Acquired hemolytic, megaloblastic and sideroblastic anemias

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CH Jolimont and ULB-Hôpital Erasme

BHS Educational Course
Red Blood Cell Disorders
November 2013
Topics

• Acquired hemolytic anemias
  – Autoimmune
  – (Microangiopathic)
  – mechanical
  – Infections
  – (Paroxysmal nocturnal hemoglobinuria)
  – Other

• Acquired megaloblastic anemias
  – Vitamin B12 deficiency
  – Folic acid deficiency

• Acquired sideroblastic anemia
Common causes of acquired extravascular destruction of red cells

- Hypersplenism
- Infections ( Bartonella bacilliformis, babesia, malaria)
- Lead, copper, snake and spider bites
- Auto-immune hemolytic anemia (warm or cold, drugs)
- Oxydant agents (dapsone, nitrites, …)
Common causes of acquired intravascular hemolysis in adults

- Microangiopathic hemolytic anemia: TTP, HUS, prosthetic valve leak
- Direct trauma (runners)
- Transfusion reactions
- Infection (clostridium perfringens sepsis with degradation of phospholipids of the red cell membrane bilayer and the structural membrane proteins, severe malaria)
- Paroxysmal nocturnal hemoglobinuria
- Liver disease with acquired alterations in red cell membrane
- Cold agglutinin disease / paroxysmal cold hemoglobinuria
- Intravenous infusion with hypotonic solutions
- Snake bites
- Compounds with high oxydant potential (copper poisoning, Wilson disease)
- IV immunoglobulins
Autoimmune hemolytic anemia (AIHA)

- Definition
  - Destruction of red blood cells (hemolysis)
  - Due to autoantibodies
  - With or without complement activation
- Detection of autoantibodies by a positive direct antiglobulin test (DAT) or direct Coombs test
- Rare incidence: 1/100000/year
- A negative direct Coombs does not exclude the diagnosis of AIHA
- Presence of microspherocytosis in blood smear may support the suspected diagnosis of AIHA with negative Coombs
- Warm or cold antibody?
  - Laboratory criteria
  - Optimal temperature for autoantibody binding to RBC
Autoimmune hemolytic anemia (AIHA)

- Normal subjects

- Positive Coombs test in normal blood donors: 1/1000 to 1/36000

- Risk factors: AIDS, drugs, age, elevated IgG, cardiolipin antibodies

- A positive Coombs test may predate a malignancy by months to years
Autoimmune hemolytic anemia (AIHA)

- Importance of isotype
  - IgM are very efficient in complement activation
    - Only one molecule of antibody is needed
  - IgG1 and IgG3 are efficient complement activators
  - IgG2 and IgA are weak complement activators
  - IgG4 does not activate complement
  - Generally, complement system not completely activated
Autoimmune hemolytic anemia (AIHA)

- Importance of isotype
  - Cold antibodies
    - Optimal binding to RBC below 30°C
    - Mostly of IgM isotype
  - Warm antibodies: 75%-80% of the cases
    - Optimal binding at 37°C
    - Mostly IgG, less commonly IgM, rarely IgA
  - Biphasic antibodies
    - Optimal binding below 30°C
    - Induce complement activation at 37°C
Autoimmune hemolytic anemia

- RBC coated with IgG with/without C3/C3d
  - preferentially removed via FC-gamma mediated phagocytosis in the spleen
- RBC coated with C3/C3d in the absence of IgG
  - destroyed via complement-receptor mediated phagocytosis in the liver
- IgM: complement activation possible until formation and introduction of the membrane attack complex leading to intravascular hemolysis

- IgG: 20-60%
- IgG+C3d: 25-65%
- C3d alone: 7-15%
- IgM+C3d: 15%
Autoimmune hemolytic anemia (AIHA)

Zeerleder S. Autoimmune haemolytic anemia – a practical guide to cope with a diagnostic and therapeutic challenge. The Netherlands Journal of Medicine 2011;69:177-184

- Indirect antiglobulin test
  - Detection of allo or autoantibodies present in the patient’s serum

- Direct antiglobulin test
  - Detection of allo or autoantibodies or complement bound in vivo to patient’s RBC

- Polyspecific anti-human globulin reagent does not contain anti-IgA

- Repeat the DAT with anti-IgA, anti-IgG, anti-IgM, anti-C3c and anti-C3d to confirm the DAT to be negative if suspicion of AIHA is strong
Autoimmune hemolytic anemia: etiology

• Idiopathic AIHA
• Congenital abnormalities of the immune system
  – Common variable immunodeficiency
• Viral infection
  – EBV, HIV
• Autoimmune diseases
  – LED
  – RA, scleroderma, ulcerative colitis…
• Lymphoma
  – CLL: 11%
  – NHL: 3%
The direct antiglobulin test: A critical step in the evaluation of hemolysis

visually

Fully automated laboratory systems
The direct antiglobulin test: A critical step in the evaluation of hemolysis

<table>
<thead>
<tr>
<th>Reactions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td></td>
</tr>
<tr>
<td>1. Normal</td>
<td></td>
</tr>
<tr>
<td>2. Autoimmune hemolytic anemia (primary and secondary causes)</td>
<td></td>
</tr>
<tr>
<td>a. Warm autoimmune hemolytic anemia</td>
<td></td>
</tr>
<tr>
<td>b. Cold autoimmune hemolytic anemia</td>
<td></td>
</tr>
<tr>
<td>c. Mixed autoimmune hemolytic anemia</td>
<td></td>
</tr>
<tr>
<td>d. Paroxysmal cold hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>3. Transfusion related</td>
<td></td>
</tr>
<tr>
<td>a. Acute hemolytic transfusion reaction</td>
<td></td>
</tr>
<tr>
<td>b. Delayed hemolytic transfusion reaction</td>
<td></td>
</tr>
<tr>
<td>c. Delayed serological reaction</td>
<td></td>
</tr>
<tr>
<td>d. Passive transfer of antibody by transfusion</td>
<td></td>
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<tr>
<td>4. Hemolytic disease of the fetus/newborn</td>
<td></td>
</tr>
<tr>
<td>5. Passenger lymphocyte syndrome</td>
<td></td>
</tr>
<tr>
<td>6. Drug-induced hemolytic anemia</td>
<td></td>
</tr>
<tr>
<td>7. Passive transfer of antibody in immunoglobulin preparations</td>
<td></td>
</tr>
<tr>
<td>a. Intravenous immune globulin (IVIG)</td>
<td></td>
</tr>
<tr>
<td>b. Rh\text{D} immune globulin</td>
<td></td>
</tr>
<tr>
<td>8. False positive</td>
<td></td>
</tr>
<tr>
<td>a. Spontaneous red blood cell agglutination</td>
<td></td>
</tr>
<tr>
<td>b. Wharton's jelly in cord blood specimens</td>
<td></td>
</tr>
<tr>
<td>c. Technical</td>
<td></td>
</tr>
<tr>
<td>i. Poor washing technique</td>
<td></td>
</tr>
<tr>
<td>ii. Improper agitation of specimen during reaction strength determination (conventional test tube method)</td>
<td></td>
</tr>
<tr>
<td>iii. Over-centrifugation</td>
<td></td>
</tr>
<tr>
<td>iv. Clotted specimens</td>
<td></td>
</tr>
</tbody>
</table>
The direct antiglobulin test: A critical step in the evaluation of hemolysis

**TABLE I. Causes of Positive and Negative Direct Antiglobulin Test (DAT) Reactions**

**Negative**
1. Nonimmune causes of hemolysis
2. Drug-induced hemolytic anemia
3. Hemolysis due to an IgA or IgM immunoglobulin
4. Low level of bound antibody and/or complement
5. Low affinity antibody
6. False negative
   a. Poor washing technique
   b. Improper agitation of specimen during reaction strength determination (conventional test tube method)
   c. Failure to add or delayed addition of antihuman globulin (AHG) reagent
   d. Inactive antihuman globulin (AHG) reagent
   e. Inappropriately concentrated red blood cell suspension
   f. Delay in testing
Proposed unifying hypothesis of drug-induced antibody reactions. The thicker, darker lines represent antigen-binding sites on the Fab region of the drug-induced antibody. (A) Drugs (haptens) bind loosely (or firmly) to cell membranes, and antibodies...

George Garratty

**Immune hemolytic anemia associated with drug therapy**

Blood Reviews Volume 24, Issues 4–5 2010 143 - 150

http://dx.doi.org/10.1016/j.blre.2010.06.004
### Table 1
Number of cases/fatalities of DIIHA encountered by us over a 10 year period.

<table>
<thead>
<tr>
<th>Drug</th>
<th>2000–2009 (10 years)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td>36 (4)</td>
<td>43</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>17 (5)</td>
<td>21</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>14 (1)</td>
<td>17</td>
</tr>
<tr>
<td>ß-lactamase inhibitors</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Other cephalospirins</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>9³</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83 (10)</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

* Columns contain number (fatalities) of cases associated with each drug.

³ Oxaliplatin (3), carboplatin (1), rifampin (1), diclofenac (1), cimetidine (1), sulfamethoxazole (1), and trimethoprim (1).
Autoimmune hemolytic anemia

- Cold antibody AIHAs
  - Cold agglutinin disease
  - Paroxysmal cold hemoglobinuria
Autoimmune hemolytic anemia: Cold agglutinins disease

- 15% of AIHA cases
- 1 per million people per year
- IgM-mediated process in 90% of patients
- Finding of agglutination without antiglobulin antisera in microtiter wells at 4°C
- Extravascular and intravascular hemolysis mediated by complement
Autoimmune hemolytic anemia: Cold agglutinins disease

- Polyclonal or monoclonal
  - Polyclonal: usually children and young adults
    - post-infectious setting: Mycoplasma, EBV, Legionelle, CMV, …
      - Usually self-resolving
    - Monoclonal: usually older patients
      - Long-term disease
      - Frequent resistance to therapy
      - May be associated with lymphoproliferative disease
Autoimmune hemolytic anemia: Cold agglutinin disease

• Clinical manifestations
  - Hemolysis
    • Transfusion: > 50% of patients
    • Therapy necessary in 70% of patients
  - Cold-induced circulatory symptoms
    • Livedo reticularis
    • Raynaud disease
    • Acrocyanosis
    • Cutaneous necrosis
  - Splenomegaly: rare
Cold agglutinin disease: clinical manifestations
Autoimmune hemolytic anemia: Cold agglutinins disease

• Diagnosis
  - Hemolytic anemia
  - Reticulocytosis
  - Hyperbilirubinemia
  - Low haptoglobin
  - LDH
  - Positive Coombs testing
    • for anti-C3
    • Classically negative anti-IgG
  - Most agglutinins clinically non significant (only 14%)
  - Titer: significant when > 1/512

• After diagnosis
  - Infection? Malignancy? Autoimmune disease?
Autoimmune hemolytic anemia: Cold agglutinins disease

Table 2. Clinical features (N = 89)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time from symptoms to diagnosis, mo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>37.4</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0-374.2</td>
<td></td>
</tr>
<tr>
<td><strong>Chief concern at diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia of undetermined origin</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>Acrocyanosis</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Fatigue</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Weakness</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Dyspnea on exertion</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Symptoms during disease course</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrocyanosis</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>Fatigue</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Dyspnea on exertion</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Weakness</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Weight loss</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><strong>Identification of triggers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Received drug therapy</td>
<td>73</td>
<td>82</td>
</tr>
<tr>
<td>Received transfusion</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td><strong>Overall survival, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.0-29.9</td>
<td></td>
</tr>
<tr>
<td>Survived 5 y after diagnosis</td>
<td>68</td>
<td>76</td>
</tr>
</tbody>
</table>

90%, in an other report

Blood 2013;122:114-1121
Autoimmune hemolytic anemia: Cold agglutinins disease

Underlying hematologic disease in 76% of patients

<table>
<thead>
<tr>
<th>Hematologic diagnosis</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGUS</td>
<td>42</td>
<td>61</td>
</tr>
<tr>
<td>Macroglobulinemia</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Unspecified lymphoproliferative disorder</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Other lymphoma†</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>CLL</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

CLL, chronic lymphocytic lymphoma.
* Disease-specific percentages were calculated by using the number of patients in the subgroup as denominators.
† Including low-grade B-cell and diffuse large B-cell lymphomas.
Autoimmune hemolytic anemia: Cold agglutinins disease

89 patients from 1970 through 2012

Cold avoidance!!!

Rituximab associated with high response rates but complete and sustained remissions are rare

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single-agent prednisone</th>
<th>Corticosteroid-containing therapy</th>
<th>Any corticosteroid-containing therapy</th>
<th>Rituximab</th>
<th>Any rituximab-containing therapy</th>
<th>Purine analog</th>
<th>Alkylating agent</th>
<th>Other therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>26</td>
<td>35</td>
<td></td>
<td>47</td>
<td>77</td>
<td>10</td>
<td>59</td>
<td>24</td>
</tr>
<tr>
<td>%</td>
<td>88%</td>
<td>85%</td>
<td></td>
<td>91%</td>
<td>93%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patients treated</strong></td>
<td>24</td>
<td>30</td>
<td></td>
<td>32</td>
<td>44</td>
<td>8</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td><strong>Used as first-line therapy</strong></td>
<td>21</td>
<td>81%</td>
<td>23%</td>
<td>14</td>
<td>30%</td>
<td>2%</td>
<td>20%</td>
<td>7%</td>
</tr>
<tr>
<td><strong>Used as part of dual- or multi-modality therapy</strong></td>
<td>---</td>
<td>7%</td>
<td>20%</td>
<td>---</td>
<td>39%</td>
<td>6%</td>
<td>60%</td>
<td>58%</td>
</tr>
<tr>
<td><strong>Duration of therapy mo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Treatments with available data</strong></td>
<td>19</td>
<td>79%</td>
<td></td>
<td>38</td>
<td>65</td>
<td>5</td>
<td>41</td>
<td>14</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>3.0</td>
<td>1.9</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>4.0</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.1-13.9</td>
<td>0.1-120.0</td>
<td></td>
<td>0.1-20.4</td>
<td>0.1-107.7</td>
<td>1.0-24.5</td>
<td>0.0-46.4</td>
<td>0.0-108.7</td>
</tr>
<tr>
<td><strong>Tolerated therapy</strong></td>
<td>22</td>
<td>85%</td>
<td>83%</td>
<td>42</td>
<td>89%</td>
<td>9%</td>
<td>90%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>Patients with known underlying hematologic disorder</strong></td>
<td>1824</td>
<td>75%</td>
<td>80%</td>
<td>3132</td>
<td>97%</td>
<td>86</td>
<td>78%</td>
<td>88%</td>
</tr>
<tr>
<td><strong>Lymphoproliferative disorder</strong></td>
<td>10</td>
<td>12%</td>
<td></td>
<td>16</td>
<td>20%</td>
<td>7%</td>
<td>17%</td>
<td>12%</td>
</tr>
<tr>
<td><strong>Other hematologic disorder</strong></td>
<td>8</td>
<td>12%</td>
<td></td>
<td>15</td>
<td>18%</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Concurrent need for transfusion</strong></td>
<td>9</td>
<td>35%</td>
<td>34%</td>
<td>6</td>
<td>13%</td>
<td>16%</td>
<td>21%</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Decreased need after therapy</strong></td>
<td>1</td>
<td>11%</td>
<td>25%</td>
<td>6</td>
<td>100%</td>
<td>10%</td>
<td>63%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Increased need after therapy</strong></td>
<td>0</td>
<td>0%</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Confirmed response to therapy</strong></td>
<td>9</td>
<td>36%</td>
<td>42%</td>
<td>39</td>
<td>83%</td>
<td>61%</td>
<td>79%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Treatments with available data</strong></td>
<td>25</td>
<td>68%</td>
<td></td>
<td>47</td>
<td>77%</td>
<td>8%</td>
<td>57%</td>
<td>23%</td>
</tr>
<tr>
<td><strong>Duration of response, mo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>2.9-210.8</td>
<td>7.0-210.8</td>
<td></td>
<td>2.0-136.6</td>
<td>24.0</td>
<td>18.5</td>
<td>11.3</td>
<td>36.6</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Further treatment required</strong></td>
<td>17</td>
<td>65%</td>
<td>69%</td>
<td>23</td>
<td>49%</td>
<td>42%</td>
<td>55%</td>
<td>60%</td>
</tr>
</tbody>
</table>

*Other therapy included azathioprine, erythropoietin, danazol, interferon alpha, plasma exchange, intravenous immunoglobulin, methotrexate, cyclosporine, or vincristine.
†Patients with "0" duration received 1 infusion or treatment (1 day) without continuation of therapy.
‡Includes chronic lymphocytic leukemia, macroglobulinemia, unspecified lymphoproliferative disorders, and other lymphomas. T-cell lymphoma was excluded.
§Includes MGUS and T-cell lymphoma.
∥Transfusion requirement was noted for each individual round of therapy, not overall patient course, to reflect the effect of the unique therapeutic agent.
*Response to therapy was noted for each round of therapy, not overall patient treatment course, to reflect the unique response to the specific therapeutic regimen.
*Further treatment was noted for each round of therapy to reflect their individual efficacies.

Blood 2013;122:114-1121
# Paroxysmal cold hemoglobinuria


<table>
<thead>
<tr>
<th>Symptoms and Signs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Usually a Child</td>
<td></td>
</tr>
<tr>
<td>History of a recent upper respiratory or “flu-like” illness.</td>
<td></td>
</tr>
<tr>
<td>Acute onset of Illness</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>Malaise</td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td></td>
</tr>
<tr>
<td>Red or Red-Brown Urine is Frequently Present</td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory Findings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td></td>
</tr>
<tr>
<td>Often Severe</td>
<td></td>
</tr>
<tr>
<td>May be Rapidly Progressive</td>
<td></td>
</tr>
<tr>
<td>Reticulocytosis (Reticulocytopenia in Some Patients)</td>
<td></td>
</tr>
<tr>
<td>Abnormal RBC Morphology</td>
<td></td>
</tr>
<tr>
<td>Spherocytosis, anisocytosis, poikilocytosis, autoagglutination, Polychromatophilia.</td>
<td></td>
</tr>
<tr>
<td>Erythrophagocytosis by neutrophils is commonly present</td>
<td></td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>Erythroid Hyperplasia or Normal Results in Bone Marrow</td>
<td></td>
</tr>
<tr>
<td>Leukocytosis (Leukopenia in Some Patients)</td>
<td></td>
</tr>
<tr>
<td>Platelet Count usually Normal or Elevated</td>
<td></td>
</tr>
</tbody>
</table>
Paroxysmal cold hemoglobinuria

Biphasic hemolysin
Sensitizes RBCs in the cold
Induces hemolysis when the RBCs reach 37°C
Normal RBC incubated with the patient’s serum at 0°C and moved to 37°C for further incubation
No lysis following incubation at 0°C
No lysis if incubation at only 37°C
**TTP**

**A**

- **Low shear stress**
  - ULVWF multimer in globular form
  - VWFCP
  - Platelet

- **High shear stress**
  - ULVWF multimer in linear form
  - VWF binding sites for platelets and VWFCP exposed
  - VWF fragments generated by VWFCP cleavage

**B**

- **Low shear stress**
  - VWFCP inactivated by inhibitory antibody

- **High shear stress**
  - No cleavage of biologically active linear ULVWF multimer in the absence of VWFCP activity
  - ULVWF multimer readily binds platelets and endothelial cell wall resulting in platelet thrombi and vessel occlusion

---

TTP: thrombotic thrombocytopenic purpura; ULVWF: ultra-large von Willebrand factor; VWFCP: von Willebrand factor cleaving protease.
Complement-mediated lysis of PNH erythrocytes.

Alternative Pathway of Complement

C3 convertase
C3bBbP
CD55

C3a
C3
C5 convertase
C3bBbC3bP
CD55

C5a
C5
Membrane Attack Complex
C5b-9n
CD59

*GPI-anchored complement regulatory proteins deficient in PNH

Complement Activation

Normal RBC
PNH RBC

Parker C J Hematology 2011;2011:21-29
Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, reticulocyte count, serum LDH concentration, bone marrow analysis

Subclinical PNH
- No specific PNH therapy—focus on underlying BMF syndrome*

PNH/BMF syndrome
- Focus on BMF†
  - Patients with large PNH clones may benefit from eculizumab¶

Classic PNH
- Treat with eculizumab§
  - Inadequate response
  - BMT, [steroids, splenectomy]**, supportive care

*Some, but not all, studies suggest a favorable response to immune-suppressive therapy (IST)
†BMT eradicates the PNH clone, and typically, treatment with IST does not affect PNH clone size
¶<10% of patients with PNH/BMF have PNH clone size >50%
§Some patients respond to Danazol as first line therapy
**Consider for patients with clinically significant extravascular hemolysis

Parker C J Hematology 2011;2011:21-29
Clinical and Laboratory Findings in Vitamin B₁₂ Deficiency.

Abnormalities in infants and children
- Developmental delay or regression, permanent disability
- Does not smile
- Feeding difficulties
- Hypotonia, lethargy, coma
- Hyperirritability, convulsions, tremors, myoclonus
- Microcephaly
- Choreaathetoid movements

Brain
- Altered mental status
- Cognitive defects
- "Megaloblastic madness": depression, mania, irritability, paranoia, delusions, lability

Spinal cord
- Myelopathy
- Spongy degeneration

Paresthesias
- Loss of proprioception; vibration, position, ataxic gait, limb weakness; spasticity (hyperreflexia); positive Romberg sign; Lhermitte's sign; segmental cutaneous sensory level

Autonomic nervous system
- Postural hypotension
- Incontinence
- Impotence

Peripheral nervous system
- Cutaneous sensory loss
- Hyperreflexia
- Symmetric weakness
- Paresthesias

Peripheral blood
- Macrocytic red cells, macroovalocytes
- Anisocytosis, fragmented forms
- Hypersegmented neutrophils, 1% with six lobes or 5% with 5 lobes
- Leukopenia, possible immature white cells
- Thrombocytopenia
- Pancytopenia
- Elevated lactate dehydrogenase level (extremes possible)
- Elevated indirect bilirubin and aspartate aminotransferase levels
- Decreased haptoglobin level
- Elevated levels of methylmalonic acid, homocysteine, or both

Bone marrow
- Hypercellular, increased erythroid precursors
- Open, immature nuclear chromatin
- Dysynchrony between maturation of cytoplasm and nuclei
- Giant bands, metamyelocytes
- Kayser-Fleischer, dysplasia
- Abnormal results on flow cytometry and cyogenetic analysis

The Normal Mechanisms and Defects of Absorption of Vitamin B₁₂.

HC: haptocorrin
TC: transcobolamin
Serum Methylmalonic Acid and Total Homocysteine Concentrations in 491 Episodes of Vitamin B$_{12}$ Deficiency.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe malabsorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia (autoimmune gastritis)</td>
<td>Intramuscular cyanocobalamin at a dose of 1000 µg administered intramuscularly daily or every other day for 1 wk, then weekly for 4 to 8 wk, and then monthly for life, or oral cyanocobalamin at a daily dose of 1000 to 2000 µg for life*</td>
<td>Administer iron and folate replacement as needed for full hemoglobin response, especially in patients with intestinal disease; perform surveillance for other autoimmune conditions, especially thyroid disease in patients with pernicious anemia; perform upper endoscopy in patients with symptoms of gastric cancer or iron deficiency</td>
</tr>
<tr>
<td>Total or partial gastrectomy</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Gastric bypass or other bariatric surgery</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Ileal resection or organ reconstructive surgery (ileal conduit diversion and ileostomy-plasty)</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Inflammatory bowel disease, tropical sprue</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Imerslund–Gräsbeck and other syndromes‡</td>
<td>Same as for pernicious anemia</td>
<td>Genetic counselling to detect vitamin B₁₂ deficiency in family members</td>
</tr>
<tr>
<td>Mild malabsorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein-bound vitamin B₁₂ malabsorption</td>
<td>Oral cyanocobalamin at a dose of 500 to 1000 µg daily or intramuscular cyanocobalamin at a dose of 1000 µg daily or every other day for 1 wk, then weekly for 4 to 8 wk, and then monthly for life</td>
<td>Perform tests for iron deficiency, anemia of chronic kidney disease, and anemia of chronic inflammation; these conditions coexist frequently in older adults, may limit the response to treatment, and may require further treatment</td>
</tr>
<tr>
<td>Mild atrophic gastritis</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
</tr>
<tr>
<td>Use of metformin†</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
</tr>
<tr>
<td>Use of drugs that block stomach acid</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
<td>Same as for protein-bound vitamin B₁₂ malabsorption</td>
</tr>
<tr>
<td>Dietary deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>Supplements containing &gt;2 µg of vitamin B₁₂ or foods fortified with vitamin B₁₂</td>
<td>Perform tests for iron deficiency, which is very common</td>
</tr>
<tr>
<td>Infants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-feeding in infants with vitamin B₁₂- deficient mothers§‡‡</td>
<td>Intramuscular cyanocobalamin at a dose of 250 to 1000 µg daily, then weekly until patient recovers; treatment of mother to enrich breast milk; oral supplementation with 1 to 2 µg of vitamin B₁₂ daily or vitamin B₁₂-enriched formula or food</td>
<td>Confirm metabolic response in infants or refer parents to genetics specialist for evaluation; provide nutritional counseling for mothers</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases similar to those causing malabsorption in adults</td>
<td>100 µg of intramuscular vitamin B₁₂ monthly or high-dose oral vitamin B₁₂ daily in younger children; treatment as per adults in older children</td>
<td>Confirm pernicious anemia or congenital malabsorption</td>
</tr>
<tr>
<td>Recreational or occupational abuse of nitrous oxide§</td>
<td>Intramuscular cyanocobalamin at a dose of 1000 µg administered on the same schedule as that for pernicious anemia above and for life if underlying pernicious anemia is present</td>
<td>Evaluate for vitamin B₁₂ malabsorption; provide addiction counseling</td>
</tr>
<tr>
<td>Nitrous oxide anesthesia in occult pernicious anemia††</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Intramuscular hydroxocobalamin can be substituted for intramuscular cyanocobalamin, but document the long-term response if it is administered at 3-month intervals.
† Experts are not in agreement about the necessity or frequency of routine upper endoscopy in patients with pernicious anemia. However, symptoms suggestive of gastric carcinoma, unexplained iron deficiency, and proven gastrointestinal blood loss should prompt a full investigation.
‡ Congenital malabsorption of vitamin B₁₂ results from mutations of the ileal cubam receptor, cubulin, or amnionless (as in the Imerslund–Gräsbeck syndrome) and from mutations in gastric intrinsic factor. These syndromes are usually manifested in infancy and early childhood, although studies have shown a delay in onset even into adolescence.§
§ Nitrous oxide inactivates the vitamin B₁₂-dependent enzyme methionine synthase and causes formation of vitamin B₁₂ analogues and gradual tissue depletion of vitamin B₁₂.
Vitamin B12 deficiency: diagnosis

- > 300 pg/ml or > 221 pmol/l:
  - cobolamin deficiency unlikely (probability 1-5%)
- 200 to 300 pg/ml or 148 to 221 pmol/l:
  - Borderline result; cobolamin deficiency possible
- < 200 pg/ml or < 148 pmol/l
  - Low; consistent with cobolamin deficiency (specificity 95 to 100%)

- When
  - Serum cobolamin value at the lower end of the normal range or in the borderline range and high degree of suspicion (unexplained neurological complaints or macrocytosis)
  - Dosage methylmalonic acid and homocysteine
# Laboratory Testing in Vitamin B$_{12}$ Deficiency

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measurement to detect deficiency</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum vitamin B$_{12}$ &lt;200 pg/ml or laboratory cutoff level</td>
<td>65–95% for proven clinical deficiency; 50% for detecting elevated level of methylmalonic acid</td>
<td>50–60% for clinical response; 80% for detecting elevated level of methylmalonic acid</td>
<td>Current vitamin B$_{12}$ assays are especially problematic in patients with anti-intrinsic factor antibodies</td>
</tr>
<tr>
<td>Serum vitamin B$_{12}$ &lt;350 pg/ml</td>
<td>90%</td>
<td>25% for detecting elevated level of methylmalonic acid</td>
<td></td>
</tr>
<tr>
<td>Holotranscobalamin &lt;20 to 45 pmol/liter</td>
<td>Insufficient data on sensitivity for clinical deficiency; 46–89% for detecting elevated level of methylmalonic acid</td>
<td>Insufficient data on specificity for clinical deficiency; 28–96% for detecting elevated level of methylmalonic acid</td>
<td>Levels of holotranscobalamin increase in renal failure; superior to measurement of total vitamin B$_{12}$ in pregnancy, when the total level decreases</td>
</tr>
<tr>
<td>Serum methylmalonic acid &gt;400 nmol/liter</td>
<td>98% for clinical deficiency</td>
<td>Poor specificity for clinical response in patients with modest elevation of level of methylmalonic acid (300–1000 nmol/liter)</td>
<td>Renal failure and volume depletion may increase level of serum methylmalonic acid, but rarely to &gt;1000 nmol/liter</td>
</tr>
<tr>
<td>Serum or plasma total homocysteine &gt;21 μmol/liter</td>
<td>96% for clinical deficiency</td>
<td>Homocysteine level also increased in clinical folate deficiency and renal insufficiency</td>
<td></td>
</tr>
<tr>
<td><strong>Test to determine cause of deficiency</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti–intrinsic factor antibodies</td>
<td>50%</td>
<td>100%</td>
<td>Must be tested &gt;7 days after vitamin B$_{12}$ injection to prevent false positive result</td>
</tr>
<tr>
<td>Anti–parietal-cell antibodies</td>
<td>80%</td>
<td>50–100%</td>
<td></td>
</tr>
<tr>
<td>Atrophic body gastritis (antral sparing)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting high serum gastrin level (&gt;100 pmol/liter)</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low level of serum pepsinogen I (&lt;30 μg/liter)</td>
<td>90%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoscopy with pentagastrin-fast hypochlorhydria</td>
<td>100%</td>
<td></td>
<td>Rarely performed</td>
</tr>
<tr>
<td><strong>Malabsorption of vitamin B$_{12}$††</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_{12}$ absorption test</td>
<td></td>
<td></td>
<td>Schilling test no longer available</td>
</tr>
<tr>
<td>Increase in serum holotranscobalamin level after oral loading</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Promising preclinical data, but still experimental</td>
</tr>
</tbody>
</table>

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* To convert the values for vitamin B$_{12}$ to picomoles per liter, multiply by 0.7378.
† Available assays are largely chemiluminescent microparticle immunoassays performed with the use of automated analyzers that in general show higher values than the radiodilution and microbiologic assays used in past studies of clinically confirmed deficiency. Thus, these tests are likely to have lower sensitivities and specificities than the older assays.
‡ The holotranscobalamin assay has been studied widely in Europe but is not yet commercially available in the United States. The appropriate lower end of the reference range is still under debate. The values for sensitivity and specificity are reviewed in Heil et al.
§ Urinary methylmalonic acid has not been extensively studied, but values greater than 2.5 μmol per millimole of creatinine suggest deficiency.
‖ Elevated levels of methylmalonic acid fall with vitamin B$_{12}$ therapy, but an associated clinical response is highly variable, depending largely on the presence of vitamin B$_{12}$–related disease. Evidence of a causal pathologic process does not confirm coexisting B$_{12}$ deficiency, since underlying gastrointestinal disease may predate the deficiency by many years.
** The relationship between atrophic body gastritis (autoimmune gastritis) and infection with *Helicobacter pylori* is variable. Antral sparing is a type of atrophic body gastritis in which the cells in the antrum can produce high levels of gastrin.
†† There is malabsorption if clinically proven vitamin B$_{12}$ deficiency is present in a patient who eats meat, receives multivitamin therapy, or both.
Folic acid deficiency

• Nutritional deficiency
  – Alcoholism
  – Poor dietary intake
  – Overcooked food
  – Depression
  – Nursing homes

• Malabsorption
  – Celiac disease
  – IBD
  – Short bowel syndrome

• Drugs
  – Methotrexate
  – Trimethprim
  – Ethanol
  – Phenytoin

• Increased requirements
  – Chronic hemolysis
  – Pregnancy
  – Lactation
Folic acid deficiency

- Small body stores small (5-10mg) in relation to daily requirements (200 to 400 ug)
- Megaloblastosis can occur in 4 to 5 months
- No neurological changes

- Serum folate concentration is reflection of short term folate balance
- Red cell folate is a time-averaged value of folate availability

- Folate > 4 ng/ml: no deficiency
- Folate < 2 ng/ml: folate deficiency
- 2 < folate < 4: borderline values: dosage of red cell folate (and homocysteine?)

- Administration of folic acid may worsen neurologic complications of untreated vit B12 deficiency
Sideroblastic anemias

• Sideroblasts
  - Nucleated red blood cell precursors (erythroblasts) with one or more iron containing granules in the cytoplasm
  - Found in the bone marrow of normal iron-sufficient subjects
  - Normal sideroblasts show random iron deposits, typically 1-5, in the cytoplasm.

Sideroblastic anemias

- Abnormal sideroblasts
  - Increase of granular iron deposits around the nucleus
  - In the most abnormal form, granules completely surround the nucleus = ring sideroblasts = diagnostic feature of sideroblastic anemia
  - Reflect aberration in the processing of iron by the erythroblast
    - Insufficient production of protoporphyrin to utilize the iron delivered to erythroblasts
    - Faults in mitochondrial functions that affect iron pathways or impair mitochondrial protein synthesis
Sideroblastic anemias

- Amount of iron in BM macrophages increased due to ineffective hematopoisis

- Iron overload
  - Present in many congenital and acquired clonal forms
  - Usually absent in the acute, reversible forms

- Ring sideroblast abnormality may be masked when concomitant iron deficiency

Refractory anemia with ring sideroblasts masked by iron deficiency anemia Blood 2011;117:5793

After iron repletion
Classification and causes of sideroblastic anemias

• Congenital
  – Genetically and clinically heterogeneous
  – Diverse underlying causes, inheritance patterns, clinical phenotypes and associated features
  – Syndromic affecting multiple systems
  – or non-syndromic

• Acquired
  – Clonal-Neoplastic
  – Metabolic/reversible
Classification and causes of sideroblastic anemias

• Acquired
  - Clonal-Neoplastic
    - Refractory anemia with ring sideroblasts (RARS)
    - Refractory anemia with ring sideroblasts and thrombocytosis (RARS-t)
    - Refractory cytopenia with multilineage dysplasia and ring sideroblast (RCMD-RS)
  - Metabolic/reversible
    - Alcoholism
    - Drugs (linezolid, isoniazid, chloramphenicol)
    - Copper deficiency
    - Hypothermia
Classification and causes of sideroblastic anemias

• Acquired
  – Clonal-Neoplastic
    • Refractory anemia with ring sideroblasts (RARS)
      – Pure sideroblastic anemia
      – Sometimes difficult to distinguish from X-linked sideroblastic anemia in female subjects
      – Best overall prognosis of all MDS variants
      – Pyridoxine: no response expected
    • Refractory anemia with ring sideroblasts and thrombocytosis (RARS-t)
      – Frequent presence of the JAK2 mutation
      – Mixed myeloproliferative and myelodysplastic syndrome
  • Refractory cytopenia with multilineage dysplasia and ring sideroblast (RCMD-RS)
    – True MDS
Acquired metabolic sideroblastic anemias

- Erythroid heme biosynthesis or mitochondrial functions adversely affected by acquired factors
- Sideroblatic anemia fully reversible when the offending factor is removed
  - Alcoholism
  - Drugs (linezolid, isoniazid, chloramphenicol)
  - Copper deficiency
  - Hypothermia
Different factors that can contribute to anemia and/or a low hematocrit in alcoholic patients

<table>
<thead>
<tr>
<th>Cause of low hematocrit</th>
<th>Possible contributing factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhage and/or iron deficiency</td>
<td>Alcoholic gastritis, Portal hypertension, Peptic ulceration</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>Chronic liver disease and/or cirrhosis, Zieve syndrome, Spur cell anemia of severe liver disease</td>
</tr>
<tr>
<td>Reduced erythropoiesis</td>
<td>Anemia of chronic disease, Nutritional (e.g. folic acid deficiency), Sideroblastic anemia, Alcohol toxicity</td>
</tr>
<tr>
<td>Hypersplenism</td>
<td>Portal hypertension</td>
</tr>
<tr>
<td>Hemodilution</td>
<td>Fluid retention of chronic liver disease, Aggressive intravenous fluid therapy</td>
</tr>
</tbody>
</table>

Lewis G et al. (2007) A case of persistent anemia and alcohol abuse
Acquired metabolic sideroblastic anemias

- Alcohol
  - Ring sideroblast abnormality
    - 25-30% of anemic alcoholic patients
    - Usually when malnutrition and folic acid deficiency
  - Inhibition of several enzymatic steps in the heme pathway
The mammalian heme biosynthetic pathway.

Inhibition by alcohol

Pyridoxal phosphate aids in the synthesis of hemoglobin, by serving as a coenzyme for the enzyme ALA synthase.
Acquired metabolic sideroblastic anemias

- Alcohol
  - Interference with vitamin B6 metabolism (pyridoxal phosphate aids in the synthesis of hemoglobin, by serving as a coenzyme for the enzyme ALA synthase)
  - Direct effect on mitochondrial protein synthesis?
  - Withdrawal of alcohol
    - Disappearance of ring sideroblasts within days to 2 weeks
    - Resolution of anemia depends on presence or not of other problems due to alcohol
Acquired metabolic sideroblastic anemias

• Drugs
  – Isoniazide (INH)
    • Moderate anemia / 1 to 10 months after initiation
    • Interference with vitamin B6 metabolism
    • Reversed by pyridoxine or by withdrawal of the drug
  – Chloramphenicol
    • Causes ring sideroblast abnormality in a dose dependent manner
    • Suppresses erythropoiesis
    • Inhibition of mitochondrial protein synthesis at the level of RNA translation
  – Linezolid
    • Inhibition of mitochondrial protein synthesis

Haematologica 2013;98:e138-140
Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Impaired intestinal absorption and mobilization of iron from reticuloendothelial cells and hepatocytes because of associated lack of ceruloplasmin (ferroxidase function)
  - Decreased heme synthesis from ferric iron and protoporphyrin because of defective reduction of ferric iron to ferrous iron (diminished activity of the copper-containing cytochrome oxydase)
The mammalian heme biosynthetic pathway.

Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Impaired intestinal absorption and mobilization of iron from reticuloendothelial cells and hepatocytes because of associated lack of ceruloplasmin (ferroxidase function)
  - Decreased heme synthesis from ferric iron and protoporphyrin because of defective reduction of ferric iron to ferrous iron (diminished activity of the copper-containing cytochrome oxydase)
  - Impaired CD34+ cell differentiation and stem cell renewal
Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Prolonged parenteral nutrition
  - Prolonged enteral feeding
  - Malabsorption
  - Bariatric surgery
  - Use of large amounts of denture cream containing zinc
  - Nephrotic syndrome (urinary losses of ceruloplasmin)

Acquired metabolic sideroblastic anemias

• Copper deficiency
  – Neurologic manifestations
    • CNS demyelination
    • Peripheral neuropathy
    • Optic neuropathy
    • Myeloneuropathy
    • Mimic subacute combined degeneration of the cord associated with B12 deficiency (! possible coexistence of the two deficiencies!)

Acquired metabolic sideroblastic anemias

• Copper deficiency
  - Hematologic manifestations
    - Anemia (sometimes very severe)
    - MCV normal or slightly increased
    - Neutropenia
    - Platelet count usually normal
    - Sometimes pancytopenia
    - Serum iron and transferrin saturation normal
    - Serum copper and ceruloplasmin levels are decreased
    - Differential diagnosis: MDS (allo-BMT in one patient!!!!!)

Acquired metabolic sideroblastic anemias

• Copper deficiency
  - Therapy:
    • parenteral or oral copper: 2 mg elemental copper/day
    • Resolution of hematologic abnormalities in < 2 months
    • Neurologic deficits may improve or only stabilize

Acquired metabolic sideroblastic anemias

- Hypothermia
  - Sensitivity of several mitochondrial functions to reduced temperature

References