Clinical Laboratory Hematology

THIRD EDITION

Shirlyn B. McKenzie • J. Lynne Williams

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Global Edition
Third Edition

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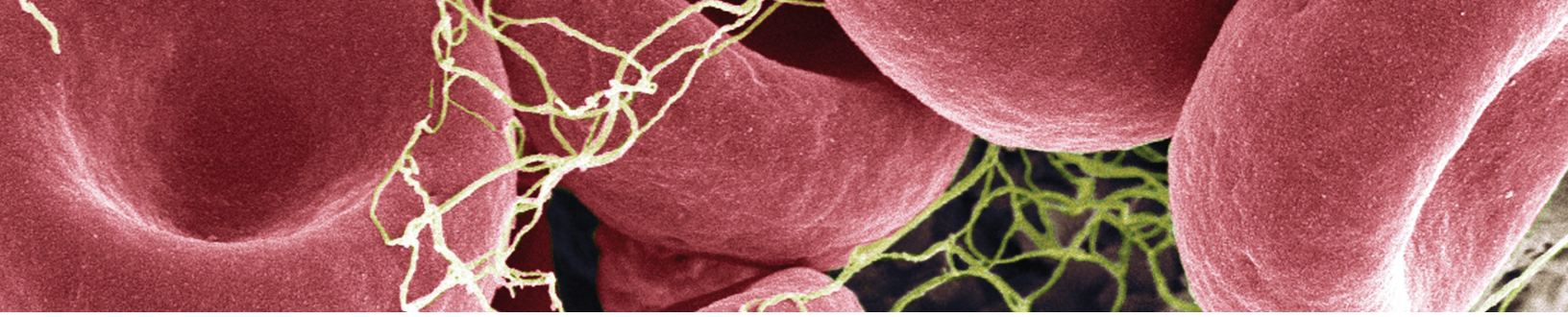

15 Megaloblastic and Nonmegaloblastic Macrocytic Anemias

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Objectives—Level I

At the end of this unit of study, the student should be able to:

1. Explain the cause and process of megaloblastic maturation in the bone marrow.
2. Describe the body's requirements for vitamin B_{12} and folate and their physiologic roles.
3. List the laboratory tests used to confirm a diagnosis of cobalamin deficiency and give expected results.
4. List the laboratory tests used to confirm a diagnosis of folic acid deficiency and give expected results.
5. Recognize the six most common disorders associated with a macrocytic anemia.
6. Name four causes of a cobalamin deficiency and give two distinguishing clinical or laboratory characteristics of each.
7. Describe the etiology and pathophysiology of pernicious anemia, including clinical symptoms and clinical subtypes.
8. Name three causes of a folate deficiency and give two distinguishing clinical or laboratory characteristics of each.
9. Differentiate the pathophysiology and peripheral blood findings of non-megaloblastic macrocytic anemia from those of megaloblastic anemias.
10. Summarize the typical blood picture seen with a folate or cobalamin deficiency.

Objectives—Level II

At the end of this unit of study, the student should be able to:

1. Summarize the process of cobalamin and folic acid metabolism and explain how a deficiency can result in megaloblastosis.
2. Compare macrocytosis associated with a normoblastic marrow and macrocytosis associated with a megaloblastic marrow on the basis of physiological defect, and differentiate them based on the laboratory blood picture.
3. Summarize the mechanism of maturation defects that lead to megaloblastosis and recognize the morphologic blood cell abnormalities.

(continued)

Chapter Outline

Objectives—Level II *(continued)*

4. Compare and contrast the various clinical forms and causes of a cobalamin and folate deficiency on the basis of clinical symptoms and laboratory results.
5. Categorize the causes and clinical variations of pernicious anemia.
6. Compare and contrast the various clinical forms and causes of a folic acid deficiency.
7. Choose and briefly explain four laboratory tests used to identify the cause of a macrocytic anemia; give the expected results of these four tests in a patient with an autoantibody directed against intrinsic factor.
8. Assess Schilling's test results and provide a differential diagnosis.
9. Compare and contrast the causes of macrocytosis that have a normoblastic marrow.
10. Construct an algorithm of laboratory testing to distinguish between a megaloblastic anemia and a macrocytic, normoblastic anemia.
11. Evaluate a case study from a patient with anemia. Determine the most probable diagnosis from the medical history and laboratory results.

Key Terms

Achlorhydria
Cobalamin
Demyelination
Dyspepsia
Folate
Folic acid
Glossitis
Intrinsic factor (IF)
Megaloblastic
Nuclear-cytoplasmic asynchrony
Pernicious anemia (PA)
Schilling test

Background Basics

The information in this chapter builds on the concepts learned in previous chapters. To maximize your learning experience, you should review these concepts before starting this unit of study:

Level I

- Describe the maturation process of erythrocytes in the marrow. (Chapter 5)
- Outline the functional and morphologic classification of anemia and list the basic laboratory tests to diagnose anemia. (Chapter 11)

Level II

- Summarize the concepts of cell development, regulation, and the process of cell division. (Chapter 2)
- List and describe the laboratory tests used in differential diagnosis of anemia. (Chapter 11)

CASE STUDY

We will refer to this case study throughout the chapter.

Kathy, a 36-year-old female, experienced a recent 35-lb weight loss. Her tongue was red and fissured. She also complained of chronic fatigue and shortness of breath upon exertion. Physical examination suggested signs of jaundice and increased numbness and a tingling sensation of fingers and toes. She was hospitalized with the general diagnosis of moderate anemia, jaundice, and neurological symptoms. Her admitting CBC demonstrated the following laboratory results:

		Differential	
WBC	4.5×10^9/L	Lymphs	36.0%
RBC	2.50×10^{12}/L	Monos	3.6%
Hb	10.0 g/dL (100.0 g/L)	Neutrophils	59.4%
Hct	31% (0.31 L/L)	Eosinophils	1.0%
MCV	124 fL	Basophils	0.0%
MCH	40.50 pg	NRBCs/100 WBCs	5.0%
MCHC	32.70 g/dL	Moderate hypersegmented neutrophils	
RDW	21.20		
PLT	155×10^9/L		

The following abnormal erythrocyte morphology was reported:

Macrocytes	2+
Anisocytosis	3+
Poikilocytosis	2+
Ovalocytes	1+
Basophilic stippling	1+
Occasional Howell-Jolly bodies	

Consider the reflex tests that might be important in identifying the etiology of this anemia.

OVERVIEW

This chapter is a study of the macrocytic anemias, which can be megaloblastic or nonmegaloblastic. The first part of the chapter discusses the megaloblastic anemias beginning with a description of the clinical and laboratory findings. Because megaloblastic anemia is most often due to deficiencies or abnormal metabolism of folate or cobalamin (vitamin B_{12}), the metabolism of these vitamins is discussed in detail. The latter part of the chapter reviews the causes of nonmegaloblastic macrocytic anemia and compares the laboratory test results in nonmegaloblastic and megaloblastic anemia. The laboratory professional can often identify diagnostic clues of megaloblastic anemia on review of a blood smear.

INTRODUCTION

Macrocytic anemias are characterized by large erythrocytes (mean MCV >100 fL) with an increased MCH and a normal hemoglobin content (MCHC). This is an important group of anemias because macrocytosis is frequently a sign of a disease process that can result in significant morbidity if left untreated.

Macrocytosis is found in 2.5–4.0% of adults who have a routine complete blood count.[1] In up to 60% of cases, macrocytosis is not accompanied by anemia,[2] but isolated macrocytosis should always be investigated. Macrocytosis without anemia can indicate early folate or cobalamin (vitamin B_{12}) deficiency because macrocytosis precedes the development of anemia in these disorders.

Macrocytosis detected by automated cell counters is not always apparent microscopically on stained blood smears. In some cases, the erythrocyte size on automated counters is falsely elevated due to hyperglycemia, cold agglutinins, and extreme leukocytosis. These causes of false macrocytosis need to be differentiated from true macrocytosis.

The most common cause of true macrocytosis is alcoholism. Other causes include folate and cobalamin deficiencies, drugs including chemotherapy, reticulocytosis due to hemolysis or bleeding, myelodysplasia, liver disease, and hypothyroidism.[2]

Macrocytic anemias are generally classified as megaloblastic or nonmegaloblastic (normoblastic), depending on morphologic characteristics of erythroid precursors in the bone marrow (Table 15-1 ★). The **megaloblastic** anemias are the result of a defect in DNA synthesis. Frequently there is an arrest in the S phase of the cell cycle and to a lesser extent during other phases of the cell cycle due to delayed nuclear development. RNA and protein synthesis, however, are relatively unimpaired. The result is unbalanced cell growth and impaired cell division characterized by erythroblasts with distinct abnormal morphologic features. The nucleus appears immature with a fine particulate chromatin pattern, whereas the cytoplasm is increased and relatively more mature (referred to as **nuclear-cytoplasmic asynchrony**). These cells are referred to morphologically as *megaloblastic*. All proliferating cells are affected.

The basis for the nonmegaloblastic anemias is not always as well defined but is often related to an increase in membrane lipids. The macrocytes in nonmegaloblastic macrocytic anemia are usually round, but in megaloblastic anemia, they are oval (Figure 15-1a, b ■). A flow chart for laboratory analysis to help distinguish causes of macrocytic anemia is shown in Figure 15-2 ■.

★ **TABLE 15-1** Conditions Associated with Megaloblastic and Nonmegaloblastic (Normoblastic) Macrocytic Anemias

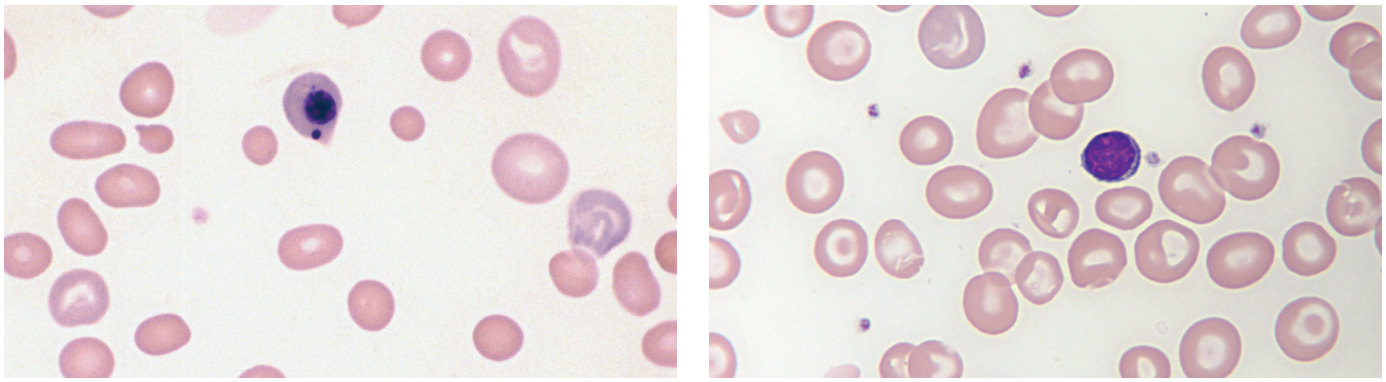
Megaloblastic	Normoblastic
Folate deficiency	Alcoholism
Nutritional deficiency	Liver disease
Increased requirement (pregnancy)	Shift reticulocytosis in hemolytic anemia or hemorrhage
Intestinal malabsorption	Hypothyroidism
Drug inhibition	Aplastic anemia
Cobalamin deficiency	Obstructive jaundice
Pernicious anemia	Splenectomy
Small bowel resection	Pregnancy
Gastrectomy	Artifactual:
Intestinal malabsorption	hyperglycemia
Nutritional deficiency	cold agglutinins
Increased requirement (pregnancy)	leukocytosis
Transcobalamin deficiency	
Nitrous oxide abuse	
Other causes	
Chemotherapy with metabolic inhibitors	
Orotic aciduria	
Congenital dyserythropoietic anemia (CDA)	

MEGALOBLASTIC ANEMIA

Although very little was known about the function or origin of blood cells before the twentieth century, some perceptive individuals began to make associations between anemia and other clinical signs in patients. In 1822, J. S. Coombe, a Scottish physician, made the initial clinical description of a patient who appeared to have megaloblastic anemia. He was the first to suggest that this anemia might be related to **dyspepsia**.[3] In 1855, Thomas Addison reported his description of a macrocytic anemia, but he made no reference to the typical microscopic blood findings.[4] The discovery and description of the abnormal erythroid precursors in the bone marrow associated with this anemia were made possible by the advent of triacid stains. Paul Ehrlich is credited with coining the term *megaloblast* in 1891 to describe the large abnormal precursors in megaloblastic anemia.[5]

Megaloblastic anemia is classified as a nuclear maturation defect. Anemia is attributed primarily to a large degree of ineffective erythropoiesis resulting from disrupted DNA synthesis. The anemia was called *megaloblastic* in an attempt to describe the giant, abnormal-appearing erythroid precursors (megaloblasts) in the bone marrow. The generic word *megaloblast* describes any maturation stage of the megaloblastic erythroid series (i.e., polychromatic megaloblast). Other nucleated cells of the marrow are also typically abnormal.

About 95% of megaloblastic anemias are caused by deficiencies of either vitamin B_{12} (**cobalamin**) or **folic acid**, vitamins necessary as coenzymes for nucleic acid synthesis. In the majority of cases, cobalamin deficiency is secondary to a deficiency of **intrinsic factor (IF)**, a protein necessary for absorption of cobalamin, rather than to a nutritional



a b

FIGURE 15-1 (a) Peripheral blood film from a patient with pernicious anemia. Note the anisocytosis with oval macrocytes and the nucleated red blood cell with a Howell-Jolly body. (Wright-Giemsa stain; 1000× magnification). (b) Peripheral blood film from a patient with normoblastic, macrocytic anemia. Compare the size of the RBCs with the lymphocyte. The RBCs are primarily round macrocytes. (Wright-Giemsa stain, 1000× magnification).

deficiency of the vitamin. Folic acid deficiency, on the other hand, is most often due to an inadequate dietary intake. Inherited disorders affecting DNA synthesis or vitamin metabolism are rare causes of megaloblastosis.

☑ CHECKPOINT 15-1

Explain why patients with cobalamin or folate deficiency have megaloblastic maturation.

Clinical Findings

The onset of megaloblastic anemia is usually insidious; because the anemia develops slowly, it produces few symptoms until the hemoglobin and hematocrit are significantly depressed. Patients can present with typical anemic symptoms of lethargy, weakness, and a yellow or waxy pallor. Dyspeptic symptoms are common. **Glossitis** with a beefy red tongue, or more commonly a smooth pale tongue, is characteristic. Loss of weight and loss of appetite are common complaints. In pernicious anemia (see "Pernicious Anemia"), atrophy of the gastric parietal cells causes decreased secretion of intrinsic factor and hydrochloric acid. Bouts of diarrhea can result from epithelial changes in the gastrointestinal tract.

Neurological disturbances occur only in cobalamin deficiency, not in folic acid deficiency. They are the most serious and dangerous clinical signs because neurological damage can be permanent if the deficiency is not treated promptly. The patient's initial complaints occasionally are related to neurological dysfunction rather than to anemia. Neurological damage has been reported to occur even before anemia or macrocytosis in some cases, particularly in elderly people. The bone marrow, however,

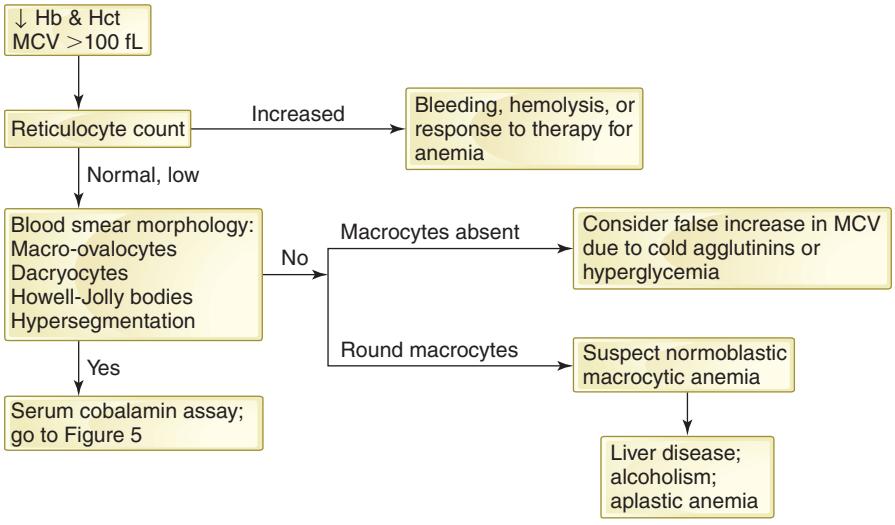


FIGURE 15-2 Algorithm for the differential diagnosis of the megaloblastic anemias from other macrocytic anemias.
Hb = hemoglobin; Hct = hematocrit; MCV = mean cell volume

usually reveals megaloblastic changes even in the absence of anemia. Tingling, numbness, and weakness of the extremities reflect peripheral neuropathy. Loss of vibratory and position (proprioceptive) sensations in the lower extremities can cause the patient to have an abnormal gait. The patient's relatives sometimes note mental disturbances such as loss of memory, depression, and irritability. *Megaloblastic madness* is a term used to describe severe psychotic manifestations of cobalamin deficiency. A patient with severe anemia occasionally is asymptomatic, which is probably a reflection of a very slowly developing anemia. It has been suggested that cobalamin deficiency should be suspected in all patients who have an unexplained anemia and/or neurological disturbances or in individuals who are at risk of developing a deficiency such as elderly people or those with intestinal diseases.[6]

☑ CHECKPOINT 15-2

Patients with megaloblastic anemia often present with a yellow or waxy pallor. What is the diagnostic significance of this clinical symptom?

Laboratory Findings

Laboratory tests are critical to a diagnosis of megaloblastic anemia. The routine CBC with a review of the blood smear gives important diagnostic clues and helps in selecting reflex tests.

Peripheral Blood

Megaloblastic anemia is typically a macrocytic, normochromic anemia. The MCV is usually >100 fL and can reach a volume of 140 fL. However, an increased MCV is not specific for megaloblastic anemia. The MCH is increased because of the large cell volume, but the MCHC is normal. In cobalamin deficiency, a macrocytosis can precede the development of anemia by months to years.[7–9] On the other hand, the MCV can remain within the reference interval. Epithelial changes in the gastrointestinal tract can cause iron absorption to be impaired. If an iron deficiency (which characteristically produces a microcytic, hypochromic anemia) coexists with megaloblastic anemia, macrocytosis can be masked, and the MCV can be in the normal range.[10] Other conditions that have been shown to coexist with megaloblastic anemia in the absence of an increased MCV include thalassemia, chronic renal insufficiency, and chronic inflammation or infection.[9] Sometimes these coexisting causes of anemia are not recognized until after the megaloblastic anemia has been treated. It has been suggested that if coexisting iron deficiency, thalassemia, or chronic disease is suspected, patient medical history, racial/ethnic background, and previous MCV should be considered.[11]

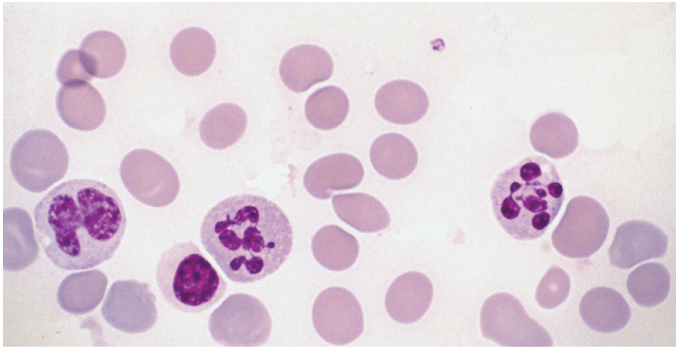
Hematologic parameters vary considerably (Table 15-2 ★). The hemoglobin and erythrocyte count range from normal to very low. The erythrocyte count is occasionally $<1 \times 10^{12}$/L. However, anemia is not always evident. In one study of 100 patients with confirmed cobalamin deficiency, only 29% had a hemoglobin of <12 g/dL.[12] This is significant because neurologic symptoms can be present even if the MCV and/or hematocrit are normal.[13] Because the abnormality is a nuclear maturation defect, the megaloblastic anemias affect all three blood cell lineages: erythrocytes, leukocytes, and platelets. This is unlike most other anemias that typically involve only erythrocytes. The leukocyte count can be decreased due to an absolute neutropenia. Platelets can also be decreased but do not usually fall below 100×10^9/L. The relative reticulocyte count (percentage) is usually normal; however, because of the severe anemia, the corrected reticulocyte count is <2%, the absolute reticulocyte count is low, and RPI is <2 (Chapter 11).

The distinguishing features of megaloblastic anemia on the stained blood smear include the triad of oval macrocytes (macro-ovalocytes), Howell-Jolly bodies, and hypersegmented neutrophils (Figure 15-1a). Anisocytosis is moderate to marked with normocytes

★ **TABLE 15-2** Comparison of Common Laboratory Values in Megaloblastic and Nonmegaloblastic Macrocytosis

Laboratory Value	Megaloblastic Macrocytosis	Nonmegaloblastic Macrocytosis
WBC count	Decreased	Normal
Platelet count	Decreased	Normal
RBC count	Decreased	Decreased
Hemoglobin	Decreased	Decreased
Hematocrit	Decreased	Decreased
MCV	Usually >110 fL	>100 fL
RBC morphology	Ovalocytes, Howell-Jolly bodies, polychromasia	Polychromasia, target cells, and stomatocytes (liver disease), schistocytes (hemolytic anemias)
Hypersegmentation of neutrophils	Present	Absent
Reticulocyte count	Normal to decreased	Normal, decreased, or increased
Serum cobalamin	Decreased in cobalamin deficiency	Usually normal
Serum folate	Decreased in folate deficiency	Normal (except in alcoholism when it can be decreased)
FIGLU	Increased in folate deficiency	Normal
MMA	Increased in B_{12} deficiency	Normal
Homocysteine	Increased	Normal
Serum bilirubin	Increased	Normal to increased
LD	Increased	Normal to increased

MCV = mean corpuscular volume; FIGLU = formiminoglutamic acid; MMA = methylmalonicacid; LD = lactic dehydrogenase



■ **FIGURE 15-3** Hypersegmented neutrophils from the peripheral blood of a patient with pernicious anemia. (Wright-Giemsa stain; 1000× magnification)

and a few microcytes in addition to the macrocytes. Poikilocytosis can be striking and is usually more so when the anemia is severe. Polychromatophilia and megaloblastic erythroblasts can be seen, especially when the anemia is severe, indicating the futile attempt of the bone marrow to increase peripheral erythrocyte mass. Cabot rings occasionally can be seen in erythrocytes.

Granulocytes and platelets can also show changes evident of abnormal hematopoiesis. Hypersegmented neutrophils can be found in megaloblastic anemia even in the absence of macrocytosis (Figure 15-3 ■). Finding 5% or more neutrophils with five lobes or one neutrophil with six or more lobes is considered hypersegmentation. This finding of hypersegmented neutrophils is considered highly sensitive and specific for megaloblastic anemia. Therefore, hypersegmented neutrophils offer an important clue to megaloblastic anemia in the face of a coexisting disease that tends to keep erythrocyte volume <100 fL. One study showed that in patients with renal disease, iron deficiency, or chronic disease with a normal or decreased MCV and 1% hypersegmented neutrophils, 94% had vitamin B_{12} or folic acid deficiency.[8] If 5% hypersegmented neutrophils were counted, the incidence of the vitamin B_{12} or folic acid deficiency increased to 98%. Hypersegmented neutrophils tend to be larger than normal neutrophils. A mild shift to the left with large hypogranular bands can also be noted. Platelets can be large, especially when the platelet count is decreased.

☑ CHECKPOINT 15-3

Why are abnormalities of leukocytes and platelets present in megaloblastic anemia?

Bone Marrow

If physical examination, patient history, and peripheral blood findings suggest megaloblastic anemia, a bone marrow examination can help establish a definitive diagnosis. In megaloblastic states, the bone marrow is hypercellular with megaloblastic erythroid precursors and a decreased M:E ratio. In a long-standing anemia, red marrow can expand into the long bones. About half the erythroid precursors typically show megaloblastic changes. Megaloblasts are large nucleated erythroid precursors that display nuclear-cytoplasmic asynchrony with nuclear maturation lagging behind cytoplasmic maturation (Figure 15-4 ■). The nucleus of the megaloblast contains loose, open chromatin that stains poorly; cytoplasmic development continues in a normal fashion. At each stage of development, the cells contain more cytoplasm with a more mature appearance relative to the size and maturity of the nucleus (resulting in a decreased nuclear:cytoplasmic [N:C] ratio).

The megaloblastic features are more easily noted in later stages of erythroid development, especially at the polychromatophilic stage in which the presence of hemoglobin mixed with RNA gives the cytoplasm the gray-blue color typical of this erythroid precursor. The polychromatophilic megaloblast nucleus, however, still has an open (lacy) chromatin pattern more typical of an earlier stage of development.

Leukocytes and platelets also show typical features of a nuclear maturation defect as well as ineffective leukopoiesis and thrombopoiesis. Giant metamyelocytes and bands with loose, open chromatin in the nuclei are diagnostic (Figure 15-4a). The myelocytes can show poor granulation as do more mature stages. Megakaryocytes can be decreased, normal, or increased. Maturation, however, is distinctly abnormal. Larger than normal forms can be found with separation of nuclear lobes and nuclear fragments.

CASE STUDY (continued from page 310)

Based on the initial CBC results, further testing was ordered with the following results:

B_{12} (cobalamin)	50 pg/mL	Low
Folate	10.3 ng/mL	Normal
Total billirubin	2.5 mg/dL	High
Direct billirubin	0.8 mg/dL	Normal
AST	35 U/mL	Normal
ALT	30 U/mL	Normal

Examination of a bone marrow aspirate revealed an erythroblastic hyperplasia with megaloblastic erythroblasts.

1. What is the morphologic classification of the patient's anemia?
2. Based on the information obtained so far, what is the most likely defect?
3. What is the significance of the AST/ALT results?
4. What further testing can be done to obtain a definitive diagnosis?

Other Laboratory Findings

If CBC results suggest megaloblastic anemia, further testing is necessary to distinguish the cause. Although no major medical organization has published guidelines for reflex testing, the most common next step is to measure serum cobalamin and serum or red cell folate. Laboratories use different methods (chemiluminescence, radioassay) to measure cobalamin, so there is no "gold standard" to use as a reference interval. Generally values <150 pg/mL are consistent with cobalamin deficiency, whereas levels >400 pg/mL suggest adequate cobalamin. Borderline levels (150–400 pg/mL) can be associated with cobalamin deficiency.