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CASE REPORT

Impact of Severe Cold Agglutination on Routine Red Blood Cell Parameters and Correction Strategies

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SUMMARY

Background: **In blood samples collected from patients with cold agglutinin disease (CAD), red blood cells (RBCs) aggregate due to antigen-antibody reactions mediated by auto-IgM-type cold agglutinins, forming reversible clumps. This phenomenon is relatively common in clinical hematology testing and can significantly interfere with routine parameter measurements by automated hematology analyzers, including red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Notably, conventional methods such as 37°C water baths often fail to correct severely cold-agglutinated specimens.**

Methods: **Cold agglutinated samples were subjected to a 30-minute 37°C water bath, then immediately analyzed using the closed-whole blood-CDR/PLT-8X mode on a Mindray BC-7500 [NR] CS hematology analyzer to obtain results for the research parameters RBC-O and HF-MCV.**

Results: **Using the formulas HCT = RBC-O x HF-MCV, MCH = HGB/RBC-O, and MCHC = HGB/HCT, the red blood cell-related parameters such as HCT, RBC, MCV, MCH, and MCHC were calculated.**

Conclusions: **Severely cold-agglutinated blood samples, when analyzed using the 37°C water bath combined with the closed-whole blood-CDR/PLT-8X mode, can effectively correct the abnormal results of red blood cells (RBCs) and their related parameters caused by cold agglutinins.**

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KEYWORDS

cold agglutination, red blood cell (RBC) parameters, reticulocyte channel

CASE PRESENTATION

An elderly female patient with a 20 year history of chronic obstructive pulmonary disease (COPD) was admitted to the hospital on April 30, 2025, due to recent exacerbation of symptoms following exposure to cold. Her clinical presentation included worsening cough and sputum production, associated with chest tightness, shortness of breath, and inability to lie supine at night. The admission diagnoses were as follows: 1) Acute exacerbation of chronic obstructive pulmonary disease (COPD); 2) Bronchial asthma; 3) Bronchiectasis with infection; 4) Type II respiratory failure; 5) Cor pulmo-

nale (pulmonary heart disease); 6. Heart failure; 7. COVID-19 infection. After admission to the department, the patient's respiratory failure worsened, accompanied by altered consciousness. She underwent endotracheal intubation and mechanical ventilation, followed by intensive care. Therapeutic interventions included meropenem and levofloxacin for anti-infection and anti-inflammatory therapy, acid-suppressive and expectorant-bronchodilator medications, anticoagulation, albumin supplementation, enteral nutrition support, fluid replacement, and maintenance of water-electrolyte balance. During the treatment period, routine blood tests were performed daily (from May 1, 2025, to May 28, 2025). Laboratory staff observed a gradual decline in the patient's RBC count, with a concurrent increase in MCHC values. By May 16, 2025, the hematology analyzer failed to generate RBC-related parameters. The trends of RBC parameters are shown in Figures 1 and 2. On May 11, 2025, the analyzer issued an alert: "RBC agglutination?" The blood sample exhibited fine sand-like particles, raising suspicion of RBC agglutination caused by cold agglutinins. The specimen was treated with 37°C water bath for 30 minutes, but RBC parameters remained uncorrected. After a 1-hour water bath at 37°C, the hematology analyzer successfully detected RBC parameters; however, compared with historical results, the RBC count remained low, while MCH and MCHC values were elevated. Additionally, immediate blood smear staining and microscopy after 1-hour 37°C water bath revealed persistent partial RBC agglutination, indicating incomplete correction of RBC parameters. Are there other effective methods to resolve RBC agglutination induced by cold agglutinins? Furthermore, the laboratory communicated with clinicians regarding the observed RBC agglutination phenomenon in the patient's blood samples.

Method: Following incubation at 37°C for 30 minutes in a water bath, the specimen was analyzed using the Mindray BC7500[NR]CS fully automated hematology analyzer under the closed-whole blood-CDR/PLT-8X mode to perform complete blood count (CBC) testing.

Analysis: Upon reviewing literature, there are currently four methods to address red blood cell (RBC) agglutination caused by cold agglutinins: 1. Water Bath Method [1]: The specimen is incubated in a water bath at 37°C for a specific duration, followed by instrumental testing. 2. Plasma Exchange Method: The sample undergoes centrifugation, and the plasma is replaced with an equal volume of diluent. This process is repeated three consecutive times for washing, after which the specimen is analyzed on the instrument. 3. Pre-Dilution Method: The instrument's pre-dilution mode is selected. In this mode, 100 μL of diluent is added to an empty tube by the instrument, followed by manual addition of 20 μL of whole blood. After thorough mixing, the instrument enters the pre-dilution testing state. 4. Formula-Based Method [2]: The instrument's closed-whole blood-CDR/PLT-8X mode is selected for testing. Using the results of the "Other Parameters" section, specifically the optical red blood cell count (RBC-O) and the highest frequency mean corpuscular volume (HF-MCV), the RBC-related parameters HCT, MCV, MCH, and MCHC are calculated using the following formulas: HCT = RBC-O x HF-MCV; MCH = HGB/RBC-O; MCHC = HGB/HCT. Thus, each of the four methods was tested once, and the test results are shown in Table 1. Data analysis revealed that the water bath method, plasma exchange method, and pre-dilution method all failed to correct RBC agglutination. For the formula-based method, since only the RBC value was available in the studied parameters and no result for MCV was obtained, the calculation of RBC-related parameters (e.g., HCT, MCH, MCHC) was also not feasible. Could a combination of the water bath method and the formula-based method be applied for detection? Additionally, further experiments were conducted: the specimen was first incubated at 37°C for 30 minutes, then analyzed using the closed-whole blood-CDR/PLT-8X mode. In the derived parameters, the optical RBC-O was recorded as 2.81 x 10^{12}/L, and the HF-MCV was 93.6 fL. Using these values, HCT, MCH, and MCHC were calculated via the formula. The computed parameters were close to the patient's initial RBC indices measured at admission.

DISCUSSION

Cold agglutinin disease represents a significant subtype of cold antibody autoimmune hemolytic anemia (cAIHA), clinically characterized by cold agglutinin (CA)-mediated hemolytic anemia and peripheral circulatory symptoms. It encompasses two main categories: primary cold agglutinin disease (CAD) and secondary cold agglutinin syndrome (CAS). CAS often develops secondary to infections such as Mycoplasma pneumoniae, influenza virus, as well as malignancies (including solid tumors) and autoimmune disorders [3]. In this patient, routine blood test results were normal at initial admission; however, as treatment progressed, the severity of RBC agglutination in the collected samples gradually worsened. This phenomenon may be associated with uncontrolled or secondary infections, autoimmune reactions induced by COPD-related inflammation or drug-induced effects of antibiotics.

RBC agglutination caused by cold agglutinins is a common phenomenon in routine blood testing. This occurs when self-reactive IgM-class cold agglutinins mediate antigen-antibody interactions, leading to reversible aggregation of RBCs. Such aggregation often results in spurious reductions in RBC count, HCT and MCH. Studies have shown [4] that RBC and HCT values are falsely decreased during cold agglutination, while hemoglobin remains unaffected. Conversely, MCV, MCH, and MCHC exhibit false elevations. When reviewing blood routine reports, identification of cold agglutinin-induced RBC agglutination is straightforward. Clues include discrepancies between RBC count and hemoglobin levels (the "rule of three"), elevated MCHC trigger-

Table 1. Measured RBC counts and related parameters under different treatment conditions.

Methods/Parameters	RBC (x 10^{12}/L)	Hb (g/L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)
Direct testing (No. pretreatment)	****	89	****	****	****	****
37°C Water bath (30 minutes)	****	89	****	****	****	****
37°C Water bath (1 hour)	1.76	90	12.3	106.2	77.7	732
37°C Water bath (2 hours)	1.22	87	12.9	105.8	71.4	674
Plasma exchange method	1.33	82	14.0	105.1	61.8	586
Plasma exchange + 37°C water bath (30 minutes)	1.52	90	12.3	106.2	58.7	497
Pre-Dilution method	1.16	98	13.9	119.6	84.5	705
Pre-Dilution + 37°C water bath (30 minutes)	1.51	95	17.0	112.8	62.9	559
Formula-Based calculation method	2.81	89	****	****	****	****
37°C water bath (30 minutes) + formula method	2.81	91	26.3	93.6	32.38	346

****** indicates no result.**

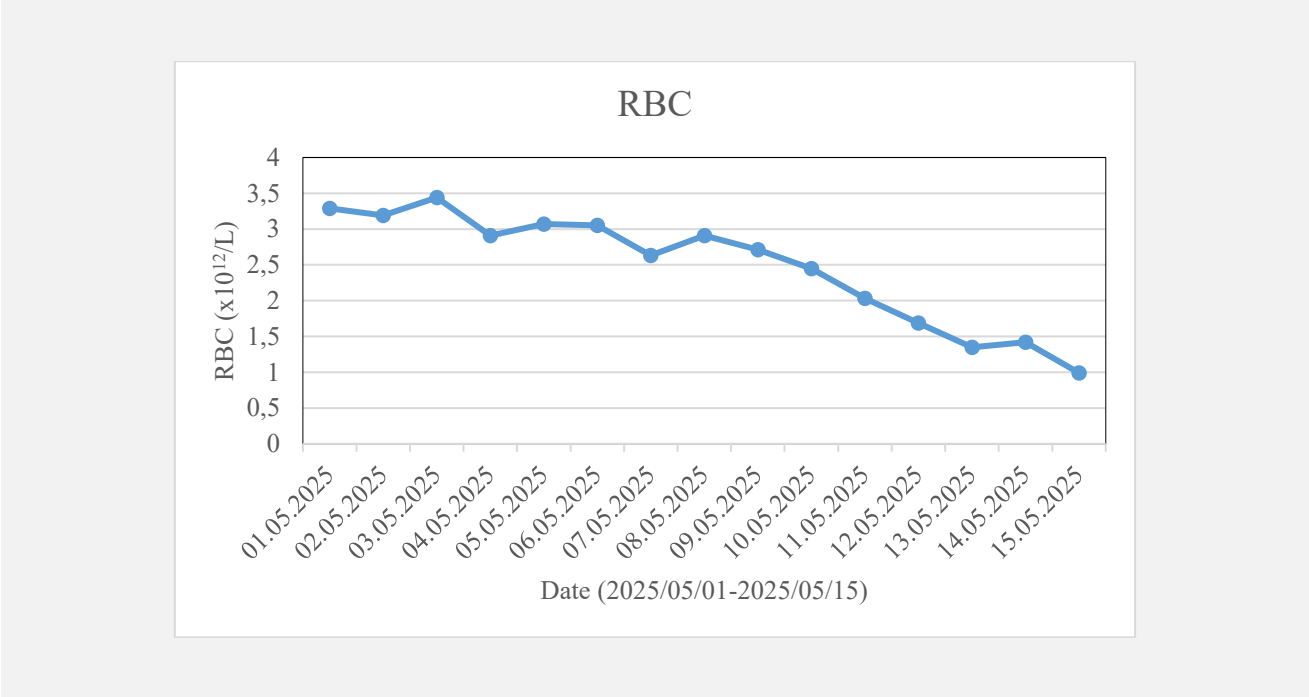

Figure 1. Serial RBC counts measured via automated hematology analyzer across different sampling dates.

ing slide review protocols, and automated instrument alerts for "RBC agglutination." Macroscopically, severely agglutinated samples exhibit a granular, sandy appearance, while microscopic examination reveals extensive RBC clumping or rouleaux formation. Mild to moderate cases can often be resolved using 37°C water bath treatment, plasma exchange, or pre-dilution methods. However, this case involved a severe agglutination specimen where conventional approaches failed. Ultimately, combining 37°C water bath treatment with detection via the reticulocyte channel yielded RBC-O and HF-MCV values. These parameters enabled formula-based calculation of HCT, MCV, MCH, and MCHC, which remained consistent with the patient's baseline indices. Regarding the validity of research parameters RBC-O and HF-MCV obtained from reticulocyte channel detection, some studies suggest that the 41°C detection temperature of the reticulocyte channel effectively

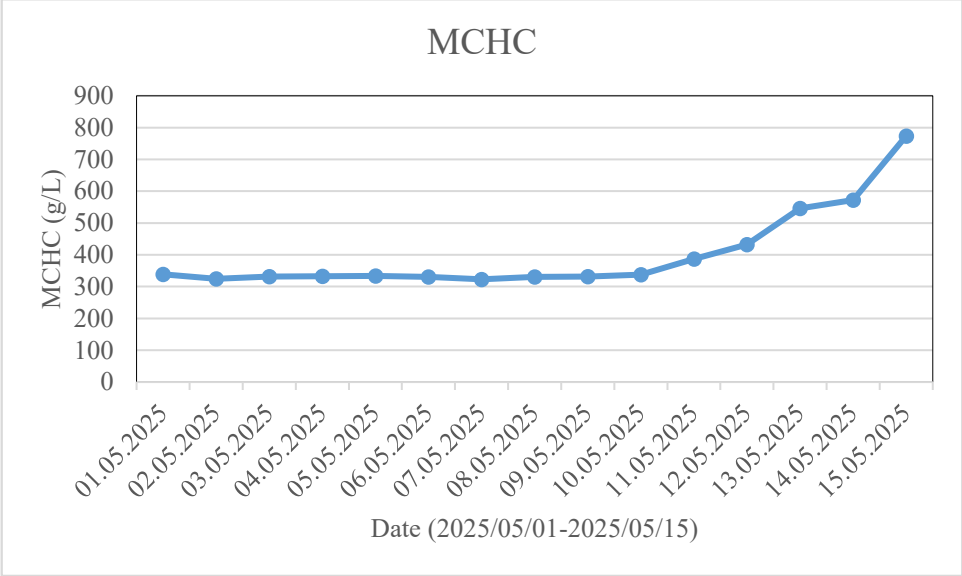

Figure 2. Serial MCHC values measured via automated hematology analyzer across different sampling dates.

resolves RBC agglutination, with the reliability of the data validated through various methods. Additionally, other studies have confirmed that severely cold-agglutinated samples, when immediately subjected to routine testing (e.g., RBC parameter measurement) after 2 minutes of incubation at 41°C, can also achieve effective correction [5,6]. Therefore, for severely cold-agglutinated samples, well-equipped laboratories can perform testing via both conventional and reticulocyte channels, while laboratories without such capabilities may incubate samples at 41°C prior to testing to correct RBC agglutination. For specialized severely cold-agglutinated samples where conventional methods (e.g., post-37°C or 41°C water bath testing, reticulocyte channel detection) fail to resolve agglutination, a combined approach - incubating samples at 37°C or 41°C followed by detection using the closed-whole blood-CDR/PLT-8X mode - can be employed. This method yields RBC-O and HF-MCV values from the research parameters, which are then used to calculate RBC-related parameters via formulas, effectively resolving issues with cold-agglutinated samples.

Declaration of Interest:

All authors declare that they have no competing interests.

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