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 2. Common Disorders Associated with Microangiopathic Hemolytic Anemia and Thrombocytopenia.*

<table>
<thead>
<tr>
<th>Disorder</th>
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<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.

Microangiopathic hemolytic anemia with thrombocytopenia

- Underlying disorders (common in adults, uncommon in children)

Kidney injury

- Sudden onset (systemic symptoms, anuria within hours)
  - Drug (immune, uncommon in children)
  - ST-HUS (Shiga toxin, more common in children)
- Acute onset (several days of illness preceding kidney injury)
  - Acquired complement (more common in children) and hereditary complement (probably equally common in children)
  - Coagulation (probably more common in children)
- Long-term onset (weeks or months of progressive kidney injury)
  - Metabolism (probably more common in children)
  - Drug (toxic, uncommon in children)
  - Acquired TTP (uncommon in children) and hereditary TTP (more common in children)

No or minimal kidney injury

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)
Case # 5

• 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

Normal Macroovalocytes
Hypersegmented neutrophil
Actions of Cobalamin and Folate

From UpToDate®
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)
- N20 anesthesia inhibits methionine synthase

**Inherited transcobalamin II deficiency**
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

Serum Cobalamin (pg/ml) vs Months of Therapy

- Oral
- Parenteral

Normal range

P < .0005

(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)

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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 $B_{12}$ 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 1. Characteristics of study sample by transcobalamin II genotype

<table>
<thead>
<tr>
<th></th>
<th>Transcobalamin II genotype</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>PP</td>
<td>PR</td>
<td>RR</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>39</td>
<td>63</td>
<td>26</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td></td>
<td>33/6</td>
<td>54/9</td>
<td>21/5</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td>66 (± 11)</td>
<td>67 (± 11)</td>
<td>67 (± 11)</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td></td>
<td>433 (± 177)</td>
<td>415 (± 168)</td>
<td>383 (± 191)</td>
</tr>
<tr>
<td>HoloTCII (pg/mL)</td>
<td></td>
<td>150 (± 81)</td>
<td>113 (± 56) *</td>
<td>104 (± 83) *</td>
</tr>
<tr>
<td>% Total B12 on TCII†</td>
<td></td>
<td>34.3 (± 9.5)</td>
<td>27.8 (± 9.9) *</td>
<td>24.6 (± 10.1) *</td>
</tr>
<tr>
<td>Methylmalonic acid (nM)</td>
<td></td>
<td>208 (± 96)</td>
<td>206 (± 80)</td>
<td>264 (± 138) ‡</td>
</tr>
<tr>
<td>Homocysteine (μM)</td>
<td></td>
<td>10.3 (± 2.6)</td>
<td>10.7 (± 2.4)</td>
<td>11.2 (± 2.8)</td>
</tr>
<tr>
<td>RBC folate (ng/mL)</td>
<td></td>
<td>363 (± 97)</td>
<td>378 (± 106)</td>
<td>398 (± 106)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td></td>
<td>1.1 (± 0.9)</td>
<td>1.1 (± 1.0)</td>
<td>1.1 (± 0.6)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td>43.4 (± 3.2)</td>
<td>43.8 (± 3.5)</td>
<td>43.6 (± 3.3)</td>
</tr>
<tr>
<td>MCV (μm³)</td>
<td></td>
<td>93.9 (± 3.2)</td>
<td>92.9 (± 3.2)</td>
<td>92.2 (± 5.1)</td>
</tr>
</tbody>
</table>

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 B\textsubscript{12}) deficiency

Ralph Carmel\textsuperscript{1}

\textsuperscript{1}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 fails 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)
• Several biological effects may explain harmful effect of B vitamins in patients with impaired renal failure
  – High levels of folic acid not metabolized to tetrahydrofolate could increase levels of asymmetric dimethylarginine, an antagonist of nitric oxide
  – Cyanide accumulates in patients given cyanocobalamin
    • Cyanide excreted as thiocyanate has role in catabolism of hydrogen sulphide, an endothelium-derived relaxing factor analogous to nitric oxide
    • In patients given methylcobalamin rather than cyanocobalamin, levels of both plasma homocysteine and asymmetric dimethylarginine were lowered

• Should patients with renal failure take methylcobalamin rather than cyanocobalamin?
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