HEMATOLOGIC ASPECTS OF AUTOIMMUNE HEMOLYTIC ANEMIAS

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Classification of Autoimmune Hemolytic Anemias

Warm antibody AIHA

Cold agglutinin syndrome

Paroxysmal cold hemoglobinuria

Unusual Immune Hemolytic Anemias

- Combined cold and warm AIHA
- AIHA with a negative direct antiglobulin (Coombs) test
- AIHA following blood transfusion
- AIHA in pregnancy
- Drug-induced AIHA
- The passenger lymphocyte syndrome
- Sickle cell hemolytic transfusion reaction syndrome (“Hyperhemolysis Syndrome”)

Characteristic Features of AIHAs

WARM ANTIBODY AIHA

Clinical manifestations: variable, usually symptoms of anemia, occasionally acute hemolytic syndrome.

Prognosis: Fair, with significant mortality.

Therapy: Steroids, splenectomy, immuno-suppressive drugs.

Characteristic Features of AIHAs

COLD AGGLUTININ SYNDROME

Clinical manifestations: Moderate chronic hemolytic anemia in a middle-aged or elderly person. Signs and symptoms may be exacerbated by cold.

Prognosis: Good, usually a chronic and quite stable anemia.

Therapy: Avoidance of cold exposure, immuno-suppressive drugs.

Characteristic Features of AIHAs

PAROXYSMAL COLD HEMOGLOBINURIA

Clinical manifestations: Acute hemolysis often with hemoglobinuria, usually in a child.

Prognosis: Excellent after initial stormy course.

Therapy: Steroids empirically and transfusions if necessary.

Characteristic Features of AIHAs

DRUG-INDUCED AIHA

Clinical manifestations: Variable; most commonly subacute in onset; occasionally severe hemolysis.

Prognosis: Excellent .

Therapy: Discontinue drug; occasionally a short course of steroids empirically.

Incidence of Various Kinds of Immune Hemolytic Anemias

- Warm antibody AIHA 70.3%
- Cold agglutinin syndrome 15.6%
- Paroxysmal cold hemoglobinuria 1.7%
- Drug-induced hemolytic anemia 12.4%

SECONDARY AIHA

1. Association of AIHA with an underlying disorder with a frequency greater than can be explained by chance alone. (CLL, SLE)
2. Reversal of AIHA with correction of the associated disease. (Ovarian tumor, ulcerative colitis)
3. Evidence of immunologic aberration as part of the underlying disorder, especially if the associated disease is thought to have an autoimmune pathogenesis (e.g., SLE).

SECONDARY AIHA

1. Lymphoid neoplasms, especially CLL, lymphomas.
2. Collagen diseases (SLE, RA, polyarteritis nodosa)
3. Ulcerative colitis.
4. Ovarian tumors.
5. Infections (*Mycoplasma pneumoniae*, infectious mononucleosis, HIV, CMV, bacterial infections)
6. Primary immunodeficiency diseases (IgA deficiency, hyper-IgM syndrome, Wiskott-Aldrich syndrome).
7. AIHA after hematopoietic stem cell transplantation.

ULCERATIVE COLITIS

1. The association of the two disorders is quite uncommon (0.6-1.7% incidence in ulcerative colitis).
2. The AIHA has almost invariably gone into remission after colectomy, even when the hemolysis is refractory to other therapeutic approaches.
3. Some authors suggest that patients with ulcerative colitis and AIHA unresponsive to steroids should undergo proctocolectomy.

OVARIAN TUMORS

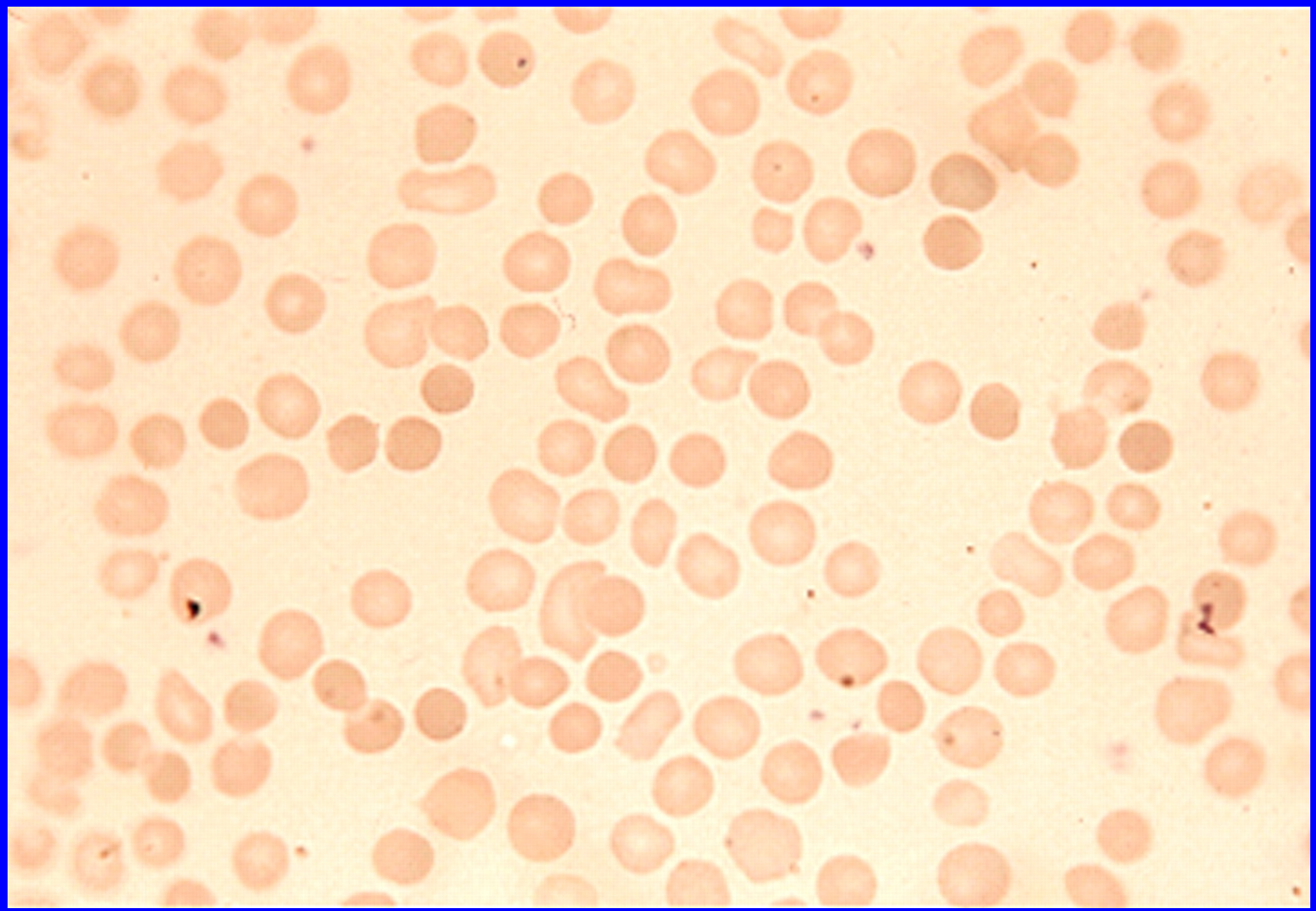
1. AIHA in association with ovarian tumors is very unusual but important.
2. Tumors include teratomas, dermoid cysts, malignant neoplasms.
3. A striking resistance of AIHA to any therapeutic approach other than surgical removal of the tumor.
4. Production of antibodies by ovarian tumors has been demonstrated.

WARM ANTIBODY AIHA

Characteristic Laboratory Findings

- Anemia.
- Abnormal RBC morphology.
  Spherocytosis, anisocytosis, poikilocytosis, polychromatophilia, autoagglutination.
- Reticulocytosis (reticulocytopenia in some patients).
- Thrombocytopenia (Evans' Syndrome).
- Leukocytosis (leukopenia in some patients).
- Urine may contain bile pigments (and/or hemoglobin in patients with severe hemolysis).
- Erythroid hyperplasia in the bone marrow.

Microspherocytes



Reticulocytopenia

- Diagnosis is more difficult.
- In one series of 109 patients with AIHA, reticulocyte counts ranged from 0.4% to 92%.
- 20% of patients had an initial counts <4%.
- Reticulocyte counts improved with therapy.
- Low initial counts thus probably represented a lag in responsiveness to hemolysis.

Reticulocytopenia

- Since there is no compensation for the short RBC survival, reticulocytopenia can be a medical emergency.
- Reticulocytopenia may persist for weeks or even months before resolving.
- The bone marrow usually demonstrates erythroid hyperplasia indicating intramedullary hemolysis.

Reticulocytopenia

Possible Mechanisms

1. RBC autoantibodies may react with nucleated RBCs and reticulocytes.
2. Parvovirus B19 binds to RBC precursors and is directly cytotoxic.

Transient aplastic crises result.

Usually resolves in 7-14 days.

IMMUNOPANCYTOPENIA

- Evans and Duane reported 5 patients with AIHA and thrombocytopenia and this has become known as “Evans’ Syndrome.”
- Two of the 5 patients also had leukopenia.
- The authors suggested the presence of a broadly reactive antibody or a separate antibody more specific for platelets and WBCs.

IMMUNOPANCYTOPENIA

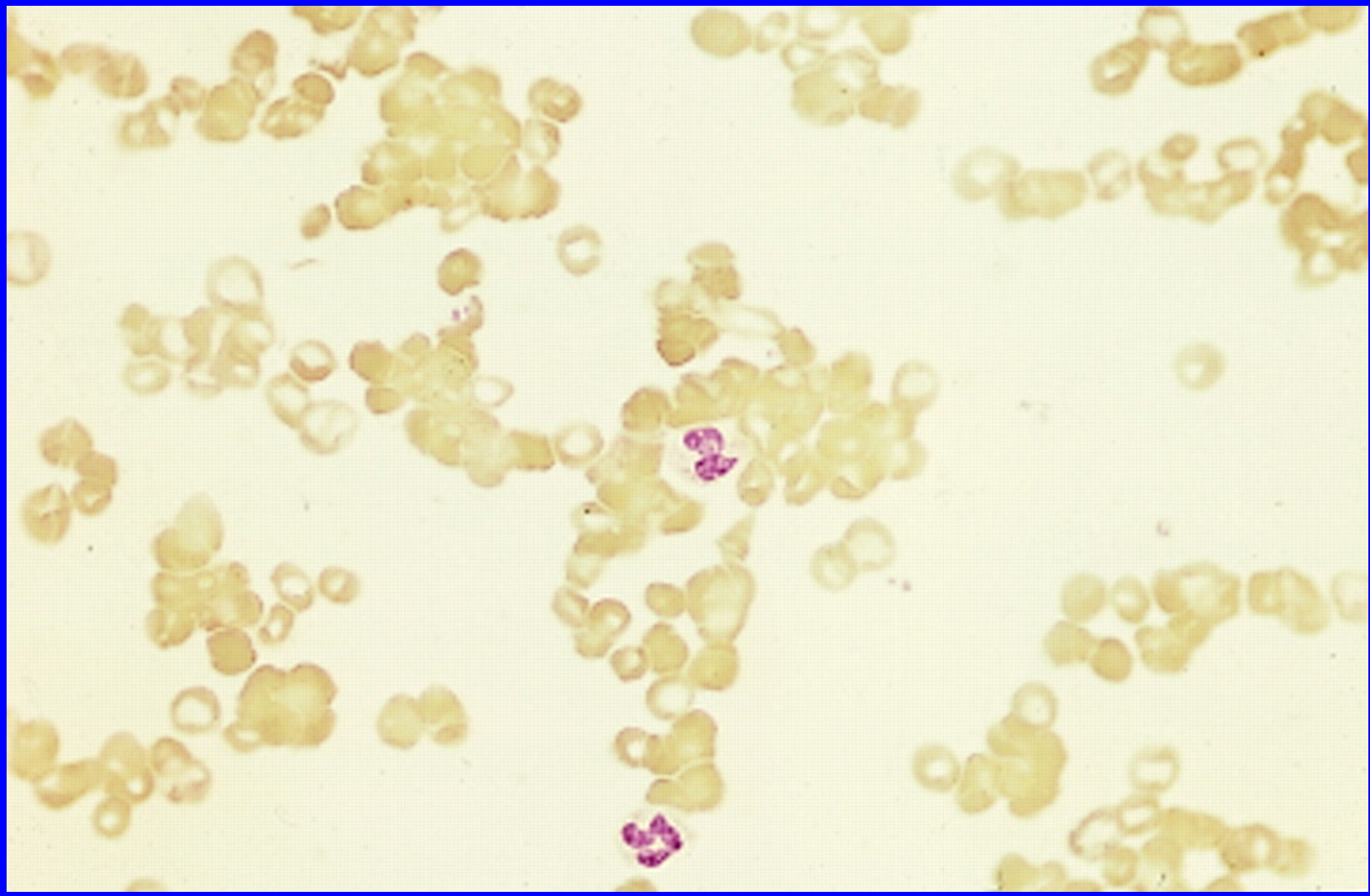
- Subsequent reports have indicated the presence of antibodies specific for granulocytes and platelets.
- Antibodies were cell specific and did not consist of a single cross-reacting antibody.
- Therefore, AIHA may be a complex autoimmune syndrome that may involve leukocytes and platelets as well as RBCs, with synthesis of autoantibodies specific for different blood cells.

COLD AGGLUTININ SYNDROME

Characteristic Laboratory Findings

- **Mild to moderate anemia in an elderly patient.**
  Prominent autoagglutination, especially at cold temperatures.
- **Abnormal RBC morphology**
  Modest degrees of spherocytosis, anisocytosis, poikilocytosis, polychromatophilia.
- **Reticulocytosis**
- **Jaundice**
- **Hemoglobinuria**
- **Erythroid hyperplasia in the marrow**

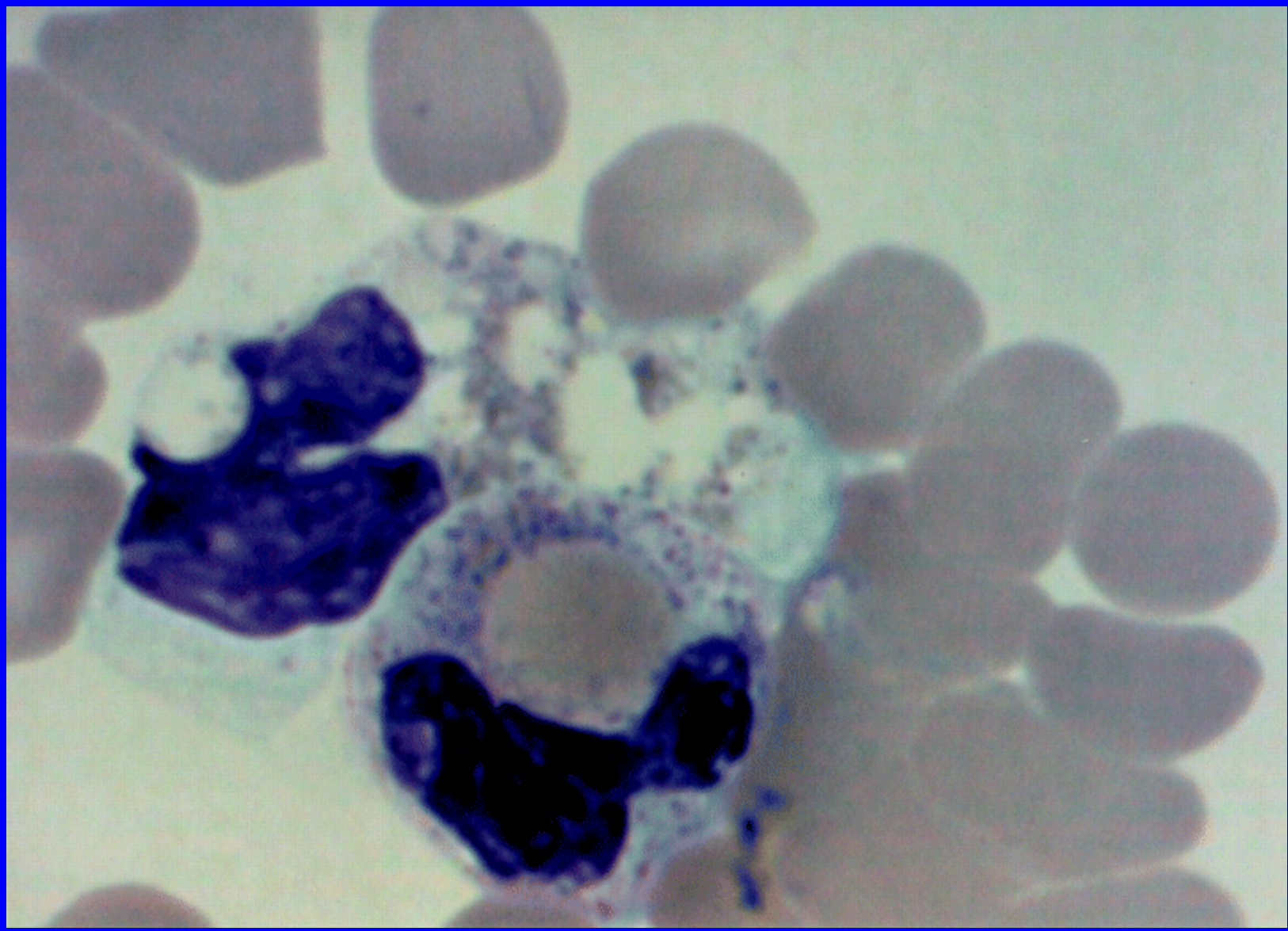
RBC CLUMPING IN COLD AGGLUTININ DISEASE



PAROXYSMAL COLD HEMOGLOBINURIA

Characteristic Laboratory Findings

- Acute hemolysis, especially in a child.
  The anemia may be rapidly progressive.
- Abnormal RBC morphology
  Spherocytosis, anisocytosis, poikilocytosis, autoagglutination, polychromatophilia.
  Erythrophagocytosis by neutrophils is commonly present.
- Hemoglobinuria
- Reticulocytosis and erythroid hyperplasia in marrow may be present.
- WBC and platelet counts normal or elevated.



Frequency of Signs and Symptoms in 42 patients

Sign/Symptom	No. of Patients
Hemoglobinuria	41
Jaundice	33
Pallor	28
Fever	23
Abdominal Pain	17
Malaise	14
Cough	13
Palpable liver/spleen	12

Serum	Blutkörperchen 3 Tropfen	Mischung durch ½ Stde. bei 5° gehalten, dann 2½ Stdn. bei 37°	Mischung durch 3 Stdn. bei 37° gehalten
Fall K. (Hämoglobinurik.) 4 Tropfen	Fall K.	rubinrot	0
	B. W.	rot	0
	Ch. G.	rot	0
	A. R.	rot	0
Fall R. (Hämoglobinurik.) 10 Tropfen	Fall R.	rubinrot	Spur Rötung
	B. W.	rubinrot	Spur „
	Ch. G.	rubinrot	Spur „
	A. R.	rubinrot	Spur „
Fall N. (Hämoglobinurik.) 7 Tropfen	Fall N.	rubinrot	0
	B. W.	rubinrot	0
	Ch. G.	rot	0
	A. R.	rubinrot	0
B. W. 6 Tropfen	B. W.	0	0
	Fall R.	0	0
	Fall N.	0	Spur Rötung
	Ch. G.	schwachrot	deutlich rot
Ch. G. 7 Tropfen	Ch. G.	0	0
	Fall K.	0	0
	Fall N.	0	0
A. R. 6 Tropfen	Fall K.	0	0
	Fall N.	0	0
	Fall R.	0	0
	B. W.	0	0
	Ch. G.	0	0

Serum	Blood Cells 3 Drops	Held for 1/2 hr at 5°, then 2 1/2 hr at 37°	Held 3 hours at 37°
Patient K (hemoglobinuria) 4 Drops	Patient K	Ruby red	0
	B.W.	Red	0
	Ch.G.	Red	0
	A.R.	Red	0
Patient R (hemoglobinuria) 10 Drops	Patient R	Ruby red	Trace of red
	B.W.	Ruby red	Trace of red
	Ch.G.	Ruby red	Trace of red
	A.R.	Ruby red	Trace of red
Patient N (hemoglobinuria) 7 Drops	Patient N	Ruby red	0
	B.W.	Ruby red	0
	Ch.G.	Red	0
	A.R.	Ruby red	0
B.W. 6 Drops	B.W.	0	0
	Patient R	0	0
	Patient N	0	Trace of red
	Ch.G.	Red tinged	Clear distinct red
Ch.G. 7 Drops	Ch.G.	0	0
	Patient K	0	0
	Patient N	0	0
A.R. 6 Drops	Patient K	0	0
	Patient N	0	0
	Patient R	0	0
	B.W.	0	0
	Ch.G.	0	0

COMBINED COLD AND WARM AIHA ("MIXED AIHA")

- Serologic findings characteristic of warm antibody AIHA while also having a cold agglutinin of high thermal amplitude.
- A cold agglutinin of high thermal amplitude (reactive at 30°C) must be documented.
- Patients characteristically have severe anemia, respond well to corticosteroids initially, but then often develop chronic hemolysis.

COMBINED COLD AND WARM AIHA ("MIXED AIHA")

Considerations in Diagnosis

- 35% of patients with warm antibody AIHA have cold agglutinins reactive to 20°C.
- Only 5% of these cold agglutinins are clinically significant and react at 37°C.
- Some patients with warm antibody AIHA have cold autoantibodies with normal cold agglutinin titers but of high thermal amplitude.

Autoimmune Hemolytic Anemia with a Negative Direct Antiglobulin (Coombs) Test

- Extensive evaluation fails to identify a non-immunlogic etiology and clinical findings are suggestive of autoimmune hemolytic anemia.
- The patients destroy transfused normal RBCs thus indicating an extrinsic mechanism for RBC destruction.
- Often autoantibodies can be detected using techniques more sensitive than standard procedures.

Acquired Hemolytic Anemia with a Negative Direct Antiglobulin (Coombs) Test

Exclude nonimmune causes of hemolytic anemia

- Oxidant drugs
- Mechanical hemolytic anemia
- Paroxysmal nocturnal hemoglobinuria
- Microangiopathic hemolytic anemia
- Infectious agents (e.g., malaria, Clostridium perfringens)

Autoimmune Hemolytic Anemia with a Negative Direct Antiglobulin (Coombs) Test

Mechanisms

- The concentration of IgG and/or C3 on RBCs is too low to be detected by routine tests.
- Presence of IgA or IgM autoantibodies which are not detected by the usual DATs.
- Presence of low-affinity IgG autoantibodies that elute from the RBCs during washing in preparation for the DAT.

The concentration of IgG and/or C3 on RBCs is too low to be detected by routine tests.

- Use sensitive methods to detect small amounts of autoantibody on RBCs.
- Detect autoantibodies in the patient's sera using agglutination potentiators (especially enzyme-treated RBCs and the direct polybrene test).
- Detect autoantibodies in concentrated eluates prepared from 50-200 ml of RBCs and concentrated to ~1ml.

Autoimmune Hemolytic Anemia with a Negative Direct Antiglobulin (Coombs) Test

- Repeat the DAT using cold saline (0-4°C) to wash patient's RBCs.
- Perform direct polybrene test.
- Indirect antiglobulin test using serum or eluate against enzyme-treated RBCs.
- Perform DAT with anti-IgA and anti-IgM and potent anti-C3, if available.
- Prepare concentrated eluate from large volume of patient's RBCs (50-200 ml concentrated to ~1 ml).
- Use column agglutination test or flow cytometry.

Autoimmune Hemolytic Anemia with a Negative Direct Antiglobulin (Coombs) Test

Therapy and Course

- Treat similarly to patients with warm antibody AIHA who have typical serologic findings.
- The response to therapy (e.g., steroids, splenectomy) is similar to that of patients with typical AIHA.
- Response likely to be associated with a reduction of cell-bound IgG as documented by sensitive tests.

Development of RBC Autoantibodies and AIHA following Transfusion.

Early Reports

- Dameshek and Levine – 1943.
- Fudenberg et al – 1958
- Allen – 1960
- Chown et al – 1971
- Cook – 1971 (Immunized Rh-negative men with Rh-positive blood. Eleven of 34 subjects developed a RBC autoantibody in addition to the expected anti-D)

Retrospective Reviews of Multiply Transfused Patients

- **Of 184 patients with sickle cell disesase who received multiple RBC transfusions, 7.6% developed warm IgG RBC autoantibodies, often in association with alloantibodies.**
- **Mean transfusion exposure at time of autoantibody formation was 24 RBC units.**
- **Clinically significant AHIA occurred in 4 patients.**

Castellino et al. BJH 1999;104:189-194

Retrospective Reviews of Multiply Transfused Patients

- **Twenty five percent of 64 transfused patients with thalassemia developed RBC autoantibodies and a positive DAT.**
- **Three patients developed severe AIHA and required prolonged treatment.**

Singer et al. Blood 2000;96:3369-3373.

The Source of Autoantibodies

- The production of alloantibodies may not be strictly specific, especially after prolonged immune stimulation. Thus the alloantibodies may expand their specificity to become autoantibodies.
- Alternatively, latent immune cells capable of producing autoantibody are stimulated to do so after prolonged immunization.

The Source of Autoantibodies

- Donor lymphocytes persist in patients for at least 1½ years following blood transfusion.
- Such microchimerism may result in antibody production by persistent donor cells.

Churchill-Livingstone, New York 2004