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CASE REPORT

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Resolution of serologic problems due to cold agglutinin mediated autoimmune hemolytic anemia and its transfusion decision

Qiming Ying[1,2] | Dingfeng Lv[1,2] | Xiaomei Fu[2] | Shuanglu Shi[1,2] | Luyan Chen[2] | Yiwen He[2] | Jing Yang[2] | Shujun Yang[3] | Qitian Mu[3]

[1]School of Medicine, Ningbo University, Ningbo, China

[2]Department of Blood Transfusion, Ningbo City First Hospital, Ningbo, China

[3]The Laboratory of Stem Cell Transplantation, Department of Hematology, Ningbo City First Hospital, Ningbo, China

Correspondence
Qitian Mu, The Laboratory of Stem Cell Transplantation, Department of Hematology, Ningbo First Hospital, Ningbo, China.
Email: muqitian0219@163.com

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Abstract

Background: Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by hemolysis caused by autoantibodies against erythrocyte surface antigen. These antibodies can be classified as warm, cold, or mixed types.

Methods: We report two cases of cold agglutinin disease (CAD), which were eventually diagnosed owing to blood group discrepancy. Resolution was achieved after washing the red blood cells (RBCs) with warm saline and absorbing the autoantibodies at 4°C with the washed RBCs. We also assessed the patient's condition and discussed the strategy of blood transfusion.

Results: The first case occurred after postoperative chemotherapy for rectal cancer, and the other manifested with anemia from the outset. Direct antiglobulin tests were positive and revealed autoantibodies against C3d only. Cold agglutinin titration was performed, and the titers of both were 1:1024. Eventually, the patient's condition stabilized without blood transfusion.

Conclusion: The serological discrepancies observed in the blood transfusion department can successfully guide blood transfusion decisions in cases of CAD.

KEYWORDS
blood transfusion decision, cold agglutinins, serologic problems

1 | INTRODUCTION

Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by hemolysis. The autoantibodies may be primary or secondary to conditions such as acute infection or cancer, which can be classified as warm, cold, or mixed types of autoantibodies.[1] Cold reactive antibodies account for 25% of all types,[2] which mediate autoimmune hemolytic anemia and are further categorized into cold agglutinin disease (CAD), cold agglutinin syndrome (CAS), and paroxysmal cold hemoglobinuria.[3] These disorders can present as a primary disorder or secondary to other malignancies or infections.[4]

Cold agglutinins (CAs) were first described by Landsteiner in 1903, and Schubothe first defined CAD in 1966.[1] CAs can be detected in healthy individuals, but their titers are low and generally do not cause clinical symptoms.[1] In contrast, significant CA activity was found in 8.5% of 172 consecutive individuals, with titers between 512 and 65,500.[5] All individuals with detectable CAs had hemolysis, C3d was usually strongly positive, and IgG was

negative.[5] Pathological cold autoantibodies are characterized by high antibody titers, which usually cause autoimmune hemolytic anemia. CAs may cause incorrect estimation of hemogram parameters in routine hemogram tests,[6] and such cases may hinder blood grouping and cross-matching, which must be solved using specialized techniques. Clinicians often directly apply for blood because of the patients' low hemoglobin level and ignore the underlying cause of this condition, which leads to blood wastage. Although standard treatment methods for patients with CAD are available, details on how to accurately diagnose CAD through sample properties, clinical signs, and laboratory test results have not been reported. We report two cases of CAD, which were found due to blood group discrepancy.

2 | MATERIALS AND METHODS

We report two cases of CAD, which were eventually diagnosed owing to blood group discrepancy. Resolution was achieved after washing the RBCs with warm saline and absorbing the autoantibodies at 4°C with the washed RBCs. We also assessed the patient's condition and discussed the strategy of blood transfusion. The study was approved by the Ethics Committee of Ningbo First Hospital. All patients provided informed consent.

2.1 | Case details

2.1.1 | Case 1

A 73-year-old male patient underwent laparoscopic radical resection of rectal cancer (T3N1M0) 6 months prior. After four rounds of chemotherapy, without obvious inducement, approximately 300 ml of dark red blood was found around the colostomy pocket. He was admitted to the emergency department of our hospital with "enterostomy bleeding." During hospitalization, because of the decrease in erythrocytes (0.26×10^{12}/L), platelets (90×10^{9}/L), and leukocytes (2.61×10^{9}/L), especially neutrocytes (1.4×10^{9}/L) and lymphocytes (0.4×10^{9}/L), the patient was considered to have bone marrow suppression after chemotherapy. After the treatment, the bleeding improved, but the hemoglobin continued to decrease progressively. Routine blood cell counts revealed erythrocyte 0.26×10^{12}/L, hemoglobin 63 g/L, hematocrit (HCT) 8%, mean corpuscular volume (MCV) 119.4 fl, mean corpuscular hemoglobin (MCH) 101.5 pg, and mean corpuscular hemoglobin concentration (MCHC) of 850 g/L. The clinical application of blood transfusion was performed on November 1, 2020.

2.1.2 | Case 2

A 56-year-old female patient felt feeble 10 days prior without obvious inducement, accompanied by yellow staining of the skin and sclera. In the emergency department of our hospital, routine blood cell counts revealed a total erythrocyte count of 1.76×10^{12}/L, HCT 9.1%, hemoglobin 62 g/L, MCV 102.2 fl, MCH 67.4 pg, and MCHC 659 g/L. The emergency department requested two units of packed RBCs, and the patient was transferred to the hematology department with anemia and jaundice.

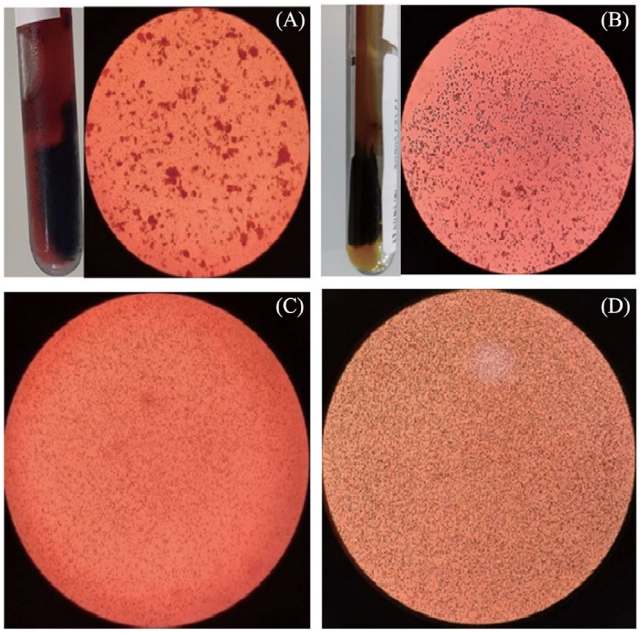

FIGURE 1 A, B, The properties of the patients' specimens in naked eyes and under the microscope (A: case 1, B: case 2). C, D, After washing with warm saline, the specimens were observed under a microscope (C: case 1, D: case)

Subsequently, two EDTA anticoagulant specimens were received in the Blood Transfusion Department (laboratory temperature, 23°C), with agglutinates visible to the naked eye. Under the microscope, the red blood cells (RBCs) agglutinated for 2–3+ (Figure 1A,B).

The blood grouping was performed using the column agglutination technique (CAT, Bioxun Biotech), and the reverse grouping of RBCs was performed with 0.8% concentration of cells (Bioxun Biotech). Forward and reverse grouping did not match, and we performed an antibody screen before all cross matches. Therefore, the indirect Coomb's test was performed using the 3-cell panel polyspecific card (Bioxun Biotech), which showed pan-reactivity (Figure 2A,B and Table 1).

Therefore, there are some questions regarding blood transfusion: What is the cause of hemolysis? How can we perform accurate blood group detection and antibody screening? Is blood transfusion the first choice for patients? If blood transfusion is necessary, how can we conduct it scientifically and reasonably?

We also evaluated the patients' medical records and examination results. The bleeding of the first patient improved after symptomatic treatment since admission, but his hemoglobin decreased gradually (Figure 3A) with total bilirubin (TBIL) 22.6 μmol/L, direct bilirubin (DBIL) 10.7 μmol/L, indirect bilirubin (IBIL) 11.9 μmol/L, and reticulocyte (0.03×10^{12}/L). Considering the possibility of

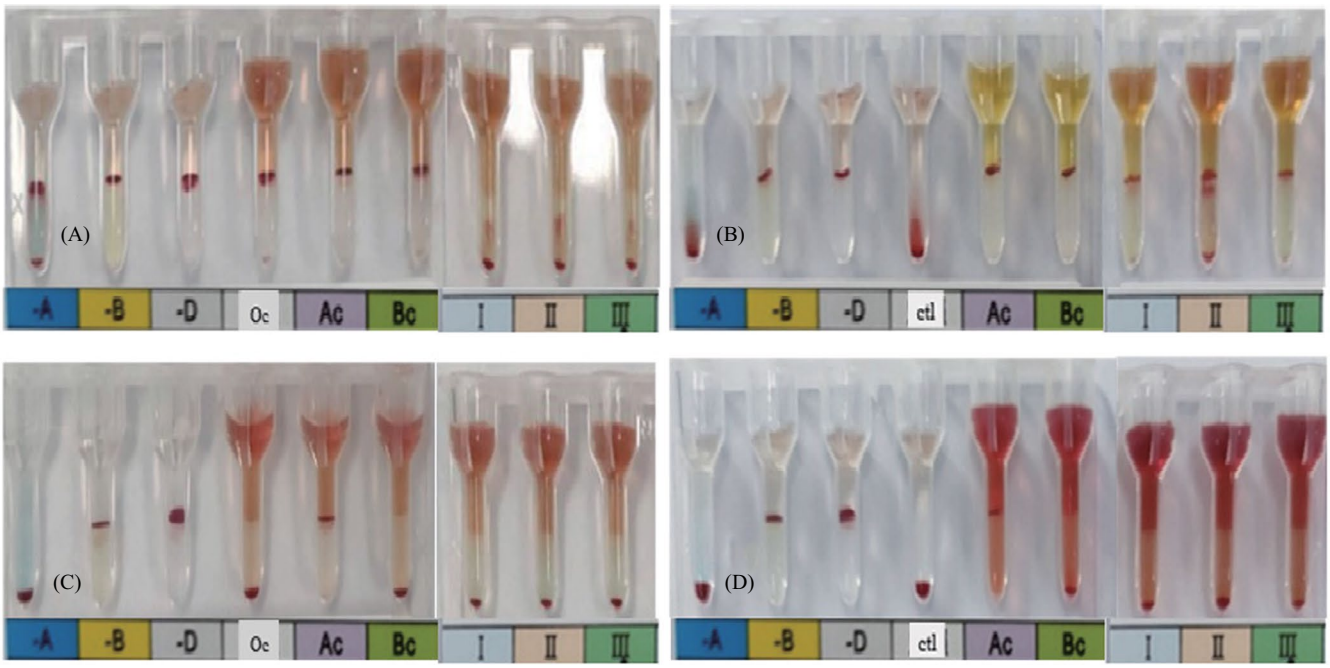

FIGURE 2 A, B, Blood group discrepancy and antibody screening (A: case 1; B: case 2). C, D, Blood grouping and antibody screening after treatment (C: case 1; D: case 2)

TABLE 1 Blood grouping and antibody screening comparison between before and after Treatment

		Forward grouping				Reverse grouping		Antibody screening		
Case		Anti-A	Anti-B	Anti-D	Ctl	A_1c	Bc	I	II	III
Case 1	Before	Dp	4+	4+	Dp	4+	4+	2+	2+	2+
	After	-	4+	4+	-	4+	-	-	-	-
Case 2	Before	2+	4+	4+	2+	4+	4+	4+	3+	4+
	After	-	4+	4+	-	4+	-	-	-	-

Abbreviations: A_1c, A_1 reagent cells; Anti-, Antibody-; Bc, B reagent cells; Ctl, control; Dp, double phenotypes.

other reasons for the low hemoglobin levels, we suggest that hematologists should postpone blood transfusion and conduct further tests.

The second patient was in a stable condition since admission, and her hemoglobin level was maintained at about 6 g/L, but she had reticulocytosis and hyperbilirubinemia: reticulocyte (0.17×10^{12}/L), TBIL 22.6 μmol/L, DBIL 10.7 μmol/L, and IBIL 11.9 μmol/L. Her lactate dehydrogenase level (579 U/L) was also high. Hemolytic anemia was considered in the hematology department, and further examinations should be completed.

Because of the patients' medical history and specimen characteristics and blood group test results, CAS was suspected, and further tests were conducted. First, the specimens were centrifuged at 1000 g for 3 min. RBCs and plasma were separated and transferred to clean test tubes. Next, the RBCs prepared in the previous step were incubated at 37°C for 15 min and washed three times with warm saline at 37°C to remove autoantibodies. The washed RBCs were used to observe agglutination under a microscope. The results showed that agglutination disappeared (Figure 1C,D). The ABO blood group was identified by reaction with anti-A, anti-B, and anti-D antibodies. We also performed the direct antiglobulin test (DAT) using the tube method with IgG, C3d, and IgG+C3d reagent (SHPBC). Finally, we mixed the plasma, washed RBCs 1:1, and absorbed the autoantibodies at 4°C for 1 h. During this period, the samples were mixed once every 10 min to facilitate complete reaction. After 1 h, the samples were centrifuged at 1000 g for 3 min, and the plasma was transferred to a clean test tube for reverse grouping and irregular antibody determination. In addition, because C3d was positive, we randomly selected packed RBCs for cross-matching using the saline and polybrene method (Baso).

Through the above experiments, the patients' blood groups were successfully identified, and the diagnosis was confirmed. After eliminating the interference of CAs, the forward and reverse grouping of the specimens were consistent: B positive, alloantibody negative, autoantibody-positive (Figure 2C,D and Table 1), C3d strongly positive, and IgG negative (Table 2). There was no hemolysis or agglutination in the primary and secondary sides of cross-matching using the saline and polybrene methods (Table 2).

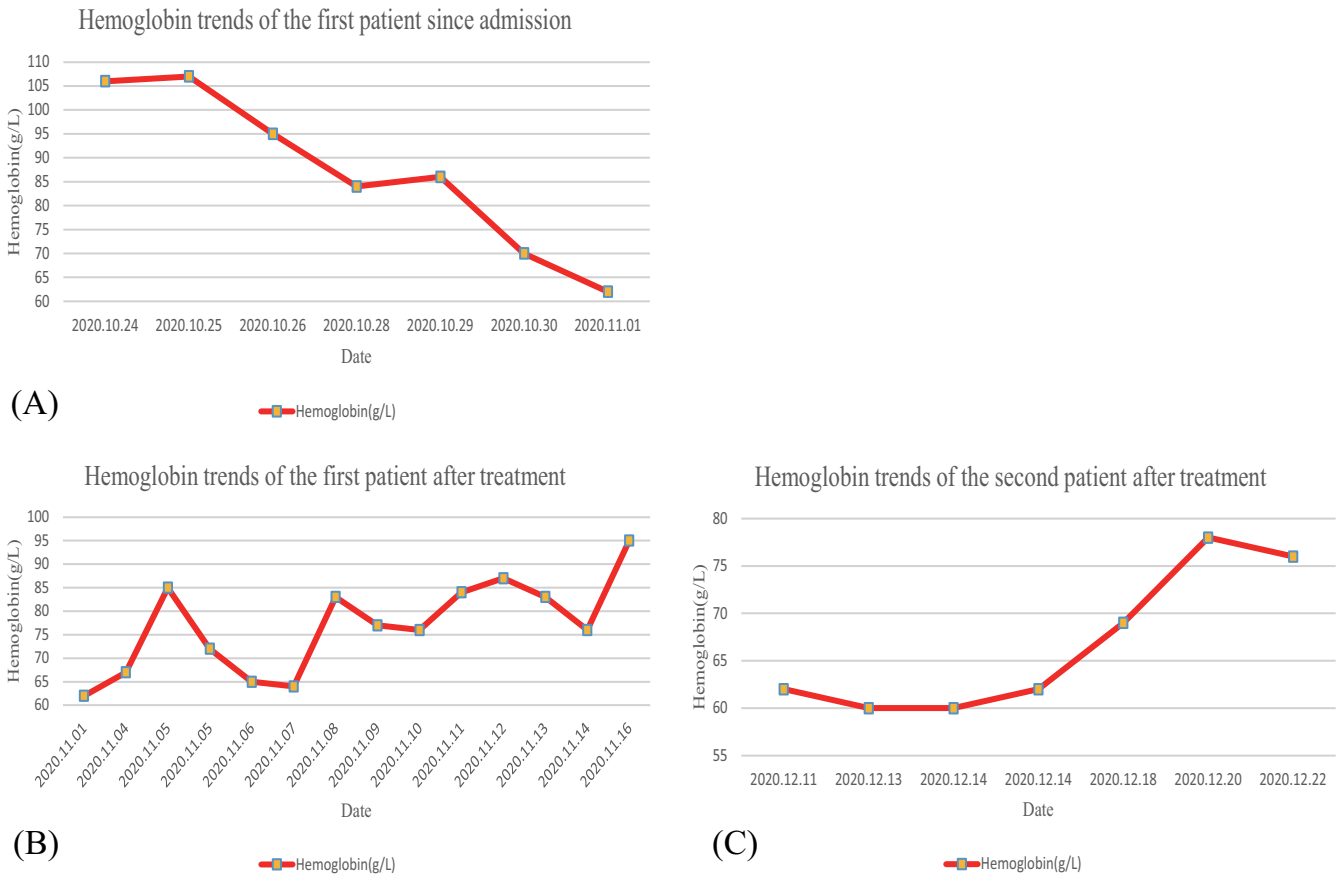

FIGURE 3 A, Hemoglobin trend of the first patient since admission. B, Hemoglobin trend of the first patient after treatment. C, Hemoglobin trend of the second patient after treatment

TABLE 2 Direct antiglobulin test and cross-match comparison between before and after treatment

		DAT			Cross-match			
					Saline method		Polybrene method	
Case		IgG	C3d	IgG+C3d	Primary side	Secondary side	Primary side	Secondary side
Case 1	Before	2+	4+	4+	3+	3+	3+	3+
	After	-	4+	4+	-	-	-	-
Case 2	Before	1+	4+	4+	3+	2+	2+	1+
	After	-	4+	4+	-	-	-	-

The cold agglutination titers of the two patients were both 1:1024. The first patient was considered to have CAS due to a malignant tumor. The second patient was considered to have primary CAD because no other primary disease was found, but she had high cold agglutinin titers, hemolytic anemia, reticulocytosis, hyperbilirubinemia, elevated lactate dehydrogenase, and positive Coombs testing for anti-C3 and negative anti-IgG.[7] High doses of intravenous immunoglobulin (IVIG) and dexamethasone were administered to both patients to improve the autoimmune hemolytic anemia, and rituximab was administered to the second patient for targeted therapy after the diagnosis of CAD. It was suggested that blood transfusion be postponed. Although bleeding occurred again in the first patient during treatment, the situation improved immediately after cessation of bleeding. Both patients' hemoglobin levels gradually increased (Figure 3B,C), without blood transfusion.

3 | DISCUSSION

Cold agglutinin disease is defined as "an AIHA characterized by a monospecific DAT strongly positive for C3d and a CA titer of ≥64 at 4°C. Patients may have a B-cell clonal lymphoproliferative disorder detectable in the blood or bone marrow but no clinical or radiological evidence of malignancy," as indicated in the recent international AIHA consensus document.[8] Compared with CAD, the

only difference in CAS is that patients have an associated condition.[8] By definition, "there may be occasional cases with a CA titer <64."[8]

As seen in our two cases, both CAD and CAS could lead to blood grouping and cross-matching difficulties due to CAs. Both patients had positive anti-C3 and negative anti-IgG, and their titers values were 1:1024. Laboratory parameters and clinical symptoms were consistent with their diagnosis. We successfully identified the patients' blood type and irregular antibodies by washing RBCs and absorbing plasma autoantibodies. Therefore, efforts should be made to eliminate the interference, and report should only be issued if the forward and reverse groupings are consistent. Unnecessary blood transfusion was avoided through effective communication with the clinic on the basis of the treatment of primary diseases.

In a study of CAD, a median hemoglobin level of 92 g/L was reported; only a quarter of them was severe (hemoglobin <80 g/L).[9] The severity of anemia is related to hemolysis markers. In our first case, the TBIL and reticulocytes were not very high, but were more evident in the second case. Therefore, the hemolysis of the second patient was more severe than that of the first, which was proved by the plasma state after absorption at 4°C (Figure 2C,D).

Because of the pentameric structure and large molecule size of IgM, RBCs can easily agglutinate after CAs bind to the cell surface. Thus, we speculated that the reason for the low RBC count in the blood routine was RBC agglutination, which led to an increase in MCV. MCH and MCHC also increased when hemoglobin levels did not decrease significantly.

Most CAs are specific to the Ii blood group system of carbohydrate antigens.[10] Only, the i antigen is expressed on neonatal RBCs, but I antigen is mainly expressed in children who are 18 months old or above.[10] Hence, the presence of anti-I and anti-i can be inferred from the agglutination of adult RBCs and umbilical cord RBCs, respectively. Unfortunately, we could not confirm the specificity of the cold antibody because of the lack of reagents.

High titers and thermal amplitude (TA) of CAs often cause distortion of the test, difficulties in blood grouping, and cross-matching. TA is defined as the highest temperature of CA reacting with antigen, and the pathogenicity of CA is mainly determined by TA rather than the titer.[11] If TA exceeds room temperature, RBCs agglutinate and are often followed by complement activation and hemolysis. In our cases, antibody screening showed that strong agglutination was still observed after incubation at 37°C for 15 min. Therefore, it was impossible to accurately determine the alloantibody or reverse grouping without an autoerythrocyte absorption test.

Regarding the treatment, steroids were considered as the first-line therapy in almost all cases of AIHA, with the exception of mild asymptomatic CAD.[12] Patients with low titers of antibodies and low TA may not need any additional treatment because the antibodies have no clinical significance. Steroids can decrease the affinity of antibodies to antigens on the surface of erythrocyte membranes, although some studies have shown that corticosteroids and other non-specific immunosuppression are generally ineffective in CAD,[8] other studies have reported that they also respond to CAD.[9,12] In addition, it has been reported that high-dose IVIG combined with the procedure can recover hemoglobin levels quickly, especially in those with lower pretreatment hemoglobin levels (<70 g/L).[13] Therefore, in our both cases, IVIG and steroids were used empirically and to improve the anemia symptoms; this treatment had a certain effect on the patients and their hemoglobin levels gradually increased (Figure 3B,C). Patients with CAD are usually treated with rituximab, which is effective. However, no therapy has been carried out for patients with secondary CAS, except for the treatment of primary disease.[8] However, since autoantibodies can also destroy the imported RBCs and aggravate hemolysis, blood transfusion should be avoided as much as possible.

Blood transfusion is only suitable for patients with a hemolytic crisis or severe anemia that may endanger life in a short time. During blood transfusion, careful monitoring, slow infusion, and maintaining warmth are necessary. The storage temperature of RBCs is 2–6°C. When RBCs are imported into the patients' bodies at this temperature, the RBCs are easily combined with high titer cold agglutinin and activate complement to produce hemolysis. Thus, the clinical medical staff should use a qualified dry heating instrument to preheat the RBCs before transfusion to maintain normal temperature. The patient, including the extremities used for transfusion, should also be kept warm. Furthermore, plasma exchange is an option for "first-aid" in critical situations that cannot wait for a specific therapeutic effect, because virtually all IgM is located intravascularly.[14]

4 | CONCLUSION

We can accurately judge the interference of CAs by sample properties, clinical signs, and laboratory test results. Avoiding unnecessary blood transfusion by communicating with the clinic is necessary. This can not only avoid the wastage of blood but also avert the secondary injury caused by adverse reactions to blood transfusion.

4.1 | Significance statement

Many studies have investigated the diagnosis and treatment of CAS and CAD. They are usually diagnosed by clinicians and use blood transfusions to improve anemia. Transfusion physicians usually play a small role. Although at present, the laboratory has mastered the common treatment methods for patients with CAD, how to accurately evaluate CAD through sample properties, clinical signs, and laboratory test results has not been reported in detail. We aim to provide clinical guidance to avoid unnecessary blood transfusions and improve the hemoglobin level of patients through medical management. I believe that the blood transfusion department is not only responsible for the transfer of blood in and out of the department, but also shoulders the important task of solving difficult blood transfusion problems for the clinic. Great attention should be paid to gain experience in routine work and implement effective communication with the clinic, and thus ensure the clinical use of blood scientifically, reasonably, and safely.

CONFLICT OF INTEREST

All authors have not reported any conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Qiming Ying https://orcid.org/0000-0002-5468-0502

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