Conflicts of Interest

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  – Section Editor & Author
  – Royalties

• I do not have other financial relationships with proprietary entities producing health care goods or services
Clinical Questions to be Addressed

• What are “don’t miss” causes of iron deficiency?
• What are “don’t miss” causes of hemolysis?
• What are diagnostic challenges in vitamin B12 deficiency?
• Who should be referred for bone marrow biopsy?
Case # 1

• A 55-year male chemist develops fatigue
• 5 years ago at age 50, he had normal screening colonoscopy with visualization of the ileocecal valve and appendiceal orifice
• With a family history of heart disease, he takes aspirin 81 mg daily and drinks a glass of wine with dinner nightly
• Family history: no known malignancies or hematologic abnormalities
• Physical exam is entirely normal
• CBC
  – RBC = 3.80 million/μL (normal, 4.5-6.2)
  – Hgb = 10.5 g/dL (normal, 14.0-18.0)
  – HCT = 30.8% (normal, 40-52)
  – MCV = 79 fL (normal, 82-98)
  – MCH = 24 pg/RBC (normal, 27-32)
  – MCHC = 34 (31-35)
  – WBC with differential and platelet count are normal
• Peripheral blood smear (see next slide)
Hypochromic, microcytic RBCs, cigars and pencils in IDA
Case # 1 continued

• Serum iron chemistries
  – Iron = 9 μ/dL (normal, 45-160)
  – Calculated TIBC = 460 μ/dL (normal, 260-470)
  – Ferritin = 15 ng/mL (normal, 30-400)
• Fecal cards obtained at home are heme-positive
• Colonoscopy is normal to cecum with visualization of appendiceal orifice
• EGD
  – Gastritis; biopsy negative for *H. pylori*
  – Duodenum appears normal; biopsy not obtained
Case # 1 continued

• He stops aspirin and begins proton pump inhibitor + non-timed-release formulation ferrous gluconate (27 mg of elemental iron) + ascorbic acid 250 mg
  – Once daily 45 minutes after his evening meal
  – 2 weeks later, he increases his iron regimen to twice daily, once after lunch and once after his evening meal
Case # 1 continued

• 6 weeks later, he returns for re-evaluation
  – He notes persistent fatigue
  – Stools are black but negative for occult blood
  – CBC remains unchanged
  – Ferritin level remains low at 15 ng/mL

• His primary care physician refers him to a hematologist
Case # 1 continued

• He denies drinking tea or taking calcium supplements with his iron supplement

• Additional labs:
  • tTG-IgA = 112 units
Case # 1: Duodenal biopsy shows celiac sprue

- Celiac sprue should be considered in any patient with IDA and documented impaired iron absorption, even without GI symptoms
  - In 1 study of pts with unexplained IDA, 11 of 93 (12%) were found to have celiac sprue on small bowel biopsy

- Tissue transglutaminic acid-immunoglobulin A
  - Generally increased with active celiac sprue
  - False normal levels in pts with IgA deficiency, which is more common in pts with celiac sprue

- At EGD, gross macroscopic duodenal findings of celiac sprue can be quite subtle
  - Duodenal biopsies should be obtained routinely at EGD in pts with unexplained IDA, with or without documented impaired iron absorption

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Case # 2

- 55-year old Russian male on disability following work-related injury in metal industry
- In 2002, underwent Roux-en-Y gastric bypass surgery for obesity management. He takes supplements of iron & vitB12
- In 2010, referred to hematology for iron deficiency anemia with ferritin = 4.7 ng/mL
  - Iron/TIBC = 47/490; vitB12 = 659 pg/mL
  - HGB = 10.9; HCT = 36.7; MCV = 77; MCH = 23.0; MCHC = 29.8; RDW = 18.4
- Colonoscopy normal to cecum
- EGD
  - Congestion and erythema in whole stomach pouch compatible with gastritis
  - Efferent jejunal limb and blind jejunal end
  - Steiner stain of gastric biopsy negative for *H. pylori*
Case # 2 has an oral iron challenge test

Following an overnight fast, blood is drawn at time 0, and he swallows 1 mL of ferrous sulfate solution (15 mg of elemental iron) admixed with a glass of orange juice. Blood samples are drawn hourly thereafter.

<table>
<thead>
<tr>
<th>Time (in minutes)</th>
<th>Serum iron level (μ/dL)</th>
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<tbody>
<tr>
<td></td>
<td>Patient</td>
</tr>
<tr>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>30</td>
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<tr>
<td>60</td>
<td>108</td>
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<tr>
<td>90</td>
<td>--</td>
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<tr>
<td>120</td>
<td>117</td>
</tr>
<tr>
<td>150</td>
<td>--</td>
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<tr>
<td>180</td>
<td>127</td>
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</table>
Case # 2 receives parenteral iron

<table>
<thead>
<tr>
<th>Date</th>
<th>Ferumoxytol</th>
<th>Ferritin</th>
<th>HGB</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
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<tbody>
<tr>
<td>03/09/10</td>
<td>---</td>
<td>4.7</td>
<td>10.9</td>
<td>36.6</td>
<td>77</td>
<td>23.0</td>
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<tr>
<td>03/17/10</td>
<td>510 mg</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>03/24/10</td>
<td>510 mg</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
</tr>
<tr>
<td>04/27/10</td>
<td>---</td>
<td>64</td>
<td>13.7</td>
<td>42.4</td>
<td>87</td>
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<td>28</td>
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<td>08/26/10</td>
<td>510 mg</td>
<td>---</td>
<td>---</td>
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<td>---</td>
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<td>43.8</td>
<td>94</td>
<td>31.4</td>
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<td>10/27/10</td>
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<tr>
<td>03/22/11</td>
<td>---</td>
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<td>15.2</td>
<td>44.5</td>
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<td>---</td>
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</tr>
<tr>
<td>03/30/11</td>
<td>510 mg</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
</tr>
<tr>
<td>06/27/11</td>
<td>---</td>
<td>107</td>
<td>14.7</td>
<td>42.9</td>
<td>92</td>
<td>31.5</td>
<td>34.3</td>
</tr>
<tr>
<td>09/13/11</td>
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<td>64</td>
<td>15.2</td>
<td>43.6</td>
<td>92</td>
<td>32.3</td>
<td>34.9</td>
</tr>
</tbody>
</table>
Iron Salts versus Polysaccharide-iron Complexes

• Iron salt formulations (NOT “time-release”)
  – Release iron in the duodenum
  – Iron is best absorbed from the duodenum and proximal jejenum

• Polysaccharide-iron complexes, enteric coated or sustained release capsules (e.g., Niferex® 150)
  – Release iron further down GI tract where iron is not absorbed
  – Counterproductive
  – More expensive than simple iron salt tablets
Factors Influencing Dietary Iron Absorption

**Positive factors**
- Ascorbic acid
- Meat or fish

**Negative factors**
- Phytate (in bran, oats, rye fiber)
- Polyphenols (in tea, some vegetables and cereals)
- Dietary calcium
- Soy protein
- Inhibition of gastric acid secretion
Optimizing Patient Acceptance in Oral Iron Therapy

- Administer small doses with or after meals
- Increase dose amounts as tolerated
  - Once daily for 2 weeks
  - Twice daily thereafter, if tolerated
  - Very few patients tolerate thrice daily iron
- Administer stool softeners proactively
Blood Loss Is the Most Common Cause of Anemia

- Obvious bleeding
  - Trauma, melena, hematemesis, menometrorrhagia
- Bleeding that is not always obvious
  - Surgical bleeding, bleeding into the upper thigh or retroperitoneum, factitious bleeding
- Iatrogenic bleeding
  - Repeated phlebotomy, hemodialysis, blood donation
- Occult bleeding
AGA Definition of Occult Blood Loss

The initial presentation of a positive fecal occult blood test result and/or iron deficiency anemia, when there is no evidence of visible blood loss to the patient or physician

Zuckerman GR et al. Gastroenterology 2000;118:201
When Bleeding Is Not Evident

- Decreased iron absorption
  - Malabsorption
  - Diet
- Intravascular hemolysis
  - Paroxysmal nocturnal hemoglobinuria
  - Malfunctioning heart valves
- Pulmonary hemosiderosis
  - Anti-glomerular basement membrane antibody disease
- Erythropoietin administration
Considerations when patients do not respond to oral iron supplements

- Poor patient adherence
  - Oral iron exacerbates symptoms of underlying disease (e.g., inflammatory bowel disease)
- Continued bleeding
- Inability to absorb iron preparation
  - Gastric by-pass for obesity
- Coexisting condition
  - Inflammation (blocking iron utilization)
  - Renal failure (erythropoietin deficiency)
- Incorrect diagnosis
How I treat unexplained refractory iron deficiency anemia

Chaim Hershko\textsuperscript{1} and Clara Camaschella\textsuperscript{2}

\textsuperscript{1}Division of Quality Assurance, Israel Ministry of Health, Jerusalem, Israel; and \textsuperscript{2}Vita-Salute San Raffaele University and San Raffaele Scientific Institute, Milan, Italy

Endoscopic gastrointestinal workup fails to establish the cause of iron deficiency anemia (IDA) in a substantial proportion of patients. In patients referred for hematologic evaluation with unexplained or refractory IDA, screening for celiac disease, autoimmune gastritis, \textit{Helicobacter pylori}, and hereditary forms of IDA is recommended. About 4\% to 6\% of patients with obscure refractory IDA have celiac disease, and autoimmune gastritis is encountered in 20\% to 27\% of patients. Stratification by age cohorts in autoimmune gastritis implies a disease presenting as IDA many years before the establishment of clinical cobalamin deficiency. Over 50\% of patients with unexplained refractory IDA have active \textit{H pylori} infection and, after excluding all other causes of IDA, 64\% to 75\% of such patients are permanently cured by \textit{H pylori} eradication. In young patients with a history suggestive of hereditary iron deficiency with serum ferritin higher than expected for IDA, mutations involving iron trafficking and regulation should be considered. Recognition of the respective roles of \textit{H pylori}, autoimmune gastritis, celiac disease, and genetic defects in the pathogenesis of iron deficiency should have a strong impact on the current diagnostic workup and management of unexplained, or refractory, IDA. (\textit{Blood}. 2014;123(3):326-333)
Proposed diagnostic workup for unexplained or refractory IDA
(Hershko C, Camaschella C. Blood 2014; 123:326-333)

<table>
<thead>
<tr>
<th>Screening</th>
<th>H pylori</th>
<th>Autoimmune gastritis</th>
<th>Celiac disease</th>
<th>IRIDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td><strong>H pylori IgG antibodies or fecal antigen</strong></td>
<td>Serum gastrin anti-parietal Abs, anti-intrinsic factor Abs</td>
<td>tTG-IgA Abs</td>
<td>Suggestive history and clinical assessment</td>
</tr>
<tr>
<td>Advanced</td>
<td>Urease breath test; gastroscopy and biopsies (optional)</td>
<td>Gastroscopy and biopsies (recommended)</td>
<td>Duodenal biopsy, HLA screening for DQ2 or DQ8 genotypes</td>
<td>Sequencing of the TMPRSS6 gene</td>
</tr>
<tr>
<td>Response to specific treatment</td>
<td><strong>H pylori eradication</strong></td>
<td>NA</td>
<td>Gluten-free diet</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable; IRIDA, iron refractory iron deficiency anemia
Clinical Questions to be Addressed

- What are “don’t miss” causes of iron deficiency?
- What are “don’t miss” causes of hemolysis?
- What are diagnostic challenges in vitamin B12 deficiency?
- Who should be referred for bone marrow biopsy?
Hemolysis: General Approach

- **Congenital vs. acquired**
  - Membrane disorders; hemoglobinopathies; enzymopathies

- **Spherocytic vs. non-spherocytic**
  - Spherocytes (membrane; immune; phospholipase)
  - Bite cells (oxidant stress; methemoglobin)
  - Schistocytes (fragmentation)
  - Spur cells (liver disease)
  - Malaria; babesiosis

- **Immune vs. non-immune**
  - Coombs testing (IgG, C3)

- **Intramedullary vs. extramedullary**
  - Intravascular (urine hemosiderin, hemoglobinuria)
  - Extravascular (spleen, RES)
Examination of the blood smear is key

Diagnosis from the Blood Smear

Barbara J. Bain, F.R.A.C.P., F.R.C.Path.

AN EXAMINATION OF THE BLOOD SMEAR (OR FILM) MAY BE REQUESTED by physicians or initiated by laboratory staff. With the development of sophisticated automated blood-cell analyzers, the proportion of blood-count samples that require a blood smear has steadily diminished and in many clinical settings is now 10 to 15 percent or less. Nevertheless, the blood smear remains a crucial diagnostic aid. The proportion of requests for a complete blood count that generate a blood smear is determined by local policies and sometimes by financial and regulatory as well as medical considerations. For maximal information to be derived from a blood smear, the examination should be performed by an experienced and skilled person, either a laboratory scientist or a medically qualified hematologist or pathologist. In Europe, only
Microcytic, hypochromic RBCs, targets and sickle forms in S-β⁺ thalassemia
Spherocytes in warm autoimmune hemolytic anemia
Macrophage Ingesting an IgG-Coated RBC

RBC aggregates in cold agglutinin disease
IgG and IgM Binding RBC Antigens
Numerous spherocyttes from phospholipase digestion of RBCs in clostridial sepsis
Erythrophagocytosis in EBV infection
Spur cell hemolytic anemia in liver disease
Diagnosis from the blood smear

Spherocytes

Bite cells

Schistocytes
Case # 3

24-yo woman develops headaches and fevers. On exam, she appears restless with dysarthric speech. T = 100.5°F.
- HCT = 28%; HGB = 9.7 g/dL; MCV = 102 fL
- WBC = 6.6 K/µL with 55% polys, 3% bands, 30% lymphs, 8% monos, 4% NRBCs
- Platelets = 5 K/µL; PT = 12.1 sec; aPTT = 24.3 sec
- Retic count = 6.6%; LDH = 546 IU/L; total bili = 1.1 mg/dL; haptoglobin < 20 mg/dL
- Creatinine = 0.5 mg/dL
Schistocytes, NRBC, decreased platelets
Thrombotic Microangiopathies
(alias Microangiopathic Hemolytic Anemias)

- Disseminated intravascular coagulation
  - Infections
  - Obstetrical disorders
  - Other causes
- Malignant hypertension
- Disseminated carcinoma
- Giant hemangioma

- TTP
  - Pregnancy
  - Autoimmune disorders
  - HIV
  - E. coli O157:H7, O104:H4
- HUS
- Immunologic vasculitis
- Antiphospholipid antibody syndrome
- Mitomycin, ticlopidine, quinine, cyclosporine
TTP: Lab Manifestations

- Red cell fragmentation can be extreme
  - Even to the extent of decreasing red cell mean corpuscular volume (MCV) and artifactually increasing reported platelet counts

- Serum lactate dehydrogenase (LDH) level is typically extremely high
  - Reflecting hemolysis and tissue damage due to systemic ischemia
Pathogenesis of Idiopathic TTP Caused by ADAMTS13 Deficiency

Sadler JE. Blood 2008; 112:11-18
ADAMTS13 Activity Predicts Relapse Risk

Kaplan-Meier curve of time to first relapse. The y-axis shows the relapse-free survival distribution function, whereas x-axis indicates years of follow-up. Forty-seven surviving patients with ADAMTS13 activity < 10% and 136 patients with ADAMTS13 ≥ 10% were analyzed.
ADAMTS13 Assays

• Assay results for the von Willebrand factor cleaving protease (ADAMTS13) and its inhibitor are not readily available

• Other reasons for decreased ADAMTS13 activity
  – HIT, severe sepsis, liver cirrhosis, chronic uremia, ITP, DIC, SLE, leukemia, pregnancy, post-op, neonatal age, advanced age
TTP: Dx and Management

- Thrombocytopenia and MAHA without another clinically apparent cause (e.g., DIC, malignant hypertension): Warrants suspicion of TTP-HUS and initiation of steroids + plasma exchange
- Alternative diagnosis: Stop plasma exchange
- ADAMTS13 activity normal: Stop steroids; debate role of plasma exchange
- ADAMTS13 inhibitor present: Continue steroids
- ADAMTS13 inhibitor present with refractory-relapsing TTP: Consider rituximab

Adjuvant rituximab to prevent TTP relapse

Adam Cuker  UNIVERSITY OF PENNSYLVANIA

In this issue of Blood, Page et al show that treatment with rituximab at the time of an initial episode of thrombotic thrombocytopenic purpura (TTP) is associated with a dramatic reduction in the risk of subsequent relapse. Their data raise the question of whether all or selected patients with a first episode of acquired TTP should be treated with adjuvant rituximab to prevent recurrence.¹

<table>
<thead>
<tr>
<th>Disease</th>
<th>Intervention</th>
<th>Absolute risk of relapse without intervention</th>
<th>Absolute risk of relapse with intervention</th>
<th>Absolute risk reduction</th>
<th>Relative risk reduction</th>
<th>Number needed to treat</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTP¹</td>
<td>Rituximab</td>
<td>43%*</td>
<td>13%*</td>
<td>30%*</td>
<td>70%*</td>
<td>3.3*</td>
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</table>

Final Comments

- Avoid platelet transfusion in patients with TTP except for management of life threatening hemorrhage.

- To date, there is no defined role for monitoring ADAMTS13 activity after recovery.
Recommended Reading

 Syndromes of Thrombotic Microangiopathy

James N. George, M.D., and Carla M. Nester, M.D.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cause</th>
<th>Clinical Features</th>
<th>Initial Management</th>
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</thead>
<tbody>
<tr>
<td><strong>Hereditary disorders</strong></td>
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<tr>
<td>ADAMTS13 deficiency-mediated TMA</td>
<td>Homozygous or compound heterozygous ADAMTS13 mutations</td>
<td>Initial presentation is typically in children but may also be in adults; possible evidence of ischemic organ injury; acute kidney injury is uncommon; patients with heterozygous mutations are asymptomatic.</td>
<td>Plasma infusion</td>
</tr>
<tr>
<td>(also called TTP)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Complement-mediated TMA</td>
<td>Mutations in CFH, CFI, CFB, C3, CD46, and other complement genes causing uncontrolled activation of the alternative pathway of complement</td>
<td>Initial presentation is often in children but may also be in adults; acute kidney injury is common; patients with heterozygous mutations may be symptomatic.</td>
<td>Plasma infusion or exchange, anti-complement agent</td>
</tr>
<tr>
<td>Metabolism-mediated TMA</td>
<td>Homozygous mutations in MMACHC (encoding methylmalonic aciduria and homocystinuria type C protein)</td>
<td>Initial presentation is typically in children &lt;1 year of age; also reported in one young adult with hypertension and acute kidney injury.</td>
<td>Vitamin B₁₂, betaine, folinic acid</td>
</tr>
<tr>
<td>Coagulation-mediated TMA</td>
<td>Homozygous mutations in DGKE; mutations in PLG and THBD also implicated</td>
<td>Initial presentation with acute kidney injury is typically in children &lt;1 year of age with DGKE mutations; clinical features of disorders associated with other mutations have not been described.</td>
<td>Plasma infusion</td>
</tr>
</tbody>
</table>

*Table 1. Primary Thrombotic Microangiopathy (TMA) Syndromes.*

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<table>
<thead>
<tr>
<th>Name</th>
<th>Cause</th>
<th>Clinical Features</th>
<th>Initial Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquired disorders</td>
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<tr>
<td>ADAMTS13 deficiency—mediated TMA</td>
<td>Autoantibody inhibition of ADAMTS13 activity</td>
<td>Initial presentation is uncommon in children; often presents with evidence of ischemic organ injury; acute kidney injury is uncommon.</td>
<td>Plasma exchange, immunosuppression</td>
</tr>
<tr>
<td>(also called TTP)</td>
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</tr>
<tr>
<td>Shiga toxin—mediated TMA</td>
<td>Enteric infection with a Shiga toxin—secreting strain of Escherichia coli or Shigella dysenteriae</td>
<td>Initial presentation is more common in young children, typically with acute kidney injury; most cases are sporadic, large outbreaks also occur.</td>
<td>Supportive care</td>
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<tr>
<td>(also called ST-HUS)</td>
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</tr>
<tr>
<td>Drug-mediated TMA (immune reaction)</td>
<td>Quinine and possibly other drugs, with multiple cells affected by drug-dependent antibodies</td>
<td>Initial presentation is a sudden onset of severe systemic symptoms with anuric acute kidney injury.</td>
<td>Removal of drug, supportive care</td>
</tr>
<tr>
<td>Drug-mediated TMA (toxic dose—related reaction)</td>
<td>Multiple potential mechanisms (e.g., VEGF inhibition)</td>
<td>Gradual onset of renal failure occurs over weeks or months.</td>
<td>Removal of drug, supportive care</td>
</tr>
<tr>
<td>Complement-mediated TMA</td>
<td>Antibody inhibition of complement factor H activity</td>
<td>Initial presentation is acute kidney injury in children or adults.</td>
<td>Plasma exchange, immunosuppression, anticomplement agent</td>
</tr>
</tbody>
</table>

* The primary TMA syndromes are described by evidence supporting a defined cause. Shiga toxin—mediated TMA (also called Shiga toxin—related hemolytic–uremic syndrome [ST-HUS]) occurs primarily in children and may be the most common of the nine primary TMA syndromes. Among adults, acquired thrombotic thrombocytopenic purpura (TTP) may be the most common primary TMA syndrome; acquired TTP is rare in children, in whom the incidence may be similar to that of hereditary TTP. The frequencies of TMAs that are mediated by complement, metabolism, coagulation, or drugs are unknown. The demonstration of antibodies that can neutralize the activity of complement factor H suggests that acquired TMA mediated by a deficiency in complement factor H may occur. DGKE denotes diacylglycerol kinase ɛ, PLG plasminogen, THBD thrombomodulin, and VEGF vascular endothelial growth factor.
Figure 1. Pathological Features of the Nine Primary Thrombotic Microangiopathy (TMA) Syndromes.

For all primary TMA syndromes, the vascular pathological abnormalities that are observed in routine specimens are the same, as illustrated in the center of the figure by the renal arteriole occlusion with endotheliosis as well as lumen and vessel-wall fibrin. Proliferation in the myocyte layer ("onion skinning") is also present in this image. TTP denotes thrombotic thrombocytopenic purpura. (Courtesy of D.G. Holanda, Department of Pathology, University of Iowa.) Additional details are provided in an interactive graphic, available at NEJM.org.
<table>
<thead>
<tr>
<th>Table 2. Common Disorders Associated with Microangiopathic Hemolytic Anemia and Thrombocytopenia.*</th>
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</thead>
<tbody>
<tr>
<td>Systemic infection</td>
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<tr>
<td>Systemic cancer</td>
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<tr>
<td>Severe preeclampsia, eclampsia, HELLP syndrome</td>
</tr>
<tr>
<td>Severe hypertension</td>
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<tr>
<td>Autoimmune disorders (e.g., systemic lupus erythematosus, systemic sclerosis, antiphospholipid syndrome)</td>
</tr>
<tr>
<td>Hematopoietic stem-cell or organ transplantation</td>
</tr>
</tbody>
</table>

*Disorders that may initially suggest primary TMA.*
Clinical Questions to be Addressed

• What are “don’t miss” causes of iron deficiency?
• What are “don’t miss” causes of hemolysis?
• What are diagnostic challenges in vitamin B12 deficiency?
• Who should be referred for bone marrow biopsy?
A 40-yo woman presents with fatigue and numerous dietary allergies. On exam, she appears thin.

- HCT = 26.0%; HGB = 9.5 g/dL; MCV = 109 fL
- WBC = 3.2 K/µL with normal differential
- Platelets = 143 K/µL
- Folate (serum) = 2.2 ng/mL (normal, 2.0-20)
- Folate (RBC) = 0
- Vitamin B₁₂ = 263 pg/mL (normal, 200-1000)
Case # 4 continued

- Methylmalonic acid level
  - 612 nmol/L (normal, 90-279)
- Homocysteine level
  - 52.8 μmol/L (normal, 0-8.9)
40-year old lab technician with increasing difficulty “focusing” and “carrying out tasks.”
- HCT, HGB, MCV, WBC, platelets normal

Over the next 2 years
- MCV begins to rise in the normal range, reaching 100 fL
- HCT and HGB levels remain normal

B12 deficiency suspected and replacement therapy begun
- Neurologic and hematologic improvement
Onset of cognitive concerns

Dx of B12 deficiency

Hypersegmented neutrophil
Actions of Cobalamin and Folate
Causes of Vitamin B12 Deficiency

**Gastric abnormalities**
- Pernicious anemia
- Gastrectomy
- Gastritis
- Autoimmune metaplastic atrophic gastritis

**Small bowel disease**
- Malabsorption syndrome
- Ileal resection or bypass
- Crohn's disease
- Blind loops

**Pancreatitis**
- Pancreatic insufficiency

**Diet**
- Strict vegans
- Vegetarian diet in pregnancy

**Agents that block absorption**
- Neomycin
- Biguanides (eg, metformin)
- Proton pump inhibitors (eg, omeprazole)
- N2O anesthesia inhibits methionine synthase

**Inherited transcobalamin II deficiency**

*From UpToDate®*
Degree of Elevation of MCV

- Often a clue to whether a vitamin deficiency is present
- Probability of folate and/or cobalamin deficiency is greater with higher MCVs
  - Normal (80-100 fL), < 25%
  - 115 to 129 fL, 50%
  - > 130 fL, 100%

Cobalamin Levels

- > 300 pg/mL
  - Cbl deficiency unlikely (1-5%)
- 200-300 pg/mL
  - Borderline result; Cbl deficiency is possible
- < 200 pg/mL
  - Consistent with Cbl deficiency (specificity, 95-100%)
- May fall during pregnancy without hematologic evidence of deficiency

Serum Methylmalonic Acid and Homocysteine Levels

- Help clarify diagnosis when cobalamin and folate levels are equivocal
- Serum MMA and homocysteine levels elevated in
  - 98 and 96% of 434 episodes of Cbl deficiency
  - 12 and 91% of 123 episodes of folate deficiency
    - Elevated MMA in all but one folate deficient patient was due to renal insufficiency or hypovolemia
  

- Hereditary homocysteinemia can raise serum homocysteine levels
Oral Replacement of Cobalamin

- 2,000 µg daily (NOT “time release”)
- Appears to be as or more effective than parenteral therapy
- Takes advantage of a second, lower efficiency transport system for Cbl that does not require intrinsic factor or a terminal ileum
- Requires patient adherence

*Kuzminski AM et al. Blood 1998;92:1191*
(Kuzminski AM et al. Blood 1998;92:1191)
(Kuzminski AM et al. Blood 1998;92:1191)
Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing

Lawrence R. Solomon

Early recognition of cobalamin (Cbl)—responsive disorders in the ambulatory care setting is essential to prevent irreversible neurologic deficits. However, diagnostic algorithms using Cbl, methylmalonic acid (MMA), and homocysteine (HCys) measurements reflect studies in academic centers, and their negative predictive values have not been established. Thus, records of 456 ambulatory patients evaluated for Cbl deficiency at a staff model HMO were reviewed. Pretherapy Cbl, MMA, and HCys values in individual patients varied by 23%, 23%, and 17%, respectively, over 2 to 6 weeks. Hematologic or neurologic responses to pharmacologic doses of Cbl occurred in 37 of the 95 evaluable patients. In these patients, pretherapy Cbl, MMA, and HCys values were normal in 54%, 23%, and 50%, respectively. If therapy had been restricted to symptomatic patients with both low or intermediate Cbl levels and increased metabolite values, 63% of responders would not have been treated. Twenty-five patients did not respond to treatment, including 5 of 11 patients (45%) with low Cbl, 22 of 49 patients (45%) with high MMA, and 13 of 30 patients (43%) with high HCys values. It is concluded that Cbl, MMA, and HCys levels fluctuate with time and neither predict nor preclude the presence of Cbl-responsive hematologic or neurologic disorders. (Blood. 2005;105:978-985) © 2005 by The American Society of Hematology

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CLINICAL OBSERVATIONS

Comment on Solomon, page 978

Unreliability of current assays to detect cobalamin deficiency: “nothing gold can stay”

Ralph Green UNIVERSITY OF CALIFORNIA, DAVIS

Measurement of vitamin B₁₂, homocysteine, and methylmalonic acid may not be the ultimate “gold standard” for diagnosis of cobalamin deficiency.
Caveats in Testing and Interpretation

• Role of dietary and vitamin supplements
  – Cobalamin deficiency “masked” by folate
  – Numerous over-the-counter $\text{B}_{12}$ supplement choices
    • 2, 25, 50, 100, 1000, and 2000 mcg
    • Not FDA-regulated

• Role of decreased GFR in determining MMA levels

• Reliability of assays and clinical labs

• Other factors influencing RBC mass, MCV, and neurologic symptoms and signs
  – Alcohol
Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults

Joshua W. Miller, Marisa I. Ramos, Marjorie G. Garrod, Margaret A. Flynn, and Ralph Green

A common polymorphism (775G>C) in the vitamin B12 transport protein, transcobalamin II (TCII), has been identified in which proline replaces arginine at codon 259. We determined the influence of TCII genotype on indices of B12 status, including total serum B12, the amount of B12 bound to TCII (holoTCII), methylmalonic acid, and homocysteine, in 128 healthy older adults (ages 40-88 years). Mean total B12 and homocysteine concentrations were not significantly different among the 3 genotypes. Mean holoTCII concentration was significantly higher in those subjects homozygous for the proline form of TCII (PP) compared with those homozygous for the arginine form (RR) and heterozygotes (PR) (P ≤ .006). In addition, mean methylmalonic acid concentrations were significantly lower in the PP and PR groups compared with the RR group (P ≤ .02). The PP genotype may be more efficient in delivering B12 to tissues, resulting in enhanced B12 functional status. TCII genotype may thus influence susceptibility to B12 deficiency. (Blood. 2002;100:718-720)

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<table>
<thead>
<tr>
<th>Table 1. Characteristics of study sample by transcobalamin II genotype</th>
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<tr>
<td>Transcobalamin II genotype</td>
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<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Sex (men/women)</td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>B12 (pg/mL)</td>
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<tr>
<td>HoloTCII (pg/mL)</td>
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<tr>
<td>% Total B12 on TCII†</td>
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<tr>
<td>Methylmalonic acid (nM)</td>
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<tr>
<td>Homocysteine (µM)</td>
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<tr>
<td>RBC folate (ng/mL)</td>
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<tr>
<td>Creatinine (mg/dL)</td>
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<tr>
<td>Hematocrit (%)</td>
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<td>MCV (µm³)</td>
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Values represent means (± SD).

†Calculated using the equation: 100 × (holoTCII/total B12).

*Significantly less than PP genotype after controlling for potential confounding by age, sex, and total B12 (P ≤ .006).

‡Significantly greater than PP and PR genotypes after controlling for potential confounding by age, sex, total B12, and creatinine (P ≤ .02).
How I treat cobalamin (vitamin B₁₂) deficiency

Ralph Carmel

Department of Medicine, New York Methodist Hospital, Brooklyn, and Weill Medical College, Cornell University, New York, NY

The challenges in medical management of cobalamin deficiency lie in attention to the unique pathophysiology that underlies cobalamin deficiency, more than in the mechanics of therapy. The central physiologic principles are that clinically important deficiency is more likely to occur (and progress) when intrinsic factor–driven absorption falls than when diet is poor and that most causes take years to produce clinically obvious deficiency. Transient defects have little clinical impact. The key management principle is the importance of follow-up, which also requires knowing how the deficiency arose. The virtues of these principles are not always fully appreciated. Recent developments have made diagnosis and management more difficult by diminishing the ability to determine cobalamin absorption status. Clinicians must also grapple with premature medicalization of isolated, mild biochemical changes that added many asymptomatic cases of still undetermined medical relevance to their caseload, often expanded by inflated cobalamin level criteria. The potential for misattribution of cobalamin-unrelated presentations to nongermane cobalamin and metabolite abnormalities has grown. Pathophysiologically based management requires systematic attention to each of its individual components: correctly diagnosing cobalamin deficiency, reversing it, defining its underlying cause, preventing relapse, managing the underlying disorder and its complications, and educating the patient. (Blood. 2008;112:2214-2221)
Clinical Questions to be Addressed

• What are “don’t miss” causes of iron deficiency?
• What are “don’t miss” causes of hemolysis?
• What are diagnostic challenges in vitamin B12 deficiency?
• Who should be referred for bone marrow biopsy?
Bone Marrow Core Biopsy
Disorders of Hematopoiesis
Peripheral Blood Clues to Myelodysplastic Syndrome

- Red blood cells
  - Prominent basophilic stippling and other sideroblastic features (e.g., hypochromic, microcytosis)
  - Macro-ovalocytes (reflecting megaloblastic maturation)
  - Tear drops (reflecting myelophthysis)
- White blood cells
  - Pelgeroid cells, hypogranulation, toxic granulation, Döhle bodies, immature myeloid forms
- Platelets
  - Giant forms, megakaryoblasts
Infiltrative Myelopathies

• Alias
  – Myelophthisic anemia
  – Leukoerythroblastic anemia

• Hallmark features include
  – Immature granulocyte precursors in the peripheral blood (bands, metas, myelos)
  – Nucleated red cells
  – Tear drops
SEM of Teardrop in Formation
Primary myelofibrosis
Aplastic Anemia
Monoclonal gammopathy of undetermined significance

- No symptoms
- No end organ damage related to plasma cell dyscrasia or a related B cell lymphoproliferative disorder
  - No lymphadenopathy, organomegaly, hypercalcemia, renal failure, anemia, bone abnormalities (skeletal survey)*
- Relatively low paraprotein level (< 3 g/dL)
- < 10% plasmacytosis in bone marrow
- Phenomenon of aging

*No role for bone scans or PET scans
Diagnostic criteria for myeloma

- **Myeloma**
  - Serum or urinary monoclonal protein
  - Clonal plasma cell in bone marrow or plasmacytoma
  - End organ damage related to plasma cell dyscrasia

- **Asymptomatic (smoldering) myeloma**
  - Serum monoclonal protein $\geq 3$ g/dL and/or bone marrow plasma cells $\geq 10$ percent
  - No end organ damage related to plasma cell dyscrasia
“Bloodline,” 1995
by Kiki Smith