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The relationship of bone marrow fibrosis at diagnosis with prognosis and survival in childhood acute lymphoblastic leukemia

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ABSTRACT

Objectives: Our aim in this study is to investigate the effect of fibrosis at diagnosis on treatment and survival in childhood acute lymphoblastic leukemia (ALL).

Methods: This study is retrospective. We evaluated the relationship between patients' age, white blood cell count at diagnosis, morphological blast percentage and flow cytometric blast percentage at diagnosis, day 15th and day 33th, absolute blast count in peripheral smear on day 8th, and the degree of fibrosis in bone marrow biopsy at diagnosis in 36 pediatric patients. The fibrosis degree in biopsy on the thirty-third day after induction therapy was measured.

Results: Twenty-eight (77.8%) cases were diagnosed B-ALL and 8 T-ALL (22.2%). There was no statistically significant difference between the groups with and without fibrosis in terms of any parameter measured at the time of diagnosis, 8th day, 15th day, and 33th day. No significant difference was found between the groups according to overall survival (OS): the mean OS was 50.22 ± 5.44 months in the fibrosis group and 49.70 ± 3.96 months in the non-fibrosis group (p = 0.557).

Conclusions: There is a high detection rate of bone marrow fibrosis in ALL pediatric cases at the time of diagnosis. Nevertheless, fibrosis does not affect survival.

Keywords: Leukemia, fibrosis, bone marrow, childhood, lymphoblastic, microenvironment

Acute lymphoblastic leukemia (ALL) is childhood cancer's most common type. Although cure rates for ALL are 80-90% with advanced chemotherapy protocols and supportive treatment, 10-20% of patients will die due to disease recurrence or treatment-related complications [1]. Patients at high risk of developing resistance to chemotherapy and relapse; are defined as patients under one-year-old, patients with BCR/ABL translocation, those with KMT2A/AFF re-arrangement, those with hypodiploidy, those with t (17;19) (q23; p13) (TCF3/HLF), IKZF1 plus mutations, those with high minimal residual disease (MRD) during induction and re-induction, and those with T immunophenotype [2].

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The cancer microenvironment is now recognized as a significant factor in cancer progression and response to treatment as it is in solid organ cancers. Consequently, the cancer microenvironment should be considered among cancer treatment targets [3-5]. Similarly, leukemia cells and the microenvironment (or niche) play a substantial role in resistance to chemotherapy and treatment failure [6]. Bone marrow microenvironment (BMM) consists of a highly complex cell population, such as mesenchymal stem cells [7], endothelial cells [8], osteoblasts [9], osteoclasts [10], adipocytes [11] and stromal cells [7]. It is important to note that the vascular and endosteal niches formed by cells in the BMM are anatomically and functionally distinct. The endosteal niche makes leukemic stem cells quiescent and chemo-resistant; in contrast, the vascular niche makes them more active and mature [6]. There is no clear understanding of the pathogenesis of bone marrow fibrosis; however, pathway-activating mutations in the JAK-STAT pathway affect the expression of cytokines produced by leukemia cells and those created by cells in the microenvironment, which leads to the development of fibrosis [12]. There is limited evidence that bone marrow fibrosis in childhood ALL is high at the time of diagnosis [13]. It is believed that fibrosis disappears during treatment in ALL pediatric cases with marrow fibrosis at diagnosis [14]. However, there still needs to be more clarity regarding the relationship between bone marrow fibrosis at the diagnosis and prognosis and treatment response in pediatric acute leukemia.

This study was aimed to make a comparison between patients treated according to the ALLIC BFM 2009 Chemotherapy protocol, which had bone marrow fibrosis detected or not detected at the time of diagnosis based on ALL risk groups and the effect of ALL treatment on bone marrow responses on days 15 and 33 and its impact on mortality.

METHODS

Thirty-six patients diagnosed with ALL were included in this study. Flow cytometric (FC) analysis, immunohistochemical studies on bios, and peripheral smears were used to diagnose ALL. All cases were divided into two groups, B-ALL and T-ALL.

The bone marrow biopsies were processed after 10% formaldehyde fixation, followed by routine tissue examination after decalcification, and embedded in paraffin. Bone marrow biopsy fibrosis was evaluated using reticulin and Masson's trichrome histochemical staining. A 4-micron thick section of a polylyzed slide was taken and stained with the ready kit by the instructions provided in the paint's instruction manual. Reticular fiber grading in the bone marrow was based on the European Consensus Report of Bone Marrow Fibrosis Grading. Accordingly, the degree of bone marrow fibrosis was evaluated between 0 and 3 (Table 1). [15]: 0 and 1 were considered as having no significant fibrosis, and 2 and 3 were supposed to have significant fibrosis. ALL-Inter Continental Berlin-Frankfurt-Münster (ALL-IC BFM) 2009 protocol was followed in all cases (Table 2) [16].

The study investigated the age of the patients, the number of white blood cells at diagnosis, morphological blast percentage at diagnosis, flow cytometric blast percentage at diagnosis, absolute blast number in peripheral blood smear on the eighth day, bone marrow morphological blast percentage on day 15 (M1; < 5%, M2; ≥ 5 – < 25, M3 ≥ 25) [16], flow cytometric

Table 1. European Consensus Report of Bone Marrow Fibrosis grading [15]

Grade	Description
Grade 0	Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow
Grade 1	Loose network of reticulin with many intersections, especially in perivascular areas
Grade 2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
Grade 3	Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis

Table 2. Parameters used to determine the risk group of patients [16]

Standard Risk Group (SR)*	Intermediate Risk Group (IR)	High Risk Group (HR)**
PB day 8: < 1,000 blasts/µL	All patients who are not stratified to SR or HR are intermediate risk patients.	IR and, if available FC MRD >10% or M3 marrow on day 15
Age ≥ 1 yr – < 6 yr		SR if available FC MRD >10%
Initial WBC < 20,000/µL		Periferik Blood on day 8: ≥ 1,000 blasts/µL
FC MRD < 0,1% or M1/ M2 marrow on day 15		M2 or M3 marrow on day 33
M 1 marrow on day 33		Translocation t(9;22) [BCR/ABL] or t(4;11) [MLL/AF4]
*All criteria must be fulfilled		Hipodiploidy ≤ 44
		**At least one criterion must be fulfilled.

PB = Peripheral Blast, SR = Standard Risk Group, IR = Intermediate Risk Group, HR = High Risk Group, FC = Flow Cytometri, MRD = Minimal Residual Disease

blast percentage on day 15, bone marrow morphological blast percentage on day 33, flow cytometric blast percentage and evaluated their relation with the degree of fibrosis in the bone marrow at diagnosis. Additionally, the extent of bone marrow fibrosis on day 33 and the effect of fibrosis on OS were evaluated.

Statistical Analysis

As descriptive statistics, numbers and percentages were used for qualitative data, and arithmetic means and standard deviation were used for quantitative data. The Mann-Whitney U test was used to compare the two groups since the Shapiro-Wilk test indicated that the continuous data were not normally distributed. The Spearman correlation coefficient was used to evaluate the correlation of quantitative data. Overall survival (OS) was estimated by the Kaplan-Meier method. Observational survival was calculated from the date of the first diagnosis to the date of any death from any cause and censored at the final follow-up date for event-free and viable patients. The log-rank test was used to compare different groups. The statistical significance level was taken as $p < 0.05$, SPSS 18.0 package program was used to evaluate the data.

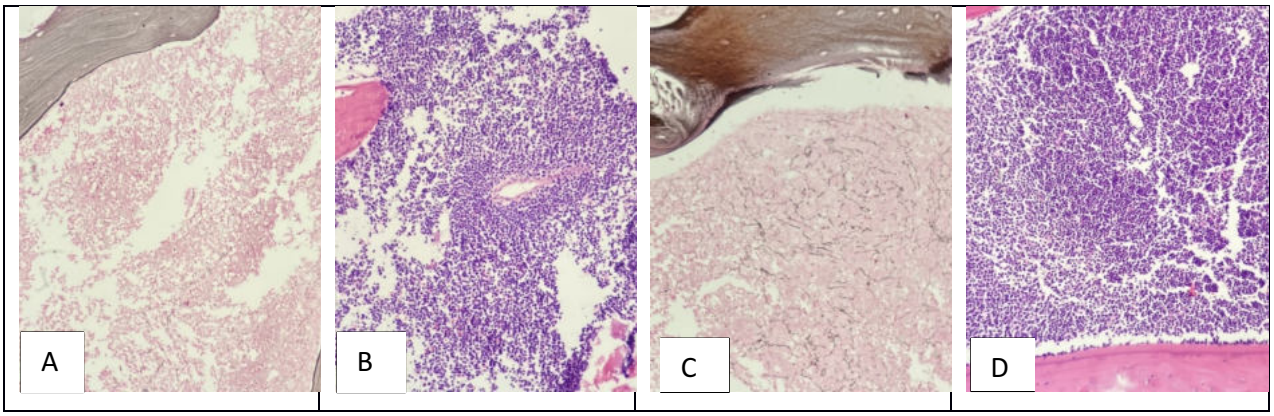

Fig. 1. Very thin short reticun fiber in the intertrabecular area, more prominent around the vessel, Grade 0 (A, ×200), infiltration of leukemia cells in the same biopsy (B, ×200 HE). Loose usually unconnected thin reticulin fiber in the intertrabecular space, Grade 1 (C, ×200), infiltration of leukemia cells in the same biopsy (D, ×200 HE).

RESULTS

Thirty-six pediatric ALL patients were included in the study. The median age at diagnosis was 4.5 years (2-18 years). Sixteen were girls (44.4%), and 20 were boys (55.6%). Twenty-eight patients were B-ALL (77.8%), and 8 were T-ALL (22.2%). According to the bone marrow biopsy examination fibrosis evaluation in diagnosis, 4 of the patients were grade 0 (11.1%), 13 patients were grade 1 (36.1%), 14 patients were grade 2 (38.9%), and five patients were grade 3 (13.9%) fibrosis. Totally 55.9% (n = 19) of the patients had fibrosis: 57.1% (n = 16) of the B-ALL patient group, and 37.5% (n = 3) of the T-ALL patient group had fibrosis (Figs. 1 and 2).

Fourteen of the patients were in the high-risk group (HR) (38.9%), 20 of them in the intermediate risk group (IR) (56.5%), and 2 of them in the standard risk (SR) (5.6%) group. Five of the patients in the HRG group were classified as T ALL (three of them were steroid unresponsive on the eighth day, and the bone marrow morphology was M3 on the 15th day, one was steroid unresponsive on the eighth day, one was steroid unresponsive on the eighth day, and M2 bone marrow was detected on the 33rd day.), nine of them were B ALL (three were evaluated as HR due to positive detection of t (9:22), M3 bone marrow detection on day 15 in four, and detection of M2 bone marrow on day 33 in two of them). According to the morphological classification made from the bone marrow aspiration smear on the 15th day, 69.4% (n = 25) were M1, 11.1% (n = 4) M2, and 19.5 (n = 7) M3. According to the morphological classification performed on the 33rd day, 94.3% (n = 33) were M1, and 5.7% (n = 2) were M2. Since a patient with reticulin fibrosis three at the diagnosis died on the 25th day, the bone marrow could not be evaluated on the 33th day. Diagnostic white blood cell count of the patients, morphological blast percentage in the bone marrow aspiration smear at diagnosis, and flow cytometric at diagnosis. Blast percentage, absolute blast number on day eight, morphological blast percentage on day 15, flow cytometric on day 15 blast percentage, morphological blast percentage on day 33, and flow cytometric on day 33 blast percentage averages are given in Table 3.

Blasts at the diagnosis (p = 0.961), the diagnosis flow cytometric between the group with and without fibrosis in the bone marrow biopsy examination at the time of diagnosis. Blast percentage (p = 0.501), absolute blast number on the eighth day (p = 0.086), morphological blast percentage on the 15th day (p = 0.856), flow cytometric on the 15th day blast percentage (p = 0.296), morphological blast percentage on day 33 (p = 0.754), flow cytometric on day 33 No statistically significant difference was found between the rate of blasts (p = 0.192) (Table 4). There was no statistically significant correlation between these values and the reticulin value (p > 0.05).

According to the leukemia type of the patients (with B-ALL fibrosis (n = 16, 57.1%), (n = 3, 37.5%) with T-ALL fibrosis (p = 0.326), according to the risk group of the patient (SR and There was no statistically

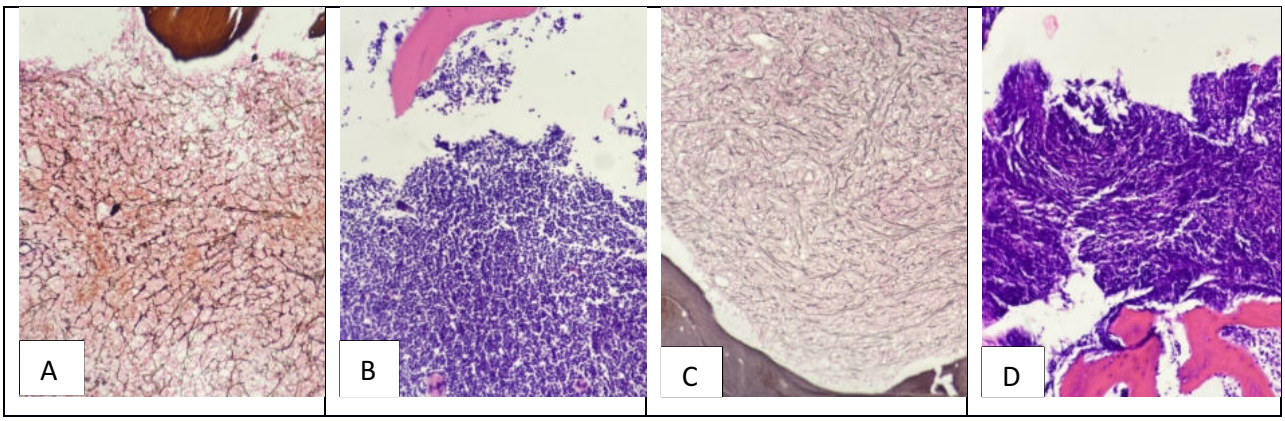

Fig. 2. Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen, Grade 2 (A, ×200), crush artifact is observed in leukemia cells compared to biopsy with lower grade reticulin (B, ×200 HE). Diffuse and very dense, thick collagen fibers, Grade 3 (C, ×200), significant crush artifact is observed in leukemia cells (D, ×200 HE).

Table 3. Demographic and laboratory characteristics of the patients

Patient data	Mean ± SD	Median	Min-Max
Diagnosis age (years)	6.78 ± 5.13	4.50	2.00-18.00
Number of white blood cell at diagnosis ($\times 10^3$)	45.7 ± 98.00	15.96	1.00-548.00
Blast rate in diagnosis (Flow cytometry) (%)	79.19 ± 19.18	85.00	29.00-99.00
Blast rate in diagnosis (Bone marrow dissemination) (%)	92.89 ± 6.15	95.00	70.00-99.00
Absolute blasts on day 8 (Peripheral spread)	23.82 ± 20.67	20.00	0.00-72.00
15. day blast rate (Bone marrow spread) (%)	11.92 ± 19.35	2.00	0.00-70.00
15. day blast rate (Flow cytometry) (%)	10.63 ± 18.11	2.54	0.00-68.00
33. day blast rate (Bone marrow spread) (%)	1.74 ± 1.62	1.00	0.00-8.00
Day 33 blast rate (Flow cytometry) (%)	4.54 ± 9.21	0.89	0.00-33.30

SD = Standard deviation, Min = Minimum, Max = Maximum

significant difference in terms of the degree of fibrosis among patients with IR fibrosis (n = 11, 50.0%), HR fibrosis (n = 8, 57.1%)) ($p = 0.676$). According to bone marrow examination, 70.6% (n = 12) of those without fibrosis were M1, 5.9 (n = 1) were M2, 2, 3.9% (n = 4) were M3, and 68.4% of those with fibrosis. It was observed that morphological blasts of the patients on the 33rd day were M1, 15.8% (n = 3) M2, and 15.8% M3, and there was no statistically significant difference between their distributions ($p = 0.764$). According to the percentages of bone marrow thinning, 100% (n = 17) of those without fibrosis were M1, 88.9% (n = 16) of those with fibrosis were M1, and 11.1% (n = 2) were M2. No significant difference was found ($p = 0.486$).

The median follow-up period was 20.15 months (min: 1.5, max: 60.67). The 18-month OS was 84.1% ± 6.7% (Fig. 3, on the left). In comparing OS between the groups with and without fibrosis, the mean OS was 50.22 ± 5.44 months in the fibrosis group and 49.70 ± 3.96 months in the non-fibrosis group. There was no statistically significant difference between them ($p = 0.557$) (Fig. 3, on the right).

While 31 patients were alive and in complete remission, five died. Two patients who died during treatment were T-ALL, and three were B-ALL. It was observed that one of the T-ALL patients was in the HR risk group and did not have fibrosis (grade 1), and the second in the IR risk group had fibrosis (grade 3). It was observed that one of the three patients with B-ALL had IR and fibrosis (grade 3), the other had IR and fibrosis (grade 1), and another had HR and fibrosis (grade 0). One of the two patients with fibrosis died due to intracranial hemorrhage after falling during induction therapy (during the first 33 days of treatment), and one died due to sepsis during the re-induction period.

In the evaluation of bone marrow fibrosis in the bone marrow biopsy on the 33rd day, one patient could not be evaluated because he died before the 33rd day. Therefore, fibrosis could be assessed on the 33rd day of 18 patients with fibrosis at the time of diagnosis, and fibrosis did not improve in only one of these patients. This patient had grade 3 fibrosis at the time of diagnosis and grade 2 fibrosis on the 33rd day of treatment.

DISCUSSION

The bone marrow is filled with leukemia cells, so much in children with acute lymphoblastic leukemia that they weaken the bone speculum [17]. As cellularity increases, reticulin fibrosis increases and serves as a support and connection between leukemia cells and bone marrow [18]. A greater degree of fibrosis prevents chemotherapy from reaching the blasts and reduces the effectiveness of chemotherapy [19]. Moreover, reticulin fibrosis has decreased adult survival [20].

Studies have reported the rate of fibrosis in ALL in childhood between 38.1% and 57% [21, 22]. Fibrosis was found in 52.8% of our patients at diagnosis, which is consistent with the literature. The incidence of fi-

Table 4. Relationship between fibrosis and other parameters

	Mean ± SD	Median	Min-Max	*p value*
Age				0.749
Fibrosis	6.8 ± 4.9	5.5	2.0-18.0	
No fibrosis	6.8 ± 5.5	4.0	2.0-17.0	
Number of white globes at diagnosis ($\times 10^3$)				0.296
Fibrosis	56.4 ± 122.3	21.7	3.0-548.0	
No fibrosis	33.8 ± 122.3	12.0	1.0-260.0	
Blast rate in diagnosis (Flow cytometry) (%)				0.501
Fibrosis	76.8 ± 20.8	83.5	29.0-95.0	
No fibrosis	81.7 ± 17.7	90.0	32.0-99.0	
Blast rate at diagnosis (Bone marrow smear) %				0.961
Fibrosis	93.3 ± 4.9	95.0	80.0-99.0	
No fibrosis	92.4 ± 7.4	95.0	70.0-98.0	
8. day white globe number ($\times 10^3$)				0.398
There is fibrosis	3.8 ± 6.8	1.6	0.5-27.5	
No fibrosis	3.9 ± 5.2	1.9	1.0-20.0	
Absolute blasts on day 8 (PS)				0.086
Fibrosis	321.8 ± 278.6	300.0	1.0-1120.0	
No fibrosis	609.2 ± 22.1	600.0	35.0-1400.0	
15. day blast (Bone marrow smear) (%)				0.856
Fibrosis	10.0 ± 16.2	2.0	0.0-60.0	
No fibrosis	14.1 ± 22.7	1.0	0.0-70.0	
15. day blast (Flow cytometry (%)				0.296
Fibrosis	7.3 ± 11.0	1.4	0.0-38.0	
No fibrosis	14.3 ± 23.5	5.0	0.0-68.0	
33. day blast (Bone marrow smear) (%)				0.754
Fibrosis	1.8 ± 2.0	1.0	0.0-8.0	
No fibrosis	1.6 ± 1.1	1.0	1.0-4.0	
33. day blast (Flow cytometry) (%)				0.192
Fibrosis	0.027 ± 0.043	0.5	0.0-12.3	
No fibrosis	0.061 ± 0.119	1.0	0.0-33.3	

SD = Standard deviation, Min = Minimum, Max = Maximum, PS= Peripheral Smear

brosis is higher in B-ALL than in T-ALL [23, 24], and B cell markers have been suggested as being the cause of this phenomenon [22]. It has also been reported that cytokines released by megakaryocytes and platelets increase fibrosis in acute megakaryoblastic leukemia. According to the same study, reticulin synthesis is thought to be stimulated by cytokines secreted by CD34 and HLA-DR expressing leukemic cells in diseases characterized by abnormal megakaryocytes [25]. According to our study, 57.1% of B-ALL patients and 37.5% of T-ALL patients had fibrosis; our findings are like those of previous studies.

There were 38.9% of HR cases in our study, similar to 33.3% of 81 cases in a previous study [24]. Fi-

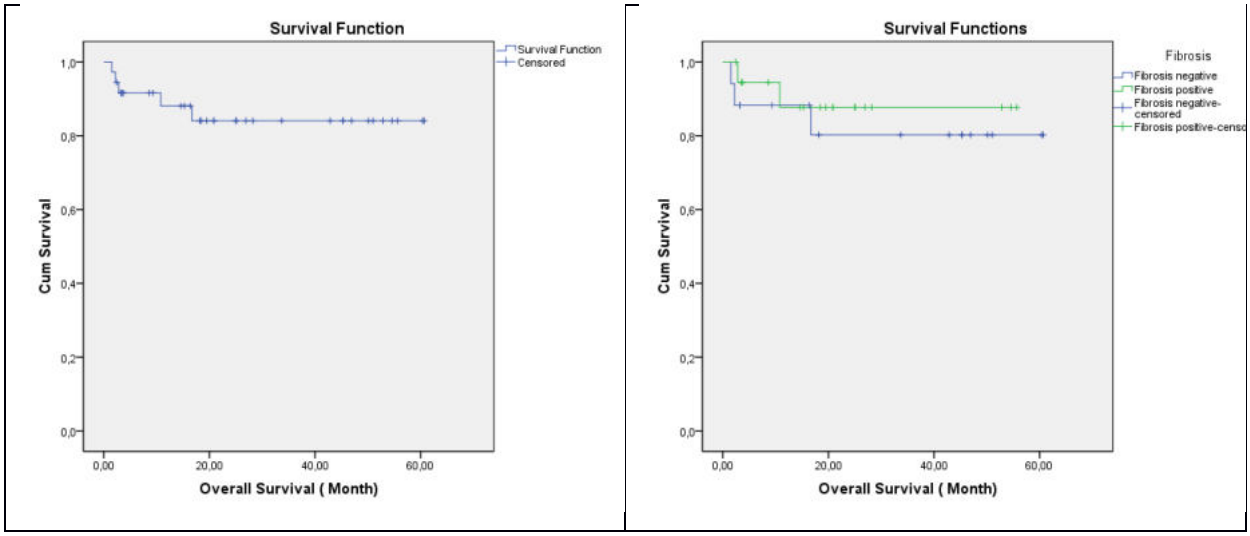

Fig. 3. Overall survival of the study population (on the left) and Overall survival of the study population according to the fibrosis (on the right).

brosis was detected in 57.1% of HR patients; in 50.0% of SR and IR patients; these findings are consistent with those from prior studies [23, 24]. We found no statistically significant difference between HR and other risk groups in bone marrow fibrosis ($p = 0.676$); however, some reports state a significantly higher level of fibrosis in the HR group [13]. This is because leukemia patients have easier access to haemato-oncologists, and treatment is initiated early in the disease process before fibrosis develops.

In our study, the median white blood cell count at diagnosis was 21.7 (×103) in the group with fibrosis, whereas it was 12.0 (×103) in the group without fibrosis. There was no significant difference ($p = 0.296$) or correlation ($p > 0.05$) between these two groups. In a previous study by Hann *et al*. [23], a negative correlation was found between the number of white blood cells at the time of diagnosis and high reticulin fiber density. Similarly, a negative correlation was found between the number of white blood cells at diagnosis and reticulin fiber density in 146 precursor B-ALL patients (r= -0.22, $p = 0.008$) [13]. We believe that our study's results depend on early access to haemato-oncologists by patients with leukemia.

An earlier study found that bone marrow fibrosis and blasts in the peripheral blood were negatively correlated (Spearman's correlation r= -0.278, $p = 0.018$) [23]. In a second study, a negative correlation was found between the number of blasts that filled the blood vessels in the bone marrow and the density of bone marrow fibrosis [18]. Nevertheless, we found that the median value of blasts in flow cytometry at diagnosis was lower in the group with fibrosis than in the group without fibrosis, 83.5% and 90.0%, respectively; neither the difference nor the correlation was significant between the two groups ($p = 0.501$, $p > 0.05$). There may be a connection between the low number of blasts detected in flow cytometry and the fact that fibrosis traps leukemia cells.

The literature reports a correlation between marrow fibrosis rate and the number of blasts, suggesting that fibrosis traps leukemia cells leading to the increase in blasts [13]. According to our study, the median blast value was 95.0% in the presence or absence of fibrosis, which was neither statistically significant ($p = 0.961$) nor correlated ($p > 0.05$).

The literature reports that high diagnostic reticulin fiber density and minimal residual disease (MRD) level on the 29th day after induction therapy are correlated and prognostic for BCP-ALL patients. Accordingly, patients with MRD values greater than 10-4 were found to have a higher degree of reticulin fibrosis (RFD) at diagnosis [13]. We did not assess MRD in our study; however, in the bone marrow examination, 100% (n = 17) of patients without fibrosis were M1, 88.9% (n = 16) of patients with fibrosis were M1, and 11.1% (n = 2) were M2. They did not differ statistically significantly ($p = 0.486$).

A total of 31 patients in our study were in remission and healthy, while five died. Fibrosis was present in only two of the Ex-patients. Among the patients with and without fibrosis, the mean OS of the patients with fibrosis was 50.22 ± 5.44 months, and the mean OS of the patients without fibrosis was 49.70 ± 3.96 months; the difference was insignificant (p = 0.557). The survival analysis of our study was similar to that of the previous study of 84 pediatric ALL patients: no difference was found between the two groups (p = 0.108) [13].

In opposite to our study, in a study of 44 adults, ALL patients found that patients with profound reductions of bone marrow fibrosis after induction therapy had longer survivals (39 months vs. 12 months) compared to patients without fibrosis or those without profound reduction at diagnosis. Reduction of fibrosis appears to be associated with better relapse-free survival [20].

The bone marrow fibrosis almost entirely returned to normal after induction treatment in other studies reported in the literature; in one study, bone marrow fibrosis was significantly reduced on the 15th day compared to when it was diagnosed. A study found that only 2.6% of patients had bone marrow fibrosis after induction therapy. Similarly, the bone marrow fibrosis of the patients in our study returned to normal except for one patient (5.5%) [14, 26].

Limitations and Strengths

Biopsy is generally not performed in the world in the diagnosis and follow-up of childhood leukemia. However, in leukemia, the bone marrow microenvironment is constantly questioned. Our study contains rare data in the world that clarifies this issue. Minimal residual disease was not detected when evaluating the response to treatment, which is the limitation of our study.

CONCLUSION

Bone marrow fibrosis can be detected during diagnosis in ALL pediatric cases. It is unclear whether fibrosis interferes with treatment or is prognostic since very few studies have clarified these issues, and their results contradict each other. Few studies have reported that bone marrow fibrosis is resolved in almost all pediatric ALL patients after induction therapy. As far as we know, this is our country's first study of its kind. In our research, the fibrosis in almost all patients returned to normal after initiating induction therapy, which suggests that the presence of fibrosis at the time of diagnosis does not contribute to predicting prognosis or survival. Based on the almost complete normalization of bone marrow fibrosis with treatment and the lack of association with prognosis, it has been hypothesized that childhood acute lymphoblastic leukemias have a different pathophysiology than childhood myeloid leukemias. The disappearance of leukemia cells with treatment and the almost normalization of fibrosis in pediatric ALL can be attributed to some cytokines expressed from leukemia cells as the cause of fibrosis. Conducting studies on a larger patient population would be necessary to uncover these factors.

Ethics Committee Approval

The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (July 2, 2021/429).

Authors' Contribution

Study Conception: ÇÖ; Study Design: ÇÖ; Supervision: YDK, İE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: NE, HSŞ; Statistical Analysis and/or Data Interpretation: YŞ; Literature Review: NE, HSŞ; Manuscript Preparation: ÇÖ, İE and Critical Review: YDK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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