The absence of peripheral blood blasts at diagnosis may predict CNS involvement or CNS relapse in pediatric acute lymphoblastic leukemia patients

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SUMMARY: Ünal Ş, Tuncer AM, Çetin M, Yetgin S. The absence of peripheral blood blasts at diagnosis may predict CNS involvement or CNS relapse in pediatric acute lymphoblastic leukemia patients. Turk J Pediatr 2008; 50: 537-541.

A high tumor burden at the time of diagnosis of childhood acute lymphoblastic leukemia has an unfavorable outcome. Peripheral white blood cell count is commonly used to reflect the leukemic burden and is used as one of the most important factors during determination of the risk-based treatment. However, peripheral blood blast count may not always reflect the tumor burden if leukocytes are not in blast nature. In the present study, we observed no central nervous system involvement at the time of diagnosis in patients with no peripheral blood blasts at the beginning, and furthermore, none of the patients with no peripheral blasts at the diagnosis had central nervous system relapse.

Key words: blasts, leukemia, central nervous system, pediatric, relapse, peripheral blood.

The outcome for children with acute lymphoblastic leukemia (ALL) has improved dramatically with current therapeutic strategies resulting in an event-free survival (EFS) exceeding 75% for most patients. However, significant challenges remain, including developing better methods to predict which patients can be cured with less toxic treatment and which will benefit from augmented therapy. In addition, since 25% of patients fail therapy, novel treatments that are focused on undermining specifically the leukemic process are urgently needed[1]. The two most important factors predictive of outcome are age and white blood cell (WBC) count at diagnosis[2,3]. A high tumor load at the time of diagnosis of childhood ALL remains a universally unfavorable prognostic factor of response to front-line treatment. Peripheral WBC counts[4-6] and the degree of hepatosplenomegaly[7] are commonly used parameters of initial leukemia cell burden[8]. In this study, we aimed to investigate whether the peripheral blood (PB) blast percentage at the time of diagnosis has a prognostic impact on the central nervous system (CNS) involvement at presentation and on relapse and survival rates in the follow-up.

Material and Methods

A total of 197 children and adolescents (up to 16 years of age at the time of diagnosis between March 1991 and July 2003) with newly diagnosed ALL at the Division of Pediatric Hematology, Hacettepe University Faculty of Medicine, were analyzed retrospectively. The diagnosis of ALL was based on morphological, cytochemical, immunophenotypic (by flow cytometric analysis; FACScan, Becton Dickinson, San Jose, CA, USA), cytogenetic and molecular genetic analysis of bone marrow aspirates. CNS involvement was determined by cytologic evaluation of the cerebrospinal fluid (CSF), whereas skeletal and mediastinal involvement was detected by plain radiographic evaluations. The patients were analyzed in three groups according to the PB blast percentage at the time of diagnosis: PB blast 0% (Group 1, n=24); PB blast between 1-50% (Group 2, n=63) and PB blast more than 50% (Group 3, n=110). Patients received St. Jude Total XI study protocol[9] between March 1991 and March 1997. Between March 1997 and July 2003, they were given modified St. Jude Total XIII study protocol[10]. Four patients underwent bone marrow transplantation, one each from Groups 1 and 2 and two patients from Group 3.

Overall survival (OS) was estimated from the date of diagnosis until the date of death. EFS was defined as time in first complete remission, until death or relapse. OS and EFS were estimated by Kaplan-Meier analysis, using the log rank test for comparisons. Differences in the distribution of variables among patient groups were analyzed using chi-square, ANOVA and Kruskal-Wallis tests. A *p*-value <0.05 was regarded as statistically significant.

Results

The characteristics of the 197 patients included in the study are shown in Table I. Of 197 patients, 67% were male and the median age at diagnosis was 60 months. The gender distribution was similar in the study groups, but Group 1 patients were older (median 96 months) at diagnosis than the other two groups (median 48 and 60 months, respectively), and the difference was statistically significant (p<0.05).

Hepatomegaly was present at the time of diagnosis in 45.8% of Group 1 patients (versus 68.3% in Group 2 and 80.0% in Group 3), and splenomegaly was found in 29.2% of Group 1 (versus 46.0% in Group 2 and 67.3% in Group 3). Hepatomegaly and splenomegaly were more commonly observed in Group 3 patients, and the difference was statistically significant (p<0.05). There was no difference in terms of skeletal and mediastinal involvement between groups. Percentages of patients who had WBC below 10000/µl at diagnosis in the three groups were 83.3%, 73.0% and 25.4%, respectively. On the other hand, no patient in Group 1 had WBC more than 50000/µl at presentation and only 3.2% of Group 2 patients were found to have more than 50000/µl WBC. However, 34.6% of Group 3 patients had more than 50000/µl WBC and this difference was statistically significant as well. The patient groups were similar in terms of French-American-British (FAB) morphologic subtype and immunophenotype by

Table I. Clinical and Laboratory Characteristics of Patients at Presentation

	Group 1 (n=24)	Group 2 (n=63)	Group 3 (n=110)	Total (n=197)
Median (range) age (month)	96 (24-168)	48 (9-192)	60 (4-180)	60 (4-192)
Male	15 (62.5%)	45 (71.4%)	72 (65.5%)	132 (67.0%)
Female	9 (37.5%)	18 (28.6%)	38 (34.5%)	65 (33.0%)
Hepatomegaly	11 (45.8%)	43 (68.3%)	88 (80.0%)	142 (72.1%)
Splenomegaly	7 (29.2%)	29 (46.0%)	74 (67.3%)	110 (55.8%)
CNS involvement	0 (0%)	3 (4.8%)	9 (8.2%)	12 (6.1%)
Skeletal involvement	7 (29.2%)	12 (19.0%)	19 (17.3%)	38 (19.3%)
Mediastinal involvement	1 (4.2%)	1 (1.6%)	12 (10.9%)	14 (7.1%)
WBC count (/µl)				
<10000	20 (83.3%)	46 (73.0%)	28 (25.4%)	94 (47.7 %)
10000-50000	4 (16.7%)	15 (23.8%)	44 (40.0%)	63 (32.0%)
50000-100000	0 (0%)	0 (%)	18 (16.4%)	18 (9.1%)
>100000	0 (0%)	2 (3.2%)	20 (18.2%)	22 (11.2%)
Hemoglobin (g/dl) (mean±SE)	9.1±2.3	8.0±2.2	7.3±2.5	7.7±2.4
Median platelet (/µl)	95.000	50.000	50.000	50.000
Median LDH (IU/L)	796	498	841	767
FAB subtype				
L1	11 (52.4%)	35 (63.6%)	63 (65.6%)	109 (63.4%)
L2	8 (38.1%)	15 (27.3%)	30 (31.3%)	53 (30.8%)
L3	2 (9.5%)	5 (9.1%)	3 (3.1%)	10 (5.8%)
Immunophenotype				
B-cell	13 (68.4%)	37 (75.5%)	67 (67.0%)	117 (69.6%)
T-cell	4 (21.1%)	3 (6.1%)	18 (18.0%)	25 (14.9%)
Mixed or biphenotypic	2 (10.5%)	9 (18.4%)	15 (15.0%)	26 (15.5%)
Duration between onset of symptoms and diagnosis (day) (median)	46	31	23	30

CNS: Central nervous system. WBC: White blood cell. LDH: Lactate dehydrogenase. FAB: French-American-British.

flow cytometric analysis. The hemoglobin levels at presentation were similar, but the platelet counts were significantly higher in Group 1 (95,000 vs 50,000). Median duration of time between onset of symptoms and diagnosis was 46, 31 and 23 days for Groups 1, 2 and 3, respectively.

Initial CSF examination at diagnosis revealed CNS involvement in 4.8% and 8.2% of patients in Groups 2 and 3, respectively. Notably, none of the patients in Group 1 was detected to have CNS involvement at diagnosis.

The relapse and survival rates are shown in Table II.

The five-year EFS rates of Group 1 patients who received low-, intermediate- and high-risk treatment protocols were 100%, 50.0±35.3% and 58.3±18.5%, respectively. Similarly, five-year EFS rates of Group 2 patients who received the same risk-based treatment protocols were 92.8±6.8%, 100% and 84.8±6.2, respectively. The five-year EFS rates of Group 3 patients who received low-, intermediate- and high-risk treatment protocols were 79.4±10.7%, 100% and 72.4±5.7%, respectively. The different treatment protocols according to risk groups were found to have no statistically significant effect on EFS in the three PB blast groups ($p>0.05$).

Table II. Relapse and Survival Rates of Patients

	Group 1	Group 2	Group 3	Total
Relapse	6 (25.0%)	10 (15.9%)	37 (33.6%)	53 (26.9%)
Type of the first relapse				
Bone marrow	6 (100%)	7 (70.0%)	22 (59.4%)	35 (66.0%)
CNS	0 (0%)	3 (30.0%)	9 (24.3%)	12 (22.6%)
Bone marrow+CNS	0 (0%)	0 (0%)	5 (13.5%)	5 (9.4%)
Testes	0 (0%)	0 (0%)	1 (2.7%)	1 (1.9%)
Mortality rate	10 (41.7%)	4 (6.3%)	34 (30.9%)	48 (24.4%)
5-year EFS (mean±SD)	50.0±0.15%	86.0±4.6%	62.0±5.4%	69.0±3.8%
5-year OS (mean±SD)	60.0±11.4%	92.8±3.4%	73.6±4.4%	78.4±3.1%

CNS: Central nervous system. EFS: Event-free survival. OS: Overall survival.

In the follow-up, relapse occurred in 33.6% of the Group 3 patients, versus in 25.0% and 15.9% in Groups 1 and 2, respectively. The relapse rate was significantly higher in Group 3 patients. The most common relapse site was bone marrow in all three groups. All of the relapses in Group 1 were in the bone marrow and none of the patients developed CNS relapse in the follow-up. In Group 2 and Group 3 patients, 30% and 24.3% of the patients, respectively, developed central neurologic involvement during the follow-up period. A cut-off PB blast rate of 53% was calculated as risky for CNS relapse.

The mortality rates were 41.7%, 6.3% and 30.9%, respectively, and mortality was significantly lower in Group 2 ($p<0.05$). The five-year EFS was 50±0.15%, 86±4.6%, and 62±5.4%, respectively, in Groups 1, 2 and 3, and the difference between groups was significant ($p=0.008$). The five-year OS was 60.0±11.4%, 92.8±3.4%, and 73.6±4.4% in Groups 1, 2 and 3, and the difference between groups was significant ($p=0.0001$).

The five-year OS of Group 1 patients who received low-, intermediate- and high-risk treatment protocols were 83.0±0.1%, 85.7±13.2% and 40.4±15.5%, respectively. The five-year OS of Group 2 patients who received the same treatment protocols were 100%, 100% and 87.9±5.6%, respectively. Lastly, five-year OS of Group 3 patients analyzed according to treatment risk groups were 77.6±9.9%, 100% and 69.1±5.4%, respectively. There were no statistically significant differences in five-year OS between the three PB blast groups in terms of low-, intermediate- and high-risk treatment protocols.

The five-year EFS of Group 1 patients with WBC less than 10000/µl at diagnosis was 50.7±16.9%, whereas it was 50.0±35.3% in patients who had a WBC between 10000 and 50000/µl. No statistically significant difference was determined between the two WBC ranges in Group 1. In Group 2, five-year EFS of patients with WBC less than 10000/µl, 10000-50000/µl and 50000-100000/µl at diagnosis was 87.4±5.2%, 85.5±9.5% and 50.0±35.3%, respectively, and the difference was statistically

insignificant (p>0.05). Lastly, in Group 3, five-year EFS of patients with WBC less than 10000/µl, 10000-50000/µl, 50000-100000/µl and more than 100000/µl at diagnosis was 55.7±11.2%, 60.3±8.3%, 61.5±13.7% and 74.4±11.0%, respectively. The difference was statistically insignificant (p>0.05).

Discussion

In vivo response to the therapy is one of the most useful predictors of outcome in ALL cases. Berlin-Frankfurt-Munster (BFM) investigators have shown that patients whose peripheral blast count drops below 1000 blasts/µl have an EFS of 61% after one week of prednisone and a single intrathecal dose of methotrexate compared to an EFS of 38% for those with a higher level of blasts in the peripheral circulation[1,11]. Investigators from St. Jude Children's Research Hospital have shown that the presence of PB blasts after one week of conventional induction chemotherapy is also an adverse prognostic feature[1,12], and it has also been suggested that use of high-dose methylprednisolone has a more potent effect on blast reduction rate and EFS when compared to the conventional dose steroid treatment[13]. A number of studies have demonstrated the value of minimal residual disease (MRD) in the assessment of EFS. Patients with no detectable MRD at the end of induction have an exceedingly good outcome (EFS >90% at 3 years)[1,14-16]. The value of determination of blast count response to treatment by the aid of any of these methods is well-studied; however, there is restricted data indicating the prognostic importance of PB blast rate in the diagnosis.

Our results suggest that a higher PB blast count is associated with a higher WBC count and higher incidence of hepatosplenomegaly, indicating the higher initial leukemic cell burden. On the other hand, none of the patients who had no PB blasts at diagnosis revealed CNS involvement at diagnosis and the CNS involvement rate increased as the PB blast rate increased (Group 2: 4.8% and Group 3: 8.2%).

The relapse rates were significantly higher in Group 3, and almost one-third of all Group 3 patients relapsed during follow-up. In Group 1, the relapses were exclusively in the bone marrow and no CNS relapse was encountered. The total CNS relapse in Group 2 was 30%; however, when CNS only and concomitant CNS and bone marrow relapses were considered together, CNS relapse was seen in 37.8% of the Group 3 patients. It is also impressive that although the highest T-cell immunophenotype was detected in Group 1 (21.1% vs 6.1% and 18%), none of these T-cell-positive Group 1 patients was found to have CNS involvement at diagnosis and also did not develop subsequent CNS relapse.

Although the relapse rates were higher in Group 3, the five-year EFS and OS were better in Group 2. When the EFS of patients was analyzed according to WBC at diagnosis, we found no difference in survivals between groups according to the different WBC ranges. The five-year EFS and OS were similar in different PB blast groups when analyzed according to treatment protocol. In this study, although we found that the PB blast rate at diagnosis was important for CNS involvement and CNS relapse, the survival rates did not change according to PB blast rate. This may indicate additional factors on survival in the follow-up of the patient, including infections. There may be further factors that impact the survivals, including undetermined cytogenetic abnormalities and socioeconomic differences among patients. Further, the patients in Group 1 were significantly older than the other two groups and the patient number in groups is limited, and these factors may have contributed to better survival results in Group 2. Cytogenetic examination results were not available in the entire study group; this may have caused some of the high-risk patients to be treated with low-risk treatment protocols, and the unexpectedly better survival in Group 2 patients may be a result of this limitation.

Leukemic involvement of the CNS results from proliferation of cells in the walls of superficial arachnoid veins, which seed at the time of diagnosis when there is a large amount of leukemic cells. These cells, which remain inaccessible to systemic chemotherapy, proliferate and destroy the arachnoid trabeculae by penetrating into the channels of CSF circulation[17]. We observed no CNS involvement at the time of diagnosis in patients with no PB blasts at the beginning, and furthermore, none of the patients with no PB blasts at the

diagnosis had CNS relapse. In this group, symptom duration before the diagnosis of leukemia was longer than in the other groups, and this may be related to the less-invasive and less-proliferative potency of the blast clone. In the patients who had blasts in the PB at the time of diagnosis, the CSF may have been contaminated by PB blasts during the initial lumbar puncture and this may have increased the relapse in CNS.

Little is known about the exact factors that control the mobilization of bone marrow blasts into the PB, although adhesion molecules, chemokines, and angiogenic factors are believed to play a role[18-20]. WBC count apparently reflects tumor load only when PB blast count is high, and the number of non-malignant leukocytes may be altered by infections[8].

As a result, WBC alone may not be helpful in predicting the outcome. Further prospective clinical trials are needed to make a more definitive suggestion regarding the prognostic impact of PB blast counts. Furthermore, further trials for the diminution of prophylactic CNS therapy in patients without PB blasts at the time of diagnosis may be conducted in order to decrease the neurotoxicity.

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