Educational Case: Warm Autoimmune Hemolytic Anemia

Ruta Arays, MD¹ and Aaron D. Shmookler, MD²

The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.

Keywords
pathology competencies, diagnostic medicine, hematology, acquired anemia, autoimmune hemolytic anemia

Received December 27, 2019. Received revised August 27, 2020. Accepted for publication September 29, 2020.

Primary Objective
Objective H4.5: Acquired Anemia. Compare and contrast the clinical features and pathophysiology acquired including mechanical trauma, toxic, and antibody-mediated anemias.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic H: Hematology; Learning Goal 4: Diagnosis of the Anemic Patient.

Patient Presentation
A 46-year-old man presents to the emergency department complaining of shortness of breath. For the past 2 weeks, he has been experiencing increased heaviness in his chest, fatigue, and palpitations; these symptoms are worsened with exertion. He denies chest pain, dizziness, and headaches. He says, “I can’t catch my breath” when he is at rest.

He has no significant past medical or surgical history. His father has hypertension, and his mother has diabetes. He does not take any prescription medications or supplements. He does not consume alcohol, tobacco products, or illicit substances. A complete review of systems is positive for mild yellow coloration of the skin and early satiety. Additionally, he says his urine has been looking a little dark in color, but he denies frank blood in his urine and stool.

His vital signs are as follows: temperature 37.2 °C, blood pressure 133/66 mm Hg, heart rate 102 beats per minute, and respiratory rate 19 beats per minute. His oxygen saturation is 99% on 2 L oxygen. His body mass index is 27.2 kg/m². On physical examination, he appears well nourished, well developed, and in mild distress. His skin appears jaundiced, sclerae icteric. Auscultation of the heart reveals a normal rhythm and mild tachycardia. There are no murmurs, rubs, or gallops. All lung fields are clear to auscultation bilaterally. An abdominal examination identifies the tip of the spleen is palpable 3 cm below the left costal margin. The liver is not enlarged. A neurologic examination is negative for deficits in sensation, motor skills, cognition, and orientation.

Diagnostic Findings, Part 1
Initial laboratory workup includes a complete blood count and reticulocyte percentage (Table 1). A basic metabolic panel is normal. An electrocardiogram demonstrates sinus tachycardia.

1 Department of Medicine, West Virginia University, Morgantown, WV, USA
2 Department of Pathology, Anatomy, and Laboratory Medicine, West Virginia University, Morgantown, WV, USA

Corresponding Author:
Aaron D. Shmookler, Department of Pathology, Anatomy, and Laboratory Medicine, West Virginia University, 1 Medical Center Drive, Morgantown, WV 26506, USA.
Email: aaron.shmookler@hsc.wvu.edu

Creative Commons Non Commercial No Derivs CC BY-NC-ND: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 License (https://creativecommons.org/licenses/by-nc-nd/4.0/) which permits non-commercial use, reproduction and distribution of the work as published without adaptation or alteration, without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
and a premature ventricular complex. A chest X-ray is unremarkable.

**Question/Discussion Points, Part 1**

**What Is the Differential Diagnosis Based on the Provided Laboratory Results?**

This patient exhibits a macrocytic anemia with reticulocytosis, the differential for which is broad and often begins with an evaluation of folate and vitamin B₁₂ levels. Deficiencies in folate and/or vitamin B₁₂ impair DNA synthesis in the nucleus but not RNA and protein synthesis in the cytoplasm. This leads to asynchronous maturation of nuclear and cytoplasmic contents consistent with megaloblastic changes. Such nutrient deficiencies can be seen with antiretrovirals, chemotherapy, hydroxyurea, and alcohol, which contributes to liver disease. In nonalcoholic liver disease, there is abnormal cholesterol deposition increasing the surface area of the red blood cell (RBC) membrane. Although the pathophysiology of macrocytosis in hypothyroidism is not well understood, possible etiologies could be associated with the lack of erythropoietin production induced by thyroid hormone or autoimmunity involving multiple organs including stomach (pernicious anemia) and thyroid. Myelodysplastic syndrome can produce a macrocytic anemia, but with a normal white blood cell (WBC) count and platelet count, in this case, it is less likely.

Additionally, macrocytosis can be seen in association with reticulocytosis as a compensatory mechanism to a drop in hematocrit seen in bleeding or in hemolytic anemia. Peripheral RBC loss or destruction results in increased ejection of immature RBCs such as reticulocytes, which contain more RNA and are thus morphologically larger compared to normal RBCs. Of note, exogenous erythropoietin can result in reticulocytosis and subsequently macrocytosis.

**Diagnostic Findings, Part 2**

Folate and vitamin B₁₂ levels and thyroid function studies are within normal limits of detection. A liver function panel is ordered and includes albumin, total bilirubin, direct bilirubin, alkaline phosphatase, total protein, alanine transaminase, aspartate transaminase, and lactate dehydrogenase. The results from these studies are normal except for total bilirubin and lactate dehydrogenase (discussed later). Given insufficient clinical evidence to suggest hemorrhage, a workup for hemolytic anemia is pursued.

**Question/Discussion Points, Part 2**

**What Laboratory Studies Are Used in the Evaluation of Suspected Hemolytic Anemia?**

Hemolytic anemia refers to the lysis of RBCs and subsequent spillage of RBC contents. As mentioned earlier, reticulocyte percentage, if increased, suggests compensation in the setting of bleeding or peripheral RBC destruction. Hemolysis can be seen visually on a peripheral blood smear by noting the presence of schistocytes or spherocytes. Additional evidence of RBC destruction is supported by laboratory studies detecting specific RBC components that are mostly unique to RBCs. Free hemoglobin binds and subsequently depletes circulating haptoglobin, which is arguably the best marker of hemolysis in the appropriate clinical context. Free hemoglobin also is broken down by macrophages into unconjugated bilirubin, which is measured as indirect bilirubin and occurs before uridine diposphogluconate glucuronosyltransferase conjugates bilirubin in the liver. The difference between total bilirubin and direct bilirubin is the indirect bilirubin. Jaundice is a clinical manifestation of increased unconjugated bilirubin. Although not unique to RBCs, there is an abundance of lactate dehydrogenase (including specifically isoenzymes 1 and 2) that can be measured beyond the normal reference range in hemolysis. Last but not least, the direct antiglobulin test (DAT) is ordered to determine whether anemia is driven by an immune response. The DAT assesses RBC antibody sensitization using antihuman globulin, also known as Coombs reagent, which binds RBC-bound immunoglobulin G (IgG) and/or complement C₃.

**Why Is There an Increase in Unconjugated Bilirubin, But Not Conjugated Bilirubin, in Hemolytic Anemia?**

Free hemoglobin is first oxidized to biliverdin and then reduced to water-insoluble bilirubin before being transported to the liver by albumin. In the liver, this form of bilirubin has a half-life of 10 minutes. Bilirubin may also be metabolized by the liver into two forms: direct conjugated bilirubin and indirect unconjugated bilirubin. Indirect unconjugated bilirubin is unable to enter the liver and is further processed to become conjugated bilirubin. In hemolytic anemia, there is increased free hemoglobin catabolism producing a transient rise in serum levels of total bilirubin, most of which is unconjugated bilirubin. This rise in serum bilirubin exceeds the rate of clearance by the liver.

**Diagnostic Findings, Part 3**

Results of the workup for suspected hemolytic anemia are summarized in Table 2 and in Figure 1. Blood cultures are negative.

---

**Table 1. Complete Blood Count.**

| Laboratory test               | Result              | Reference interval                  |
|------------------------------|---------------------|------------------------------------|
| White blood cell count       | 8.9                 | 3.7-11.0 cells × 10^9/μL          |
| Red blood cell count         | 2.68                | 4.50-6.10 cells × 10^9/μL         |
| Hemoglobin                   | 9.9                 | 13.4-17.5 g/dL                    |
| Hematocrit                   | 27.2                | 38.9%-52.0%                       |
| Mean corpuscular volume      | 101.5               | 78.0-100.0 fl                     |
| Mean corpuscular hemoglobin  | 36.9                | 26.0-32.0 pg                      |
| Mean corpuscular hemoglobin concentration | 36.4 | 31.0-35.5 g/dL |
| Red blood cell distribution width | 22.9               | 11.5%-15.5%                       |
| Platelet count               | 222                 | 150-400 cells × 10^9/μL           |
| Reticulocyte percentage      | 11.81               | 0.5%-2.20%                        |
Table 2. Additional Laboratory Studies in the Evaluation of Hemolytic Anemia.

| Laboratory test             | Result | Reference interval |
|-----------------------------|--------|--------------------|
| Total bilirubin             | 5.9    | 0.3-1.3 mg/dL      |
| Direct bilirubin            | 0.2    | <0.3 mg/dL         |
| Lactate dehydrogenase       | 805    | 125-220 U/L        |
| Haptoglobin                 | <3     | 32-197 mg/dL       |
| Direct antiglobulin test     | Positive* | Negative         |

Abbreviation: IgG, immunoglobulin G.

*Agglutination seen in the presence of antihuman globulin antibodies to IgG and C3.

Figure 1. Peripheral blood smear (Giemsa, ×100) is remarkable for many polychromatophilic red blood cells (arrowhead) and spherocytes (arrow). Schistocytes are not identified. Printed with permission of Suzanne Venskoske, MT(ASCP)SH.

What Causes Red Blood Cell Hemolysis?

Accelerated RBC turnover may be classified as either hereditary or acquired. Hereditary causes are often secondary to intrinsic RBC abnormalities such as membrane defects (eg, hereditary spherocytosis, hereditary elliptocytosis), hemoglobinopathies (eg, Sickle disease, thalassemia), and enzyme defects (eg, glucose-6-phosphate dehydrogenase deficiency). Acquired causes are typically extrinsic and subclassified as either immune or nonimmune. Immune causes include infections such as malaria and antibodies that either specifically or nonspecifically implicate the RBC membrane. Nonimmune causes include mechanical damage from heart valves or other foreign implantation materials and physiochemical damage such as burns. Such damage causes RBC shearing into fragments known as schistocytes.

What Is the Pathophysiology of Immune-Mediated Hemolysis?

Immune-mediated hemolysis is driven predominantly by IgG and immunoglobulin M (IgM) antibodies recognizing a pathogenic attribute of or on the RBC as either self (autoantibody) or foreign (alloantibody). Immunoglobulin G is typically monomeric and binds RBCs with high affinity, whereas IgM can form pentamers and bind with high avidity. Thus, IgG effectively opsonizes pathogens, whereas IgM efficiently activates the complement system. The 3 currently known complement pathways—classical, alternate, and lectin—coalesce to produce complement C3. Complement C3 is the nidus from which subsequent reactions produce the membrane attack complex, culminating in intravascular hemolysis characteristic of schistocytes. Extravascular hemolysis occurs when circulating immunoglobulin-coated RBCs are processed by the spleen: macrophages partially ingest these RBCs, and the undigested RBC remnants become spherocytes. Splenomegaly is a clinical manifestation of extravascular hemolysis.

Pathogenic attributes include infections, drugs, and antigens. Given the novelty of most infections (not otherwise introduced as a vaccine), IgM plays an important role in controlling them as the earliest humoral immune response. This, in part, explains why schistocytes can be seen in disseminated intravascular coagulation secondary to septic shock. Drugs can mediate a variety of immunologic reactions that are mediated mostly by IgG. Penicillin, for example, classically acts as a...
Table 3. Distinguishing Laboratory Characteristics of Autoimmune Hemolytic Anemia.

| Laboratory characteristic | Warm autoimmune hemolytic anemia | Cold autoimmune hemolytic anemia | Paroxysmal cold hemoglobinuria |
|---------------------------|---------------------------------|---------------------------------|-------------------------------|
| Direct antiglobulin test   | IgG and C3 (most frequently)     | C3 only                         | C3 only                       |
| Pathologic immunoglobulin | IgG                             | IgM                             | IgG                           |
| Temperature reactivity     | 37 °C                           | 4 °C                            | 37 °C*                        |

Abbreviation: IgG, immunoglobulin G; IgM, immunoglobulin M.

* IgG binds red blood cells at 4 °C but does not result in clinical hemolysis until the temperature rises to 37 °C.

hapten by binding to the RBC membrane, drawing the corresponding antibody to the RBC that results in reticuloendothelial sequestration. As another example, nonsteroidal anti-inflammatory agents such as aspirin result in the formation of antigen–antibody complexes that bind RBCs leading to complement-mediated lysis. The RBC surface alone is coated with over 250 antigens grouped into systems such as ABO, RH, and Kell.11 Although some antibodies to these antigens are naturally occurring (eg, anti-A and anti-B), most are alloantibodies acquired after exposure to a corresponding antigen recognized as foreign from a blood transfusion, hematopoietic stem cell transplant, or fetus (during pregnancy). Occasionally, autoimmune antibodies (discussed later) may be directed against one’s own RBC antigens, usually e antigen or another Rh system antigen.5,11

What Are the Different Types of Autoimmune Hemolytic Anemia?

As discussed earlier, immune-mediated hemolytic anemia occurs when the body responds humorally to a pathogenic attribute of or on its own RBCs such as infection, drugs, and antigens. The diagnosis requires both clinical and laboratory correlation. The DAT is especially important as it is often positive. When the clinical history does not suggest evidence of drug-induced immune-mediated hemolytic anemia, consider autoimmune hemolytic anemia (AIHA). The different types of AIHA depend on the class and temperature reactivity of the immunoglobulins driving the reaction (Table 3).12 In general, AIHA is uncommon, seen in up to 3 per 100 000 patients annually.13

Although the exact pathophysiology of warm autoimmune hemolytic anemia (WAIHA) is not known, this disease is typically thought to be caused by autoantibodies against a patient’s own RBCs, resulting in hemolysis at an excessive or uncompensated rate. Warm autoimmune hemolytic anemia is typically mediated by IgG autoantibodies, which fix complement and also results in the partial phagocytosis of RBCs by splenic macrophages and subsequent formation of spherocytes. The DAT is most commonly positive for both IgG and C3.12 Approximately half of all cases of WAIHA are idiopathic (primary); secondary cases are associated with B-cell malignancies, systemic autoimmune diseases, and drugs.13

Cold autoimmune hemolytic anemia (CAIHA), or cold agglutinin disease, is mediated by IgM reacting in colder temperatures. Clinically, patients who are exposed to the cold can exhibit acrocyanosis and hemoglobinuria as evidence of hemolysis. Cold autoimmune hemolytic anemia most commonly is seen in the setting of Mycoplasma pneumoniae, and in association with autoantibodies against I antigen on RBCs, the titers for which are often very high (greater than or equal to 1000).12 It has also been observed in patients with hematologic malignancies, such as chronic lymphocytic leukemia and Waldenstrom macroglobulinemia, and with Epstein-Barr virus in the setting of infectious mononucleosis and in association with autoantibodies against i antigen on RBCs.12 Of note, RBC agglutination can be seen on the peripheral blood smear and can suggest CAIHA in the appropriate clinical and laboratory context.

Paroxysmal cold hemoglobinuria (PCH), the rarest of AIHAs, is perhaps now more commonly seen in children and in association with viral and bacterial infections; historically, PCH was noted to have been closely associated with syphilis.12,14 This unique AIHA is characterized by a biphasic hemolysin whereby an IgG autoantibody binds P antigen on RBCs in colder temperatures and only causes hemolysis in warmer temperatures; this is the basis for the diagnostic Donath-Landsteiner test.12

What Is This Patient’s Diagnosis?

This patient has WAIHA. To summarize, the initial workup revealed a macrocytic anemia with reticulocytosis (Table 1). Additional laboratory workup (Table 2 and Figure 1) suggests RBC destruction. The DAT notably is positive for both IgG and C3 (Table 3), which, along with a negative family history of blood disorders, argues against hereditary spherocytosis. There is no evidence suggesting drugs, infection, or blood transfusion as a cause for immune-mediated hemolytic anemia.

What Is the Management of Warm Autoimmune Hemolytic Anemia?

Management, including relapse and refractory cases, depends on whether WAIHA is primary or secondary.13 A thorough workup to exclude potentially treatable underlying conditions secondarily causing WAIHA should be investigated. These conditions are believed to induce or promote hemolysis.13 The main focus of management in WAIHA is decreasing the production of autoantibodies as well as decreasing splenic clearance of RBCs from circulation. First-line treatment is typically glucocorticoids such as prednisone (this is not the case for cold-mediated disease).13 Glucocorticoids can decrease macrophage ability to clear IgG or complement-coated erythrocytes which...
in turn reduces antibody production. The starting dose of steroid is usually 1 mg/kg/d and is given as a prolonged taper over many weeks. The goal is to achieve stabilization of hemoglobin, and if possible, a negative DAT. Other management options include the anti-CD20 antibody rituximab and splenectomy. Less commonly, further immunosuppressive agents such as cyclophosphamide, azathioprine, and mycophenolate mofetil have to be considered if first-line approaches fail or are ineffective.

Teaching Points

- The differential diagnosis of macrocytic anemia includes folate and/or vitamin B₁₂ deficiency, alcoholic and nonalcoholic liver disease, hypothyroidism, myelodysplastic syndrome, and drugs.
- Macrocytic anemia with splenomegaly and jaundice suggest extravascular hemolysis.
- Laboratory studies supporting hemolytic anemia include reticulocytosis, decreased haptoglobin, increased total and indirect bilirubin, increased lactate dehydrogenase, peripheral blood smear showing schistocytes (intravascular hemolysis) or spherocytes (extravascular hemolysis), and DAT positive for IgG and/or C3 (if due to antibodies).
- Increased MCHC is morphologically compatible with hyperchromia and is usually only associated with spherocytosis.
- The DAT is a test of humoral immunity: it detects the in vivo coating of RBCs by immunoglobulin (IgG) and/or complement C3.
- Primary (idiopathic) and secondary WAIHA are typically mediated by autoantibodies directed against a patient’s own RBCs. These are typically IgG in nature and cause extravascular hemolysis leading to anemia.
- Management of WAIHA includes glucocorticoids in the first line.

Acknowledgments

The authors acknowledge Suzanne Venskoske, MT(ASCP)SH for contributing the slide from which the figure was obtained.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The article processing fee for this article was funded by an Open Access Award given by the Society of ‘67, which supports the mission of the Association of Pathology Chairs to produce the next generation of outstanding investigators and educational scholars in the field of pathology. This award helps to promote the publication of high-quality original scholarship in Academic Pathology by authors at an early stage of academic development.

ORCID iD

Aaron D. Shmookler https://orcid.org/0000-0001-7789-7184

References

1. Knollmann-Ritschel BEC, Regula DP, Borowitz MJ, Conran R, Prystowsky MB. Pathology competencies for medical education and educational cases. Acad Pathol. 2017;4. doi:10.1177/2374289517715040
2. Nagao T, Hirokawa M. Diagnosis and treatment of macrocytic anemias in adults. J Gen Fam Med. 2017;18:200-204. doi:10.1002/jgf2.31
3. Kjeldsberg CR. Practical Diagnosis of Hematologic Disorders. 4th ed. American Society for Clinical Pathology Press; 2006.
4. Yang J, Yan B, Yang L, et al. Macrocytic anemia is associated with the severity of liver impairment in patients with hepatitis B virus-related decompensated cirrhosis: a retrospective cross-sectional study. BMC Gastroenterol. 2018;18:161. doi:10.1186/s12876-018-0893-9
5. Fein HG, Rivlin RS. Anemia in thyroid diseases. Med Clin North Am. 1975;59:1133-1145. doi:10.1016/s0025-7125(16)31963-0
6. d’Onofrio G, Chirillo R, Zini G, Caenaro G, Tommasi M, Micciulli G. Simultaneous measurement of reticulocyte and red blood cell indices in healthy subjects and patients with microcytic and macrocytic anemia. Blood. 1995;85:818-823.
7. Fisher JW. Erythropoietin: physiology and pharmacology update. Exp Biol Med. 2003;228:1-14. doi:10.1177/153537020322800101
8. McPherson RA, Pincus MR. Henry’s Clinical Diagnosis and Management by Laboratory Methods. 23rd ed. Elsevier; 2017.
9. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. Immunobiology: The Immune System in Health and Disease. 5th ed. Garland Science; 2001.
10. Lesesve JF, Martin M, Banasiak C, et al. Schistocytes in disseminated intravascular coagulation. Int J Lab Hematol. 2014;36:439-443. doi:10.1111/ijlh.12168
11. Reid ME, Oyen R, Marsh WL. Summary of the clinical significance of blood group alloantibodies. Semin Hematol. 2000;37:197-216. doi:10.1016/s0037-1963(00)90044-1
12. Fung MK, Eder A, Spitalnik SL, Westhoff CM. Technical Manual. 19th ed. AABB; 2017.
13. Go RS, Winters JL, Kay NE. How I treat autoimmune hemolytic anemia. Blood. 2017;129:2971-2979. doi:10.1182/blood-2016-11-693689
14. Shanbhag S, Spivak J. Paroxysmal cold hemoglobinuria. Hematol Oncol Clin North Am. 2015;29:473-478. doi:10.1016/j.hoc.2015.01.004