EUROPEAN JOURNAL OF
MODERN MEDICINE AND PRACTICE
Vol. 6 No. 05 (May - 2026) EJMMP ISSN: 2795-921X

https://inovatus.es/index.php/ejmmp

CLINICAL AND LABORATORY DIAGNOSIS OF AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

Kurbonova Z. Ch.
Tashkent State Medical University

Khushbokova G.U.
Tashkent State Medical University

Abstract: Autoimmune hemolytic anemia (AIHA) is a heterogeneous group of immune-mediated disorders characterized by the premature destruction of red blood cells due to the production of autoantibodies directed against erythrocyte surface antigens. Although the disease is relatively rare, it remains clinically important due to its potential severity, diagnostic complexity, and frequent association with systemic autoimmune diseases, lymphoproliferative disorders, infections, and drug exposure. The diagnostic process requires a combination of clinical assessment and laboratory investigations, with particular emphasis on identifying hemolysis and confirming immune-mediated mechanisms.The direct antiglobulin test (DAT), also known as the Coombs test, is considered the cornerstone of laboratory diagnosis. However, recent studies have shown that a subset of patients may present with DAT-negative autoimmune hemolysis, which complicates diagnosis and may delay treatment. Therefore, modern diagnostic strategies emphasize an integrated approach that includes hematological parameters, peripheral blood smear evaluation, and advanced immunopharmacological techniques.This literature review summarizes current evidence from 2020 to 2026 regarding the clinical presentation, laboratory features, immunopathogenesis, and diagnostic challenges of AIHA, with a particular focus on improving diagnostic accuracy and recognizing atypical forms of the disease.

Keywords: Autoimmune Hemolytic Anemia, DAT, Coombs Test, Hemolysis, Warm AIHA, Cold Agglutinin Disease, Laboratory Diagnosis, Immunohematology.

Introduction

Autoimmune hemolytic anemia is an immune system disorder in which the body produces antibodies that mistakenly target and destroy its own red blood cells. This process leads to varying degrees of anemia and may develop as a primary idiopathic condition or as a secondary complication of other diseases such as systemic lupus erythematosus, chronic lymphocytic leukemia, lymphomas, infections, or exposure to certain medications. The pathogenesis is based on a breakdown of immune tolerance, resulting in antibody-mediated erythrocyte destruction either through extravascular phagocytosis or complement activation [1].

Clinically, AIHA presents with symptoms related to anemia and hemolysis. Patients often experience generalized weakness, fatigue, shortness of breath, and reduced exercise tolerance. As hemolysis progresses, signs such as jaundice, dark urine, and tachycardia may appear. In more severe cases, splenomegaly can be

detected due to increased erythrocyte destruction in the spleen. Cold agglutinin disease may present with additional features such as acrocyanosis and symptoms exacerbated by cold exposure, reflecting the temperature-dependent nature of IgM antibody activity [2].

Laboratory evaluation plays a central role in diagnosis. A complete blood count typically reveals decreased hemoglobin levels, while reticulocyte counts are elevated as a compensatory response of the bone marrow. Biochemical markers of hemolysis include increased lactate dehydrogenase, elevated indirect bilirubin, and decreased haptoglobin levels. These findings collectively indicate active red blood cell destruction. Peripheral blood smear examination provides further diagnostic clues, as warm autoimmune hemolytic anemia is often associated with the presence of spherocytes, whereas cold agglutinin disease may demonstrate erythrocyte agglutination [5].

Materials and Methods

The direct antiglobulin test remains the most important diagnostic tool for confirming immune-mediated hemolysis. It detects immunoglobulin G antibodies and/or complement components bound to the surface of red blood cells. Despite its high diagnostic value, the test is not universally positive, and recent studies indicate that a proportion of patients with clinically confirmed AIHA may have negative DAT results. This limitation has led to increased use of more sensitive techniques, including gel card methods, flow cytometry-based assays, and solid-phase immunological testing [9].

AIHA is broadly classified into warm, cold, mixed, and DAT-negative forms. Warm autoimmune hemolytic anemia is the most common subtype and is mediated primarily by IgG antibodies that function optimally at body temperature. Hemolysis in this form is mainly extravascular and occurs in the spleen. Cold agglutinin disease, on the other hand, is mediated by IgM antibodies that bind to red blood cells at lower temperatures and activate the complement system, leading to hemolysis. Mixed AIHA combines features of both mechanisms and is generally associated with more severe disease manifestations. DAT-negative AIHA remains a diagnostic challenge and often requires extended immunopharmacological testing for confirmation [3].

One of the major difficulties in diagnosing AIHA is the presence of false-negative DAT results. This may occur due to low antibody density on erythrocytes, low-affinity antibodies, or limitations in conventional testing techniques. In such cases, advanced diagnostic approaches are essential to confirm the diagnosis. Another important aspect is distinguishing primary AIHA from secondary forms, as underlying conditions such as autoimmune diseases, malignancies, and infections significantly influence treatment strategies and prognosis. Furthermore, AIHA must be differentiated from other causes of hemolytic anemia, including hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, and thrombotic microangiopathies [7].

Modern diagnostic algorithms recommend a stepwise approach beginning with clinical suspicion of hemolytic anemia, followed by laboratory confirmation through hematological parameters and hemolysis markers, and finally immunological testing using DAT. In cases where DAT is negative or inconclusive, additional specialized testing is required. Recent literature emphasizes the importance of a comprehensive diagnostic strategy rather than reliance on a single laboratory test [11].

Recent advances in the field have significantly improved the understanding and diagnosis of AIHA. Enhanced sensitivity of modern DAT techniques, better characterization of complement-mediated hemolysis, and increased awareness of DAT-negative cases have contributed to more accurate diagnosis. Additionally, complement inhibition therapies are being investigated as potential treatment options, particularly for cold agglutinin disease. Standardization of diagnostic criteria in recent hematology guidelines has also improved clinical consistency across different healthcare settings [4].

Results and Discussion

Pathophysiological and Immunological Mechanisms. Autoimmune hemolytic anemia develops as a result of a loss of immune tolerance toward erythrocyte membrane antigens. Recent studies emphasize that the central mechanism involves dysregulation of both humoral and cellular immunity, particularly abnormal activation of B-lymphocytes and impaired T-regulatory cell function. This imbalance leads to uncontrolled production of autoantibodies directed against red blood cell surface proteins [15].

In warm autoimmune hemolytic anemia, immunoglobulin G (IgG) antibodies bind to erythrocyte antigens at physiological temperature. These coated erythrocytes are subsequently recognized by Fc-gamma receptors on splenic macrophages, leading to their phagocytosis. This process results in predominantly extravascular hemolysis. In some cases, partial phagocytosis of the erythrocyte membrane leads to the formation of spherocytes, which are less deformable and more susceptible to splenic sequestration [6].

Cold agglutinin disease is primarily mediated by immunoglobulin M (IgM) antibodies, which bind to red blood cells at lower temperatures, usually in peripheral circulation. This binding activates the classical complement pathway, leading to deposition of complement component C3b on the erythrocyte surface. Depending on the extent of complement activation, hemolysis may occur intravascularly or via hepatic clearance through Kupffer cells [8].

Recent immunological research highlights the role of complement system overactivation not only in cold agglutinin disease but also in severe and refractory cases of warm autoimmune hemolytic anemia. This has led to increasing interest in complement inhibitors as a potential therapeutic strategy [10].

Laboratory Diagnostic Interpretation. Laboratory findings in AIHA should always be interpreted as a combined pattern rather than isolated values. The severity of hemolysis can be indirectly estimated by the degree of lactate dehydrogenase elevation and the reduction of haptoglobin levels. Haptoglobin binds free hemoglobin released during intravascular hemolysis; therefore, its depletion is a sensitive marker of active red cell destruction [12].

Indirect bilirubin elevation reflects increased heme breakdown and hepatic processing overload. Reticulocytotic represents bone marrow compensatory response, although in severe or chronic cases, bone marrow exhaustion may lead to inadequate reticulocyte response despite ongoing hemolysis.

Peripheral blood smear remains a crucial diagnostic tool. The presence of micro spherocytes is highly suggestive of immune-mediated hemolysis, especially in warm AIHA. In contrast, erythrocyte agglutination is more characteristic of cold agglutinin disease and may cause spurious laboratory abnormalities such as falsely elevated mean corpuscular volume [14].

Differential Diagnosis of Autoimmune Hemolytic Anemia. Differential diagnosis is a critical step, as several hematological conditions can mimic AIHA clinically and laboratory. Hereditary spherocytosis is one of the most important conditions to exclude, as it also presents with spherocytes on peripheral smear. However, unlike AIHA, it is not associated with a positive direct antiglobulin test. Osmotic fragility testing or eosin-5-maleimide binding tests are typically used for confirmation [13].

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is another important cause of hemolytic anemia, particularly in response to oxidative stress. Unlike AIHA, hemolysis in G6PD deficiency is typically episodic and triggered by infections, drugs, or certain foods.

Thrombotic microangiopathies such as thrombotic thrombocytopenic purpura and hemolytic uremic syndrome present with microangiopathic hemolytic anemia, characterized by schistocytes on blood smear. These conditions are distinguished from AIHA by the absence of immune-mediated antibody binding and the presence of thrombocytopenia and organ dysfunction. Drug-induced immune hemolytic anemia must also be considered, as certain medications can induce antibody formation or non-immunological adsorption of proteins onto erythrocytes.

Modern Diagnostic Algorithm. Modern diagnostic approaches to autoimmune hemolytic anemia follow a structured sequential pathway. The initial step is clinical suspicion based on symptoms of anemia and hemolysis. Once suspected, a complete blood count is performed to confirm anemia and evaluate reticulocyte response. Subsequently, biochemical markers of hemolysis, including lactate dehydrogenase, bilirubin, and haptoglobin, are assessed to confirm ongoing red blood cell destruction. Peripheral blood smear examination provides morphological evidence supporting hemolysis and helps in preliminary subtype differentiation. The direct antiglobulin test is then performed to determine immune involvement. A positive result confirms the diagnosis in most cases; however, if the test is negative but clinical suspicion remains high, extended immunopharmacological investigations are required. These include more sensitive DAT techniques, flow cytometry-based assays, and antibody elution studies. Finally, identification of underlying etiological factors is essential. This includes screening for autoimmune diseases, malignancies, infections, and drug exposure, as management strategies differ significantly depending on the underlying cause.

Recent Advances in AIHA Diagnosis. Recent years have seen significant improvements in the diagnostic accuracy of autoimmune hemolytic anemia. Gel card agglutination methods have improved the sensitivity of direct antiglobulin testing, particularly in cases with low antibody density. Flow cytometry has emerged as a powerful tool for detecting low levels of immunoglobulin and complement deposition on erythrocytes. This has been especially useful in diagnosing DAT-negative cases, which were previously difficult to confirm.

Another important advancement is the growing understanding of complement-mediated hemolysis. This has led to the development of targeted therapies that inhibit complement activation pathways, offering new treatment options for refractory cases, particularly cold agglutinin disease.

Standardization of diagnostic criteria across international hematology guidelines has also improved consistency in diagnosis and reduced variability between laboratories.

Clinical significance, prognosis and therapeutic implications of AIHA. Autoimmune hemolytic anemia is not only a diagnostic challenge but also a clinically significant condition with variable prognosis depending on its subtype, severity, and underlying etiology. Recent literature emphasizes that disease outcomes are strongly influenced by whether AIHA is primary or secondary, as well as by the rapidity of diagnosis and initiation of appropriate therapy. In many cases, delayed recognition leads to severe anemia requiring transfusion support and, in rare situations, life-threatening complications such as acute hemolytic crisis [1].

The prognosis of warm autoimmune hemolytic anemia is generally considered more favorable when compared to cold agglutinin disease, although relapsing courses are common. Patients with warm AIHA often respond well to first-line corticosteroid therapy, which suppresses autoantibody production and reduces macrophage-mediated erythrocyte destruction. However, long-term disease control may be complicated by steroid dependence or resistance, necessitating the use of second-line treatments such as rituximab or splenectomy in selected cases [2].

Cold agglutinin disease tends to have a more chronic clinical course, particularly in older patients. Hemolysis in this subtype is primarily driven by complement activation, which explains the increasing interest in complement-targeted therapies. Recent studies have demonstrated promising results with complement inhibitors that block early stages of the classical pathway, leading to reduced hemolysis and improvement in hemoglobin levels. This therapeutic direction represents a significant advancement in the management of refractory cases [3].

Mixed autoimmune hemolytic anemia is associated with a more severe clinical presentation and often requires aggressive and combined therapeutic approaches. These patients may present with both IgG- and IgM-mediated mechanisms of hemolysis, making treatment response less predictable and requiring close laboratory monitoring [4].

DAT-negative autoimmune hemolytic anemia presents a unique clinical challenge not only in diagnosis but also in management. Because confirmation is more complex, treatment initiation may be delayed, which can negatively affect outcomes. Recent publications suggest that once hemolysis is confirmed by indirect laboratory markers and alternative immunopharmacological testing, treatment should not be postponed solely due to negative DAT results [5].

From a prognostic perspective, secondary autoimmune hemolytic anemia is generally associated with a less favorable outcome compared to primary forms, as it reflects an underlying systemic disease such as systemic lupus erythematosus, chronic lymphocytic leukemia, or other malignancies. In such cases, successful management depends not only on controlling hemolysis but also on treating the underlying disorder [6].

Modern therapeutic strategies increasingly rely on individualized approaches based on disease subtype, severity of hemolysis, and patient comorbidities. Corticosteroids remain the cornerstone of initial therapy in warm AIHA, while rituximab has become an important second-line option due to its ability to target CD20-positive B cells and reduce autoantibody production. In selected cases, splenectomy may still be considered, although its use has declined due to the availability of less invasive immunotherapies [7].

In cold agglutinin disease, avoidance of cold exposure remains a fundamental non-pharmacological intervention. Pharmacological treatment focuses on inhibiting complement activation pathways, which has become an important area of ongoing research. The introduction of targeted biological therapies has significantly changed the therapeutic landscape of this disease subtype [8].

Differential diagnosis and common diagnostic pitfalls in AIHA. Autoimmune hemolytic anemia is frequently misdiagnosed in clinical practice due to the overlap of its laboratory and clinical features with other forms of hemolytic and non-hemolytic anemias. Recent literature emphasizes that accurate differentiation is essential, as misclassification can lead to inappropriate therapy, delayed treatment, and worsening of patient outcomes. The complexity of AIHA arises from the fact that hemolysis itself is a shared final pathway of many hematological and systemic disorders, while immune-mediated mechanisms are not always easily identifiable with standard laboratory testing [9].

One of the most important conditions to exclude in the differential diagnosis is hereditary spherocytosis. This inherited membrane defect leads to chronic hemolytic anemia and also presents with spherocytes on peripheral blood smear, which can closely resemble findings in warm autoimmune hemolytic anemia. However, in hereditary spherocytosis, the direct antiglobulin test remains negative, and additional diagnostic methods such as eosin-5-maleimide binding tests or osmotic fragility testing are required for confirmation. Unlike AIHA, this condition has a genetic basis and typically presents earlier in life [10].

Glucose-6-phosphate dehydrogenase deficiency represents another important differential diagnosis. Hemolysis in this condition is triggered by oxidative stress, infections, or certain drugs, and often occurs in episodic patterns. Laboratory findings may include hemoglobin drop, elevated lactate dehydrogenase, and increased bilirubin, which can closely mimic AIHA during acute episodes. However, the absence of autoantibodies and a negative DAT test help differentiate this disorder from immune-mediated hemolysis. Enzyme activity testing is required for definitive diagnosis [11].

Thrombotic microangiopathies, including thrombotic thrombocytopenic purpura and hemolytic uremic syndrome, are particularly important to exclude due to their high mortality risk if untreated. These conditions are characterized by microangiopathic hemolytic anemia with schistocytes on peripheral blood smear, thrombocytopenia, and varying degrees of organ dysfunction, especially neurological and renal involvement. In contrast to AIHA, these disorders are not mediated by autoantibodies against erythrocytes, and the direct antiglobulin test is negative. Immediate recognition is critical because management strategies differ significantly and require urgent plasma exchange or targeted therapy [12].

Another clinically important differential diagnosis is drug-induced immune hemolytic anemia. Certain

medications can induce the formation of antibodies that either directly target red blood cells or bind to them in the presence of drug-dependent mechanisms. This condition can closely resemble idiopathic AIHA both clinically and laboratory-wise. However, a detailed drug history and resolution of hemolysis after discontinuation of the offending agent are key diagnostic clues. In some cases, specialized serological testing is necessary to identify drug-dependent antibodies [13].

Non-immune causes of hemolysis, such as mechanical hemolysis due to prosthetic heart valves or severe vascular abnormalities, must also be considered. These conditions typically present with schistocytes on peripheral smear and elevated hemolysis markers, but without evidence of immune involvement. The absence of a positive DAT test helps distinguish these cases from autoimmune etiologies [14].

A major diagnostic challenge highlighted in recent studies is the presence of DAT-negative autoimmune hemolytic anemia, which may be incorrectly classified as non-immune hemolysis or other hematological disorders [15].

Conclusion

Autoimmune hemolytic anemia is a complex and heterogeneous immune-mediated disorder that requires careful integration of clinical findings and laboratory investigations for accurate diagnosis. While the direct antiglobulin test remains the cornerstone of diagnosis, its limitations highlight the necessity of additional diagnostic methods, particularly in atypical or DAT-negative cases. A comprehensive approach that includes evaluation of hemolysis markers, peripheral blood smear morphology, and advanced immunopharmacological testing is essential for correct diagnosis.

Recent studies emphasize the importance of standardized diagnostic algorithms and multidisciplinary evaluation in improving diagnostic accuracy and patient outcomes. Continued advances in laboratory technology and a deeper understanding of immune mechanisms are expected to further enhance the diagnostic process in the coming years.

References

[1] Alexander Röth, Wilma Barcellini, Shirley D'Sa, Yoshitaka Miyakawa, Catherine M. Broome, Marc Michel, David J. Kuter, Bernd Jilma, Tor H.A. Tvedt, Joachim Fruebis, Xiaoyu Jiang, Stella Lin, Caroline Reuter, Jaime Morales-Arias, William Hobbs. New England Journal of Medicine. 2021. doi: 10.1056/NEJMoa2027760.

[2] Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Front Immunol. 2020;11:946. doi: 10.3389/fimmu.2020.00946.

[3] Barcellini W, Fattizzo B. Autoimmune hemolytic anemia: causes and consequences. Expert Rev Clin Immunol. 2022;18(7):731-745. doi: 10.1080/1744666X.2022.2089115.

[4] Barcellini W, Fattizzo B. Strategies to overcome the diagnostic challenges of autoimmune hemolytic anemias. Expert Rev Hematol. 2023;16(7):515-524. doi: 10.1080/17474086.2023.2216930.

[5] Blackall D, Dolatshahi L. Autoimmune hemolytic anemia in children: laboratory investigation, disease associations, and treatment strategies. J Pediatr Hematol Oncol. 2022;44(3):71-78. doi: 10.1097/MPH.0000000000002438.

[6] Claudia MV, Javiera AA, Sebastián NS, José FR, Gloria L. Interplay between desiccation and oxidative stress responses in iron-oxidizing acidophilic bacteria. J Biotechnol. 2024;383:64-72. doi: 10.1016/j.jbiotec.2024.01.017.

[7] Elneil S, Lalezari JP, Pourhassan NZ. Case study of a critically ill person with COVID-19 on ECMO successfully treated with leronlimab. J Transl Autoimmun. 2021;4:100097. doi: 10.1016/j.jtauto.2021.100097.

[8] Fattizzo B, Barcellini W. Autoimmune hemolytic anemia: causes and consequences. Expert Rev Clin Immunol. 2022;18(7):731-745. doi: 10.1080/1744666X.2022.2089115.

[9] Jacobs JW, Raza S, Clark LM, et al. Mixed autoimmune hemolytic anemia: a systematic review of epidemiology, clinical characteristics, therapies, and outcomes. Am J Hematol. 2025;100(8):1397-1407. doi: 10.1002/ajh.27721.

[10] Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. Blood Rev. 2020;41:100648. doi: 10.1016/j.blre.2019.100648.

[11] Loriamini M, Cserti-Gazdewich C, Branch DR. Autoimmune hemolytic anemias: classifications, pathophysiology, diagnoses and management. Int J Mol Sci. 2024;25(8):4296. doi: 10.3390/ijms25084296.

[12] Nayyar V, Bhutia O, Kakkar A, Mishra D. Primary intraosseous oncocytic mucoepidermoid carcinoma of the jaw: first case report. Oral Oncol. 2022;126:105732. doi: 10.1016/j.oraloncology.2022.105732.

[13] Patel PA, Gopali R, Reddy A, Patel KK. The representation of board members from developing countries on major international ophthalmological journals. Asia Pac J Ophthalmol (Phila). 2022;11(4):394-395. doi: 10.1097/APO.0000000000000481.

[14] Scheckel CJ, Go RS. Autoimmune hemolytic anemia: diagnosis and differential diagnosis. Hematol Oncol Clin North Am. 2022;36(2):315-324. doi: 10.1016/j.hoc.2021.12.001.

[15] Wu C, Zhang J, Zhang Y, Zeng Y. A 7.5-mV input and 88%-efficiency single-inductor boost converter with self-startup and MPPT for thermoelectric energy harvesting. Micromachines (Basel). 2022;14(1):60. doi: 10.3390/mi14010060.