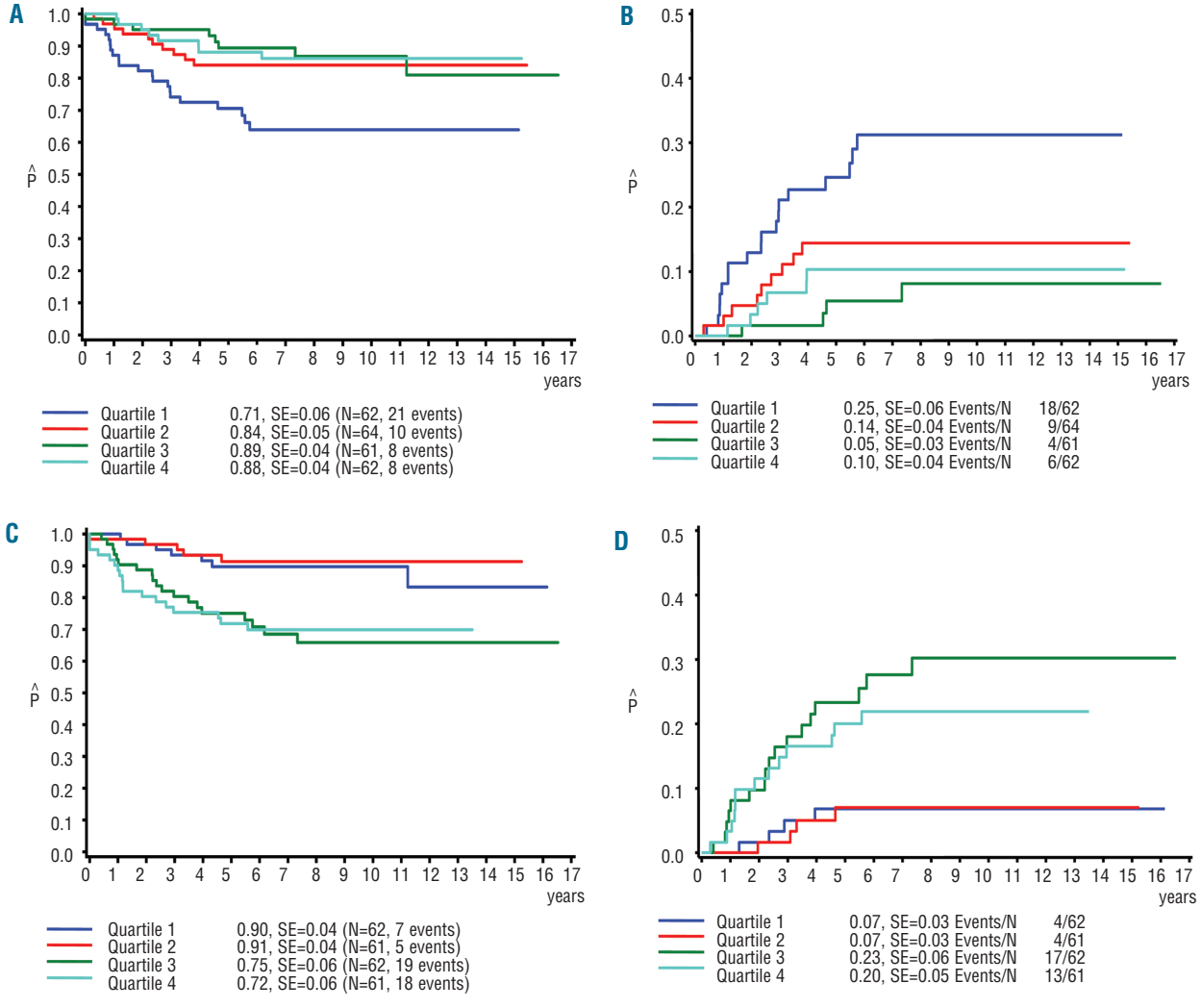

Figure 1. Kaplan-Meier estimates of 8-year event-free survival (EFS) and cumulative incidences of relapse (CI) of all evaluable patients with childhood ALL treated on trials ALL-BFM 90, 95 and 2000 at Hannover Medical School; SE, standard error; quartiles based on cut-points shown in Table 3. (A) EFS according to quartiles of platelet count at treatment day 33; Log-rank test: quartile 1 *versus* quartiles 2 to 4, *P*=0.0002, the probability of EFS at 8 years is given in the rows at the bottom of the figure directly after the respective quartile. (B) CI according to quartiles of platelet count at treatment day 33; Gray's test: quartile 1 *versus* quartiles 2 to 4, *P*=0.0002; the incidences at 8 years are given in the rows at the bottom of the figure directly after the respective quartile. (C) EFS according to quartiles of neutrophil count at treatment day 8; Log-rank test: quartiles 1 and 2 *versus* quartiles 3 and 4, *P*=0.0001; the probability of EFS at 8 years is given in the rows at the bottom of the figure directly after the respective quartile. (D) CI according to quartiles of neutrophil count at treatment day 8; Gray's test: quartiles 1 and 2 *versus* quartiles 3 and 4, *P*=0.0001; the incidences at 8 years are given in the rows at the bottom of the figure directly after the respective quartile.

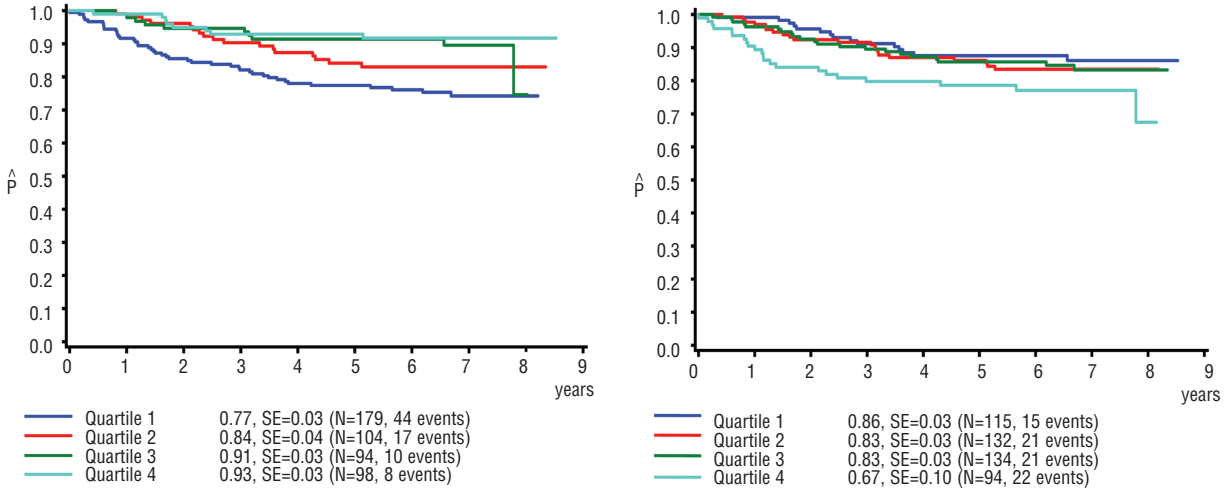

Figure 2. Kaplan-Meier estimates of 8-year event-free survival (EFS) of patients with childhood ALL from the replication cohort treated in trial ALL-BFM 2000; SE, standard error; quartiles based on the cut-points shown in Table 3. (A) EFS according to quartiles of platelet count at treatment day 33; Log-rank test: quartile 1 *versus* quartiles 2 to 4, *P*=0.0002, the probability of EFS at 8 years is given in the rows at the bottom of the figure directly after the respective quartile. (B) EFS according to quartiles of neutrophil count at treatment day 8; Log-rank test: quartiles 1 and 2 *versus* quartiles 3 and 4, *P*=0.18; the probability of EFS at 8 years is given in the rows at the bottom of the figure directly after the respective quartile.

hood ALL. When comparing our results on neutrophil counts at treatment day 33 to those described by Laughton *et al.* after induction treatment, we saw a similar effect in our study population, although the magnitude of the effect was far smaller in our study. This may be explained by differences in treatment. Although both study groups received similar drugs for induction treatment, the BFM protocols are more intensive than the Australian and New Zealand Children's Cancer Study Group protocols. This is not only reflected by higher glucocorticoid (60 mg/m^2/day prednisone or 10 mg/m^2/day dexamethasone *versus* 40 mg/m^2/day prednisolone) and anthracycline doses (4×30 mg/m^2 daunorubicin *versus* 4×25 mg/m^2), but also by lower median normal blood counts after induction (e.g., median platelet count after induction of 236×10^9/L *versus* 319×10^9/L). It might, therefore, be possible that more intensive treatment on BFM protocols modulates the prognostic role of neutrophil counts after induction treatment leading to a reduced effect on treatment outcome.

A third study on normal hematopoiesis was published by De Angulo *et al.*[26] They analyzed the prognostic significance of absolute lymphocyte, neutrophil, and platelet counts evaluated weekly during induction chemotherapy for acute myeloid leukemia and ALL. In their study, De Angulo *et al.* found that absolute lymphocyte counts of <0.35×10^9/L on induction day 15 were significantly and independently associated with poor treatment outcome in ALL. None of the other normal blood values evaluated in that study was associated with outcome. In our study, we only found that patients with lymphocyte counts on treatment day 8 in quartiles 2 and 3 did significantly better than those in quartile 1 or 4. Differences between the results of the study by De Angulo *et al.* and those of our study can most likely be explained by differences in treatment and differences in the populations of patients. An absolute lymphocyte count of 0.35×10^9/L on induction day 15 indicates very intensive treatment. For example, in our population of patients the upper boundary for the first quartile of lymphocyte counts on day 15 was 0.70×10^9/L. These differences may be explained by the fact that the population of ALL patients studied by De Angulo *et al.* was older (median age =11 years) and 25 out of 89 patients received intensive hyper-CVAD treatment (dexamethasone, vincristine, doxorubicin, cyclophosphamide, then methotrexate, cytarabine, x 8 cycles). Overall, the published studies in context with our results presented here indicate that the prognostic role of normal hematopoietic blood counts during and after induction varies with the intensity and/or type of treatment applied.

Among our patients, we observed the strongest effects regarding poor treatment outcome for low platelet count on treatment day 33 and high neutrophil count on day 8. A poor platelet recovery after induction – as already described above – may simply function as a surrogate marker for an overall poor treatment response to induction treatment with slow clearance of leukemic cells from the bone marrow and the associated compromised hematopoietic recovery. On the other hand, a high neutrophil count after 1 week of treatment may indicate differential responsiveness to glucocorticoid treatment relat-

Table 4. Platelet count on treatment day 33 quartiles[a] and minimal residual disease[b] (MRD) in 475 patients with childhood acute lymphoblastic leukemia from the replication cohort treated according to protocol ALL-BFM 2000.

	MRD standard-risk n (%)	MRD intermediate-risk n (%)	MRD high-risk n (%)	*P*[b]
Platelet count on day 33 in quartile 1	57 (27.7)	90 (39.8)	32 (74.4)	
Platelet count on day 33 in quartiles 2 to 4	149 (72.3)	136 (60.2)	11 (25.6)	<0.00001

[a]Based on the cut-points shown in Table 3; [b]Standard-risk patients were MRD-negative on treatment days 33 (TP1) and 78 (TP2), high-risk patients had residual disease (≥10^{-3}) at TP2, MRD intermediate-risk patients had positive MRD detected at either or both time points but at a level of <10^{-3} at TP2; [b]P χ^2.

ed to interindividual variation regarding host factors. An alternative explanation for a negative impact of a high neutrophil count on treatment day 8 may be that it reflects highly proliferative disease with no visible impact yet of impaired marrow capacity on peripheral blood counts during very early treatment.

The most striking and important finding of our study is probably the strong association of platelet counts after induction treatment with MRD risk group distribution. The strong prognostic impact of platelet counts on treatment day 33 especially with non-MRD-based treatment and the fact that 74% of MRD high-risk patients had platelet counts in quartile 1 after induction treatment make this variable a strong candidate prognostic factor for the improvement of therapeutic risk stratification in trials not using any MRD analyses. The potential benefit of such a strategy supports further studies on the impact of platelet counts after induction treatment of childhood ALL as a surrogate maker for MRD and could be of particular interest in countries with limited financial resources.

Authorship and Disclosures

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