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VIA MEDICA

Relationship between reticular fibrosis with platelet surface markers (CD41A, CD42A, CD42B, CD61) and prognostic markers (WBC, PLT) in acute promyelocytic leukemia

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Abstract

Introduction: Acute promyelocytic leukemia is a type of leukemia in which abnormal promyelocytes predominate in the peripheral blood and bone marrow. Its clinical course and treatment differ from those of other acute myeloid leukemias. It is necessary to elucidate bone marrow fibrosis in acute promyelocytic leukemia.

Material and methods: Our study included 44 patients who were followed up and treated for acute promyelocytic leukemia in the adult hematology clinic of Turgut Özal Medical Center, Malatya, Türkiye. The relationship between CD 41A, CD 42A, CD 42B, and CD61 levels in flow cytometry and prognostic markers (WBC, PLT) was studied at diagnosis, and the fibrosis grade in the bone marrow pathology taken at diagnosis was examined.

Results: The relationship between the fibrosis grade in bone marrow biopsy at diagnosis and PLT, WBC, CD41A, CD42A, CD42B and CD61 values was statistically insignificant (p >0.05). There was no statistical difference between genders according to fibrosis results in bone marrow biopsy at diagnosis (p >0.05). There was a statistically significant age difference (p <0.05). At the time of diagnosis, the bone marrow fibrosis grade of 12 patients was found to be 2 or higher. We observed an increase in the fibrosis grade in bone marrow in three patients, a decrease in fibrosis in five patients, and no change in the fibrosis grade in six patients after treatment. There was no relationship between platelet surface markers and risk groups during the diagnosis of acute promyelocytic leukemia. While no correlation was detected between disseminated intravascular coagulation and platelet surface markers after treatment, a negative correlation was observed with pre-treatment INR.

Conclusions: Reticular fibrosis may be seen in patients diagnosed with acute promyelocytic leukemia. The cause of reticular fibrosis is unclear. Elevation of flow cytometric platelet surface markers in blasts at diagnosis are not directly related to reticular fibrosis. There are conflicting results in the regression of reticular fibrosis after treatment.

Keywords: acute promyelocytic leukemia, reticular fibrosis, platelet surface markers, flow cytometry

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Introduction

Acute promyelocytic leukemia (APL) is a type of leukemia in which abnormal promyelocytes predominate in the peripheral blood and bone marrow, and its clinical presentation and treatment differ from other acute myeloid leukemias. In acute promyelocytic leukemia, making a quick decision and starting urgent treatment is essential. It constitutes 5–20% of all patients with acute myeloid leukemia. It is a medical emergency, the incidence of which increases in adolescence, is not seen in childhood, and can be encountered even in old age [1].

Acute promyelocytic leukemia most commonly presents with weakness, fatigue resulting from anemia, infections due to neutropenia, and bleeding due to thrombocytopenia or disseminated intravascular coagulation [2]. More than 92% of acute promyelocytic leukemia cases have the balanced translocation t(15;17)(q24.1;q21.1), which includes RARA and PML, the retinoic acid receptor alpha gene on chromosome 17, which guides the clinician in his or her diagnosis [3].

The PML/RARA fusion gene is formed due to the translocation of the promyelocytic leukemia protein (PML) gene region on the long arm of chromosome 15 and the RARA (retinoic acid receptor alpha gene region) long arm of chromosome 17.RARA fusion gene product proteins strongly suppress transcription of retinoic acid signals, resulting in arrest of differentiation of myelocytes at the promyelocyte stage. This fusion gene is expressed as t(15;17)(q24.1;q21.2) [4]. In diagnosing acute promyelocytic leukemia, reverse transcriptase polymerase chain reaction for PML/RARA RNA is considered by many to be the gold standard method for confirming a diagnosis. However, diagnosis can also be made by detecting t(15;17) chromosomal translocation using conventional cytogenetics or the FISH technique (fluorescence in situ hybridization) [5].

Acute promyelocytic leukemia malignant cells typically express strongly CD13 and CD33 on flow cytometry, are usually negative or weakly positive for CD34, and express little or no CD15, CD117, HLA-DR, or CD11b [6]. While the expression of CD41 (glycoprotein IIb), CD61 (glycoprotein IIIa), and CD42b (glycoprotein Ib), known as platelet surface markers in flow cytometry, increases especially in megakaryoblastic leukemia, it can sometimes be seen in other leukemias. Increased surface expression of these markers is not usual in acute promyelocytic leukemia [7]. In flow cytometry, platelet surface markers (CD41,42,61) are evaluated using a specific monoclonal IgM antibody [8].

Bone marrow pathology preparations are made for fibrosis with reticulin and trichrome dyes. Increasing reticular paint involvement may be associated in malignant and benign cases, while the rise in trichrome paint involvement is more likely to be seen in malignant procedures. In recent studies, reticular fibrosis has not been shown to correlate with disease severity, whereas collagenous fibrosis appears to be associated with a poor prognosis. Also in recent studies, the idea that pathologies in the bone marrow cells cause an increase in stromal fibers in the bone marrow, and develop as a result of stockins released from hematopoietic cells, has gained importance. Though the increase in bone marrow stromal fibers is caused by stem cells and microenvironment disorders, recent research has shown that this fibrosis results from cellular anomalies rather than a single anomaly. Studies have indicated that stromal fibrosis occurs due to transforming growth factor-beta and cytokines released from megakaryocytes [9].

Reticular fibrosis can be seen in many hematological pathologies in bone marrow pathological examinations. It is likely to be seen in 33% of acute leukemias at diagnosis. It is estimated that fibrosis develops in the bone marrow under the influence of cytokines released from blasts. While widespread fibrosis is seen in acute megakaryoblastic leukemia, it is not an expected condition in acute promyelocytic leukemia, and is rarely seen [10].

Determining basic parameters such as cellularity and fiber content in bone marrow biopsy material is important for survival, treatment initiation, and treatment evaluation. European pathologists established a consensus for bone marrow evaluation in 2005. As a result of the study, opinions were expressed regarding the evaluation of bone marrow pathology reports. This study is particularly important to avoid the false impression that fiber content is reduced in fatty or edematous bone marrow samples after treatment. Bone marrow pathology preparations are stained using silver impregnation to evaluate reticulin fibers [11].

Grading of fibrosis in bone marrow pathology preparations should be defined by scaling the fibrosis grade due to trichrome and silver staining. After the bone marrow biopsy materials have been stained with reticulin dye, they are stained with trichrome dye in cases of significant involvement. Most pathology centers accept the evaluation of bone marrow pathology preparations in fibrosis developed by the European pathology experts in their consensus report of 2005 (*i.e.* graded between 0 and 3) [12].

It has been a noteworthy aspect of our study that significant reticular fibrosis was present in the bone marrow in some patients with acute promyelocytic leukemia. It has been determined that some patients with reticular fibrosis have elevated platelet surface markers. Our study was undertaken on the prediction that bone marrow reticular fibrosis may be related to platelet surface markers. The literature contains limited data. Reticular fibrosis seems to be an expected condition in acute megakaryocytic leukemia. It is reported in the literature that reticular fibrosis may occur in acute leukemias due to the effect of blasts and active molecules released from blasts.

Therefore, we felt that bone marrow reticular fibrosis during the diagnosis of acute promyelocytic leukemia, the relationship between platelet surface markers and bone marrow reticular fibrosis in flow cytometry, and the

frequency of platelet surface markers in flow cytometry, required clarification.

This study examines the frequency of platelet surface markers in flow cytometry conducted at the time of diagnosis in patients with acute promyelocytic leukemia, the relationship of this frequency with reticular fibrosis, and the frequency of reticular fibrosis in bone marrow pathology at diagnosis. Our aim was to elucidate bone marrow fibrosis in acute promyelocytic leukemia.

Material and methods

Study design

Forty-four patients who were followed up and treated with a diagnosis of acute promyelocytic leukemia in the adult hematology clinic of İnönü University's Turgut Özal Medical Center, Malatya, Türkiye were included in the study. The patients included in the study were adults diagnosed with acute promyelocytic leukemia between 2012 and 2022. A total of 70 patients were scanned in the hospital electronic system with the diagnosis code ICD C92.4 (acute promyelocytic leukemia). Twenty-six patients were not included in the study because they were followed up in pediatric hematology (due to lack of data).The relationship between the patients' flow cytometry levels (CD41A, CD42A, CD42B, CD61), prognostic markers (white blood cell count, platelet level) at diagnosis whit the degree of fibrosis in bone marrow pathology was examined. The patients' preparations were taken from the archive and re-evaluated for reticular fibers by an experienced pathologist in the pathology clinic. Since the diagnosis of three patients had been made at an external center, bone marrow pathology preparations were unavailable. After treatment, bone marrow pathological preparations of only 14 patients were obtained. Data could not be collected from three patients for CD42A and from 21 patients for CD42B who were examined at diagnosis, as there was no flow cytometry analysis.

Analysis of tissue materials

Flow cytometry studies

Bone marrow and peripheral blood samples of patients CD41A (Beckman Coulter, Brea, CA USA), CD42A (IMMUNOTECH SAS, Marseilles, France), CD42B (BD Pharmingen mouse anti-human), and CD61 (IMMUNOTECH SAS) kits were analyzed on a Beckman Coulter life sciences Noviosex device.

Examination of pathology preparations

The paraffin blocks prepared before dyeing were soaked in 0.05% potassium permanganate (BDH Chemicals, Poole, UK) for 1 minute and rinsed in tap water for 2 minutes and with distilled water twice. In the next stage, the prepared material was soaked in 0.2% potassium meta sulfate (Merck, Darmstadt, Germany) for 1 minute, then rinsed in tap water for 2 minutes, and then with distilled water twice. In the next stage, the prepared material was soaked in 0.2% ammonium ferric sulfate (Mrck) for 2 minutes, then kept in tap water for 2 minutes and rinsed twice with distilled water. In the next stage, the sample prepared was kept in the prepared material silver nitrate (Advanced Diagnostics & Research, İstanbul, Türkiye) solution for 1 minute, rinsed twice with distilled water, and then kept in formalin for 3 minutes. Then, the sample was kept in tap water for 3 minutes and rinsed twice with distilled water. In the next stage, fading was done with the prepared material gold chloride (Cyclex, Manchester, UK) (adequate fading time was determined by examining it under a microscope) and rinsed twice with distilled water. The sample, which was kept in sodium thiosulphate for 1 minute, was kept in tap water for 2 minutes, then covered with alcohol and xylene (covered with a coverslip on the slide), and the preparation was ready for examination.

Management of patients

In our clinic, ATRA (all trans-retinoic acid) + chemotherapy is used as a standard 28-day cure for low/intermediate and high-risk patients (low/intermediate: idarubicin 12 mg/m^2/day for three days, ATRA 45 mg/m^2/day for 15 days), high: cytarabine 1 g/m^2/day for three days, idarubicin 12 mg/m^2/day for three days, ATRA 45 mg/m^2/ /day for 15 days).

Consolidation treatment is given as ATRA+ chemotherapy in three cycles of 28 days (1st consolidation low/intermediate: idarubicin 5 mg/m^2/day for three days, ATRA 45 mg/m^2/day for 15 days), high: cytarabine 1g/m^2/day for three days. Induction ATO 0.15 mg/m^2/day for four weeks and consolidation days, idarubicin 5 mg/m^2/day for three days, ATRA 45 mg/m^2/day for 15 days); (2nd consolidation low/intermediate: mitoxantrone 10 mg/m^2 /day for three days, ATRA 45 mg/m^2/day for 15 days), high: cytarabine 1 g/m^2/day for three days, mitoxantrone 10 mg/m^2/day for three days, ATRA 45 mg/m^2/day for 15 days); (3rd consolidation low/intermediate: idarubicin 12 mg/m^2 /day on 1st day, ATRA 45 mg/m^2/day for 15 days), high: cytarabine 1 g/m^2/day for 2–5 days, idarubicin 12 mg/m^2 /day for three days, ATRA 45 mg/m^2/day for 15 days).
Maintenance treatment was administered to the patients as 6-mercaptopurine 50 mg/m^2/every day, methotrexate 15 mg/m^2/week, and ATRA 45 mg/m^2/day for 15 days every three months for 1–2 years.

In cases of side effects due to ATRA, patients were switched to an arsenic trioxide (ATO) regimen (induction ATO 0.15 mg/m^2/day for maximum eight weeks and consolidation). The consolidation cycle consisted of one . A cycle consisted of five weeks. The patient was given ATO for five days and not given ATO for two days each week.

Arsenic trioxide cannot be given in the first line treatment of acute promyelocytic leukemia because it is not reimbursed. It is used in patients with relapses or in patients

who develop side effects. In two of our patients, ATO was used with the Turkish government's approval due to its side effects; the bone marrow reticular fibrosis level in these two patients was graded <2 on diagnosis and after treatment.

Statistical analysis

The suitability of the data for normal distribution was determined using visual and analytical methods (Shapiro-Wilk test). Levene test was used for the homogeneity of the variables. Data that did not comply with normal distribution was expressed as median, minimum/maximum, and parameters that did comply with normal distribution were expressed as mean ± standard deviation. To compare the two groups (parametric test assumptions positive groups) an Independent Sample T test was used. To compare the two groups (parametric test assumptions negative groups) a Mann-Whitney U test was used. Qualitative parameters were expressed as frequency (n) and percentage (%), and the relationship between them was evaluated with the Chi-square test. In all analyses, p <0.05 values were accepted as the threshold for statistical significance. In additional analyses, since the data did not meet the assumption of normal distribution, the Kruskal-Wallis test was used for group comparison and the Spearman rho correlation analysis was used to examine the relationships between quantitative data. Data was studied using an IBM SPSS Statistics Base Grad Pack 28.0 Academic Package program.

Results

In flow cytometric analyses studied at diagnosis, the mean PLT and WBC values for patients with grade <2 fibrosis were 37 (10^3/microlitre) and 2.6 (10^3/microlitre), respectively. For patients with grade ≥2 fibrosis, the mean PLT and WBC values were 38 (10^3/microlitre) and 4.6 (10^3/microlitre), respectively. The maximum CD41A value for patients with grade ≥2 fibrosis was 67.4. In patients with grade <2 fibrosis, the maximum values for CD41A, CD42A, and CD61 were 93.2%, 82.8%, and 95.2%, respectively. The maximum CD61 level was 72.7% in patients with grade ≥2 fibrosis. According to fibrosis results in bone marrow biopsy at diagnosis, PLT, WBC, CD41A, CD42A, CD42B and CD61 values were statistically insignificant p >0.05 (see Tab. I).

At the time of diagnosis, there were five female and six male patients with bone marrow fibrosis of grade 2 and above, and 16 female and 17 male patients with bone marrow fibrosis of below grade 2. The patients' average age was 55.455 ± 15.965 for grade 2 and above, and 43.879 ± ± 13.722 for those below grade 2. There was no statistical difference between genders according to fibrosis results in bone marrow biopsy at diagnosis p >0.05. There was a statistically significant difference in age p <0.05 (see Tab. II).

Bone marrow biopsy was performed in 44 patients at the time of diagnosis, and bone marrow grade of fibrosis was found to be 2 or above in 12 patients. We observed an increase in the grade of fibrosis of bone marrow in three patients after treatment, a decrease in the grade of fibrosis of bone marrow in five patients, and no change in the grade of fibrosis in six patients. Since no bone marrow was evaluated after treatment in 31 patients, the fibrosis level could not be examined (see Tab. III).

Although elevated platelet surface markers were detected in a few patients during the diagnosis of acute promyelocytic leukemia, there was no statistical relationship with the risk groups that are determinants of disease prognosis (see Tab. IV).There was no statistically significant difference in CD41a, CD42a, CD42b, CD61, INR, aPTT or fibrinogen levels between the 'increased', 'decreased' and 'unchanged' groups (p >0.05) (see Tab. V).

Correlation analysis results revealed several significant relationships among the measured variables.

Table I. The relationship between the degree of bone marrow fibrosis at diagnosis and flow cytometric and prognostic parameters

	Bone marrow biopsy fibrosis grade at diagnosis		*p*-value*
	Grade <2	Grade ≥2	
	Median (Min–Max)	Median (Min–Max)	
PLT	37 (8–117)	38 (13–107)	0.626
WBC	2.6 (0.45–52.8)	4.6 (1.25–107.9)	0.364
CD41A	5.8 (0.9–93.2)	15 (2.1–67.4)	0.159
CD42A	2.3 (0.2–82.8)	5 (0.3–22)	0.090
CD42B	3.3 (1–21.4)	4.9 (1.8–24.8)	0.259
CD61	6.6 (0.9–95.2)	13.9 (1.8–72.7)	0.329

*Mann-Whitney U test. Abbreviation; PLT-platelet (103/microlitre), WBC-white blood count (103/microlitre)

Table II. Age and gender distribution of bone marrow fibrosis grade

Variable Grade <2		Bone marrow biopsy fibrosis at diagnosis Grade ≥2		*p*-value
Gender*	female	16 (48.48)	5 (45.45)	0.86
	male	17 (51.52)	6 (54.55)	
Age**		43.879 ± 13.722	55.455 ± 15.965	0.02

*frequency (%), chi-squared test; **mean ± standard deviation, independent samples t test

Table III. Grade of bone marrow fibrosis at diagnosis and after treatment

Variable	Category	n	%
Bone marrow biopsy fibrosis at diagnosis	Grade <2	33	75.0
	Grade ≥2	11	25.0
Bone marrow biopsy fibrosis after treatment	Grade <2	12	85.7
	Grade ≥2	2	14.3

Table IV. Relationship of platelet surface markers with acute promyelocytic leukemia risk groups during diagnosis

Variable*	Risk group			*p*-value**
	Low	İntermediate	High	
CD41a	10.2 (14.5)	8 (38.9)	8.4 (23.825)	0.906
CD42a	2.6 (7.5)	4.75 (17.05)	2.05 (2.75)	0.637
CD42b	3.3 (2.95)	4.9 (3.6)	4.2 (1.5)	0.664
CD61	7.4 (9)	12.7 (27.8)	4.1 (15.075)	0.538

*Variables are summarized as 'median (interquartile range)'; **Kruskal-Wallis test

Table V. Relationship between platelet surface markers and disseminated intravascular coagulation parameters and post-treatment bone marrow fibrosis change

Variable*	Group			*p*
	Increased	Decreased	Unchanged	
CD41a	8 (13.1)	22.7 (27.5)	9.15 (51.075)	0.7345
CD42a	0.8 (8.5)	3.95 (3.625)	7.6 (14.55)	0.42671
CD42b	4.55 (1.45)	4.9 (11.2)	1 (0)	0.34252
CD61	6.5 (12.2)	20.7 (30)	5.4 (11.85)	0.68256
INR	1.19 (0.065)	1.17 (0.2)	1.22 (0.203)	0.51142
aPTT	27.1 (1.35)	26.9 (2)	24.45 (6.325)	0.5288
Fibrinogen	121.67 (26)	85 (20.73)	124.515 (66.44)	0.23069

*Kruskal-Wallis test, aPTT — active partial thromboplastin time; INR — International Normalized Ratio

Table VI. Correlation of platelet surface markers and disseminated intravascular coagulation parameters

		CD41A	CD42A	CD42B	CD61	INR	aPTT	Fibrinogen
CD41A	rho	1.000	0.368	0.943	0.411	–0.654	0.138	0.266
	p-value		0.216	0.005	0.144	0.011	0.637	0.358
CD42A	rho	0.368	1.000	0.700	0.401	–0.355	–0.308	0.500
	p-value	0.216		0.188	0.174	0.234	0.306	0.082
CD42B	rho	0.943	0.700	1.000	0.943	–0.899	0.143	0.371
	p-value	0.005	0.188		0.005	0.015	0.787	0.468
CD61	rho	0.411	0.401	0.943	1.000	–0.498	–0.165	0.204
	p-value	0.144	0.174	0.005		0.070	0.573	0.483
INR	rho	–0.654	–0.355	–0.899	–0.498	1.000	–0.099	–0.328
	p-value	0.011	0.234	0.015	0.070		0.736	0.252
aPTT	rho	0.138	–0.308	0.143	–0.165	–0.099	1.000	–0.077
	p-value	0.637	0.306	0.787	0.573	0.736		0.794
Fibrinogen	rho	0.266	0.500	0.371	0.204	–0.328	–0.077	1.000
	p-value	0.358	0.082	0.468	0.483	0.252	0.794	

aPTT — active partial thromboplastin time; INR — International Normalized Ratio; *Spearman rho correlation analysis

CD41A showed a strong positive correlation with CD42B ($p = 0.943$, $p = 0.005$) and a significant negative correlation with INR ($p = –0.654$, $p = 0.011$). CD42B strongly correlated positively with CD61 ($p = 0.943$, $p = 0.005$) and significantly negatively with INR ($p = –0.899$, $p = 0.015$).

These findings indicate important interdependencies between CD41A, CD42B, CD61, and INR, highlighting INR's inverse relationship with CD41A and CD42B (see Tab. VI).

Discussion

Increased fibrosis in bone marrow pathology preparations is common in hematological malignancies. Bone marrow reticular fibrosis can be seen with a frequency of c.33% in acute leukemias. This fibrosis is thought to result from blasts densely present in the circulation. Fibrosis is seen more frequently in megakaryoblastic leukemia than in other

types of leukemia. Bone marrow reticular fibrosis is not an expected condition in acute promyelocytic leukemia. There have been only rare case reports of bone marrow fibrosis in promyelocytic leukemia [9].

Abou Dalle et al.'s [10] study summarized the clinicopathological features, treatments, and outcomes to date of three cases of classic acute promyelocytic leukemia with increased reticulin fibrosis in the bone marrow, identifying six cases with the presence of bone marrow fibrosis in acute promyelocytic leukemia.

In their study, grade 2 and above reticular fibrosis was observed in a total of 12 patients in the pathological evaluation of bone marrow biopsy material performed at the time of diagnosis of acute promyelocytic leukemia. In some cases, high platelet surface markers were observed in flow cytometry analyses. The relationship of these markers with reticular fibrosis was not statistically significant. Statistical analyses may have been affected by the study's limited patient population. Comprehensive studies are needed to elucidate the relationship between platelet surface marker abnormalities observed in blasts during the diagnosis of acute promyelocytic leukemia and reticular fibrosis.

The case report by Mori et al. [13] featured a patient with acute premyelocytic leukemia with advanced bone marrow fibrosis. In this patient, a regression of fibrosis was observed after treatment with chemotherapy. Bone marrow fibrosis regressed in parallel with the decrease in blasts in the periphery and bone marrow. Bone marrow fibrosis was thought to be caused by blasts and cytokine release genes detected in the blasts. It was studied before and after treatment. It was shown that the expression of this gene decreased after treatment. While the gene expression that causes the release of transforming growth factor-beta (1), which causes bone marrow fibrosis, was high at diagnosis, a decrease in gene expression was observed after treatment. Transforming growth factor-beta (1) has been suggested as a possible cause of bone marrow fibrosis [13].

Judging by the analysis of this study, transforming growth factor-beta(1) gene expression was not studied. At diagnosis, 25% grade ≥2 bone marrow fibrosis was detected in 12 patients in bone marrow pathology preparations. It was observed that there was an increase in the grade of fibrosis in four patients, a decrease in the grade of fibrosis in four patients, and no change in the grade of fibrosis in six patients after treatment. Since no bone marrow was evaluated after treatment in 31 patients, fibrosis level could not be examined [13]. All trans-retinoic acid and multiple chemotherapies were used in the induction treatment of the patients. In this study, there was no regression in bone marrow reticular fibrosis after treatment in every patient. It is thought that bone marrow reticular fibrosis may be caused by different factors other than the cytokines released from blasts.

In the study by Lam et al. [14] on platelet debridement, it was determined that the cytoplasmic fragments of blasts in leukemic patients were evaluated as platelets, and the device incorrectly expressed the number of platelets as high. Blue cytoplasmic particles were observed in the peripheral blood smears of the patients.

In the data from this study, flow cytometry analyses on myelomonocytic and granulocytic cells in acute promyelocytic leukemia at diagnosis revealed that the maximum CD41A value was 67.4% for patients with grade ≥2 fibrosis. In patients with grade <2 fibrosis, the maximum values for CD41A, CD42A, and CD61 were 93.2%, 82.8%, and 95.2 %, respectively. The maximum CD61 level was 72.7% in patients with grade ≥2 fibrosis. Platelet surface marker elevations are among the less likely possibilities of platelet debridement. In flow cytometric analyses performed on blasts, platelet residues may be seen, although with a low probability. Laboratories operate according to international standards to ensure this situation does not affect the analyses. Our laboratory performs flow cytometry procedures according to international standards.

In the study by Losada et al. [15] on bone marrow pathology preparations of 51 patients with acute promyelocytic leukemia at the time of diagnosis, fibrosis was not observed in 13.7% of the patients. In contrast, mild fibrosis was observed in 9.8% and severe fibrosis in 3.9%. As a result, when the treatment responses in patients with fibrosis were examined, it was observed that fibrosis regressed after treatment.

This study detected 25% grade ≥2 bone marrow fibrosis in 12 patients diagnosed with acute promyelocytic leukemia [15]. It was observed that there was an increase in the grade of fibrosis in four patients, a decrease in the grade of fibrosis in four patients, and no change in the grade of fibrosis in six patients after treatment. Patients were given induction, consolidation and maintenance treatment for the first year. Patients were evaluated for translocation t(15;17) every three months. PML/RARA fusion gene negativity occurred in all patients, and a complete hematological response was achieved. As a result, no difference was observed between clinical and treatment responses in patients with and without fibrosis.

Beckman et al. evaluated the amount of reticulin in normal bone marrow; the grade of fibrosis was found to be low in normal patients without any hematological pathology. While the amount of reticulin evaluated according to the Bauermeister scale was found to be grade 1 in c.33% of the patients and grade 2 in 4%, no advanced (grades 3 and 4) reticular fibrosis was observed in any patient [16].

This study, bone marrow pathology preparations at diagnosis were graded according to the European pathology consensus criteria (0, 1, 2, 3) scale. Patients with grade 2

and above fibrosis were considered to be significantly fibrotic. Patients with significant fibrosis accounted for 25%. Reticular fibrosis values below grade 2 were accepted as the normal range, and statistical analyzes were performed on patients with grade 2 and above.

Platelet integrin GPIIb/IIIa (CD41A) plays an important role in thrombus formation through interactions with adhesive ligands, and it has emerged as a primary target for developing antithrombotic agents. Receptor activation is tightly controlled by activators, inhibitors, and signaling mechanisms that control its conformation [17]. The glycoprotein GPIb-IX (CD42b complex), a large number of receptor complexes expressed explicitly on the surface of platelets and megakaryocytes, plays an important role in hemostasis and thrombosis. Bleeding symptoms can develop in patients with this defect [18]. Our study detected a negative correlation between CD41 AND CD42b and the international normalized ratio (INR). The decrease in these surface markers was determined to increase the INR level. There needs to be more explanatory data regarding this in the literature. The decrease in platelet surface markers caused an increase in the time until the fibrin clot formed, and this effect was associated with an increase in the INR level.

Conclusions

Reticular fibrosis may be seen in patients diagnosed with acute promyelocytic leukemia. The cause of reticular fibrosis is unclear. Elevation of platelet surface markers in blasts at the time of diagnosis is not associated with reticular fibrosis of bone marrow. There are conflicting results in the regression of reticular fibrosis after treatment.

Article information and declarations

Data availability statement

Informed consent of the patients is available in the hospital file. Patient files are stored in the hospital service archive.

Ethics statement

Ethical approval was received by the scientific research and publication ethics board of Inönü University of the Republic of Türkiye: health sciences non-invasive clinical research ethics board decision number 2022/4216. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Authors' contributions

AK — conducting study and article writing; MAE — supervision of study and article writing; İK — departmental approval and providing support for study and article writing; EK, İB, AS, SB, SA — providing support for study and article writing; ZB — re-examination of pathology preparations and providing pathological support during writing phase; FHY — providing biostatistical support for study and article.

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Conflicts of interest

The authors declare no conflict of interest.

Supplementary material

Four auxiliary materials will be uploaded to the system. Uploading supplementary material to the system is convenient for authors.

References

1. Sharma A, Yang J, Singh V. Epidemiology and early mortality patterns of acute promyelocytic leukemia in the United States. Ann Hematol. 2023; 102(5): 1053–1062, doi: 10.1007/s00277-023-05154-0, indexed in Pubmed: 36918414.
2. Mantha S, Tallman MS, Soff GA. What's new in the pathogenesis of the coagulopathy in acute promyelocytic leukemia? Curr Opin Hematol. 2016; 23(2): 121–126, doi: 10.1097/MOH.0000000000000221, indexed in Pubmed: 26760586.
3. Lin J, Han LX, Qian J, et al. Expression patterns of specific promyelocytic/retinoic acid receptor-alpha transcripts in patients with acute promyelocytic leukemia. Int J Lab Hematol. 2010; 32(3): 344–350, doi: 10.1111/j.1751-553X.2009.01190.x, indexed in Pubmed: 19863682.
4. Arber D, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127(20): 2391–2405, doi: 10.1182/blood-2016-03-643544.
5. Gagnon MF, Berg HE, Meyer RG, et al. Typical, atypical and cryptic t(15;17)(q24;q21) (PML::RARA) observed in acute promyelocytic leukemia: A retrospective review of 831 patients with concurrent chromosome and PML::RARA dual-color dual-fusion FISH studies. Genes Chromosomes Cancer. 2022; 61(10): 629–634, doi: 10.1002/gcc.23070, indexed in Pubmed: 35639830.
6. Dimov ND, Medeiros LJ, Kantarjian HM, et al. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody: the M. D. Anderson Cancer Center experience of 349 patients. Cancer. 2010; 116(2): 369–376, doi: 10.1002/cncr.24775, indexed in Pubmed: 19950129.
7. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022; 36(7): 1703–1719, doi: 10.1038/s41375-022-01613-1, indexed in Pubmed: 35732831.

8. Frelinger AL, Spurgeon BEJ. Clinical Cytometry for Platelets and Platelet Disorders. Clin Lab Med. 2023; 43(3): 445–454, doi: 10.1016/j.cll.2023.04.008, indexed in Pubmed: 37481322.
9. Nakayama S, Yokote T, Hiraoka N, et al. Transforming growth factor β- and interleukin 13-producing mast cells are associated with fibrosis in bone marrow. Hum Pathol. 2017; 62: 180–186, doi: 10.1016/j.humpath.2017.01.007, indexed in Pubmed: 28159675.
10. Abou Dalle I, Nassif S, Bazarbachi A. Acute promyelocytic leukemia with increased bone marrow reticulin fibrosis: Description of three cases and review of the literature. Hematol Oncol Stem Cell Ther. 2018; 11(2): 99–104, doi: 10.1016/j.hemonc.2016.08.001, indexed in Pubmed: 27614232.
11. Izuta M, Kuwamoto S, Kamiya T, et al. Imaging of Liver Tissues Using a Combination of Silver Impregnation and Low-Vacuum Scanning Electron Microscopy; A Simple Method for the High-Resolution Visualization of Reticulin Structures with Applicability to a Quantitative Analysis. Yonago Acta Med. 2023; 66(1): 78–86, doi: 10.33160/yam.2023.02.009, indexed in Pubmed: 36820283.
12. Thiele J, Kvasnicka HM, Facchetti F, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005; 90(8): 1128–1132, indexed in Pubmed: 16079113.
13. Mori A, Wada H, Okada M, et al. Acute promyelocytic leukemia with marrow fibrosis at initial presentation: possible involvement of transforming growth factor-beta(1). Acta Haematol. 2000; 103(4): 220–223, doi: 10.1159/000041054, indexed in Pubmed: 11014898.
14. Lam WK, Law YF, Yip SF. Resolution of platelet count interference due to cytoplasmic fragments of leukaemic cells by flow cytometry in acute myeloid leukaemia. Int J Lab Hematol. 2022; 44(6): 983–985, doi: 10.1111/ijlh.13859, indexed in Pubmed: 35504732.
15. Losada R, Cabrera H, Hernández P, et al. Bone marrow reticulin fibrosis at diagnosis in promyelocytic leukaemia treated with all-trans retinoic acid has no adverse prognosis. Acta Haematol. 2002; 108(2): 111–112, doi: 10.1159/000064753, indexed in Pubmed: 12187033.
16. Beckman EN, Brown AW. Normal reticulin level in iliac bone marrow. Arch Pathol Lab Med. 1990; 114(12): 1241–1243, indexed in Pubmed: 2252420.
17. Hagemeyer CE, Peter K. Targeting the platelet integrin GPIIb/IIIa. Curr Pharm Des. 2010; 16(37): 4119–4133, doi: 10.2174/138161210794519255, indexed in Pubmed: 21247395.
18. Li R. Recent advances on GPIb-IX-V complex. Platelets. 2022; 33(6): 809–810, doi: 10.1080/09537104.2022.2075146, indexed in Pubmed: 35543611.