

Remember the Blood Smear: A Clinical Laboratory Vignette

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We describe four patients with cold agglutinins, causing artifactually low red blood cell counts, and high mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration in the automated blood count. The diseases generating the cold agglutinins were pneumonia in two cases, cytomegalovirus mononucleosis in one, and B cell lymphoma in one. We discuss several mechanisms for the artifactually pathologic values, and emphasize the importance of the manual blood smear examination.

Brittin et al. [1] reported that erroneous results for red cell parameters can be obtained using the Coulter model A in patients with autoantibodies due to microagglutination. Lawrence and Zozicky [2] described a patient showing the same characteristics using the Coulter model S Plus. In all cases mentioned, the autoantibodies caused microaggregates that the instruments measured as single cells, resulting in an extremely low red blood cell count and hematocrit, and high MCV, MCH and MCHC. Hemoglobin concentrations were reliably measured in all cases. In at least some cases, since the incorrect results were caused by cold autoantibodies, warming of the sample corrected the pathologic values.

Recently, we had occasion to observe four patients in whom the extremely pathologic red blood cell indices, measured by our newest model Coulter STKS, enabled us to quickly diagnose the presence of cold agglutinins. Confirmation of this phenomenon was made by manually examining the blood smear.

Patient Descriptions

Case 1

A 35 year old man was admitted with fever

and cough. The patient was in good health until one month prior to admission, when he developed a non-productive cough and fever. He was treated by his family physician with oral erythromycin, without improvement. Physical examination upon admission revealed a profusely sweating man, without shortness of breath, and temperature of 37.7°C. Auscultation to the lungs revealed diminished breath sounds over the base of the left lung. Laboratory biochemistry tests results were in the normal range except for a mildly elevated lactate dehydrogenase hormone level. Erythrocyte sedimentation rate was 15 mm/hour. The complete blood count results, shown in Table I, demonstrated leukocytosis with neutrophilia, and a normal platelet count. There was a discrepancy between normal hemoglobin, and hematocrit and RBC count, which indicated severe anemia. The MCV was 106, the MCH 68 and the MCHC 64. A blood smear showed normal white blood cells, RBC aggregates, schistocytes and normal thrombocytes. Cold agglutinin titer was 1:2048, direct Coombs' was ++, haptoglobin 236 mg/dl; sputum culture revealed normal flora; anti-*Mycoplasma* antibodies were negative. Chest X-ray showed a mild consolidation in the base of the left lung. The patient was treated with eryth-

romycin intravenously with a very good overall response. A convalescent CBC was within the normal limits [Table I] and the cold agglutinin titer had declined to 1:128.

Case 2

A 47 year old man was seen in the emergency room, complaining of a non-productive cough and fever. The symptoms began 5 days previously, and did not respond to amoxicillin-clavulanic acid. The patient was a smoker of 25 pack-years. Temperature was 38.0°C. Auscultation to the lungs revealed bronchial breathing at the base of the right lung. Chest X-ray showed infiltrate in the base of the right lung. Blood biochemistry was normal except for LDH 813 IU (normal < 618), alanine aminotransferase 205 IU (normal < 56), and aspartate aminotransferase 98 IU (normal < 50). The CBC results [Table I] demonstrated a major discrepancy between hemoglobin, which was normal, and hematocrit and total RBC count indicating severe anemia. Results of the MCV, MCH and MCHC, plus visible gross agglutination in the test tube strongly suggested the presence of cold agglutinins. A repeat sample was analyzed within 5 minutes of being drawn, with very minimal normalization of values. Repeat physical

Table I. Blood count data, patients 1–4

	CBC	WBC	Hemo- globin	Hemato- crit	RBC	MCV	MCH	MCHC	Plate- lets
Patient 1	Acute	16,200	15.6	24	2.26	106	68	64	374
	Convalescent	5,600	14.9	43	5.23	82	28	35	340
Patient 2	Acute	13,500	16.1	11	0.58	195	276	142	102
	Acute	10,900	15.7	13	0.93	139	169	121	219
Patient 3	Acute	5,500	13.1	22	2.3	97	57	59	282
	Convalescent	5,100	12.3	36	3.8	89	32	37	246
Patient 4	Pre-warming	30,500	8.2	4.1	0.35	118	234	200	253
	Post-warming	24,300	8.3	24	2.7	91	31	34	243

MCV = mean cell volume
MCH = mean cell hemoglobin

MCHC = mean cell hemoglobin concentration
RBC = red blood cell

CBC = complete blood count
LDH = lactate dehydrogenase hormone

examination did not reveal clinical signs of hyperviscosity and the patient was discharged on oral clarithromycin, aspirin, and recommendations to maintain a warm environment at home and to drink warm liquids. The patient defervesced, but was lost to our follow-up.

Case 3

A 38 year old woman presented with fever up to 39.5°C, cough and myalgia of 4 days duration. The patient was treated with cefuroxime axetil. As there was no improvement, oral roxithromycin was added. Upon admission 3 days later the patient was clinically worse with continuing fever of 39.0°C. Auscultation to the lungs was normal; enlarged spleen was palpated 3 cm below the costal margin. Blood biochemistry was normal except for elevated LDH 976 IU (normal < 618) and alkaline phosphatase 156 IU (normal < 130). Chest X-ray was normal, and abdominal ultrasound revealed fatty liver. CMV serology for immunoglobulin G (negative upon admission) and CMV IgM (borderline upon admission) became positive 4 days later. The CBC results [Table I] demonstrated a discrepancy between hemoglobin, which was normal, and hematocrit and total RBC count indicating anemia. Results of the MCV, MCH and MCHC, plus the presence of erythrocyte aggregates on peripheral blood smear suggested the presence of cold agglutinins, which were subsequently found to be 4+. Atypical lymphocytes were also observed in the blood smear. The patient recovered gradually within 2 months. The results of repeat blood count are shown in Table I.

Case 4

Upon routine laboratory examination, a 63 year old white woman had leukocytosis of 23,000/mm³, hemoglobin 9.4 g/dl, and platelet count 260,000/mm³. Her past medical history included hypothyroidism treated with L-thyroxine during the previous 6 months, and excision of two basal cell carcinomas. Physical examination revealed a pale, obese female in good condition with mild pedal edema. Blood biochemistry was normal except for total bilirubin of 3.4 mg/

dl (normal < 1.5), almost completely indirect. Protein electrophoresis was normal. Haptoglobin was < 4 mg/dl (normal 60–270 mg/dl), and anti-*Mycoplasma* antibodies were negative. The CBC results shown in Table I demonstrated a major discrepancy between hemoglobin, which was 8 g/dl, hematocrit 4.1%, and RBC count 0.35x10⁶/mm³. The MCV, MCH and MCHC were high: 118, 234 and 200, respectively. Results of a warmed specimen, resolving the discrepancies, are shown in Table I. Direct Coombs' test was positive with a titer of 1:512, composed of anti-IgM, anti-C3c and anti-C3d. The reticulocyte count was 9.9%. A peripheral blood smear revealed large areas of red cell agglutination and an abnormal white cell population, suggestive of a lymphoproliferative disorder. A bone marrow core biopsy showed focal involvement of a lymphoproliferative process, compatible with small lymphocytic lymphoma. Computerized tomography revealed deep axillary and inguinal lymphadenopathy and a significant splenomegaly of 18 cm.

Diagnosis of cold agglutinin hemolytic anemia secondary to a B cell indolent lymphoma was made, based upon the bone marrow core biopsy results and immunophenotyping of the peripheral blood and bone marrow. The patient was treated with chlorambucil and prednisone resulting in an improvement in the blood count and reduction of transfusion requirements, despite the persistence of cold agglutinins and Coombs' positivity.

Comment

We report four patients with cold agglutinins whose red blood cell parameters demonstrated incompatible and unreasonable results. RBC counts and hematocrits were very low as compared to hemoglobin concentration, resulting in impossibly high MCV and MCHC. MCV was also elevated, and, in one case, impossibly elevated. The phenomenon of cold agglutinins and cryoglobulins causing erroneous results in RBC and CBC parameters has previously been reported using older models of cell counters [1–4]. This is the first report in the English medical literature of this phenomenon with the latest Coulter STKS model.

The presence of cold autoantibodies appears to cause microaggregates of erythrocytes, which are counted as single cells. This results in spuriously high MCV values and lowers the measured RBC count. The apparent increase in MCV does not correspond to the more severe erroneous decrease in RBC count. As a result, the instrument-derived hematocrit (RBC count x MCV) can fall far below the actual microhematocrit value, and as the measured hemoglobin concentration is unaffected by cold agglutinins the calculated MCH (hemoglobin/RBC count) and MCHC (hemoglobin/hematocrit) will be markedly elevated. All our patients had cold agglutinins and not cryoglobulins, and the discrepancies we observed were mainly in RBC but not white blood cell or platelet parameters [5].

In summary, physicians should be aware of the consequences of the presence of cold agglutinins on RBC parameters as measured by even the newest cell counters. Awareness of the described phenomenon can sometimes assist in diagnosis, and certainly is necessary for prevention of unneeded patient work-ups for anemia diagnosed on the basis of low hematocrit alone. The importance of sample pre-warming and the manual examination of prepared blood smears in cases of suspected cold agglutinins cannot be overemphasized.

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CMV = cytomegalovirus
Ig = immunoglobulin