Case Presentation

Introduction

Macrocytic anemia is a common clinical finding invoking a broad differential diagnosis including vitamin deficiencies (Vitamin B₁₂/folate), systemic diseases, drug effects, and myelodysplastic syndromes. Because of the broad differential, careful attention to clinical features, CBC data, and blood morphologic features can help to direct cost-effective work up.¹,²,³

Part 1

An 82-year-old woman with an eight-month history of increasing fatigue and pallor is seen by her primary care physician for her annual physical. Table 1 lists the CBC results obtained at this appointment.

Table 1: CBC results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>8.75 x 10⁹/L</td>
<td>4.8-10.8 x 10⁹/L</td>
</tr>
<tr>
<td>RBC</td>
<td>2.98 x 10¹²/L</td>
<td>3.90-5.20 x 10¹²/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>5.2 g/dL</td>
<td>13-17 g/dL (men)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-15 g/dL (women)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>29.2%</td>
<td>40%-52% (men)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36%-47% (women)</td>
</tr>
<tr>
<td>MCV</td>
<td>121 fL</td>
<td>80-100 fL</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.1 g/dL</td>
<td>32 to 36 g/dL</td>
</tr>
<tr>
<td>RDW</td>
<td>26%</td>
<td>11.1%-14.9%</td>
</tr>
<tr>
<td>PLT</td>
<td>180 x 10⁹/L</td>
<td>150-450 x 10⁹/L</td>
</tr>
</tbody>
</table>

Question #1

In an older patient who presents with macrocytic anemia, what would be the initial differential diagnostic considerations?

Short Answer to Question #1

The differential diagnostic considerations for macrocytic anemia include a wide variety of both nonneoplastic and neoplastic disorders. Nonneoplastic causes of macrocytosis include:

- Deficiencies of vitamin B₁₂ or folate
- Drug effects
- Liver disease
- Hypothyroidism
- Alcohol abuse
- Reticulocytosis

Neoplastic considerations include myelodysplastic syndromes.

Discussion

Macrocytic anemia is defined as an anemia that has a mean corpuscular volume (MCV) greater than 99 fL (99 µl/m²) although the mean corpuscular hemoglobin concentration (MCHC) is often normal. There are many causes of macrocytic anemia (see Table 2 and discussion).¹,²,³
Clinical Pathology Improvement Program (CPIP) – 2017-A Case 1: Hematology – Accurate and Cost-effective Diagnosis of Megaloblastic Anemia

Table 2: Differential diagnosis of macrocytic anemia.

<table>
<thead>
<tr>
<th>Nonmegaloblastic</th>
<th>Megaloblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver disease</td>
<td>Vitamin B₁₂ deficiency</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>Folate deficiency</td>
</tr>
<tr>
<td>Alcohol, drugs, chemotherapy</td>
<td>Due to inhibition of DNA synthesis during RBC production</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td></td>
</tr>
<tr>
<td>Copper deficiency</td>
<td></td>
</tr>
<tr>
<td>Increased reticulocytes</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td></td>
</tr>
</tbody>
</table>

Often macrocytic anemias are divided into those that display morphologic megaloblastic features or are nonmegaloblastic. Megaloblastic anemias arise due to deficiencies in either vitamin B₁₂ (cobalamin) or folate leading to an inhibition of DNA replication and cellular proliferation in all cells, including erythroid precursors. The biochemical reactions requiring vitamin B₁₂ as a cofactor include formation of methionine from homocysteine and formation of succinate from methylmalonate (Figure 1), whereas folate acts as a cofactor in DNA pyrimidine synthesis. Erythroid cells must undergo extensive proliferation in the bone marrow to maintain sufficient numbers of red cells to allow for adequate oxygen transport. Hence, anemia is often one of the primary manifestations of a vitamin B₁₂ (cobalamin) or folate deficiency.³

Figure 1: Biochemical reactions requiring vitamin B₁₂ (cobalamin).

1. Homocysteine \[\xrightarrow{\text{methylcobalamin}}\] Methionine
   
   5-methyl THF \[\xrightarrow{\text{THF}}\] THF
   
   2. Methylmalonate \[\xrightarrow{\text{Mutase (coenzyme B₁₂)}}\] Succinate

THF = tetrahydrofolate

Numerous drugs can also cause a macrocytic anemia that may simulate cobalamin/folate-induced megaloblastic anemia. Drugs like aminopterin and methotrexate, that are structurally similar to folate, may enter cells by the folate carriers and inhibit dihydrofolate reductase. This results in a decrease in nucleotide biosynthesis and a block in DNA synthesis. Zidovudine, which is widely used in the treatment of human immunodeficiency syndrome, may be complicated by megaloblastic anemia, which may be severe and along with neutropenia are common reasons for discontinuation of this drug. Hydroxyurea is commonly employed for a variety of conditions including myeloproliferative neoplasms and psoriasis, and can induce megaloblastic changes by inhibiting the conversion of ribonucleotides to deoxyribonucleotides, which is reversible upon discontinuation of the drug. Omeprazole has been associated with decreased serum cobalamin levels, possibly due to the drug’s effects on parietal cells.
In contrast to megaloblastic anemia associated with cobalamin/folate deficiency, drug-induced megaloblastic anemias disproportionately affect the erythroid components of the bone marrow. This morphologic clue, in addition to a drug history, can assist in determining the cause of a patient’s megaloblastic anemia. Biochemical testing for cobalamin and folate are normal in drug-induced cases.

Macrocytic anemia may also be seen in a variety of clinical settings without associated megaloblastic changes (also referred to as nonmegaloblastic macrocytic anemia). Reticulocytosis, liver disease, alcohol abuse, and hypothyroidism are the most common forms of nonmegaloblastic macrocytosis. These entities can often be distinguished from megaloblastic anemia on morphologic grounds, since the marked anisopoikilocytosis and hypersegmentation of neutrophils that are among the hallmarks of megaloblastic anemia is largely lacking. Other entities that could be considered in the differential diagnosis include disorders associated with dyspoiesis in one or more cell lineages resulting in peripheral cytopenias and/or macrocytosis (myelodysplastic syndromes). A comprehensive clinicopathologic approach must be employed to distinguish these conditions from one another, as they often have overlapping morphologic findings, laboratory features, and clinical presentations.

**Question #2**

What is reticulocytosis and how is it determined in the peripheral blood smears?

**Short answer to Question #2**

Reticulocytosis is defined as an increase in circulating reticulocytes (or young red blood cells) in excess of 1% of circulating red blood cells. Reticulocytes are the final immature stage in red blood cell maturation and contain certain organelles (mitochondria and ribosomes) and RNA. They can be identified in Wright-Giemsa stained peripheral blood smears as increased polychromasia and can be confirmed using a supravital stain such as new methylene blue, in which the affected red blood cells contain multiple blue punctate structures distributed throughout the cytoplasm. It is important to remember that other red cell inclusions may be positive with the supravital such as Pappenheimer bodies, and Howell-Jolly bodies. Heinz bodies, which represent precipitated hemoglobin and require a supravital stain for visualization, are encountered in some hemolytic anemias. Pappenheimer bodies represent mitochondria-bound hemosiderin.

Pappenheimer bodies are seen in sideroblastic anemia, thalassemia, congenital dyserythropoietic anemia, and post splenectomy, and their presence can be confirmed with a Prussian blue or other stain for iron. Howell-Jolly bodies are nuclear fragments that generally occur as single intracellular structures in red blood cells. Although most commonly associated with a hyposplenic or asplenic state, they may also be rarely encountered in severe hemolysis and congenital dyserythropoietic anemia.

**Discussion**

Most currently used CBC analyzers can provide accurate reticulocyte enumeration, decreasing the need for manual counts. They use dyes (including thiazole orange) or fluorescence techniques to detect residual mRNA in young erythrocytes. Automated techniques can provide accurate reticulocyte counts expressed as a percentage of RBCs or as an absolute number. An example of this is the immature reticulocyte fraction (IRF), which is used by one major instrument manufacturer, and is defined as the sum of the fraction of high-fluorescence intensity regions plus the fraction of middle-fluorescence intensity regions. Anemic specimens with an IRF below the normal range imply that the bone marrow is nonresponsive or underresponsive to the anemia. Anemic specimens with an increased IRF may require further examination to determine the etiology of the anemia.
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**Question #3**
What are the expected laboratory findings in anemias associated with liver disease and alcoholism?

**Short answer to Question #3**
Morphologic changes in the red blood cells in patients with liver disease include increased numbers of target cells and occasionally acanthocytes. Peripheral blood specimens from patients who chronically abuse alcohol may have a mild macrocytosis in which the macrocytes have a round rather than an oval configuration, not accompanied by hypersegmentation of neutrophil nuclei.

**Discussion**
Patients with liver disease often have abundant target red blood cells in the peripheral blood, a feature generally not prominent in megaloblastic anemia. Red blood cell changes may vary depending on the degree and type of liver pathology. Patients with intra- and extrahepatic jaundice often have increased red blood cell cholesterol and phospholipid levels, and the predominant circulating red blood cell types are target/leptocyte red cells. Patients with more advanced liver disease may have increased numbers of circulating acanthocytes, which result from an increase in red blood cell cholesterol over phospholipids.

Anemia in patients who chronically abuse alcohol is frequently multifactorial in origin and may include anemia related to nutritional deficiencies (including folate deficiency), chronic gastrointestinal bleeding, and liver disease. But ethanol itself has a direct toxic effect on red blood cells and platelets. The majority of alcoholics have a mild macrocytosis in the range of 100-110 fL, generally with a normal hemoglobin level. Microscopic examination reveals these macrocytes to have a round rather than an oval configuration, not accompanied by hypersegmentation of neutrophil nuclei. Prolonged abstinence from alcohol ingestion results in a normalization of MCV in approximately two to four months. Although bone marrow analysis is generally not indicated, review of the bone marrow aspirates of chronic alcoholics often reveals clear vacuoles in the cytoplasm of red blood cell precursors. This picture may be complicated by nutritional deficiencies, most commonly for folate and iron, particularly in hospitalized malnourished alcoholics. Folate deficiency is particularly noted in consumers of wine and whiskey; chronic abusers of beer generally obtain sufficient amounts of folate from the latter beverage.7,8

**Question #4**
What are the expected features of anemias associated with hypothyroidism?

**Short answer to Question #4**
Mild to moderate anemia which can be microcytic, normocytic, or macrocytic may be seen in hypothyroid individuals.

**Discussion**
*In vitro* evidence suggests a role for thyroid hormones in normal erythropoiesis. Anemia is a recognized complication of hypothyroidism and may also be identified in patients following thyroidectomy. It is generally described as mild to moderate, with hemoglobin levels in the range of 8 to 9 g/dL. The range of reported MCVs in hypothyroid individuals is broad, and includes microcytic, normocytic, and macrocytic anemias, possibly reflecting the confounding issues of concomitant deficiencies of iron, cobalamin, and folate. Patients with macrocytic anemia may have uncomplicated anemia due to hypothyroidism, although marked elevations of MCV suggest deficiency of cobalamin and/or folate. Another potential point of confusion in the differential diagnosis of macrocytic anemia in hypothyroidism is due to the established association between hypothyroidism and pernicious anemia. Diagnosis of anemia of hypothyroidism requires exclusion of all potential confounders. In patients with uncomplicated anemia of hypothyroidism, thyroid
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Hormone replacement has been shown to improve hemoglobin levels when given for several months.3

Another important consideration in the differential diagnosis is myelodysplastic syndromes (MDS). MDS shares certain clinical features with megaloblastic anemia, including peripheral cytopenias and dyspoietic changes. In patients with a suspected MDS, cytogenetic evidence of an MDS-associated abnormality confirms the diagnosis, although in cases of MDS without an increase in blasts cytogenetic abnormalities are identified in only a minority of patients. Newer molecular testing, such as next generation sequencing (NGS), may also indicate molecular abnormalities in patients with MDS, although use of this data remains somewhat controversial in establishing a diagnosis, and requires careful integration with clinical and pathologic findings. In contrast to megaloblastic anemia, patients with myelodysplasia generally have normal results for testing for serum/plasma folate, red blood cell folate, serum cobalamin, methylmalonic acid, and homocysteine.9

Case Presentation
Part 2
An initial laboratory chemistry panel was performed which was within normal limits for all analytes including liver enzymes. A peripheral smear was examined for possible clues into the etiology of the patient’s cytopenias.

Question #5
Which morphologic features in the peripheral blood smear may help narrow the differential to allow for cost-effective use of laboratory testing in identifying the cause of the patient’s macrocytic anemia?

Short Answer to Question #5
Identification of megaloblastic related changes, particularly hypersegmentation of neutrophils and ovalocytes help to narrow the differential diagnostic considerations to entities such as B12 or folate deficiency or some drugs. In contrast, identification of dysplastic changes, such as hyposegmentation and hypogranulation of neutrophils may suggest the possibility of a myelodysplastic syndrome.

Discussion
Because of the rapid cellular proliferation needed to maintain red cell mass, adequate levels of folate and vitamin B12 are required for normal erythropoiesis. When there is decreased availability of either folate or vitamin B12 there will be inhibition of DNA synthesis due to the lack of appropriate enzymatic activity. The deficiency-related inhibition of DNA synthesis is not associated with an inhibition of RNA synthesis, leading to characteristic nuclear and cytoplasmic maturational dyssynchrony in the cells of a deficient patient resulting in immature nuclear features but mature cellular levels of hemoglobin in the cytoplasm. The uncoupling of erythroid cell nuclear and cytoplasmic maturation leads to increased ineffective hematopoiesis. This is characterized by high rates of erythroid apoptosis in the bone marrow and development of anemia despite bone marrow hypercellularity and increased numbers of red cell precursors. In addition, vitamin B12 or folate deficiency also affects other proliferating cell lines and is manifested, for example, by increased size of late myeloid precursors (metamyelocytes, myelocytes), hypersegmentation of neutrophils, and increased megakaryocytic size.

In a patient with severe vitamin B12 or folate deficiency, there will usually be a moderate to severe macrocytic anemia with MCVs ranging from 100 to 150 fL with normal MCHCs. Vitamin B12 or folate deficiency is most likely when the MCV exceeds 120 fL, particularly when there is no history of medications that may cause macrocytosis. It should be noted that some patients with folate or vitamin B12 deficiency may have a normal MCV due to associated deficiencies, including iron
deficiency, or other disease processes (renal failure or inflammatory disorders leading to abnormal iron transport). Usually, there is marked anisocytosis and the red cell distribution width (RDW) is elevated.

**Question #6**
What are the expected morphologic features of the peripheral blood of patients with folate or vitamin B₁² deficiency?

**Short answer to Question #6**
Morphologic features associated with folate or vitamin B₁² deficiency include the presence of numerous oval macrocytes and the presence of hypersegmented neutrophils in the blood.

**Discussion**
Oval macrocytes (Image 1) may be useful in distinguishing vitamin B₁² or folate deficiency from other causes of macrocytic anemia (such as myelodysplasia or drug effects), which are more likely to have round macrocytic red cells. Basophilic stippling and occasionally Howell-Jolly bodies may be present. In patients with very low hematocrits, circulating nucleated red blood cell precursors may be seen which show characteristic nuclear cytoplasmic dysynchrony and megaloblastic maturation.

**Image 1**: Oval macrocyte. Wright-Giemsa stain, 1000x magnification.

Often, a useful clue that one is dealing with macrocytic anemia due to vitamin B₁² or folate deficiency is the presence of hypersegmented neutrophils (Image 2), which occurs early in the development of deficiency and reflects the abnormality in nuclear maturation.

**Image 2**: Hypersegmented neutrophil with normal-appearing platelet. Wright-Giemsa stain, 1000x magnification.

Hypersegmentation is defined as a mature neutrophil with six or more distinct nuclear lobes or an elevation in the mean neutrophil lobe count (increased numbers of neutrophils containing greater
than four distinct lobes or greater than 5% of neutrophils containing five lobes). Other causes of macrocytic anemia will not have hypersegmentation as a prominent feature.

As previously stated, reticulocytosis, liver disease, alcohol abuse, and hypothyroidism are the most common forms of nonmegaloblastic macrocytosis. These entities can often be distinguished from megaloblastic anemia on a morphologic basis, since the marked anisopoikilocytosis and hypersegmentation of neutrophils that are among the hallmarks of megaloblastic anemia are largely lacking.

**Case Presentation**
**Part 3**
Based on the reported morphologic findings of hypersegmentation of normally granulated neutrophils, identification of numerous oval macrocytes with a very high MCV and normal platelet granulation, the clinician contacts you to advise him on additional testing and to set up a bone marrow procedure and cytogenetic testing to further work up the patient’s pancytopenia.

**Question #7**
With megaloblastic features identified in the blood smear, what laboratory testing should be ordered next to address the differential diagnostic considerations?

Should a bone marrow examination be performed?

**Short Answer to Question #7**
In patients with a presumptive diagnosis of megaloblastic anemia, initial laboratory analysis should be directed toward assessment of the individual's serum vitamin B\textsubscript{12} and folate levels. Bone marrow analysis is generally not necessary at this stage.

**Discussion**
The common method of assessing cobalamin status is via the serum cobalamin assay. The original method, which was a microbiologic technique that used an organism (\textit{Euglena gracilis}) that required cobalamin for growth, was reliable but due to suboptimal turnaround time (approximately 48 hours) has been superseded by radioisotope dilution and chemiluminescence assays. The major limitation of the modern techniques is a false normal result in ~10% of cobalamin deficient individuals. Therefore, testing for other metabolites (Table 3), including serum or plasma methylmalonic acid and plasma homocysteine levels are felt to be better indicators of low tissue cobalamin stores and are particularly useful in detecting early deficiency.

**Table 3**: Tests useful in diagnosis of vitamin B\textsubscript{12} deficiency.

<table>
<thead>
<tr>
<th>Test</th>
<th>Expected Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B\textsubscript{12} levels</td>
<td>May be normal or slightly decreased in early deficiency</td>
</tr>
<tr>
<td>Methylmalonic acid</td>
<td>Increased in B\textsubscript{12} deficiency</td>
</tr>
<tr>
<td>Total homocysteine</td>
<td>Increased in B\textsubscript{12} deficiency</td>
</tr>
<tr>
<td>IF/parietal cell antibodies</td>
<td>Useful to identify pernicious anemia</td>
</tr>
<tr>
<td>Folate</td>
<td>May be decreased in vitamin B\textsubscript{12} as B\textsubscript{12} required for folate entry into cells</td>
</tr>
</tbody>
</table>

Measurement of serum holotranscobalamin II may be useful in early vitamin B\textsubscript{12} deficiency, if a patient has normal renal function but is usually a send out test. In addition, patients with suspected B\textsubscript{12} deficiency should be tested for the presence of autoantibodies to parietal cells and intrinsic
factor to rule out possible pernicious anemia (discussed further below). The deoxyuridine suppression test, which measures the capacity of in vitro cultivated marrow cells to utilize deoxyuridine in DNA synthesis, is another technique to identify cobalamin/folate deficiency even in patients who have not yet developed anemia or macrocytosis but currently is of little value in the average clinical scenario. The test takes advantage of the fact that the majority of thymidine used in DNA synthesis is derived from deoxyurididine, which requires cobalamin and folate enzymes. In patient specimens with deficient cobalamin and folate, greater than 10% of added tritium-labeled thymidine is incorporated DNA.10,11

A microbiologic assay using Lactobacillus casei has been the traditional method for assessing folate levels, although this technique has lately been eclipsed by radioisotope dilution and chemiluminescence assays. False normal results can be problematic, and occur in up to 40% of folate deficient patients. Folate levels may be determined in the serum and plasma; however, red blood cell folate is a more specific measure of body folate levels. Red blood cell folate is usually assessed by the radioisotope dilution and chemiluminescence assays rather than the microbiologic method. It should be noted that folate levels may also be decreased in ~10% of cases of vitamin B12 deficiency as vitamin B12 is required for folate entry into the cells. Thus, vitamin B12 deficiency may lead to decreased folate levels. Folate levels in the serum also rapidly fluctuate with intake and a fasting specimen may be more representative of folate levels. In fact, serum folate may be increased in up to 20% of cobalamin deficient patients.11

Folate is commonly supplemented in a variety of different foods in the USA and dietary deficiency is becoming increasingly rare unless there is a marked increase in metabolic needs due to growth, malignancy, or rapid cell turnover. Therefore, unless there are clear indications (eg, small bowel resection, symptomatology of sprue, or intake of specific drugs), testing for folate levels may not be indicated. Additionally, as noted above, severe vitamin B12 deficiency may cause decreases in folate due to secondary effects on folate metabolism that are NOT indicative of a coexisting folate deficiency.

Bone marrow examination in patients with vitamin B12 deficiency is usually NOT required for diagnosis. The marrow, if examined, is usually hypercellular with marked erythroid and associated myeloid hyperplasia. The erythroid cells will show evidence of nuclear and cytoplasmic dyssynchrony with normal levels of hemoglobin production (Image 3 and Image 4).

**Image 3:** Bone marrow aspirate. Wright-Giemsa stain, 1000x magnification.

Bone marrow aspirate showing megaloblastic maturation of erythroid precursors characterized by enlarged cell size, immature nuclear features, and more mature cytoplasmic features (nuclear/cytoplasmic dyssynchrony). Erythroid dyspoiesis is present.
Image 4: Bone marrow core. H&E stain, 400x magnification.

Bone marrow core showing the predominance of megaloblastic erythroid precursors giving rise to a hypercellular marrow for age.

The myeloid series may show giant metamyelocytes and myelocytes as well as hypersegmentation of neutrophils (Image 5).

Image 5: Bone marrow aspirate. Wright-Giemsa stain, 1000x magnification.

Bone marrow aspirate showing giant metamyelocytes indicative of megaloblastic maturation seen in vitamin B₁₂/folate deficiency.

Thus, most of the cells in the bone marrow aspirate may appear larger than normal and have nuclei that appear less mature than appropriate for the degree of cytoplasmic maturation. Occasionally, large megakaryocytes may be seen. If there is coexistent iron deficiency or other processes that impact red cell development or maturation, the red cells may show features intermediate between iron deficiency and vitamin B₁₂ deficiency. However, the myeloid line will continue to show hypersegmentation. Cytogenetics should be normal, and ordering cytogenetics in this clinical context is usually not warranted unless there are additional clinical or morphologic features that suggest the possibility of a deficiency superimposed upon an underlying myeloid malignancy, such as MDS, or if there is no response to vitamin replacement therapy.

An important differential diagnostic consideration, particularly in an older patient with macrocytic anemia and other associated cytopenias is a MDS. MDSs are defined by the World Health
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Organization (2008) as a group of clonal hematopoietic stem cell diseases characterized by cytopenias, dysplasia in greater than or equal to one hematopoietic lineage, ineffective hematopoiesis (ie, peripheral cytopenias), and variably increased risk of development of acute myeloid leukemia. They may occur sporadically or as a consequence of prior radiotherapy, chemotherapy, or environmental exposure. There is considerable clinical and laboratory overlap between MDS and nonneoplastic causes of macrocytic anemia. However, certain clues in the hemogram and the morphology of bone marrow and peripheral blood elements may aid in the distinction of MDS from a cobalamin/folate-induced megaloblastic anemia. For example, patients with very high MCVs (>120fL) more likely have a cobalamin/folate-induced megaloblastic anemia. Although dyspoiesis is seen in MDS and cobalamin/folate-induced megaloblastic anemia, the extent of dyspoiesis is often much more pronounced in MDS. In addition, certain dyspoietic features are more commonly associated with cobalamin/folate-induced megaloblastic anemia. These include nuclear hypersegmentation of neutrophils and giant band forms and metamyelocytes. In contrast, cytoplasmic hypogranularity and nuclear hyposegmentation of neutrophils and their precursors, nuclear hyposegmentation of megakaryocytes, hypogranularity of platelets, and increased blasts support diagnosis of an MDS. In addition, identification of a cytogenetic abnormality by conventional metaphase cytogenetic analysis or fluorescence in situ hybridization (FISH) may be helpful in establishing the diagnosis of MDS.

Question #8
What are the pathophysiologic mechanisms underlying development of vitamin B₁₂ or folate deficiency?

Short Answer to Question #8
Vitamin B₁₂ is widely available in food and body stores; most cases of B₁₂ deficiency arise due to cases of long-standing inhibition of normal absorption, most frequently due to pernicious anemia. Folate stores are much more sensitive to changes in diet.

Discussion
Vitamin B₁₂ is synthesized by bacteria and is readily available in fish, meat, and dairy products where it exists as a heat stable protein. The body is able to store 3,000 to 5,000 mg of cobalamin in the liver, so stores may be available for two to five years in patients with a deficient diet. Because of the wide availability of vitamin B₁₂ in food and body stores, most cases of B₁₂ deficiency arise due to cases of long-standing inhibition of normal absorption, most frequently due to pernicious anemia (Table 4).
Table 4: Mechanisms of vitamin B\textsubscript{12} deficiency.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Cause</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate intake</td>
<td>Strict (lacto-ovo) vegetarianism</td>
<td>Occasional</td>
</tr>
<tr>
<td>Increased requirement</td>
<td>Growth, high cell turnover</td>
<td>Rare</td>
</tr>
<tr>
<td>Decreased absorption</td>
<td>Pernicious anemia (decreased IF)</td>
<td>Common</td>
</tr>
<tr>
<td></td>
<td>Congenital IF deficiency</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Atrophic gastritis</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>Decreased pancreatic enzymes/pancreatititis</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>Defect in ileal absorption (sprue, regional enteritis, surgical resection)</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>Parasitic or bacterial overgrowth</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>Drug interference with absorption (alcohol, colchicine)</td>
<td>Occasional</td>
</tr>
<tr>
<td></td>
<td>Mutation in B\textsubscript{12}-IF receptor (Imerslund-Grasbeck syndrome)</td>
<td>Rare</td>
</tr>
<tr>
<td>Defective transport</td>
<td>Congenital deficiency of transcobalamin II</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Normally, ingested vitamin B\textsubscript{12} will complex with intrinsic factor (IF), a protein produced by the stomach parietal cells. The B\textsubscript{12}-IF complex will then bind to receptors on mucosal cells in the terminal ileum. Inadequate intake leading to vitamin B\textsubscript{12} deficiency may be seen in some patients with a very strict vegetarian diet (no milk or egg products) over many years. Occasionally, increased requirements for growth and development in combination with inadequate absorption due to atrophic gastritis may contribute to development of vitamin B\textsubscript{12} deficiency. For vitamin B\textsubscript{12} to be released and absorbed in the small intestine there must be normal amounts of pancreatic enzymes to degrade the B\textsubscript{12}-IF complex that has bound to the ileal mucosa. Diseases of the ileum including parasitic or bacterial overgrowth, regional enteritis, sprue, or surgical resection of the ileum may also decrease the body’s ability to effectively absorb vitamin B\textsubscript{12}. Some drugs, including alcohol and colchicine, also inhibit B\textsubscript{12} absorption. Very rarely, genetic mutations in IF protein, cobalamin transport proteins, or the ileal receptor for the B\textsubscript{12}-IF complex result in B\textsubscript{12} deficiency. Pernicious anemia is by far the most common cause of vitamin B\textsubscript{12} deficiency seen in Western nations. Pernicious anemia is an autoimmune disorder associated with development of chronic atrophic gastritis. Autoantibodies to either intrinsic factor and/or parietal cells lead to decreased levels of intrinsic factor production or levels by either direct destruction of IF or decreased production secondary to parietal cell destruction. When no IF is present, vitamin B\textsubscript{12} cannot bind nor be absorbed.

Once absorbed, vitamin B\textsubscript{12} circulates in the peripheral blood as a protein complex. Approximately 30% of vitamin B\textsubscript{12} binds to transcobalamin II, which delivers the vitamin directly to the liver, bone marrow, and other body sites where cellular proliferation occurs. The remainder (70%) of vitamin B\textsubscript{12} will bind to transcobalamin I, transcobalamin III, and other proteins that deliver the vitamin exclusively to the liver for storage. These binding proteins attach to specific membrane receptors at the target organs to allow transfer of vitamin B\textsubscript{12} into the cell. The transcobalamin protein complex will then be degraded and the active forms of vitamin B\textsubscript{12} (methylcobalamin and 5-deoxyadenosylcobalamin) will be released into the cell to act in the formation of methionine and succinate. In patients with vitamin B\textsubscript{12} deficiency, both megaloblastic anemia and neurologic disorders secondary to defective myelination appear to be linked to impaired synthesis of methionine. Excretion of vitamin B\textsubscript{12} can occur in both the urine and bile.
Most patients have extensive stores of vitamin B$_{12}$. The time intervals to development of deficiency may be prolonged. It may take one to two years before vitamin B$_{12}$ levels in the serum are decreased and early blood and/or bone marrow abnormalities such as macrocytosis and hypersegmentation to appear, which may precede the development of anemia. Early myelin damage of nerves may cause such symptoms as paresthesias, numbness and tingling in the hands and feet, decreased vibration sense, and decreased sense of position. After two to three years of deficiency, blood levels of vitamin B$_{12}$ will be markedly decreased and there will also be less than 10% saturation of vitamin B$_{12}$ binding proteins. Because of secondary effects on folate metabolism, there may be an associated decrease in red blood cell folate with normal to increased serum folate levels. Patients will develop worsening anemia over time and will have evidence of florid morphologic megaloblastic maturation. In addition, there may be clinical evidence of deficiency in other mucosal surfaces, including atrophy of the tongue, gastrointestinal tract atrophy with secondary malabsorption, as well as vaginal atrophy. In young infants there may be a failure to thrive. Severe damage to myelin may lead to axonal degeneration involving the posterior and lateral columns of the spinal cord with ataxia or even symmetrical paralysis. There may be progression to cerebral involvement with mental status changes, paranoia, and depression.

Folate is required for the rate-limiting step in pyrimidine synthesis involving conversion of deoxyuridine monophosphate to deoxythymidine monophosphate. Most folate deficiencies arise from inadequate intake secondary to dietary deficiency or increased requirements. Occasionally defective absorption or metabolic disorders may lead to deficiency. Dietary deficiencies are most commonly seen with inadequate diets (lacking fresh fruits or vegetables) or in severe chronic alcoholism. Increased folate is required in phases of rapid growth including infants, pregnant and lactating women, and in patients who have a malignancy or rapid cellular turnover (such as in chronic hemolytic anemia). In particular, premature infants may have very low folate stores as do breast-fed infants of mothers who are on restricted diets, making them more susceptible to developing a deficiency. Folate is rapidly destroyed by heat and overcooking may adversely impact available folate levels in vegetables and fruits.¹²

Unlike vitamin B$_{12}$, there are very low level body folate stores and decreased serum folate levels will be seen as early as three to four weeks after a poor diet or increased requirements are manifested (Table 5).

**Table 5: Sequence of development of symptoms of vitamin B$_{12}$ deficiency.**

<table>
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<th>Time Interval of Deficiency</th>
<th>Findings</th>
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| 1-2 years                  | • Mild to moderate decrease in vitamin B$_{12}$ level  
• Hypersegmentation of neutrophils  
• Megaloblastic/macrocytic maturation of bone marrow cells  
• Early myelin damage, paresthesia, numbness, tingling |
| 2-5 years                  | • Marked decrease in vitamin B$_{12}$ levels  
• Vitamin B$_{12}$ binding proteins show <10% saturation  
• Megaloblastic/macrocytic maturation in bone marrow  
• Hypersegmentation, oval macrocytes in blood and marrow  
• Myelin damage with posterior and lateral column damage, cerebral changes |

After five to seven weeks, hypersegmentation of neutrophils will occur with megaloblastosis being seen in the bone marrow in 10 to 12 weeks. The presence of macroovalocytes and florid megaloblastosis with megaloblastic macrocytic anemia occurs at 18 to 20 weeks after deficiency. Folate deficiency may also occur due to defective absorption. As folate is absorbed in the small bowel, surgical resection or diseases that affect the small bowel mucosa, such as sprue, may
impact absorption. In addition, anticonvulsants, antituberculosis drugs, oral contraceptives, antimetabolites such as methotrexate (commonly used in treating cancers) may inhibit folate absorption or utilization. In contrast to vitamin B₁₂ deficiency, folate deficiency is not associated with development of abnormalities in myelination or associated neurologic symptoms. The primary source of folate is in plants and the most common dietary source is from green leafy vegetables and fresh fruits.

Case Presentation

Part 4

Additional testing showed a decreased vitamin B₁₂ level of <150 pg/ml (normal range 240 to 930 pg/ml) and the presence of anti-intrinsic factor antibodies. The patient was treated with parenteral supplementation of vitamin B₁₂ with resolution of anemia and other CBC abnormalities. Patient felt increased energy over the next six weeks. No bone marrow biopsy was obtained and the patient continues to do well one year after the initial diagnosis of macrocytic anemia.

Conclusion

In summary, the laboratory findings along with the pathologist’s review of the initial blood smear can provide the initial direction to the clinical team in the appropriate work up of a macrocytic anemia.

The clinical laboratory plays a central role in the assessment of patients with suspected vitamin B₁₂/folate deficiency. Information from hematology (CBC, reticulocyte parameters), clinical chemistry (serum and urine levels of cobalamin, folate, and other metabolites), and morphologic features are essential to making the diagnosis in a cost-effective manner. In rare cases (particularly those with atypical features or cases that do not respond to vitamin supplementation), bone marrow examination together with cytogenetics can be incorporated into the evaluation of these patients.

Summary/Key Points

- Evaluation of the CBC parameters and peripheral blood morphology can provide important clues in the assessment of patients with macrocytic anemias.
- Clinical laboratory evaluation of cobalamin and folate levels can be challenging and the currently available techniques are subject to limitations in sensitivity.
- The differential diagnosis of megaloblastic anemia is broad and includes nonneoplastic and neoplastic entities. Repeat analysis may be necessary in some individuals.
- Bone marrow aspirate and biopsy are generally not required in most patients with macrocytosis, but may be indicated in individuals with prolonged unexplained macrocytosis accompanied by peripheral cytopenias, circulating blasts, or other atypical features.

Key “Go To” References


References/Resources


