

Cold-Reactive Erythrocyte Agglutination Causing Analytical Interference in Automated Hematology: A Case Report

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ABSTRACT

Cold-reactive erythrocyte agglutination is an important cause of analytical interference in automated hematology testing. We report a 55-year-old man with severe anemia, leukocytosis, thrombocytopenia, and suspected hematologic malignancy in whom hematological parameters changed substantially following specimen warming. The blood specimen had a sandy appearance and was incubated at 37°C before repeat analysis. Before incubation, WBC was 32,270/ μ L, RBC 0.29×10^6 / μ L, hemoglobin 8.3 g/dL, hematocrit 2.8%, MCH 283.3 pg, and MCHC 295.8 g/dL. Following incubation, WBC was 19,630/ μ L, RBC 1.93×10^6 / μ L, hemoglobin 6.1 g/dL, hematocrit 17.8%, MCH 31.7 pg, and MCHC 34.3 g/dL. Peripheral blood smear demonstrated erythrocyte agglutination and approximately 10% immature cells resembling myeloid blasts. This case highlights the importance of recognizing cold-reactive erythrocyte agglutination, appropriate specimen warming, and peripheral blood smear examination to improve interpretation of automated hematology results.

Keywords: Cold-reactive erythrocyte agglutination; peripheral blood smear; specimen warming

Introduction

Cold-reactive erythrocyte agglutination is a clinically and laboratorily important phenomenon in which red blood cells aggregate in response to cold-reactive antibodies, most commonly immunoglobulin M (IgM). This phenomenon may be appropriate occur in cold autoimmune hemolytic anemia (AIHA), primary cold agglutinin disease, or secondary cold agglutinin syndromes associated with infections, autoimmune conditions, and lymphoproliferative disorders [1–4]. When clinically significant, cold-reactive antibodies may contribute to complement-mediated hemolysis and anemia. However, the demonstration of erythrocyte agglutination alone does not establish a diagnosis of Cold-reactive erythrocyte agglutination, which requires correlation with evidence of hemolysis and appropriate immunohematological investigations, particularly direct antiglobulin testing (DAT) and, when indicated, cold agglutinin characterization [2,3].

From a laboratory perspective, Cold-reactive erythrocyte agglutination is particularly important because it can interfere with automated hematology analysis. Aggregated RBCs may be counted as single or abnormally large cellular particles, resulting in falsely decreased RBC counts and hematocrit values and disproportionately increased calculated erythrocyte indices, particularly mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) [5–7]. Such discrepancies may produce physiologically implausible results and potentially lead to misinterpretation if the specimen characteristics are not recognized. Prewarming the blood specimen to approximately 37–38°C is recommended when Cold-reactive erythrocyte agglutination interferes with hematological analysis because warming may disperse cold-induced RBC aggregates and improve the interpretability of the results [6,8,9]. Recent evidence has further demonstrated that severe Cold-reactive erythrocyte agglutination can substantially affect routine RBC parameters and that conventional warming procedures may not always completely correct the analytical interference [9].

Peripheral blood smear examination remains an important complementary laboratory method for recognizing RBC agglutination and evaluating other hematological abnormalities. Direct visualization of erythrocyte aggregates may provide an important clue to Cold-reactive erythrocyte agglutination, particularly when automated hematology results show marked discrepancies between RBC count, hematocrit, MCH, and MCHC [5,7]. In addition, examination of peripheral blood morphology may reveal immature or abnormal circulating cells that raise suspicion of an underlying hematologic disorder and indicate the need for further investigation [2,4].

This case report aims to describe the laboratory characteristics of suspected cold - reactive erythrocyte agglutination and its effect on automated hematology parameters before and after specimen incubation at 37°C, while highlighting the diagnostic value of peripheral blood smear examination in a setting where confirmatory immunohematological and hematologic investigations were unavailable. This case highlights the importance of recognizing suspected Cold-reactive erythrocyte agglutination-related analytical interference, appropriate specimen handling, and peripheral blood smear examination to obtain clinically interpretable hematological results.

Material and Methods

This case report describes the laboratory evaluation of a 55-year-old male patient presenting with severe anemia, leukocytosis, thrombocytopenia, and suspected hematologic malignancy. The evaluation focused on changes in automated hematological parameters following specimen warming and the morphological findings identified by peripheral blood smear examination. Clinical and laboratory data were obtained from the patient's medical and laboratory records, and patient confidentiality was maintained throughout the preparation of the case report.

Venous blood was collected into an ethylenediaminetetraacetic acid (EDTA) tube for complete blood count (CBC) analysis. The initial specimen was analyzed using a Mindray BC-700 automated hematology analyzer according to the laboratory's routine operating procedure. The parameters evaluated included white blood cell (WBC) count, differential leukocyte count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count. During pre-analytical evaluation, the blood specimen exhibited an abnormal sandy appearance, raising suspicion of cold-related erythrocyte agglutination and possible analytical interference. The specimen was subsequently incubated at 37°C for 30 minutes and reanalyzed using the same Mindray BC-700 analyzer under the same analytical conditions. Hematological parameters obtained before and after incubation were compared, with particular attention to RBC count, Hct, MCV, MCH, and MCHC, which may be substantially affected by cold-induced erythrocyte agglutination.

Peripheral blood smear examination was performed using the EDTA blood specimen. A blood film was prepared, air-dried, and stained using Wright staining according to the

laboratory's standard operating procedure. The stained smear was examined microscopically for erythrocyte morphology, including erythrocyte agglutination and other morphological abnormalities. Leukocyte morphology was also evaluated, with particular attention to immature or blast-like cells. Approximately 10% immature cells morphologically resembling myeloid blasts were identified on peripheral blood smear examination. The findings obtained before and after specimen warming were descriptively compared to assess changes in automated hematological parameters associated with specimen warming. The interpretation was based on the concordance between the physical appearance of the specimen, automated hematology results, and peripheral blood smear morphology.

Results

Clinical and Initial Laboratory Findings

A 55-year-old man presented with a three-day history of severe weakness, poor oral intake, and decreased consciousness. On admission, the patient was critically ill, with a Glasgow Coma Scale of 11, blood pressure of 60/26 mmHg, heart rate of 145 beats/minute, respiratory rate of 22 breaths/minute, body temperature of 37°C, and oxygen saturation of 95%. Physical examination revealed marked pallor and cold extremities. The patient had a previous history of anemia and had recently been hospitalized for severe anemia, suspected acute-on-chronic kidney disease, and septic shock, for which he had received three units of packed red blood cells.

Initial laboratory examination demonstrated severe anemia, leukocytosis, and thrombocytopenia. Blood urea was markedly elevated at 423 mg/dL, while serum creatinine was 8.8 mg/dL. During pre-analytical evaluation, the EDTA blood specimen showed an abnormal sandy appearance. This finding prompted further evaluation for possible cold-related erythrocyte agglutination and its potential effect on automated hematology results.

Hematological Findings Before and After Specimen Warming

The initial analysis performed using the Mindray BC-700 analyzer demonstrated marked abnormalities in several erythrocyte-related parameters. Before incubation, the WBC count was 32,270/ μ L, with neutrophils of 52.7%, lymphocytes of 42.0%, monocytes of 3.9%, eosinophils of 0.4%, and basophils of 1.0%. The RBC count was 0.29×10^6 / μ L, hemoglobin was 8.3 g/dL, and hematocrit was 2.8%. The MCV was 95.8 fL, whereas MCH and MCHC were markedly abnormal at 283.3 pg and 295.8 g/dL, respectively. The platelet count was 63,000/ μ L.

The specimen was subsequently incubated at 37°C for 30 minutes and reanalyzed

using the same hematology analyzer. Following incubation, the WBC count decreased to 19,630/ μ L, with an increase in neutrophil proportion to 86.5% and a decrease in lymphocyte proportion to 10.3%. The RBC count increased to $1.93 \times 10^6/\mu$ L and hematocrit increased to 17.8%, while hemoglobin decreased to 6.1 g/dL. The MCV remained relatively similar at 92.5 fL. In contrast, MCH and MCHC changed markedly to 31.7 pg and 34.3 g/dL, respectively. The platelet count was 54,000/ μ L. The comparison of hematological parameters before and after specimen warming is presented in Table 1.

Tabel 1. Comparison of Hematology Analyzer Result Before and After Incubation

Parameter	Before incubation	After incubation	Observation
WBC	32,270/ μ L	19,630/ μ L	Decreased
Neutrophils	52.70%	86.50%	Increased
Lymphocytes	42.00%	10.30%	Decreased
Monocytes	3.90%	3.00%	Slightly decreased
Eosinophils	0.40%	0.20%	Decreased
Basophils	1.00%	0%	Decreased
RBC	$0.29 \times 10^6/\mu$ L	$1.93 \times 10^6/\mu$ L	Markedly increased
Hemoglobin	8.3 g/dL	6.1 g/dL	Decreased
Hematocrit	2.80%	17.80%	Markedly increased
MCV	95.8 fL	92.5 fL	Relatively unchanged
MCH	283.3 pg	31.7 pg	Markedly decreased
MCHC	295.8 g/dL	34.3 g/dL	Markedly decreased
Platelet	63,000/ μ L	54,000/ μ L	Decreased

Peripheral Blood Smear Findings

Peripheral blood smear examination using Wright staining demonstrated erythrocyte agglutination consistent with findings suggestive of cold-reactive erythrocyte agglutination. In addition to the erythrocyte abnormalities, approximately 10% immature cells morphologically resembling myeloid blasts were identified. These findings were considered abnormal and raised suspicion of an underlying hematologic disorder requiring further hematological evaluation.

Overall, the most prominent changes following specimen warming were observed in the RBC count, hematocrit, MCH, and MCHC. The marked discrepancy between these parameters before warming and their substantially different values following incubation at 37°C, together with the observed erythrocyte agglutination on peripheral blood smear,

supported the presence of a significant specimen-related effect on automated hematological measurements. Peripheral blood smear examination demonstrated marked erythrocyte agglutination before specimen warming (Figure 1A), with extensive clustering of erythrocytes and reduced visualization of individual cells. Following incubation at 37°C for 30 minutes, the erythrocyte aggregates were markedly reduced, and individual erythrocytes became more clearly separated (Figure 1B). This morphological change was consistent with the substantial changes observed in the automated RBC-related parameters following specimen warming.

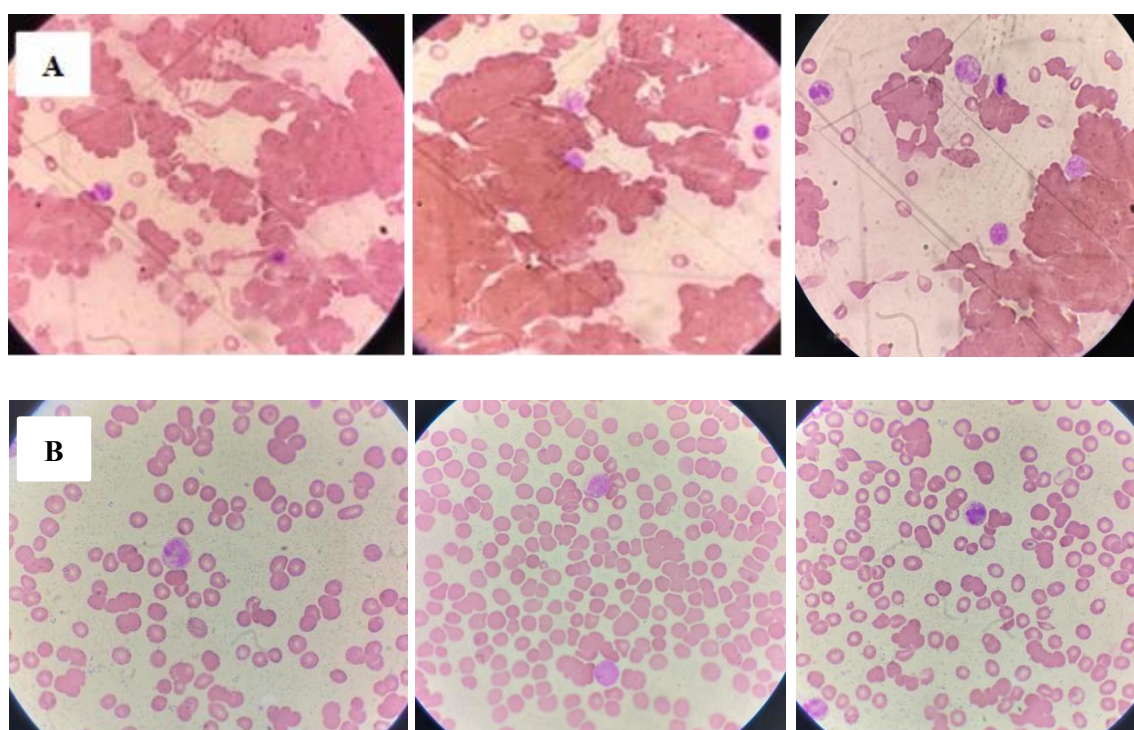


Figure 1. Peripheral blood smear before and after specimen warming. (A) Before incubation, marked erythrocyte agglutination was observed. (B) After incubation at 37°C for 30 minutes, erythrocyte agglutination was markedly reduced. Wright stain, 1000x oil immersion

This case report was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent for publication of the case report, including the clinical and laboratory data and peripheral blood smear images, was obtained from the patient's legally authorized representative. Patient confidentiality was maintained throughout the preparation and publication of this report, and no personally identifiable information is disclosed.

Discussion

Suspected cold-reactive erythrocyte agglutination is an important pre-analytical and analytical challenge in hematology testing because it can substantially interfere with

automated red blood cell measurements. In the present case, the combination of an abnormal sandy appearance of the EDTA specimen, marked erythrocyte agglutination on peripheral blood smear, and substantial changes in RBC-related parameters after incubation at 37°C strongly supported the presence of temperature-dependent erythrocyte agglutination. However, because direct antiglobulin testing (DAT) and specific cold agglutinin studies were not performed, the findings should be interpreted as suggestive of cold-reactive erythrocyte agglutination rather than definitive evidence of cold autoimmune hemolytic anemia (AIHA).

Cold autoimmune hemolytic anemia (AIHA) is an immune-mediated disorder in which cold-reactive antibodies, predominantly immunoglobulin M (IgM), bind to erythrocytes at temperatures below physiological body temperature and may induce erythrocyte agglutination and complement-mediated hemolysis [1,2,4,7]. In the present case, the combination of an abnormal sandy appearance of the EDTA blood specimen, marked erythrocyte agglutination on peripheral blood smear, and substantial changes in automated hematological parameters following incubation at 37°C strongly suggested the presence of cold-reactive erythrocyte agglutination. The case is clinically relevant because Cold-reactive erythrocyte agglutination may not only contribute to hemolytic anemia but can also produce significant analytical interference in routine hematology testing [5,7].

The most prominent laboratory finding in this case was the marked discrepancy in RBC-related parameters before and after specimen warming. Before incubation, the analyzer reported an extremely low RBC count of $0.29 \times 10^6/\mu\text{L}$ and hematocrit of 2.8%, accompanied by markedly elevated MCH of 283.3 pg and MCHC of 295.8 g/dL. After incubation at 37°C for 30 minutes, the RBC count increased to $1.93 \times 10^6/\mu\text{L}$ and hematocrit to 17.8%, while MCH and MCHC decreased to 31.7 pg and 34.3 g/dL, respectively. This pattern is consistent with the recognized analytical effect of Cold-reactive erythrocyte agglutination on automated cell counting. When erythrocytes aggregate, multiple RBCs may be recognized by the analyzer as a single larger cellular event, leading to an apparent reduction in RBC count and distortion of calculated erythrocyte indices [5,7,10]. Previous reports have similarly demonstrated falsely decreased RBC and hematocrit values with disproportionately increased MCH and MCHC in specimens containing cold agglutinins [5,10].

The peripheral blood smear findings provide important morphological support for this interpretation. Before warming, extensive erythrocyte aggregation was observed, whereas after incubation at 37°C for 30 minutes, the aggregates were markedly reduced and individual erythrocytes became more clearly separated. This morphological change parallels

the changes in automated RBC parameters and supports the hypothesis that cold-dependent erythrocyte aggregation contributed substantially to the pre-warming analytical abnormalities. Similar findings have been described in previous laboratory case reports, in which erythrocyte clusters observed on peripheral blood smear disappeared or were markedly reduced after warming the specimen to 37°C [5,8]. Thus, peripheral blood smear examination remains an important complementary method when automated hematology results show physiologically implausible RBC indices.

The response to warming in this case is also consistent with previous studies evaluating prewarming as a corrective laboratory procedure. Xie et al. 2021., reported that preheating specimens containing high-titer cold agglutinins improved RBC-related parameters, including RBC count, hematocrit, MCH, and MCHC [11]. Similarly, studies using alternative analytical channels or warmed specimens have demonstrated that Cold-reactive erythrocyte agglutination can be recognized through abnormal MCHC values and discrepancies among RBC-related parameters [6,10]. More recently, Yang et al. demonstrated that severe Cold-reactive erythrocyte agglutination can significantly interfere with routine RBC parameters and evaluated a 30-minute warming procedure at 37°C as part of a strategy to obtain more reliable results [9]. These findings are particularly relevant to the present case because the same warming duration of 30 minutes was used before repeat analysis on a Mindray BC-700 analyzer.

Although warming produced substantial changes in RBC count, hematocrit, MCH, and MCHC, the hemoglobin concentration in the present case decreased from 8.3 g/dL before incubation to 6.1 g/dL after incubation. This finding should be interpreted cautiously. Hemoglobin measurement is analytically different from the calculation of RBC indices and is not expected to change in the same manner as RBC count solely because of erythrocyte agglutination. Therefore, the decrease in hemoglobin should not be interpreted as evidence that warming caused hemolysis or as a direct corrective effect of warming. The discrepancy may reflect differences in specimen characteristics, analytical conditions, or the patient's underlying severe anemia. Consequently, the post-warming RBC-related indices, particularly the normalization of MCH and MCHC, should be interpreted together with the peripheral blood smear rather than relying on a single parameter.

The WBC count also decreased from 32,270/ μ L to 19,630/ μ L after warming, while the differential count changed from a relatively balanced neutrophil and lymphocyte distribution to predominant neutrophilia. These findings indicate that temperature-dependent or specimen-related effects may not be restricted exclusively to RBC parameters. However,

the biological significance of the WBC change cannot be determined from this single case, and it may also reflect the patient's critical clinical condition, including suspected infection or systemic stress. Therefore, the principal analytical interference in this case remains the marked alteration in RBC-related parameters and their correlation with the morphological findings.

Another important finding was the presence of approximately 10% immature cells morphologically resembling myeloid blasts on peripheral blood smear. In the context of severe anemia, leukocytosis, and thrombocytopenia, this finding raised suspicion of an underlying hematologic disorder. Cold-antibody-mediated AIHA may occur in association with neoplastic disorders, particularly lymphoproliferative diseases, and appropriate evaluation for an underlying hematologic condition is recommended when Cold-reactive erythrocyte agglutination is suspected [2,12]. Primary cold agglutinin disease itself is now recognized as a clonal B-cell lymphoproliferative disorder, whereas cold agglutinin syndrome may occur secondary to other diseases, including malignant lymphoproliferative disorders [12,13]. Nevertheless, the immature cells observed in the present case were described as cells resembling myeloid blasts rather than confirmed blasts. Morphological assessment alone is insufficient to establish a diagnosis of acute leukemia or another specific hematologic malignancy. Further investigations, including flow cytometry and bone marrow examination, would therefore be required to characterize these cells and determine the underlying hematologic disease.

The present case also emphasizes the importance of pre-analytical observation in laboratory hematology. The abnormal sandy appearance of the specimen was an early warning sign that prompted additional investigation before the hematological results were interpreted. Cold-reactive erythrocyte agglutination can produce results that are physiologically implausible, and failure to recognize this phenomenon may lead to inappropriate clinical interpretation of severe anemia or abnormal RBC indices [5,7]. A practical laboratory approach includes reviewing the analyzer flags and RBC histograms, correlating RBC count with hematocrit and hemoglobin, inspecting the specimen visually, examining the peripheral blood smear, and repeating the analysis after appropriate warming when Cold-reactive erythrocyte agglutination is suspected [9-11]. Recent literature emphasizes that temperature control during specimen handling is particularly important in the laboratory evaluation of cold agglutinin disease [4,14].

An important limitation of this case is the absence of confirmatory immunohematological investigations, including direct antiglobulin testing (DAT), cold

agglutinin titer, thermal amplitude testing, and immunoglobulin characterization. These examinations are important for establishing and classifying Cold-reactive erythrocyte agglutination and distinguishing cold agglutinin disease from secondary cold agglutinin syndrome [2,4,13]. In addition, flow cytometry and bone marrow examination were not available in the present evaluation to confirm the nature of the approximately 10% immature cells resembling myeloid blasts. Therefore, the findings should be described as suggestive of cold-reactive erythrocyte agglutination and suspected underlying hematologic malignancy rather than as definitive diagnoses.

This case demonstrates that cold-reactive erythrocyte agglutination can produce profound analytical discrepancies in automated hematology testing and that these abnormalities may be recognized through the combination of specimen appearance, internal consistency of CBC parameters, specimen warming, and peripheral blood smear examination. The marked reduction in the implausibly elevated MCH and MCHC and the improvement in RBC aggregation after warming support the presence of temperature-dependent analytical interference. Future studies should evaluate standardized laboratory algorithms for detecting Cold-reactive erythrocyte agglutination, including predefined MCHC or RBC/Hct discrepancy thresholds, analyzer flags and histograms, optimal warming temperature and duration, and the use of alternative analytical channels. Prospective studies involving larger numbers of specimens would also be valuable for determining the most reliable and reproducible strategy for correcting Cold-reactive erythrocyte agglutination interference in different hematology analyzer platforms.

Conclusion

In conclusion, this case demonstrates that cold-reactive erythrocyte agglutination can cause substantial analytical interference in automated hematology testing. The combination of abnormal specimen appearance, peripheral blood smear findings, implausible RBC-related parameters, and improved analytical accuracy after warming at 37°C provides a practical laboratory approach to recognizing this phenomenon. Although the findings raised suspicion of cold antibody-mediated hemolysis and a possible underlying hematologic disorder, definitive classification as Cold-reactive erythrocyte agglutination or a specific hematologic malignancy was not possible because confirmatory immunohematological and hematological investigations were unavailable.

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