Original Investigation | Özgün Araştırma

Gazi Med J 2025;36(3):250-254

DOI: http://dx.doi.org/10.12996/gmj.2025.4226

Autoimmune Hemolytic Anemia in the Course of Pediatric Acute Leukemia and After Hematopoietic Stem Cell Transplantation

Pediatrik Akut Lösemi Tedavisinde ve Hematopoetik Kök Hücre Nakli Sonrasında Gelişen Otoimmün Hemolitik Anemi

Sırma Karamercan, Serap Kırkız Kayal, Büşra Topuz Türkcan, Zühre Kaya, Ülker Koçak

Department of Pediatric Hematology-Oncology, Division of Pediatric Hematology, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Autoimmune hemolytic anemia (AIHA), thrombocytopenia, neutropenia and some other autoimmune diseases can be observed in the context of lymphoproliferative diseases and after hematopoietic stem cell transplantation (HSCT). Among these, AIHA is a rare red blood cell disorder; however, the cause cannot be determined in almost half of the cases.

Methods: This retrospective study analyzed nine pediatric leukemia patients who developed hemolytic anemia during chemotherapy or following HSCT. Patients were diagnosed with hemolysis based on laboratory markers, including hemoglobin (Hb), haptoglobin, reticulocyte count, lactate dehydrogenase (LDH), and direct antiglobulin test (DAT), and peripheral blood smear findings. Infectious and genetic causes of hemolytic anemia were also investigated.

Results: The mean age of the patients was 9.5±4.5 years (2.2-16.7 years). Six patients were being followed with acute lymphoblastic leukemia (ALL) whereas three had biphenotypic leukemia. Three of the ALL patients underwent allogeneic HSCT. During AIHA attacks, reticulocyte and LDH were high, whereas Hb and haptoglobin levels were low. While indirect bilirubin elevation was not detected in one case, the direct antiglobin test was positive at different rates in all, except three cases. Significant hemolysis findings were observed in all peripheral smears. The hemolytic attacks were thought to be related to chemotherapy, infection, and drugs. Steroids, intravenous immunoglobulin, rituximab were used for treatment.

Conclusion: In the clinical follow-up of leukemia patients, the significant increase in reticulocytes with low levels of Hb and haptoglobin and the increase in LDH and/or indirect bilirubin levels along with DAT positivity should suggest AIHA. Diagnosis and management planning must be undertaken accordingly.

Keywords: Leukemia, hemolytic anemia, autoimmune

ÖZ

Amaç: Otoimmün hemolitik anemi (OİHA), trombositopeni, nötropebi ve diğer otoimmün hastalıklar lenfoproliferatif hastalıklar sürecinde ve hematopoetik kök hücre nakli (HKHN) sonrası gelişebilir. Bunlardan OİHA olgularının yaklaşık yarısının etiyolojisi net değildir.

Yöntemler: Bu çalışmada lösemi tedavi sırasında ve HKHN sonrasında OİHA gelişen dokuz hasta değerlendirildi. Hastalarda hemoliz parametreleri olan hemoglobin (Hb), retikülosit, haptoglobulin, laktat dehidrogenaz (LDH), direkt antiglobulin test değerlendirildi. Ayrıca hastalarda enfeksiyöz ve genetik nedenler de tetkik edildi.

Bulgular: Hastaların ortalama yaşı 9,5±4,5 (2,2-16,7) idi. Altı hasta akut lenfoblastik lösemi, 3 hasta bifenotipik lösemi tanılıydı. Üç hastaya allojenik HKHN yapıldı. Otoimmün hemolitik anemi atakları sırasında retikülosit ve LDH yüksek, Hb ve haptoglobin düzeyleri düşük idi. Bir olguda indirekt bilirubin yükselmesi saptanmadı ve üç hasta dışında tüm olgularda direkt antiglobin testi farklı oranlarda pozitifti. Periferik yayma incelemesinde hemoliz bulguları gözlendi. Hemolitik atakların kemoterapi, enfeksiyon ve ilaçlarla ilişkili olduğu düşünüldü. Tedavi için steroidler, intravenöz immünoglobulin ve rituksimab kullanıldı.

Sonuç: Lösemi takibi sırasında gelişen retikülositteki anlamlı artış, hemoglobin düşüşü ve diğer hemoliz parametlerindeki değişimlerin tespiti ile otoimmün hemolitik anemi düşünülerek tedavi hızla yapılmalıdır.

Anahtar Sözcükler: Lösemi, hemolitik anemi, otoimmün

Cite this article as: Karamercan S, Kırkız Kaya S, Topuz Türkcan B, Kaya Z, Koçak Ü. Autoimmune hemolytic anemia in the course of pediatric acute leukemia and after hematopoietic stem cell transplantation. Gazi Med J. 2025;36(3):250-254

Address for Correspondence/Yazışma Adresi: Sırma Karamercan, MD, Department of Pediatric Hematology-Oncology, Division of Pediatric Hematology, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: karamercanesirma@gmail.com
ORCID ID: orcid.org/0000-0001-8852-7875

Received/Geliş Tarihi: 03.06.2024
Accepted/Kabul Tarihi: 07.07.2025
Publication Date/Yayınlanma Tarihi: 11.07.2025

INTRODUCTION

Autoimmune hemolytic anemia (AIHA) is a condition characterized by acute hemolysis caused by autoantibodies that develop against red blood cell (RBC) surface antigens. Clinically, fatigue, pallor and jaundice are frequent symptoms. While hemoglobin (Hb) and haptoglobin levels decrease, reticulocyte, bilirubin, and lactate dehydrogenase (LDH) levels are increased. The direct antiglobulin test (DAT) is usually positive. However, it may not be positive in all cases (1,2). The incidence of AIHA has been reported as 0.8/100,000 for those under 18 years of age (1). AIHA can be due to warm, cold, or mixed autoantibody types. Warm autoantibodies, which are the most common cause of AIHA, are typically a member of the IgG class. The DAT identifies IgG antibodies and/or C3, attached to the patient's RBCs after the introduction of the antihuman globulin reagent. Cold autoantibodies are usually of the IgM class and activate complement (usually C3), which is found on the RBC membrane. Despite being a routine and effective diagnostic tool for AIHA, the DAT is falsely negative in 3-11% of patients (3,4). Immunodeficiency, infections, medications, and malignancies can cause AIHA. Lymphoproliferative diseases are known to be accompanied by hemolytic anemia. AIHA has been reported to develop in acute lymphoblastic leukemia (ALL), acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, some lymphomas, and after hematopoietic stem cell transplantation (HSCT) (2,4,5). The association of AIHA with chronic lymphocytic leukemia has been clearly described, in which apoptosis of B lymphocytes, lack of adequate immunoglobulin release, deterioration of T lymphocytes involved in antigen presentation, and loss of immune tolerance have been involved in the pathogenesis (2). AIHA is rarely observed in solid tumors. Passenger lymphocyte syndrome is implicated in the etiology of AIHA after solid organ transplantation. It is thought to be caused by lymphocyte dysfunction (6). Anemia is an expected side effect during leukemia treatment and follow-up. However, attributing it solely to myelosuppression is not sufficient. Therefore, in this study, etiological factors (immune, non-immune) of anemia and the development of AIHA, which developed during the course and treatment of acute leukemia and after HSCT in children, were investigated and discussed.

MATERIALS AND METHODS

This study included leukemia patients who were diagnosed, treated, and continued to be followed up in our pediatric hematology unit between May 1 and November 30, 2021. All the records of these patients were reviewed retrospectively. Of these records, the patients who developed anemia (<-2 standard deviation for age) were selected and their peripheral blood smears were evaluated for the hemolysis indicators (anisocytosis, poikilocytosis, polychromasia, etc.). Complete blood count, bilirubin, LDH, haptoglobin, and DAT of the patients were also recorded.

The DAT was analyzed using the Grifols diagnostic DG gel system with the Erytra® Automated System Devices. Additionally, infectious agents [Epstein-barr virus (EBV), cytomegalovirus (CMV), hepatitis, Brucella, mycoplasma] and genetic causes of hemolytic anemia [thalassemia, deficiencies of glucose 6 phosphate dehydrogenase, pyruvate kinase (PK), etc.] that could assist in the etiology of anemia were also investigated. Patients without hemolysis indicators in peripheral blood smears were excluded.

The study was conducted through retrospective file review and was approved by the Gazi University Ethics Committee (approval number: 2025-1105, date: 17.06.2025).

RESULTS

Peripheral blood smears of 54 patients with anemia (<-2 standard deviation for age) who had acute leukemia were evaluated. Records of nine patients (5 females, 4 males) with hemolysis indicators: (anisocytosis, poikilocytosis, polychromasia, etc.) were included in the study.

The mean age of the patients was 9.5±4.5 years (2.2-16.7 years). Six patients were followed with ALL; three had biphenotypic leukemia (BL). Three of the ALL patients underwent HSCT. Laboratory values showed a mean Hb level of 9±0.5 g/dl (8.6-10). Reticulocyte count increased in all cases with a mean value of 8.6±2.6% (6.4-14.5%); LDH was also increased in the range of 269-889 IU/L. Indirect hyperbilirubinemia was detected in all cases except in one patient. All patients had anisocytosis, poikilocytosis (mostly spherocytes and schistocytes) and polychromasia in the peripheral blood smear. The direct antiglobin test was positive in six patients. In the three DAT negative patients, although they have typical laboratory and clinical findings for AIHA, cold agglutinins were also negative. Haptoglobin levels were low in seven patients, and high levels in the remaining two cases were thought to result from systemic infections. Serological tests showed positivity for EBV in two patients and CMV in one patient. One patient had a DAT positive anemia concomitant with PK deficiency. PK deficiency was incidentally identified when tests were conducted due to a sudden drop in Hb levels in this particular patient. In terms of treatment modalities, antiviral therapy was administered for CMV in patients with infection, while rituximab was used to treat EBV. Four patients who developed autoimmune anemia received steroid treatment (2 mg/kg/day for 3 days and 1 mg/kg/day for four days). In three of these patients who did not respond, an increase in hemoglobin was observed following intravenous immunoglobulin (IVIG) treatment (1g/kg/day for 2 days). For a patient with AIHA who was febrile and neutropenic, IVIG was preferred over steroid treatment. Table 1 shows the demographic and clinical findings of the patients.

Discussion

Leukemia patients suffer from anemia due to the disease itself, bleeding, chemotherapy, loss of appetite, and vomiting. Autoimmune hemolysis might also contribute to the severity of anemia, for several reasons, such as malignant disease, chemotherapy, immune deficiency, and infections (1-3). Reticulocyte, indirect bilirubin, and LDH levels are expected to be high in hemolytic anemia, while hemoglobin and haptoglobin levels are decreased, along polychromasia in the blood smear (2-5). However, none of these tests is essential for diagnosing hemolysis. Liver disease can lead to increased LDH and decreased haptoglobin; milder hemolysis can cause normal bilirubin levels. In 20% of patients, reticulocytopenia might be seen despite erythroid hyperplasia (7). Moreover, DAT is usually positive except in 3-11% of the cases (4). Direct antiglobin

Table 1. Patient characteristics

Patient	Age (years)	Gender	Diagnosis	Time	Co-morbidity	Hb (10.8-13.3 gr/dL)	Ret (%)	LDH (120-250 ıu/L)	Hapto (30-200 mg/dL)	IDB (<0.8 mg/dL)	DAT	Cold agglutinin	Peripheral blood smear	Treatment
1	13	F	ALL	After HSCT (3th month)	EBV	8.2	9.5	654	4	0.45	+	N/A	Acanthocytes, schistocytes, spherocytes	IVIG
2	13.6	F	ALL	After HSCT (1stmonth)	CMV	9	6.8	352	2	0.46	+	N/A	Anisocytosis, acanthocytes, spherocytes	Antiviral treatment
3	16.7	M	ALL	After REZ-BFM-ALL F2 block	Down syndrome	9.6	6.9	889	3	1.08	++	N/A	Spherocytse, polychromasia	IVIG
4	11.3	M	ALL	Maintenance CT (17th.wks)	PK defiency	8.8	14.5	563	7	2.5	+	-	Schistocytes	Stop CT
5	2.2	F	ALL	After CT (Bortezomib-VCR-Doxo-dexamethazone)	Infection (neutropenia)	9	6.4	269	187	0.38	+	N/A	Acanthocytes, polychromasia	IVIG-rituximab
6	8	F	BL	After REZ-BFM-ALL F2 block	-	8.6	7.9	373	28	0.32	+++	N/A	Polychromasia, schistocytes	Steroid
7	7.5	M	BL	Interfant protocol (Octatad-day 22	Infection (neutropenia)	10	7.5	389	246	1.17	-	-	Anisocytosis, polychromasia	IVIG
8	5	M	BL	Interfant protocol (MARMA- day 10	-	9	11.2	270	2	2.68	-	N/A	Polychromasia, schistocytes	IVIG
9	8.5	F	ALL	After HSCT (Day 47)	EBV	9.5	6.8	330	25	0.4	-	-	Acanthocytes, schistocytes, spherocytes	Rituximab

ALL: Acute lymphoblastic leukemia, BL: Biphenotypic leukemia, CT: Chemoterapy, EBV: Ebstein-Barr virus, CMV: Cytomegalovirus, PK: Pyruvate kinase, Hb: Hemoglobin, Ret: Reticulocyte, LDH: Lactate dehydrogenase, Hapto: Haptoglobin, IDB: Indirect bilirubin, DAT: Direct antiglobin test, IVIG; Intravenous immunglobulin, HSCT: Hematopoetic stem cell trasnplantation

IgG was found to be positive in only six of our cases. The negative DAT results in our three cases may be due to antibody types, such as IgA, that are not detected by DAT or the low-affinity antibody titer of IgG (3). In these situations, specific tests need to be performed for confirmation, which unfortunately are not available at most centers. Although the positivity of cold agglutinin can also cause DAT negativity, this was not the case in any of our DAT negative patients. AIAHs and their subtypes are defined by typical laboratory and clinical findings in the absence of standard diagnostic criteria. Clinical studies indicate that the use of different criteria for diagnosis can lead to varying treatment responses (8).

Children and adults with leukemia have been reported to develop AIHA similar to our ALL and BL patients (9,10). Three of our patients had developed AIHA secondary to infections while on immunosuppressive therapy after HSCT. The etiology of AIHA in these patients consists of both immunosuppression and infections due to chemotherapy and/or HSCT. Posttransplant EBV-associated AIHA has been reported to develop years after kidney and heart transplantation (11,12). AIHA after HSCT has been defined in transplants from mismatched unrelated donors, chronic graft versus host disease (GVHD) and non-malignant primary disease (13). Two of our three patients who developed AIHA after HSCT had been transplanted from an unrelated donor. In all three, GVHD of different severities had also been developed. One of these cases who was DAT negative received intensive immunosuppressive therapy for grade IV acute gastrointestinal GVHD with an additional EBV positivity. Moreover, CMV positivity accompanied AIHA in another transplant patient. Wang et al. (14) examined 553 adult HSCT patients. AIHA was detected in 19 of them. Although CMV positivity was detected in 10 of these cases, the difference was not statistically significant. However, CMV-associated AIHA has been reported in the past in immunosuppressed patients (15). Two of our three patients with hemolytic anemia after chemotherapy had concomitant neutropenic fever. IVIG treatment was given because the patient had both culture-negative neutropenic fever and AIHA. Chemotherapy-associated anemia develops through immune, microangiopathic and/or oxidative mechanisms (16). Three of our cases were DAT negative, but there were no other findings supporting microangiopathy or oxidative hemolysis. An increase in hemoglobin value following IVIG supported our diagnosis of immune hemolytic anemia. In AIHA, low haptoglobin, high LDH, and indirect hyperbilirubinemia are expected (4). Haptoglobin was elevated in two of our patients with febrile neutropenia and hemolysis. Since it is an acute phase indicator, this elevation was not considered significant.

The main treatment modalities of AIHA are, removal of the etiological factors, corticosteroids, IVIG and rituximab. If there is no response with these treatments, other immunosuppressive agents are used as line therapy (17). Our patients improved with the above treatments accompanied by removal of etiological factors. In conclusion, effective management of AIHA was achieved through a comprehensive approach that involved IVIG, corticosteroids, and addressing underlying factors, highlighting the importance of tailored therapeutic strategies in these cases.

Study Limitations

The study's retrospective design, relying on the review of past patient records, introduces inherent limitations. Moreover, the findings and conclusions are dependent on the availability and completeness of patient records. Incomplete or missing data could impact the accuracy and reliability of the results. As this is a pilot study, it will inform multicentric prospective studies. The study included a limited number of patients from a single center. These findings may not be generalized to a larger population or other healthcare settings. Another important limitation of this study is that it was focused on leukemia patients who developed AIHA during the course of their disease. This selection may introduce bias, as patients with specific characteristics or conditions might be overrepresented or underrepresented. Determining the exact etiological factors contributing to AIHA can be complex. The study aimed to investigate both immune and non-immune causes, but some factors, such as low-titer antibodies and IgA DAT, were not fully explored. External factors such as changes in treatment protocols, new developments in medical practices, or variations in patient demographics over time were also not considered in the study.

CONCLUSION

Although the diagnosis of AIHA typically involves a stepwise approach focused on identifying both laboratory and clinical evidence of hemolysis, it is important to note that the interpretation of laboratory parameters can occasionally be misleading. Besides, differential diagnosis could be challenging in leukemia and stem cell transplanted patients. Pediatric hemato/oncologists should be aware of hemolysis in leukemia patients, as detailed examination, prompt diagnosis, and immediate treatment have paramount importance to decrease morbidity and mortality. This would also contribute to the survival of the children with leukemia.

Ethics

Ethics Committee Approval: The study was conducted through retrospective file review and was approved by the Gazi University Ethics Committee (approval number: 2025-1105, date: 17.06.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., Ü.K., Concept: S.K., Ü.K., Design: S.K., S.K.K., Supervision: Z.K., Ü.K., Resources: S.K., Material: S.K., Data Collection or Processing: S.K., B.T.T., Analysis or Interpretation: S.K., S.K.K., B.T.T., Literature Search: S.K., S.K.K., Writing: S.K., Critical Review: S.K., Ü.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Voulgaridou A, Kalfa TA. Autoimmune hemolytic anemia in the pediatric setting. J Clin Med. 2021; 10.
2. Barcellini W, Giannotta JA, Fattizzo B. Autoimmune complications in hematologic neoplasms. Cancers (Basel). 2021; 13.
3. Miller J, Cai W, Andrews J, Narla A. A case series of pediatric patients with direct antiglobulin test negative autoimmune hemolytic anemia. Transfusion. 2019; 59: 2528-31.

4. Arora S, Dua S, Radhakrishnan N, Singh S, Madan J, Nath D. Autoimmune hemolytic anemia in children: clinical presentation and treatment outcome. Asian J Transfus Sci. 2021; 15: 160-5.
5. Fattizzo B, Giannotta JA, Serpenti F, Barcellini W. Difficult cases of autoimmune hemolytic anemia: a challenge for the internal medicine specialist. J Clin Med. 2020; 9.
6. Nadarajah L, Ashman N, Thuraisingham R, Barber C, Allard S, Green L. Literature review of passenger lymphocyte syndrome following renal transplantation and two case reports. Am J Transplant. 2013; 13: 1594-600.
7. Hill A, Hill QA. Autoimmune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2018; 2018: 382-9.
8. Hill QA, Hill A, Berentsen S. Defining autoimmune hemolytic anemia: a systematic review of the terminology used for diagnosis and treatment. Blood Adv. 2019; 3.
9. Tarkun P, Hacıhanefioğlu A, Demirbağ E, Turgut T. Development of autoimmune hemolytic anemia during the treatment of a patient with acute myelomonocytic leukemia. Turk J Haematol. 2005; 22: 95-9.
10. Deeren D. B-lymphoblastic leukaemia presenting as autoimmune haemolytic anaemia. Ann Hematol. 2009; 88: 499.
11. Castillo DR, Sheth P, Nishino K, Stevens WT, Nguyen A, Romagnolo A, et al. Successful treatment of autoimmune hemolytic anemia concomitant with proliferation of Epstein-Barr virus in a post-heart transplant patient. Hematol Rep. 2022; 14: 261-4.
12. Hamilton AJ, Webb LH, Williams JK, D'Souza RJ, Ngu LS, Moore J. Autoimmune haemolytic anaemia associated with Epstein-Barr virus infection as a severe late complication after kidney transplantation and successful treatment with rituximab: case report. BMC Nephrol. 2015; 16: 108.
13. Holbro A, Passweg JR. Management of hemolytic anemia following allogeneic stem cell transplantation. Hematology Am Soc Hematol Educ Program. 2015; 2015: 378-84.
14. Wang M, Wang W, Abeywardane A, Adikarama M, McLornan D, Raj K, et al. Autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation: analysis of 533 adult patients who underwent transplantation at King's College Hospital. Biol Blood Marrow Transplant. 2015; 21: 60-6.
15. Yacoub MS, Doraji M, Yadlapalli S. Cytomegalovirus-induced Coombs-positive hemolysis or drug-induced hemolysis in an immunocompetent young adult. Cureus. 2022; 14.
16. Doll DC, Weiss RB. Hemolytic anemia associated with antineoplastic agents. Cancer Treat Rep. 1985; 69: 777-82.
17. Go RS, Winters JL, Kay NE. How I treat autoimmune hemolytic anemia. Blood. 2017; 129: 2971-9.