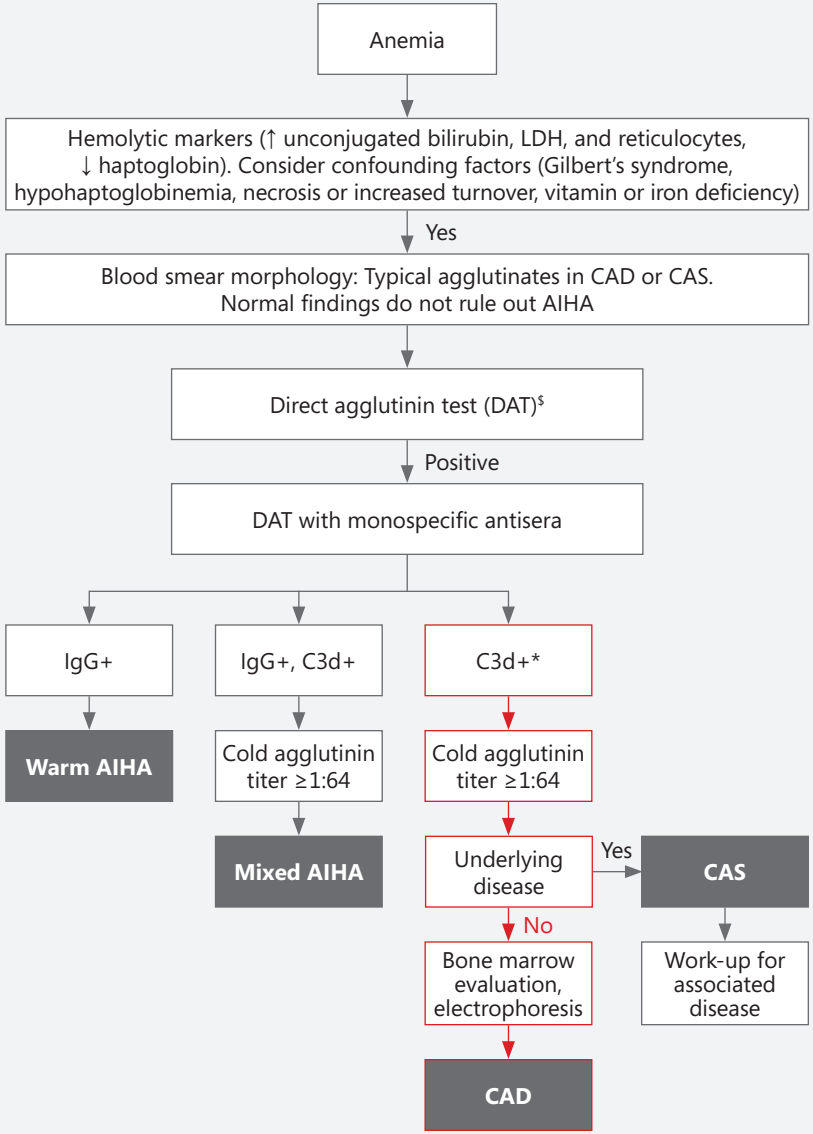
AIHA Diagnosis Tool

Autoimmune Hemolytic Anemias[1]

	Cold-Antibody Type		Warm-Antibody Type	Mixed Type	PCH
	CAD	CAS			
Incidence and age at onset	Up to 16 people/10^6 are impacted by CAD. Median age of onset is ~67 years	Rare, at any age	5-10 cases/ 10^6 persons/year; occurs at any age but frequently in the elderly	Rare, depending on definition	Rare in children, ultrarare in adults
Cause	Low-grade lymphoprolifer-ative bone marrow disorder	Secondary*	Unknown in <50% cases; secondary in ≥50% of cases*	Unclear	Post-viral (in children); tertiary syphilis, hematologic cancers (in adults)
Pathogenesis					
Autoantibody	Cold-agglutinin, anti-I (in rare cases, anti-Pr or anti-IH), monoclonal	Cold-agglutinin, anti-I or anti-i, polyclonal or monoclonal	Warm-reactive, panreactive, polyclonal	Both warm- and cold-reactive antibodies	Non-agglutinating, biphasic anti-P, polyclonal
Immunoglobulin class	IgM (in rare cases, IgG)	IgM or IgG	IgG (in rare cases, IgM or IgA)	IgG plus IgM	IgG (in rare cases, IgM)
Complement activation†	Classical pathway (+++), terminal pathway (+)	Classical pathway (+++), terminal pathway (+)	Frequently none; classical pathway (++), terminal pathway (+)	Present, details not established	Classical pathway (+++), terminal pathway (+++)
Predominant type of hemolysis	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Extravascular (mainly in the liver); intravascular (in acute exacerbations)	Extravascular (mainly in the spleen)	Not established	Intravascular
Typical findings					
Direct antiglobulin test	Monospecific DAT+; IgG positive; C3d positive; In rare cases, IgG or IgM positive; IgA negative	C3d positive; IgG positive or negative; In rare cases, IgM positive; IgA negative	IgG positive; C3d negative or positive; In rare cases, IgA or IgM positive	IgG and C3d positive; In rare cases, IgM positive; IgA negative	C3d positive; In rare cases, IgG or IgM positive; IgA negative
Cold agglutinin	High titer; ≥64 at 4°C	High titer	Absent	High titer	Absent

*Warm-antibody type is secondary to immunologic or lymphoproliferative disorders (e.g., chronic lymphocytic leukemia, systemic lupus erythematosus, or common variable immunodeficiency), and cold agglutinin syndrome is secondary to infection (e.g., *Mycoplasma pneumoniae* or Epstein–Barr virus infection) or cancer (e.g., aggressive lymphoma) and caused by auto-immune disorders.
†The designation (+) denotes weak or absent, (++) moderate, and (+++) strong

AIHA Diagnostic Algorithm[1,2]



§Consider that 3–10% of AIHAs are DAT negative. A false positive DAT may occur in several conditions hypergammaglobulinemia, paraproteins, therapy with IVIG or daratumumab. The DAT may be positive for alloantibodies in recently transfused patients. Consider Donath–Landsteiner test (PCH) in cases with a DAT positive for C3 only and no detectable cold agglutinin.
*Weakly positive for IgG in up to 20% of cases
Figure adapted from Berentsen S and Barcellini 2021[1]

AIHA Diagnosis Tool

CAD: Handling of Samples[1,3]

Analysis	Material	Sampling	Handling of Sample
LDH, unconjugated bilirubin, haptoglobin Cold agglutinin titer Immunoglobulin class quantification	Serum or plasma	Blood is drawn into prewarmed vacutainers without additive. Place in warming cabinet or water bath at 37–38°C	**Keep at 37–38°C until serum has been removed** from the clot, after which the sample can be handled at room temperature
Hemoglobin, blood cell counts Polyspecific (simple) DAT Monospecific (extended) DAT	Blood	EDTA vacutainer	**Prewarm at 37–38°C before analysis** if problems with agglutination
Bone marrow aspirate (Flow cytometry)	Bone marrow	Add EDTA or heparin	**Prewarming before analysis** will often be sufficient. If not, wash cells at 37–38°C

Pathophysiology of CAD[4]

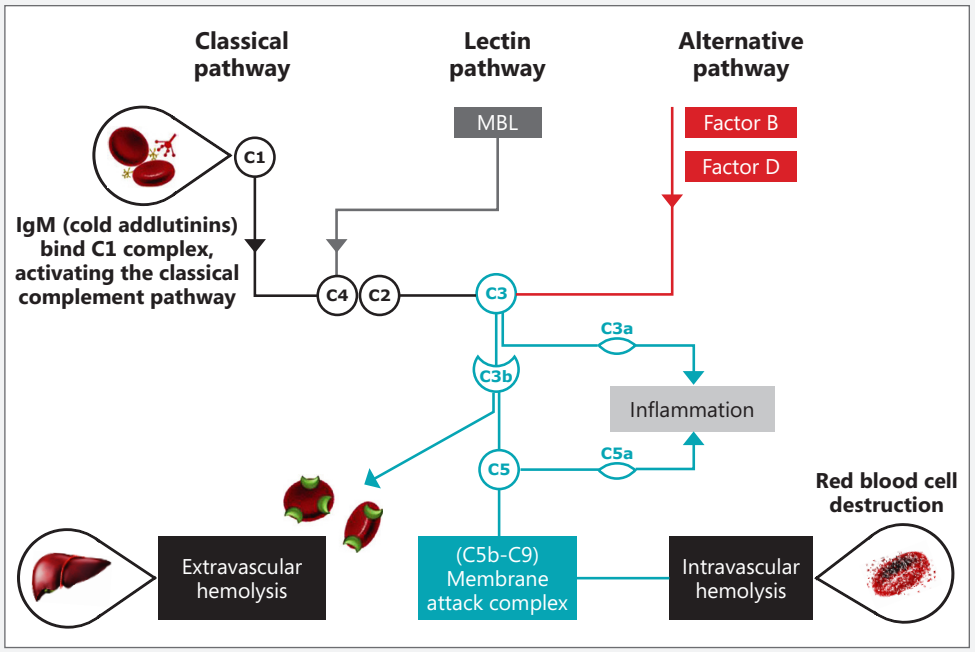


Figure adapted from Röth et al. 2021[4]

CAD: Signs and Symptoms[5-9]

- In CAD, IgM autoantibodies (cold agglutinins) preferentially bind to the "I" antigen on erythrocytes at temperatures ≤37°C and may result in erythrocyte agglutination
- Binding of the IgM-RBC complex to complement protein C1 leads to the activation of the classical complement pathway
- Hemolysis in CAD is entirely dependent on complement activation by the classical pathway
- All patients with CAD have chronic hemolysis with or without anemia

Complement-mediated symptoms

Hemolysis · Fatigue · Jaundice · Anemia · Dyspnea · Hemoglobinuria

Cold-induced circulatory symptoms

Acrocyanosis · Raynaud's disease · Other (ischemia, livedo reticularis, gangrene)

Abbreviations

AIHA, autoimmune hemolytic anemia; C, complement; C1, complement component 1; C2, complement component 2; C3, complement component 3; C3a, complement component 3a; C3b, complement component 3b; C3d, degradation product of complement component 3; C4, complement component 4; C5, complement component 5; C5a, complement component 5a; C5b, complement component 5b; C9, complement component 9; CAD, cold agglutinin disease; CAS, cold agglutinin syndrome; DAT, direct antiglobulin test; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IVIg, intravenous immunoglobulin; LDH, lactate dehydrogenase; MBL, mannan-binding lectin; PCH, paroxysmal cold hemoglobinuria; RBC, red blood cell; wAIHA, warm AIHA.

References

1. Berentsen S, Barcellini W. Autoimmune hemolytic anemias. *N Engl J Med*. 2021;385(15):1407–1419. doi:10.1056/NEJMra2033982; **2.** Barcellini W, et al. Fast facts: cold agglutinin disease. S. Karger Publishers Ltd. 2023. doi:10.1159/isbn.978-3-318-07233-4; **3.** Berentsen S, et al. Cold agglutinin disease: current challenges and future prospects. *J Blood Med*. 2019;10:93–103; doi:10.2147/JBM.S177621; **4.** Röth A, et al. Sutimlimab in cold agglutinin disease. *N Engl J Med*. 2021;384(14):1323–1334. doi:10.1056/NEJMoa2027760; **5.** Climent F, et al. Cold agglutinin disease: a distinct clonal B-cell lymphoproliferative disorder of the bone marrow. *Hemato*. 2022;3(1):163–173. doi:10.3390/hemato3010014; **6.** Jager U, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. *Blood Rev*. 2020;41:100648. doi:10.1016/j.blre.2019.100648; **7.** Berentsen S. Complement activation and inhibition in autoimmune hemolytic anemia: focus on cold agglutinin disease. *Semin Hematol*. 2018;55(3):141–149. doi:10.1053/j.seminhematol.2018.04.002; **8.** Berentsen S. How I manage patients with cold agglutinin disease. *Br J Haematol*. 2018;181(3):320–330. doi:10.1111/bjh.15109. **9.** Berentsen S, et al. Cold agglutinin-mediated autoimmune hemolytic anemia. *Hematol Oncol Clin North Am*. 2015;29(3):455–471. doi:10.1016/j.hoc.2015.01.002;

RECORDATI RARE DISEASES