CASE REPORT

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Case Report: Auto Anti-D, Auto-IgG, and Cold Agglutinins in a Rh(D) Positive Patient with CD5-Negative B Lymphoproliferative Disorder Causing Autoimmune Haemolytic Anaemia: A Single-Centre Experience

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ABSTRACT

Background: Autoimmune hemolytic anemia (AIHA) is defined by premature red blood cell destruction mediated by autoantibodies against self-antigens. Autoantibodies with specificity to the D antigen (auto-anti-D) are exceedingly uncommon. Their presence creates substantial diagnostic and transfusion-related challenges, particularly in patients with prior transfusion exposure or partial D variants, where differentiation between auto anti-D and allo anti-D is problematic. This distinction is clinically critical, as transfusion of Rh(D)-positive red cells in patients with unrecognized allo anti-D may precipitate severe hemolytic transfusion reactions.

Case Presentation: We describe a Rh(D)-positive male with a CD5-negative lymphoproliferative disorder who experienced a relapse of AIHA, having a prior history of auto-IgG–mediated hemolysis. The patient presented with typical manifestations of hemolysis, including fatigue, dyspnoea, and transfusion-refractory anemia. Notably, serological evaluation revealed the emergence of auto-anti-D antibodies in a Rh(D)-positive individual—an infrequent finding that may reflect immune dysregulation associated with disease progression. The coexistence of auto-IgG and newly detected cold agglutinins further underscores the complex immunopathogenesis of AIHA in this setting.

Conclusion: Although auto-anti-D antibodies are often regarded as having limited clinical significance, their presence alongside other autoantibodies may intensify hemolysis and worsen clinical outcomes. Awareness and accurate identification of this rare antibody profile are essential for optimizing transfusion strategies and guiding overall disease management.

Keywords: AIHA, auto anti-D, auto-IgG, cold agglutinins, CD5-negative lymphoproliferative disorder.
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INTRODUCTION

Autoimmune haemolytic anaemia (AIHA) is an acquired immune-mediated disorder characterized by premature destruction of red blood cells (RBCs) due to the formation of autoantibodies directed against erythrocyte surface antigens. These autoantibodies shorten RBC survival through Fc receptor–mediated phagocytosis and/or complement activation, leading to extravascular or intravascular hemolysis. The clinical spectrum of AIHA is heterogeneous and depends on the immunoglobulin class, thermal reactivity, and complement-fixing properties of the antibodies involved.[1]

AIHA is broadly classified based on the temperature at which autoantibodies optimally bind to erythrocytes. Warm antibody haemolytic anaemia (WAIHA) is mediated predominantly by IgG autoantibodies reacting at body temperature (≥37 °C) and accounts for approximately 70–80% of AIHA cases.[1,2] In contrast, cold agglutinin disease (CAD) is caused mainly by IgM autoantibodies that react at lower temperatures and activate the classical complement pathway, resulting in intravascular or mixed hemolysis. CAD is considerably less common, with an estimated annual incidence of approximately 1 per million individuals.[3]

Antibodies directed against Rh blood group antigens are rarely implicated in AIHA. Anti-D antibodies are typically alloantibodies formed in Rh(D)-negative individuals following exposure to the D antigen through pregnancy or transfusion of Rh(D)-positive red blood cells.[4,5] In exceedingly rare circumstances, autoantibodies with apparent anti-D specificity may develop in Rh(D)-positive individuals, targeting their own D antigen.[6] The true prevalence of auto anti-D is unknown due to its rarity and the diagnostic challenges in distinguishing it from allo anti-D, particularly in patients with a history of transfusion or those with variant or partial D phenotypes.[6,7]

The identification of auto anti-D antibodies has important clinical and transfusion implications. Failure to differentiate auto anti-D from allo anti-D may result in inappropriate transfusion of Rh(D)-positive blood and potentially severe hemolytic transfusion reactions.[8] This distinction becomes especially complex in patients with underlying lymphoproliferative disorders, where immune dysregulation may lead to the production of multiple autoantibodies and atypical serological patterns.

Auto-IgG antibodies are central to the pathogenesis of WAIHA and are frequently observed in secondary AIHA associated with autoimmune diseases, infections, and lymphoproliferative malignancies.[2,9] Cold-reactive antibodies, typically of the IgM class, are characteristic of CAD and may also be detected in post-infectious conditions, chronic lymphocytic leukaemia (CLL), and other B-cell lymphomas.[3,10] Although low-titre cold agglutinins may occasionally be present in healthy individuals, they are generally clinically insignificant unless associated with a wide thermal amplitude or high antibody titre.[10]

The coexistence of warm and cold autoantibodies, along with rare specificities such as auto anti-D, reflects a profound disruption of immune tolerance and highlights the complexity of AIHA pathophysiology, particularly in the setting of lymphoproliferative disorders. Accurate recognition of these atypical antibody profiles is essential for appropriate diagnostic interpretation, transfusion strategy, and overall patient management.

Based on those mentioned above, this case report aims to describe the rare occurrence of auto anti-D antibodies in a Rh(D)-positive patient with a CD5-negative lymphoproliferative disorder presenting with relapsed AIHA, to highlight the associated diagnostic and transfusion challenges, and to emphasize the clinical significance of recognizing complex autoantibody profiles, including the coexistence of auto-IgG and cold agglutinins, in guiding optimal patient management.

CASE PRESENTATION

An 85-year-old man with a known history of CD5-negative B-cell lymphoproliferative disorder presented with a relapse of autoimmune haemolytic anaemia (AIHA). The haemolytic process was mediated by an autoantibody exhibiting anti-D specificity, in conjunction with auto-IgG and suspected cold agglutinins. The patient was admitted from the outpatient clinic during a scheduled haematology follow-up. He reported a two-week history of fever, generalized lethargy, progressive dyspnoea, dizziness, and bilateral lower limb oedema.

Initial haematological evaluation demonstrated profound anaemia, with a haemoglobin concentration of 4.0 g/dL and a haematocrit of 14.4%, accompanied by a reticulocyte count of 2.86%, indicating an inadequate marrow compensatory response. The white blood cell count was markedly elevated at 195.03 $\times 10^3/\mu L$, consistent with the underlying lymphoproliferative disorder, while the platelet count remained within normal limits. Biochemical investigations revealed indirect evidence of haemolysis, including mildly elevated total bilirubin (36.9 µmol/L) and lactate dehydrogenase (LDH) levels (485 U/L).

Given the poor haemoglobin increment despite repeated packed red blood cell transfusions, further immunohematological investigations were performed to assess the presence of newly developed alloantibodies. The polyspecific direct antiglobulin test (DAT) was strongly positive (3+), with monospecific testing demonstrating 3+ reactivity for both anti-IgG and anti-C3d. Acid elution studies revealed pan-agglutination, with antibody identification indicating autoantibody activity with anti-D specificity. Red cell phenotyping confirmed a CDe/cde (R1r) phenotype. This serological profile represented a new finding, as previous antibody identification had demonstrated only auto-IgG. The concomitant presence of cold agglutinins could not be excluded, as indirect antiglobulin testing (IAT) showed persistent weak reactivity (1+) at 4 °C. Collectively, these findings support a diagnosis of mixed-type AIHA, likely exacerbated by progression of the underlying lymphoproliferative disorder. The summary of laboratory findings is depicted in **Table 1**.

Table 1. The laboratory results of the patient

Parameter	Results	Commentary
Haemoglobin	4.0 g/dL	Severe anaemia
Haematocrit	14.4%	Markedly reduced
Reticulocyte count	2.86%	Inadequate response relative to severity
White blood cell count	195.03 × $10^3/\mu L$	Markedly elevated; consistent with lymphoproliferative disorder
Platelet count	Within normal limits	Preserved megakaryopoiesis
Total bilirubin	36.9 µmol/L	Mildly elevated; haemolysis
Lactate dehydrogenase (LDH)	485 U/L	Elevated; haemolysis
Direct antiglobulin test (DAT)	Positive (3+)	Anti-IgG (3+), Anti-C3d (3+)
Acid elution	Pan-agglutination	Autoantibody with anti-D specificity
Red cell phenotype	CDe/cde (R1r)	Rh(D) positive
Cold agglutinin screening (IAT at 4 °C)	Weakly positive (1+)	Cold agglutinins not excluded

DISCUSSION

The clinical significance of auto anti-D antibodies lies in their heterogeneous haemolytic potential and the substantial diagnostic and transfusion challenges they introduce. Although auto anti-D is traditionally regarded as less clinically aggressive than allo anti-D, accumulating evidence indicates that it may contribute meaningfully to haemolysis, particularly when present alongside other autoantibodies such as warm-reactive IgG or complement-fixing cold agglutinins. This evolving understanding reflects the broader conceptual shift in AIHA, in which disease severity is increasingly viewed as a spectrum rather than a binary classification based solely on antibody type.[11]

Recent literature emphasizes that the clinical course of AIHA is strongly influenced by the complexity of the autoantibody profile and the underlying immune milieu. Patients with secondary AIHA, especially those with lymphoproliferative disorders, frequently exhibit mixed serological patterns and demonstrate poorer responses to transfusion and immunosuppressive therapy.[11-16] In this context, the emergence of auto anti-D may represent not an isolated serological phenomenon, but a marker of progressive immune dysregulation driven by clonal B-cell expansion and impaired immune tolerance.

Accurate identification of antibody specificity remains central to safe transfusion practice. Differentiating auto anti-D from allo anti-D is particularly challenging in patients with a history of transfusion or those harbouring variant or partial D antigens, as conventional serological testing may not reliably distinguish antibody origin.[12,13] Misclassification carries important clinical implications, as transfusion of Rh(D)-positive red blood cells to a patient with unrecognized allo anti-D can precipitate severe haemolytic transfusion reactions.[12] Consequently, careful integration of clinical history, serological findings, and, where available, molecular blood group genotyping is increasingly advocated in complex cases.[14]

A major challenge in the present case was the difficulty in identifying serologically compatible red cell units during cross-matching, as auto-anti-D may react with most Rh(D)-positive donor units, resulting in widespread incompatibility. Despite this, contemporary transfusion practice recognizes that complete serological compatibility should not delay transfusion in clinically unstable patients. Evidence suggests that transfusion of Rh(D)-positive red blood cells is generally safe in warm AIHA mediated by autoantibodies, provided that clinically significant alloantibodies have been reasonably excluded.[12,17] This principle underscores the need to prioritize physiological necessity over laboratory perfection in life-threatening anaemia.

In patients with severe haemolysis or transfusion refractoriness, and in whom differentiation between auto anti-D and allo anti-D remains uncertain, particularly those with prior transfusion exposure, it may be reasonable to consider the use of Rh(D)-negative red blood cells to minimize the risk of alloimmunization.[13] However, this strategy must be balanced against the limited availability of Rh(D)-negative blood units and the broader principles of blood stewardship. Close collaboration between clinicians and transfusion services is therefore essential to ensure judicious use of scarce resources while maintaining patient safety.[12]

In the present case, transfusion with Rh(D)-positive red blood cells was deemed appropriate based on several factors, including the patient's advanced age, confirmation of a CDe/cde (R1r) phenotype, and evidence of progressive lymphoproliferative disease. The coexistence of auto-IgG and suspected cold agglutinins further supports the diagnosis of mixed-type AIHA, a subtype increasingly recognized in association with lymphoid malignancies and often linked to more severe disease manifestations.[11,16] Mixed-type AIHA is also associated with higher rates of thromboembolic complications and transfusion dependency, reinforcing the need for heightened clinical vigilance.[15]

Overall, this case illustrates the dynamic nature of autoantibody evolution in AIHA and highlights the importance of repeated immunohematological assessment in patients with lymphoproliferative disorders. Recognition of rare autoantibody specificities such as auto anti-D is critical not only for transfusion decision-making but also for understanding disease progression and immune dysregulation in secondary AIHA.

This report is limited by its single-case design, which restricts the generalizability of the findings. Molecular RHD genotyping was not performed and could have provided additional insight into the presence of partial or variant D alleles. Furthermore, the transient nature and thermal amplitude of the suspected cold agglutinins could not be fully characterized due to the lack of serial testing. These limitations prevent definitive attribution of the relative haemolytic contribution of each autoantibody.

Future studies should focus on systematic characterization of rare autoantibody specificities, including auto anti-D, in patients with secondary AIHA. Integration of molecular blood group genotyping into routine practice may enhance diagnostic precision and transfusion safety. Multicentre registries and longitudinal studies are needed to better define the clinical course, transfusion outcomes, and optimal management strategies for mixed-type AIHA, particularly in the setting of lymphoproliferative disorders.

CONCLUSION

The development of auto-anti-D antibodies in Rh(D)-positive individuals is an infrequent finding. In this case, antibody identification demonstrated anti-D specificity consistent with auto-anti-D formation. The presence of an underlying CD5-negative lymphoproliferative disorder, together with a prior history of auto-IgG–mediated haemolysis, likely contributed to the emergence of this atypical serological profile. The concurrent detection of suspected cold agglutinins suggests a dynamic and evolving immune dysregulation, which may have been transient or previously undetected. While auto anti-D is often regarded as having limited clinical significance, its coexistence with other autoantibodies, particularly auto-IgG and cold-reactive antibodies, may amplify haemolytic activity and adversely affect clinical outcomes.

Comprehensive immunohematological evaluation is therefore essential to guide appropriate transfusion strategies and overall patient management in complex cases of autoimmune haemolytic anaemia.

ETHICS CONSIDERATION

This case report was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from the patient for the publication of this case and any accompanying laboratory data. Patient anonymity was strictly maintained, and no identifying information has been disclosed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this publication.

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AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the case report. The authors performed clinical data collection and laboratory investigations. Data interpretation, literature review, and manuscript drafting were undertaken collaboratively. All authors critically revised the manuscript for important intellectual content and approved the final version for publication.

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