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Neoplastic Diseases of the Blood

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13

CLASSIFICATION OF THE ACUTE LEUKEMIAS: CYTOCHEMICAL AND MORPHOLOGIC CONSIDERATIONS

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DEFINITION AND CLASSIFICATION

The acute leukemias may be defined as neoplastic transformations of uncommitted or partially committed hematopoietic stem cells. The origin of the malignant neoplasm is almost invariably within the bone marrow. Replacement of the marrow pulp or the repression of normal hematopoietic cells results in variable degrees of anemia, neutropenia, and thrombocytopenia.

Historically, the term *acute* implied not only a poorly differentiated blast population, but also a clinical syndrome that led to a rapidly fatal outcome. Since it is apparent that, with modern chemotherapy, including bone marrow transplantation (BMT), patients with acute leukemia of several morphologic types can enjoy complete remission (CR), and indeed cure, the term *acute* can be maintained only for nosologic reasons.

Traditionally, the different involved lineages were identified by morphology and cytochemistry. During the past 10 years, major advances in our knowledge of the nature of leukemia consequent to the application of the techniques of immunology (monoclonal antibodies [mAbs]); cytogenetics (chromosomal abnormalities), and molecular genetics (gene rearrangements) have

taken place. The demonstration of membrane and cytoplasmic antigen or enzyme by immunologic or immunocytochemical methods provides supplementary arguments for accurate classification.

Identification of subclasses of acute leukemia is important for three reasons. First, some leukemias have clinical features that influence the therapeutic approaches; that is, the central nervous system (CNS) is more frequently involved in acute lymphocytic leukemia (ALL) than in acute myelocytic leukemia (AML). Hypergranular acute promyelocytic leukemia is associated with intravascular coagulation¹ and acute monocytic leukemia with skin and gum infiltration.² Second, differences in response rate and survival are observed in the treatment of acute leukemias; that is, the percentage of CR rate is higher in childhood ALL than in adult ALL. Third, classification greatly facilitates communication and cooperation around the world, and comparisons of results are possible only through reproducible definitions of acute leukemias.

Examination of both peripheral blood and bone marrow smears is necessary for the diagnosis and classification of acute leukemias. The most useful cytochemical stains are myeloperoxidase (MPO); Sudan Black B (SBB); specific and nonspecific esterases, such as chlo-

roacetate esterase, naphthol ASD acetate esterase (Plates 13-1 and 13-2) with and without sodium fluoride (NaF), α -naphthyl acetate esterase (ANAE), or butyrate esterase; periodic acid-Schiff (PAS); and acid phosphatase.

There are three instances in which the diagnosis of acute leukemia can be made on the basis of histologic material. The first occurs when there is an abundance of reticulin in the bone marrow, resulting in a so-called dry tap.³ This can be present in any of the acute leukemias and is recognized most commonly associated with acute megakaryoblastic leukemia. Although touch preparation smears may be of help in such cases, well-prepared hematoxylin and eosin (H&E) stains of paraffin-embedded material, PAS, chloroacetate esterase stains, and the immunologic methods identifying antigens on the cell surface (factor VIII) can often establish the diagnosis. The second example is hypocellular (hypoplastic) acute myeloid leukemia, in which bone marrow aspirations from several sites and biopsy from one site are necessary for diagnosis.⁴ The correct diagnosis depends on a representative biopsy specimen. The third example is that of an uncommon localized extramedullary mass of cells of the granulocytic-monocytic series (granulocytic sarcomas).⁵ These tumors can be seen in an established diagnosis of AML, either at presentation or as the first manifestation of relapse.

DIAGNOSIS OF ACUTE LEUKEMIAS

The most widely used classification of acute leukemias is that proposed by the French-American-British (FAB) Cooperative Group.⁶ The classification is based exclusively on the morphology of the bone marrow and peripheral blood leukemic cells in Romanowsky-stained smears. The initial intent was to separate ALL and AML into easily identifiable cell types. In attempting to define boundaries between overlapping groups, major attention is given to the predominant cell type present. An essential cytochemical stain is one that demonstrates MPO, an enzyme restricted to the primary granules of granulocytes and monocytes.⁷ This classification has stood the test of time, since only a few cases do not fit the FAB criteria. The distribution of the FAB cell types is indicated in Table 13-1. Today the morphologic definition must be complemented by immunophenotyping and cytogenetics.

ACUTE LYMPHOCYTIC LEUKEMIAS

ALL is one of the most common malignancies observed in the pediatric age group. It derives from the clonal proliferation of lymphoid progenitors in the bone marrow. ALL represents about 80 percent of cases of acute leukemia in children but only 20 percent in adults. The consequence of bone marrow infiltration is various cytopenias in the peripheral blood and is associated with the appearance of peripheral blast cells. In some instances, leukemic cells are not seen in the pe-

Table 13-1. Classifications of the Acute Leukemias

Morphologic Type	Subtypes (Common Terms)
Lymphocytic	ALL (FAB) CD10 (CALLA) (L1-L2) T-ALL (L1-L2) B-ALL (Burkitt; L3)
Myelocytic	AML Myeloblastic without cytologic maturation (M0) Myeloblastic with minimal maturation (M1) Myeloblastic with significant maturation (M2) Hypergranular promyelocytic (M3) Hypogranular promyelocytic (M3 variant)
Myelomonocytic	AMML (M4)
Monocytic	Poorly differentiated (M5A) AMoL Differentiated (M5B) AMoL
Erythroid	AEL (M6)
Mast cell	Acute mast cell leukemia
Megakaryocyte	Acute megakaryocytic leukemia (M7)

Abbreviations: AEL, acute erythroleukemia; ALL, acute lymphoid leukemia; AML, acute myelocytic leukemia; AMML, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; CALLA, common ALL; FAB, French-American-British (Cooperative Group).

ripheral blood. Thus, examination of the bone marrow is certainly necessary to confirm the diagnosis. Since the CNS is infiltrated at diagnosis in 5 percent of patients, examination of the cerebrospinal fluid (CSF) by cytocentrifuge is also necessary.

Morphologic Classification of Acute Lymphocytic Leukemia

The current classification of ALL was proposed by the FAB group in 1976.⁶ The FAB classification was based on the observation that approximately 80 percent of children had a uniform population of small blast cells with a high nuclear/cytoplasmic (N/C) ratio, a small inconspicuous nucleolus, and a regular nucleus. These were designated L1 (Plate 13-1). In a few patients, the cells were described as more heterogeneous; more than 20 percent had large cells with irregular nuclear membranes, one or more nucleoli, more cytoplasm, and a lower N/C ratio; this subtype was called L2 (Plate 13-2). A rare type seen in approximately equal percentages in children and adults (about 3 to 5 percent), referred to as L3, is morphologically identical to the cell characteristics of Burkitt's lymphoma. The cells are large and homogenous with round to oval nuclei. The chromatin is finely dispersed with prominent nucleoli. The cytoplasm is intensely basophilic with or without vacuoles (Plate 13-3). Of the three types described, only L3 is constantly associated with surface immunoglobulin (sIg) (mature B-ALL).⁸ In addition, an identical nonrandom chromosome abnormality [t(8;14) translocation] has been described in both Burkitt's lymphoma and L3 ALL.⁹ In about 50 percent of patients CD10 (CALLA) positivity has been reported along with the sIgs.¹⁰ In rare instances, terminal deoxynucleotidyl transferase-positive (TdT+) ALL with sIgs has been re-

Classification of the Acute Leukemias: Cytochemical and Morphologic Considerations

193

Table 13-2. Simplified Scoring System for FAB L1 and L2

Criteria	Score
High N/C ratio $\geq 75\%$ of cells	+
Low N/C ratio $\geq 25\%$ of cells	-
0-1 nucleoli (small)	+
1 or more nucleoli (prominent)	-
Irregular nuclear membrane $\geq 25\%$ of cells	-
Large cells $\geq 50\%$ of cells	-
Scoring range: +2 to -4 (L1: 0 $\rightarrow$ +2; L2: -1 $\rightarrow$ -4)	

Abbreviations: N/C, nuclear/cytoplasmic (ratio) other abbreviations as in Table 13-1.

ported.¹¹ These cases do not have the typical chromosomal translocation or the L3 morphology.

Despite the accurate definition of these subtypes, observers registered discordance about their appreciation. The original FAB proposal was later modified in 1981,¹² eliminating some of the discordance among the observers and improving clinical correlations. The new scoring system was focused on three essential features of the blast cell population: N/C ratio, nucleoli, and irregular nuclear membrane. Cell size was another component when less than 50 percent of leukemic cells were larger than twice the size of small lymphocytes. A simplified scoring system is given in Table 13-2. In this system, the sum may vary from +2 to -4. A total score of 0 to +2 defines L1, while a score of -1 to -4 establishes L2. With this revised system, concordance rates were close to 85 percent in clinical studies.¹³

Overall, L1 morphology is associated with a better event-free survival and induction rate than L2,¹⁴ but this fact must be tempered because approximately 85 percent of the L1 cases observed are in the pediatric age group. By contrast, L3 morphology has the worst prognosis.

Cytochemistry

By standard definition, the peroxidase reaction is totally negative in leukemic lymphoblasts. SBB stain is also negative. However, it has been suggested that SBB is less specific than MPO because positive reactions have been reported in ALL.¹⁵ It should be remembered that in case of a negative MPO or SBB reaction, megakaryocytic, erythroid, some monocytic, and AML M0 subtype, as well as lymphoid lineage, may be involved.

The PAS reaction is useful in supporting the diagnosis of ALL (Table 13-3), but its importance has declined, as immunologic markers have become more important for this purpose. Although block-like PAS positivity is considered characteristic of ALL, only 15 percent of cases have this type PAS reaction⁶ (Plate 13-4). This pattern of PAS positivity correlates with the immunologic phenotype, which is more common in B-lineage cases. The presence of both vacuolated blasts and PAS positivity correlates with reactivity of CD10 antigen.¹⁶ It should be noted that block-like PAS positivity can be seen in monoblasts in AML M5 and in erythroblasts in

Table 13-3. Reaction of Acute Leukemic Cells to Cytochemical Stains

Stain	Cell Types			
	AML (FAB M1-M3)	AMML (M4)	AMoL (M5)	ALL (1-2)
PAS	$\pm$	+	++	++
Acid phosphatase	-	$\pm$	++	+++
Peroxidase	+	+	$\pm$	-
Chloroacetate esterase	+	+	-	-
α -Naphthyl ester	$\pm$	+	++	+++

Abbreviations: PAS, periodic acid-Schiff; other abbreviations as in Table 13-1; -, absent; $\pm$, occasional activity; +, moderate activity; ++, strong activity; +++, focally strong activity in T cells; + + + +, focally strong activity at acid pH in T cells.

AML M6.¹⁷ Moreover, fine granular activity in blocks of PAS material can be identified in all AML cell types, (Plate 13-5). PAS stain is therefore a poor discriminator of various cell types, but it may be useful as a part of battery of stains, particularly when many of the reactions are negative or nonspecific.

A strong focal (paramuclear) acid phosphatase (ACP) activity (Plate 13-6) or a strong focal ANAE activity greater than 75 percent of blasts are characteristic of T-ALL.¹⁸ However, it should be remembered that a localized ACP and ANAE reaction is also a feature of rare forms of AML: M7 and M6.^{19,20}

Immunocytochemistry

Indirect immunoperoxidase and indirect alkaline phosphatase anti-alkaline phosphatase (APAAP) techniques are applied to fixed cells in blood and bone marrow smears or cytospin preparations.²¹ These methods permit the detection of surface antigens (i.e., CD19, CD10), cytoplasmic antigens (i.e., cytoplasmic μ chain or MPO), and nuclear antigens (e.g., TdT).

PHILADELPHIA-POSITIVE ALL

Although detailed cytogenetics are covered in Chapter 15, this special form of ALL merits discussion. The detection of the Philadelphia chromosome is more difficult in ALL than it is in chronic myelocytic leukemia (CML). The success rate of cytogenetic techniques is lower, but molecular genetic methods are also difficult because the translocation breakpoints are more heterogeneous than they are in CML.²² The true incidence of the Philadelphia chromosome in ALL is unknown; cytogenetic studies suggest that it occurs in 3 to 10 percent of children with ALL,²³ but it is as high as 30 percent in adults.²⁴ Thus, this chromosome appears to be the single most common genetic abnormality in adult ALL. Moreover, the presence of the Philadelphia chromosome identifies a subgroup of ALL with a poor prog-

nosis.²⁵ In addition to the incidence of the Philadelphia chromosome in ALL, questions have been raised about its relationship to CML. These patients, by definition, have acute leukemia without the prior chronic phase characteristic of CML, and have a variable course. Some patients are refractory to treatment; others have a short but true hematologic remission with partial or complete loss of the Philadelphia chromosome clone. By contrast, lymphoid blast crisis of CML with appropriate therapy reverts to a second Philadelphia-positive chronic phase. It has been suggested that cases of Philadelphia-positive bcr-positive ALL expressing p210, may be CML in lymphoid blast crisis; bcr-negative cases expressing a variant p190 may be true *de novo* ALL.²⁶ However, no clinical data have been reported to substantiate this claim. One characteristic of Philadelphia-positive ALL is the occasional presence of both myeloid and lymphoid features in the same leukemia. Both biphenotypic and bichlonal variants have been described.²⁷ This feature supports the theory that this subtype of ALL arises from pluripotential progenitors.²⁸ The morphology of the blasts can be either L1 or L2. Although the mixed-lineage phenomenon was initially described in some patients with lymphoid blast crisis of CML, its actual incidence is relatively low.²⁹

ACUTE MYELOCYTIC LEUKEMIAS

AMLs result from a neoplastic transformation of a single pluripotential hematopoietic stem cell. Evidence to support this statement is morphologic,³⁰ immunologic,³¹ and chromosomal,³² as well as from *in vitro* cell culture studies.³³ These studies have demonstrated trilineage dysplastic features or lymphoid antigen expressions in AML, monocytic and erythroid precursors in cultures, and elaboration of colony-stimulating factors (CSFs) from monocytes.

Four major types of AML have long been recognized: acute myeloid, acute myelomonocytic, acute monocytic, and acute erythroleukemia. Despite these subtype definitions, Auer rods are the only consistent neoplastic marker demonstrating that a blast is myeloid and of leukemic origin. Auer rods are abnormal azurophilic crystalline-like granules that represent the coalescence of primary lysosomal granules of myeloid precursors, as documented by cytochemical and ultrastructural studies.^{34,35} We have demonstrated that the addition of a peroxidase or another specific stain for primary granules increases the percentage of positive blast cells that contain Auer rods from 21 percent to 60 percent. The clinical relevance of these observations is uncertain at the present time.

Morphologic Classification

The minimal requirements for morphologic specification of each case of AML are well-prepared peripheral blood and bone marrow smears prepared with Wright-Giemsa stain. To prove lineage, some routine cyto-

Table 13-4. Acute Leukemia Subtype Distribution

FAB	%
M1	20
M2	30
M3	10
M4	25
M5A	4
M5B	6
M6	5

Abbreviations: As in Table 13-1.

chemical stains can be applied. These techniques are discussed below.

A uniform reproducible subtype classification in AML is necessary for several reasons. First, it permits comparison among various therapeutic regimens from different groups and from program to program within the same institution or cooperative group. Second, it allows for the potential identification of different clinical features and laboratory aspects that may be unique to certain subtypes. Finally, with the increasing interest and sophistication of chromosomal abnormalities in AML, accurate description of myeloid subsets may permit a meaningful association of nonrandom rearrangements and translocations.

Morphologic classification of AML was proposed by the FAB Cooperative Group in 1976,⁶ based on the morphologic and cytochemical features of the blast cells. The original proposal was revised and expanded in 1985.³⁷ This classification is widely accepted and has been incorporated with immunologic and cytogenetic results in numerous studies (Table 13-4).

The FAB classification currently accepts two types of blasts: Type I blasts are myeloblasts with open chromatin, distinct nucleoli, and immature cytoplasm without granules. Type II blasts are similar to type I, except that they contain up to 20 delicate cytoplasmic azurophilic granules or Auer rods, or both. Recently, Goasguen et al.³⁸ distinguished another type of blasts from promyelocytes, referred to as type III blasts. These lack major characteristics of the promyelocyte, especially the Golgi zone and eccentric nuclear location, but they have many azurophilic granules. Type III blasts are characteristically identified in AML M2 associated with t(8; 21). They can also be seen in secondary AML and AML developing from myelodysplastic syndrome (MDS).³⁹ Neoplastic promyelocytes of M3, promonocytes and monoblasts of M5, and megakaryoblasts of M7 leukemias are also recommended for inclusion in the total blast percentage.

Once the diagnosis of AML has been established by identifying 30 percent or more blast cells out of all nucleated cells, then to assess the percentage of differentiating granulocytes or monocytes, the erythroblasts should be eliminated from consideration. A diagnosis of AML is compatible with an erythroid cell count of up to 50 percent of all the nucleated cells. Beyond this (more than 50 percent) the diagnosis is acute erythroleukemia (FAB M6).

Cytochemistry

Myeloperoxidase and Sudan Black B

An essential cytochemical stain is one that demonstrates the presence of MPO, an enzyme restricted to the primary granules of granulocytes and monocytes.⁷ This enzyme can be identified by performing a benzidine base peroxidase reaction or SBB staining or by diaminobenzidine reaction. The benzidine method is the best available method against which other methods should be compared. However, because of the concern for its potential carcinogenicity, it could not be adopted as the reference method.⁴⁰

The SBB reaction, a general stain for intracellular lipids, can be used in the same way. This stain is present in both primary and secondary granules. Sudanophilia and MPO activity are closely parallel.⁴¹

Specific and Nonspecific Esterases

The chloroacetate esterase reaction can also be used to identify myeloid lineage. Although it is less sensitive than either MPO or SBB in detecting myeloid differentiation, it can be useful, when combined with ANAE in a double esterase stain, in confirming a monocytic component. Because ANAE is positive both in neutrophils and in monocytes, inhibition by NaF in monocytes should be used to identify these cells.^{40,41}

Periodic Acid-Schiff Reaction

All glycogen-containing cells as well as neutrophils and granulocytes at all stages of maturation stain with this reaction. Our experience in more than 200 patients has shown that fine granular PAS reaction can be identified in all AML subtypes, ranging from 60 percent in AML (M1 to M3), 70 percent in AML M4, and 80 percent in AML M5. Moreover, the latter often demonstrates a block-like reaction pattern characteristic of ALL (Bennett J.M. unpublished observations). The block-like pattern of PAS reactivity is seen in erythroid precursors of M6.

Other Cytochemical Techniques

Electron Microscopy

Electron microscopy is used to demonstrate either MPO or platelet peroxidase (PPO). These ultrastructural studies are useful in the confirmation of myeloid (M0) or megakaryocytic (M7) origin of blasts. MPO can be demonstrated by electron microscopy in blast cells that previously had negative results (less than 3 percent positive blasts) by conventional criteria. It is also useful to define the myeloid component in biphenotypic leukemias.^{42,43} In megakaryoblasts, the PPO reaction is localized exclusively on the nuclear membrane and the endoplasmic reticulum, whereas in myeloblasts it occurs in the Golgi area and cytoplasmic granules.

Immunocytochemistry

Cytospin preparations or direct bone marrow or peripheral blood smears are used to demonstrate the presence of specific lineage antigens using a secondary technique (e.g., APAAP) to show antibody fixation.²¹ With this technique, it is possible to demonstrate the topographic distribution of the antigen on the cell surface, as well as morphologic recognition of the cells in the same specimen. The determination of MPO and CD41, CD34, and CD13 or CD33 antigens are possible by this method. Moreover, in biphenotypic leukemias, the presence of myeloid and lymphoid antigens can be demonstrated by using a double staining system (i.e., alkaline phosphatase for one antibody and peroxidase for the second). It is also possible to demonstrate the presence of p-170 glycoprotein (multidrug resistance [MDR] phenotype) on the cell surface, using cytospin preparations.

ACUTE MYELOBLASTIC LEUKEMIA WITHOUT CYTOLOGIC MATURATION (M0)

The use of mAbs as a diagnostic aid in acute leukemia has resulted in the recognition of some cases that do not fit the FAB criteria for AML (M1 to M7) but that do fit the FAB categories described for ALL L2, but without evidence of lymphoid differentiation by immunologic analysis. These cases are now termed M0.⁴⁴ Morphologically, blasts are large with open chromatin and prominent single or multiple nucleoli. The N/C ratio is generally low. Cytoplasm is moderately basophilic. Azurophilic granules or Auer rods are not seen (Plate 13-27). MPO- or SBB-positive blasts are less than 3 percent by light microscopy and can only be demonstrated by electron microscopy or by anti-MPO antibodies, using the APAAP technique. Moreover, lymphoid differentiation antigens should be absent, except for TdT, which is expressed in one-half of cases.⁴⁵ The incidence of AML M0 has been reported to be 5 to 8 percent in different series.^{46,47,48,50,51} All these patients share certain features. They were found to be negative for MPO by light microscopy but positive for MPO by electron microscopy, and all had myeloid antigens (CD13, CD33) on the cell surface; many of them would once have been diagnosed as either ALL L2 or acute undifferentiated leukemia (AUL). In each series, there is evidence that patients with the AML M0 subtype have a poorer response to combination therapy.

It is important to stress that a diagnosis of AML M0 cannot be made on morphologic grounds alone and that it always requires confirmation by immunologic techniques. Since CD13 antigen is expressed in the cytoplasm of myeloblasts earlier than on the surface, CD13 should be tested by immunocytochemistry whenever the results of flow cytometry on the cell suspensions are negative. Some studies suggested that the ultrastructural demonstration of MPO in very early myeloid

cells is slightly more sensitive than by immunocytochemistry.⁵¹

ACUTE MYELOBLASTIC LEUKEMIA WITH MINIMAL DIFFERENTIATION (M1)

Poorly differentiated myeloblasts are the predominant nonerythroid cell type (types I and II). Rarely, a case of M1 may present with the majority of blasts type III. Auer rods may be present and consistent with the diagnosis (Plate 13-8). More than 3 percent of these blast cells are MPO or SBB positive by conventional cytochemistry (Plate 13-9). AML M1 should be differentiated from ALL L2, acute monoblastic leukemia without differentiation (M5A), and acute megakaryoblastic leukemia. Cytochemical stains and immunophenotyping are necessary for the differential diagnosis. The low percentage of MPO-positive (3 to 10 percent) M1 cases may constitute up to 25 percent of all M1 cases.⁵² In such cases of M1, immunophenotyping using a classic panel (CD13, CD33, CD14, CD15, and CD34) to confirm the myeloid nature of blasts is recommended. CD13, CD33, and CD14 can be done by the APAAP method.

In AML M1, low MPO positivity cases were found to coexpress lymphoid and myeloid antigens.⁵³ Moreover, discrepancy between immunologic MPO detection and light cytochemistry was found to be more frequent in patients with mixed immunophenotype than in patients without lymphoid markers.⁵⁴

MPO reaction may have a prognostic significance in AML M1. In our laboratory, 72 patients with AML M1 were investigated for MPO activity.⁵² Two groups of patients were distinguished according to MPO-positive blast cells (less or 50 percent or greater MPO-positive blast cells). Although no remarkable morphologic difference of blast cells between these two groups was observed, patients with low MPO reactivity showed a significantly lower CR rate (52.6 percent) than that if patients with high MPO reactivity (CR rate 85.3 percent). These results suggest that the low MPO group has a poorer result with the induction of remission.

ACUTE MYELOBLASTIC LEUKEMIA WITH SIGNIFICANT MATURATION (M2)

The defining characteristic that separates M2 from M1 is clear evidence of significant differentiation in all cells beyond the promyelocyte state (Plate 13-10). The sum of the blast cells (types I+, II+, and III) is greater than 30 percent but less than 90 percent, and generally there is a large predominance of type II blasts. Type III blasts are seen in AML M2 with t(8;21). Monocytic precursors cannot exceed 20 percent. The MPO reaction is strongly positive. However, M2 cases with a partial MPO deficiency in the granulocytic precursors and the mature granulocytes have been reported.⁵⁵ Esterase

reactions identify less than 20 percent of monocytic precursors. Pseudo-Pelger-Huët cells and hypogranular neutrophils, as well as dysmorphic features in the erythroid and megakaryocytic lineages, are usually identified in AML M2. These abnormalities are the components of the trilineage dysplasia and are discussed later.

The t(8;21) (q22;q22) is a frequent karyotypic abnormality seen in approximately 10 percent of patients with AML M2. The translocation interrupts two genes—AML1 on chromosome 21 and ETO on chromosome 8—that are consequently fused in the der (8) chromosome to produce a novel chimeric gene and message. Selected genomic DNA probes from chromosomes 21 and 8 near the breakpoint junction detect rearrangements in the DNA of about 80 percent of patients with the rearrangements at diagnosis and in relapse.⁵⁶⁻⁵⁸ It has been reported that this translocation is associated with characteristic morphologic features.⁵⁹ Blasts are large cells with a distinctive nucleolus. The cytoplasm contains abnormal heavy azurophilic granulations (type III blasts) with tiny needle-like Auer rods (Plate 13-11).

On occasion, basophilic differentiation of the granulocyte precursors can be demonstrated by electron microscopy. Basophilic differentiation can be suspected by light microscopy because of the presence of few but distinct basophilic granules in their cytoplasm. Although M2 basophilia is associated with t(6;9) or t(12;12), it has been shown that these aberrations are not as closely correlated as those previously mentioned.⁵⁵ M2 basophilia must be separated from CGL in blast crisis.⁵¹ Another variant of M2, characterized by immature eosinophilic precursors (M2 Eo), should be separated from the more common M4Eo.⁵⁹

ACUTE PROMYELOCYTIC LEUKEMIA (M3)

Acute promyelocytic leukemia (APL) is a distinct class of AML characterized by specific morphologic, ultrastructural, cytogenetic, and clinical features. In most series, M3 accounts for about 10 percent of cases in adults and for 4 to 8 percent in children.⁶⁰ Typical APL is characterized by the presence of hypergranular blast cells, which have the appearance of abnormal promyelocytes. The cytoplasm is dense with coarse dark staining granules that often obscure the nucleus. The nuclear border is generally irregular and has a folded or reniform appearance (Plate 13-12). Cells contain bundles of delicate needle-like Auer rods, giving a meshwork appearance (so-called Faggots), found in some of the leukemic promyelocytes. These abnormal promyelocytes are strongly positive for the MPO or SBB reactions. In contrast to other AML subtypes, dysplastic features of the myeloid series associated with leukemic proliferation are not seen.

The FAB classification recognizes a variant of APL (FAB M3v),⁶¹ characterized by bilobate cells or by cells with reniform nucleus and cytoplasm with minimal or no granulations (Plate 13-13). Typical M3 cells are in-

frequent in the bone marrow. Hypogranular promyelocytes are strongly positive both for MPO and for SBB, as in typical APL. The immunophenotype of M3v is identical to that described for the typical APL (expression of myeloid antigens in the absence of HLA-DR reactivity).⁶² Special attention should be given to differentiate these cases from M2 and M4 subtypes. The distinction is important because of the well-known association of M3 with disseminated intravascular coagulation (DIC).¹ The DIC syndrome is present equally in the M3v. In adults, the microgranular variant constitutes 25 percent of APL cases.⁶³ It may also occur in children.⁶⁴ The hematologic characteristics of M3v at onset, in addition to DIC, is hyperleukocytosis (greater than 20,000/mm³). The CR rate is lower, reflecting early hemorrhagic deaths.^{63,64}

Besides the clinical and morphologic features of M3, a specific association with a chromosomal abnormality has been described, referred to as t(15;17). It is present in at least 90 percent of all cases of M3, including M3v. This translocation fuses the *PML* gene of the chromosome 15 to the retinoic acid receptor- α (*RARA*) gene, producing a chimeric *PML/RARA* gene.⁶⁵⁻⁶⁷ The involvement of *RARA* at the translocation breakpoints may explain the clinical response to all-*trans*-retinoic acid (ATRA).^{68,69}

A rare variant of APL is associated with basophilic differentiation.^{70,71} Patients with this variant show basophilic maturation during remission induction with cytotoxic chemotherapy or ATRA. However, it is not clear whether the basophils are reactive or derive from the leukemic clone. It has been suggested that basophilia in APL⁷¹ is not related to the t(15;17) but occurs as a result of translocations known to be associated with basophilia (translocations involving genes at 9q34, 16p13, or 16q22).

ACUTE MYELOMONOCYTIC LEUKEMIA (M4)

AMML accounts for 20 to 30 percent of adult AML cases, depending on the application of esterase staining with fluoride inhibition. Its diagnosis and separation from M2 and M5 require assessment of both the peripheral blood and bone marrow smears. In bone marrow, both granulocytic and monocytic precursors exist in varying proportions (Plate 13-14). The blast cells (types I, II, III) are greater than 30 percent of the nonerythroid component. The sum of the myeloblasts, promyelocytes, and maturing granulocytes is 30 percent or more, but less than 80 percent, of the nonerythroid cells. The marrow monocytic component must account for 20 percent or more of the nonerythroid nucleated cells but cannot exceed 80 percent (otherwise, the diagnosis is M5). Auer rods are occasionally present. When these findings are present with a peripheral blood monocytosis of 5,000/mm³ or greater, the diagnosis of M4 is not in question. If the peripheral monocytosis is less than 5,000/mm³, a diagnosis of M4 can still be made if the presence of a significant monocytic component (greater than 20

percent) is confirmed by cytochemical stain (e.g., double esterase reaction with NaF or other esterase combinations). Cytochemical stains are also necessary for confirming the diagnosis of M4 in the presence of hypogranular neutrophils that resemble monocytes. Dysplastic features involving granulocytic, erythroid, and megakaryocytic lineages can be identified in approximately 20 percent of patients. The monocytic component can be identified by immunophenotyping using CD14 in conjunction with other granulocyte-restricted antibodies.

M4 With Eosinophilia (M4Eo)

A small percentage of patients (about 5 percent) with M4 are characterized by myelomonocytic bone marrow infiltration, eosinophils with abnormal immature granules positive for chloroacetate esterase, an inversion or translocation of chromosome 16, and an increased risk of meningeal relapse. Eosinophils account for 5 percent or more of nonerythroid cells. These eosinophils are abnormal; in addition to the specific eosinophilic granules, they have large basophilic granules and demonstrate chloroacetate esterase and PAS (coarse granules) positivity. Often the nuclei have pseudo-Pelger features (Plate 13-15). Arthur and Bloomfield⁷² described a unique chromosomal abnormality with this cell type. The most common abnormality involves a pericentric inversion of chromosome 16 (p13q22).⁷³ In some cases, a deletion is seen instead of the inversion. Both abnormalities are characterized by an involvement of band q22. However, most patients with del 16 do not have typical features of M4Eo and tend to have a worse prognosis.⁷⁴ The favorable prognosis of M4Eo has been confirmed in several studies.⁷⁵ Recently, Haeflacker et al.⁷⁶ reported that 91.7 percent of their 24 patients with M4Eo reached complete remission, with a relapse-free survival rate of 49 percent after 3 years, compared with 35 percent for other types combined. In our experience with 600 adult AML patients from Eastern Cooperative Oncology Group (ECOG) studies, 11 patients (5 percent of all M4 patients) had typical features that described for M4Eo. All these patients achieved CR, compared with 65 percent of patients with M4 in this study (Cantin G., Bennett J.M., unpublished observations).

Acute Monocytic Leukemia (M5A and M5B)

Acute monocytic leukemia (AMoL) accounts for about 10 percent of the AML and is clonally expressed in cells committed to differentiation to the monocytic pathway.⁷⁷ AMoL is divided into two morphologic subtypes: M5A and M5B.

M5a, or the poorly differentiated subtype, is frequently observed in children; it can be confused with the ALL L3 type. In the bone marrow, 80 percent or more of all monocytic cells are monoblasts. Blast cells display abundant deep basophilic cytoplasm, which is often vacuolated and has no or few azurophilic granules

and no Auer rods. The nuclei are round to oval, with one or more prominent nucleoli (Plate 13-16). Since the peroxidase reaction may be negative in 40 to 50 percent of such cases and the PAS reaction is often strongly positive with a block-like pattern, it is very useful to use a nonspecific esterase (NSE) stain in differential diagnosis. This stain will be strongly positive in more than 90 percent of cases (Plate 13-17). Occasionally, the SBB reaction will be positive in the absence of a peroxidase reaction. Cells with monocytic differentiation can express CD14 antigen on the cell surface.

M5B, or the well-differentiated subtype, is defined by the presence of 20 percent or more of abnormal cells being (promonocytes) with twisted or folded nuclei, gray-blue cytoplasm, and scattered azurophilic granules (Plate 13-18). Rarely, a few cells will contain Auer rods. The percentage of mature monocytes is often much higher in the blood than in the bone marrow. Monocytes with dysplastic features are present both in blood and in marrow, which can be difficult to distinguish from blast cells. Dysplastic features involving the other lineages can also be seen and have an adverse prognostic effect. Although karyotypic abnormalities involving chromosome 11, t(11)(q23) have been described,⁷⁸ they are not specific to AMoL.

ACUTE ERYTHROLEUKEMIA (M6)

Erythroleukemia accounts for 4 to 5 percent of de novo and 10 to 20 percent of therapy-induced AML (t-AML). In 1976, the FAB Cooperative Group included erythroleukemia within the classification system of AML as M6 and agreed on a quantitative standard to be used in the diagnosis. The standards were revised in 1985 to the form in use today.³⁷ The proposed criteria for the diagnosis of M6 are different from those used for the other types of AML. A minimum of 30 percent blasts among the nonerythroid cells (NECs) and more than 50 percent erythroid precursors with dysplastic features are necessary for the diagnosis. Howell-Jolly bodies and ringed sideroblasts are present. The erythroid proliferation is often megaloblastoid. PAS stain can demonstrate diffuse or blue staining of the cytoplasm (Plate 13-19). M6 must be distinguished from refractory anemia with excess blasts (RAEB) or refractory anemia with excess blasts in transformation (RAEB-t). This may be difficult when most marrow cells are erythroid precursors. In such instances one can arbitrarily increase the percentage of leukoblasts by dividing the percentage of absolute leukoblasts by the percentage of the nonerythroid marrow, thus fulfilling the criteria of 30 percent to establish a diagnosis of AML.

M6 is a clonal disorder arising from a multipotential stem cell that has broad myeloid potential.⁷⁹ Colony formation studies suggested that the leukemic blast progenitors in M6 originated at the stage of bipotential hematopoietic precursors capable of differentiating into myeloid and erythroid lineages.⁸⁰ In some rare cases, a pure proliferation of erythroid precursors has

been identified. In these cases, the diagnosis was established by using immunologic markers such as carbonic anhydrase I (CAI) and CD36 mAb, which detect an earlier antigen than glyophorin A in erythroid lineage.⁸¹ Patients with erythroleukemia present with pancytopenia, peripheral blood nucleated red blood cells, dysmorphic erythrocytes, and no, or few, peripheral blood blast cells. Cytogenetic and clinical characteristics of 26 patients with erythroleukemia have been described by Olopade et al.⁸² Clonal chromosomal abnormalities were found in 77 percent of their patients. These workers defined two distinctive subgroups: one group tended to be older, had complex cytogenetic abnormalities especially of chromosomes 5 and 7, and shared biologic and clinical features of t-AML; the other group, with simple or no detectable cytogenetic abnormalities, had a more favorable prognosis when treated with intensive chemotherapy.

ACUTE MEGAKARYOBLASTIC LEUKEMIA (M7)

Acute megakaryoblastic leukemia (AMkL) is a rare form of AML. It accounts for 3 to 5 percent of all instances of leukemia,⁸³ but it is not so rare in children younger than 3 years especially those with Down syndrome.⁸⁴ Thus, AMkL has two peaks of distribution: one in adults accompanied by myelofibrosis, and the other in young children aged 1 to 2 years. AMkL in children is often accompanied by leukocytosis and organomegaly, which are not observed in adults.⁸⁵ Although AMkL is believed to be resistant to treatment and associated with poor prognosis, several reports in children indicate that the disease responds well to treatment.^{85,86} Thus, the features of AMkL differ between children and adults, suggesting that there may be several variants of the disease.⁸⁷ Moreover, by electron microscopic analysis, leukemic megakaryoblasts differed between children with M7 with and without Down syndrome, adults with M7, and patients with CGL in megakaryoblastic blast crisis.⁸⁸

In contrast to other types of AML, bone marrow biopsy is invaluable in helping establish the diagnosis of AMkL.⁸³ The reason is that in only a few cases does the bone marrow aspirate show more than 30 percent of blast cells. By contrast, bone marrow biopsy may show many clusters of micromegakaryoblasts, as well as more mature megakaryoblasts (Plate 13-20). This is associated with an increase in reticulum formation and a corresponding decrease in normal hematopoietic precursors. In the peripheral blood, megakaryocyte fragments with changes in red cell morphology and circulating small blast cells resembling either type I blasts or L2 cells can be found. The morphology of the blast cells reveals cells that are very pleomorphic that may vary from very small with dense nuclear chromatin to somewhat larger with a fine reticulated nuclear chromatin and one to three prominent nucleoli. Cytoplasmic blebs or actual platelet shedding may be found surrounding some blasts. Standard cytochemistry can

be helpful but is not specific. The blasts are MPO or SBB negative, and the ANAE reaction shows distinct localized positivity, compared with the more diffuse staining in monocytes. PAS and ACP reactions can be positive and show a localized pattern reaction. Thus, a diagnosis of AMkL must be confirmed by immunologic and electron microscopic cytochemistry studies. The PPO reaction by electron microscopic cytochemistry has been found to be more sensitive and to detect more immature cells than mAbs directed against platelet glycoprotein IIb/IIIa (CD41).⁸⁹ However, PPO is not specific to megakaryocytes and platelets, because the reaction is seen in immature erythroblasts⁹⁰ and mast cell precursors.⁹¹ By contrast, mAbs also react with hematopoietic stem cells.⁹² Thus, electron microscopic and immunologic techniques must be combined to diagnose AMkL to exclude cells other than megakaryoblasts. However, CD41 positivity is taken as sufficient to confirm the diagnosis of M7 when associated with other distinctive features, such as myelofibrosis.

Marrow fibrosis is one of the characteristics of AMkL.⁹³ Abnormal platelets are postulated to release platelet-derived growth factor (PDGF) from α -granules, in turn stimulating the bone marrow fibroblasts to produce reticulin, and producing fibrosis in the marrow cavity. The *c-SIS* gene encodes a chain of PDGF. The presence of *c-SIS* mRNA has been demonstrated in M7 cells.

Cytogenetic studies are not performed easily in AMkL cases. However, a new chromosomal abnormality was recently described with M7 in infants, an association that occurs with high frequency $t(1;22)$.⁹⁴ AMkL has occurred either as a *de novo* form of acute leukemia in all age groups or as a part of the leukemic transformation in blast crisis of CGL or secondary acute leukemia. In these cases, often a mixed population of blast cells is present.⁹⁵ A prominent megakaryoblastic component is considered when more than 20 percent of cells express the CD41 antigen.

ACUTE UNDIFFERENTIATED LEUKEMIA

In general, the diagnosis of acute leukemia is based on morphology and standard cytochemistry. In most cases, immunologic marker studies support the diagnosis. However, a low percentage (about 1 percent) of cases remain difficult to interpret. In these cases, morphology is undifferentiated with inconclusive or totally negative cytochemical stainings. It is now possible to describe new entities, such as FAB M0, FAB M7, and hybrid leukemia, using numerous mAbs and double or triple fluorescence analysis by combined flow cytometry. Moreover, electron microscopy aids in further classification of AML-M0 and hybrid leukemias.^{42,43}

REPRODUCIBILITY OF CLASSIFICATION

The FAB classification is based on morphologically defined criteria and standardized cytochemical reactions. Its first task, trying to establish objective criteria

for separation of AML from ALL, was successfully met as the concordance between observers was subsequently 95 percent or higher.⁹⁶ However, the reproducibility of the FAB parameters for the subdivision of AML into subtypes was within the range of 60 to 70 percent.⁹⁷ Most interobserver discrepancies were the result of failure to separate M1 from M2, M2 from M4, and M4 from M5b. Applying strict criteria with appropriate cytochemistry can help eliminate these difficulties. A recent study, designed to avoid these difficulties, has given excellent results; the concordance was 85 percent.^{98,99}

Certain types of acute leukemia, such as acute undifferentiated leukemia, AML-M0, and cases of mixed-lineage leukemias, are not described in the FAB classification. In the diagnosis of such cases, immunocytochemical and/or immunologic methods are absolutely necessary.

Finally, morphology remains the primary technique for establishing the diagnosis of AML and ALL and will continue to play a major role in classifying the main groups of acute leukemia. However, for the subclassification of acute leukemia, the interpretation of morphologic and cytochemical data should be incorporated with complementary methods, such as immunophenotyping, cytogenetics, and molecular biology.

RARE FORMS OF ACUTE MYELOCYTIC LEUKEMIA

Acute Myelocytic Leukemia With Trilineage Myelodysplasia

Ten to 15 percent of cases of primary AML show marked myelodysplastic features involving erythroid, granulocytic, and megakaryocytic lineages. This finding has suggested that some *de novo* AML cases could be derived from undiagnosed MDS.^{100,101} Acute myelocytic leukemia with trilineage myelodysplasia (AML/TMDS) could be seen in all FAB types of AML except M3; it is more common in M6 and M7 and rare in M1. In a number of these patients, a unique type of relapse with MDS, and without excess of blasts, has been documented.¹⁰² Moreover, the response rate of AML/TMDS is lower than in AML without these features.¹⁰⁰

In another study,¹⁰³ which focused only on dysmegakaryopoiesis (DysM), 12 percent of 95 patients with AML had DysM, with 75 percent occurring in FAB M4. Again, the CR rate was lower in cases with DysM (14 percent) than in those without DysM (82 percent). However, in a study by Goasguen et al.,¹⁰⁴ when the effects of DysM, dysgranulopoiesis (DysG), and dyserythropoiesis (DysE), alone or in combination, were assessed, only DysG was found to have an adverse effect on CR rate. The CR rate for patients with DysG was 57 percent, compared to 71 percent for patients without DysG.

Chromosome information is not available in most of these retrospective series. However, it is noteworthy that the remission rate is quite low and parallels the remission rate expected from AMLs that present with

karyotypic abnormalities characteristic of MDS (i.e., -5, 5q-, -7, 7q-).³¹

Hypocellular (Hypoplastic) AML

Hypocellular AML is a diagnostic dilemma. It does not fit the FAB classification of acute leukemia and often is mistaken for myelodysplasia or even aplastic anemia. Several investigators have reported on groups of such patients and have called them "hypoplastic" or "hypocellular" AML.¹⁰⁵⁻¹⁰⁷ In general, these patients are elderly, most having longstanding hematologic complaints before diagnosis and lacking physical findings. Most of them are leukopenic or pancytopenic and have few blasts in the peripheral blood. The bone marrow is hypocellular, with more than 30 percent poorly differentiated type I blasts. The incidence of hypocellular AML is reported to be 8 to 14 percent. According to most investigators, a case of acute leukemia is considered hypocellular when the bone marrow exhibits cellularity of less than 40 percent or 50 percent. However, since most patients are elderly and have a low cellularity because of age, the cutoff value of bone marrow cellularity is controversial. In a recent study conducted at our laboratory,¹⁰⁸ bone marrow cellularity of 40 percent would seem to present the upper limit to include such cases into hypocellular AML for clinical comparisons. In this study, all cases were classifiable with the FAB criteria. FAB classification demonstrated preponderance of the M1 category, followed by M2 and M6 types. Most blasts are type I, and the median MPO positivity was 14 percent. Immunophenotyping of bone marrow cells showed expression of myeloid antigens (CD13, CD33). Significant dysplasia involving erythroid and megakaryocytic lineages were seen in most of the cases. Trilineage dysplasia was observed in 36 percent of cases. Most of these patients underwent aggressive induction chemotherapy. Median survival for the entire group was 1.5 months. The CR rate was 72.6 percent, and remission duration was 14.5 months. Higher frequencies of trilineage dysplasia and M6 type together with low percentage of peripheral blasts and presence of antecedent hematologic disorders suggest that some of these cases might represent the hypocellular form of AML with trilineage dysplasia.

Granulocytic Sarcoma

Granulocytic sarcomas are solid myelogenous tumors that can occur in 3 to 7 percent of AML,^{109,110} most frequently observed in children. These tumors either appear green or may become green in diluted acid because of their high MPC content (chloroma). Granulocytic sarcomas can be localized in a number of locations, including the skin, breast, gastrointestinal tract, ovaries, and brain. However, most of these tumors arise adjacent to bony or neural structures. Granulocytic sarcomas can be recognized in three types of clinical setting³: (1) *de novo* presentation without generalized marrow involvement; (2) in association with a recognized CGL; and (3) in an established diagnosis of

AML, either at presentation or as the first manifestation of relapse.

The histologic diagnosis remains very difficult and demands careful examination of tissue sections that are cut thinly. The morphologic features are highly variable, ranging from little to no differentiation, where the differential diagnosis includes large cell lymphomas (Plate 13-21). When the suspected tumor has been excised, imprint preparations should be performed. The presence of azurophilic granules or Auer rods confirms the diagnosis. In the presence of more immature cells, the demonstration of MPO activity or immunocytochemical detection of myeloid antigen is necessary to show a myeloid origin. A newly described polyclonal antibody for MPO is also available that will strongly stain myeloblasts and immature myeloid cells in formalin, B5, or Zenker-fixed paraffin-embedded tissues.¹¹¹ A more traditional stain is the specific granulocyte esterase reaction, naphthol ASD chloroacetate esterase stain on air-dried touch preparations or non-Zenker-fixed sections (Plate 13-22).

Although karyotype analyses were not reported in many cases of granulocytic sarcoma in the literature, the vast majority of abnormal karyotypes involved either t(8;21) or t(9;22). Recently, Tallman et al.¹¹² reviewed a large series of patients with AML and t(8;21) in whom granulocytic sarcomas had developed. Analysis of these cases demonstrated that granulocytic sarcomas occur in approximately 18 percent of this population. These patients may have a less favorable prognosis than other patients with t(8;21).

ACUTE MAST CELL LEUKEMIA

Mast cell leukemia is a rare and rapidly fatal disorder. It is not included in the FAB classification. Mast cell leukemia develops in approximately 15 percent of patients with systemic malignant mastocytosis.¹¹³ The definition of mast cell leukemia is accepted when more than 30 percent of mast cells are circulating in the blood. Most cases are those of tissue well-differentiated mast cells with dense uniform basophilic granules that may obscure the nucleus. Both heparin and histamine are contained within the granules, but the major basic protein is not present. MPO is negative, and toluidine blue stain demonstrates metachromatic granules. The naphthol ASD chloroacetate esterase and PAS reactions can be positive as well. A less differentiated form has been described and can be separated from other acute leukemias by cytochemical methods.¹¹⁴ One problem that can occur is the confusion between acute mast cell leukemia and the blast crisis of CGL. The cells may be confused with abnormal promyelocytes because of the basophilic granules, but the cytoplasm is more elongated, and the granules tend to cluster about the nucleus (Plate 13-23). However, the distinction can be resolved by cytochemistry.

Mast cell leukemia should be distinguished from cases of AML that arise against a background of a systemic mast cell disease. In a study by Travis et al.,¹¹⁵

22 of 66 patients with systemic mast cell disease were found to have other hematologic disorders.

CENTRAL NERVOUS SYSTEM LEUKEMIA

Without CNS prophylaxis against CNS leukemia in ALL, isolated CNS relapse occurs in 50 percent of patients and is associated with subsequent systemic relapse. Therefore, CNS prophylaxis is routinely used in ALL. With appropriate CNS treatment, the incidence of CNS leukemia has been reduced to less than 10 percent. However, approximately 5 percent of patients with ALL present with an initial diagnosis of CNS leukemia.¹¹⁶ The incidence of CNS leukemia is lower in AML, and CNS prophylaxis is not usually used. Analysis of two ECOG trials in AML¹¹⁷ showed that the incidence of CNS leukemia was 5 percent (30 of 569 patients) overall and 3 percent (10 of 331) in patients in CR. Male gender and white blood cell (WBC) counts higher than 44,000/mm³ were found to be associated increased risk of CNS leukemia. By FAB type, the highest frequency is with FAB M5 (10 percent of cases). It should be remembered that CNS leukemia occurs frequently (35 percent) in patients with inversion or deletion of chromosome 16, which is present in M4Eo.

The most likely explanation for CNS leukemia is direct extension from bone marrow in the skull, extending along the dura mater. Additional venules provide a direct route for leukemic infiltration of the brain parenchyma.¹¹⁸

Diagnostic lumbar puncture is recommended for all patients with ALL and for patients with AML who have WBC counts higher than 40,000/mm³.¹¹⁷ Careful cytologic examination of CNS fluid is essential to establish a diagnosis of involvement.

A cerebrospinal fluid (CSF) leukocyte count greater than 5 leukocytes/ml with the presence of unequivocal blasts on cytocentrifuge preparation is accepted as minimum cytologic criteria for CNS leukemia.¹¹⁹ The two most common techniques include a cytocentrifuge preparation and a Wright-Giemsa stain or Millipore filtration technique and the Papanicolaou method. It is important to distinguish atypical but reactive cells secondary to drug-related arachnoiditis from leukemia blasts.

PERIPHERAL ACUTE LEUKEMIA

According to current FAB criteria, the presence of less than 30 percent blasts in the bone marrow aspirate is not considered diagnostic of acute leukemia. Recently, Cason et al.¹²⁰ reported on five patients who had more than 30 percent peripheral blasts and less than 30 percent marrow blasts. Four of these five patients progressed to acute leukemia within 1.5 months of the development of more than 30 percent peripheral blasts. Two of these four patients had evidence of acute leukemia by criteria other than bone marrow involvement

at presentation. One of these patients had evidence of multifocal dermal involvement; and the other had a cytogenetic abnormality, t(8;21). In the fifth patient, acute leukemia developed 2 years after initial presentation with more than 30 percent peripheral blasts. After this report, the National Cancer Institute (NCI)-sponsored workshop³⁹ recommended diagnosing patients who present with less than 30 percent bone marrow blasts and more than 30 percent peripheral blasts as having acute leukemia.

EVALUATION OF REMISSION

In a patient treated for acute leukemia, CR is considered when peripheral blood cell counts approach the normal range, the bone marrow is normocellular and shows orderly maturation of hematopoietic cells with less than 5 percent blast cells. The criteria for remission of less than 5 percent bone marrow blasts is arbitrary. The leukemic blasts in AML M3, ALL L3, or blasts containing Auer rods are quite distinctive and can be readily identified, even when present in small numbers. In other types of leukemia, a definitive diagnosis of residual leukemia may not be possible by morphologic examination. Persistence of dysplastic maturing granulocytes, erythrocytes, and megakaryocytes in a patient who might otherwise fulfill the criteria for CR results in a much higher relapse rate. It should be remembered that the bone marrow regeneration occurs more quickly in pediatric patients (3 or 4 weeks). Therefore, one can observe a modest increase in myeloblasts and promyelocytes during recovery that may persist for 1 to 2 weeks. This should not be overinterpreted as a regrowth of leukemic cells.

The definition of partial remission is accepted when less than 25 percent leukemic cells are counted in the bone marrow smears. Relapse is defined by the presence of more than 25 percent leukemic cells in the bone marrow aspirate and is usually associated with peripheral cytopenias and sometimes the presence of blasts. In the case of relapse, the leukemic cells often lose certain antigens, becoming more undifferentiated cells. Detection of the p-170 antigen related to MDR phenotype is important in these situations.

MYELOYDYSPLASTIC SYNDROME

MDS is a clonal proliferation of multipotential hematopoietic stem cells. Clinically, MDS represents a condition of bone marrow failure, usually of the elderly (primary MDS), or of patients previously exposed to prior chemotherapy or radiation, or both (secondary MDS). The correlation of the biology of this clonal disorder with its clinical presentation of cytopenias is varied, ranging from an incidental mild anemia stable for years to a rapidly evolving leukemia.

Prior to widespread use of the term MDS such terms as preleukemic acute leukemia, smoldering acute leukemia, refractory anemia, RAEB, and sideroblastic ane-

mia were used. The term *preleukemia* was used to suggest the high risk of leukemic transformation. Yet, it is well known that only 20 to 30 percent of MDS evolves into acute leukemia.¹²¹

Clonality of MDS was first suspected by Dacie et al.¹²² and was verified by glucose 6-phosphate-dehydrogenase (G6PD) isoenzyme studies.¹²³ Furthermore, several studies using X-linked restriction fragment length polymorphism (RFLP)^{124,125} demonstrated that MDS arises from a multipotential hematopoietic stem cell with the potential for both myeloid and lymphoid differentiation. Tsukamoto et al.¹²⁶ recently confirmed these results by explaining the coexistence of lymphoid or plasmacytic neoplasms with MDS. However, by using fluorescence in situ hybridization (FISH), it could be demonstrated that the initial trisomy 8 observed at diagnosis was present in peripheral granulocytes, monocytes, eosinophils, and basophils, but never in the lymphocytes of three analyzed MDS patients.¹²⁷

By definition, MDS is characterized by hypercellular marrow with trilineage dysplasia. Recently, the bone marrow cellularity at diagnosis was assessed in different stem cell disorders by our group.¹²⁸ The estimation of bone marrow cellularity for each group was used both with and without age adjustment, based on normal marrow biopsies. Without correcting the marrow cellularity for age, it was observed that median marrow cellularity was estimated at 40 percent for MDS. The age-corrected data disclosed that all patients with chronic myeloproliferative disorders, 63 percent of patients with AML, and only 35 percent of patients with MDS had hypercellular bone marrow according to their age.

The diagnosis of MDS requires minimal descriptions described by the FAB Cooperative Group in 1976.⁷ The original proposal was revised in 1982¹²⁹ and in 1985.³⁷ Five subgroups are defined by the FAB Cooperative Group, based on the recognition of dysplastic features common to all groups and other criteria that distinguish them. Secondary MDS and MDS in young patients and children are diagnosed using the same morphologic criteria.

Morphologic Characteristics

The following discussion highlights the morphologic features used to define the MDS.¹³⁰⁻¹³³ In general, these features should be present in at least 10 percent or greater of cells of the respective lineage under consideration.

Dyserythropoiesis

Morphologic bone marrow DysE may include the presence of ringed sideroblasts, multinuclear fragments, bizarre nuclear shapes, internuclear bridging, mitosis, abnormal intensity of the chromatin or fine chromatin with asynchronous cytoplasm, and abnormal cytoplasmic features (intense basophilia, Howell-Jolly bodies, ghosted cytoplasm). Marked macrocytosis (at least 100 fmoles/L), basophilic stippling, anisocytosis, and

poikilocytosis may be observed in the peripheral blood smear. Quantitative changes include the presence of a significant percentage of ringed sideroblasts (exceeding 15 percent of all nucleated erythroid cells), and a number of megaloblastic erythroid precursors (5 to 50 percent).

Normal marrow can have occasional erythroblasts, typically with fewer than five granules. Pathologic sideroblasts have five or more granules and are termed "ringed sideroblasts" if the granules cover more than one-third of the nuclear rim (Plate 13-24). Ringed sideroblasts and increase iron stores can be found in any of the MDS. But they are characteristic of refractory anemia with ringed sideroblasts (RARS). With appropriate nuclear counter stains (neutral red), the lower limit of ringed sideroblasts was chosen at 15 percent to include such cases in RARS.

Dysgranulopoiesis

In dysgranulopoiesis (DysG), the peripheral blood can be notable for hypogranulation and hyposegmentation of the polymorphonuclear leukocytes (PMLs) with chromatin condensation (Pelger-Huët-like abnormality). Hypogranulation is most commonly noted and can be associated with a negative MPO reaction. The cells can be devoid of all granules, often in the more immature forms. Nuclear sticks can be seen, particularly in cases of secondary MDS or therapy-related MDS. These morphologic features may explain in part the frequency of infection in these patients. Moreover, the function of neutrophils (i.e., phagocytic adhesion, chemotaxis, and microbicidal capacities) may be impaired, but no correlation has been found between the loss of granules and impaired function.^{14,33}

Dysmegakaryopoiesis

Qualitative findings are more common in dysmegakaryopoiesis (DysM) because the number of megakaryocytes is usually normal, although hypoplasia or hyperplasia can occasionally be seen. In the peripheral blood, large hypogranular or hypergranular platelets can be found. In the bone marrow, morphologic abnormalities of the megakaryocytic precursors can be seen in half of the patients. Commonly, there are micromegakaryocytes (dwarf forms) (Plate 13-25), megakaryocytes with multiple small round, and separate nuclei and large mononuclear forms. Micromegakaryocytes can be further recognized by using CD41 and antifactor VIII antibodies on bone marrow smears with the APAAP technique. Small mononuclear megakaryocytes with single eccentric nucleus and many granulations are strongly correlated with 5q- syndrome.¹³⁴

Dysmonocytopoiesis

Identification of the monocyte precursors in the bone marrow is sometimes difficult, and the use of double esterase staining may be necessary. CD14 mAbs can also be used to identify the monocytic population.

Blast Cell Characteristics

One of the most important criteria for MDS subclassification is the number of blast cells. An area of confusion has been the definition of blasts in MDS. The FAB group had established the definition of blast cells distinguishing blast type I from type II, based on the presence or absence of primary azurophilic granules in the cytoplasm (Plate 13-25). Type II blasts were defined as containing a few or moderate number of primary granules or an Auer rod. However, some "blasts" have more than 20 granules without a Golgi zone and have been considered as an abnormal promyelocyte. Inclusion of these cells in the blast count (e.g., as type III blasts) seems reasonable based on the paper by Goasguen and co-workers.³⁸

Bone Marrow Histology

MDS is characterized by architectural disorganization. Therefore, a bone marrow biopsy is required for evaluation. Bone marrow histology provides useful information on cellularity, relative proportions of three hematopoietic cell lines, architectural disorganization and increase in the reticulin fibers. Architectural disorganization affects the spatial localization of the hematopoietic cells so that erythroblasts and megakaryoblasts are found at the trabecular surface. Moreover, small clusters of blast cells may be identified in central marrow regions referred to as abnormal localization of immature precursors (ALIP). Tricot et al.¹³⁵ showed that patients presenting with ALIP experienced early leukemic transformation and short survival, irrespective of FAB type. After this, the prognostic significance of ALIP was confirmed by several studies.¹³⁶⁻¹³⁸ However, it is difficult to distinguish ALIP from immature erythroid and megakaryocytic aggregates (pseudo-ALIP) on H&E or Giemsa-stained sections. Mangi and Muftic¹³⁹ recommended the use of immunocytochemistry to confirm both the presence and lineage of these abnormally localized immature cells.

Classification

Refractory Anemia

Refractory anemia (RA) is anemia (hemoglobin below 11 g/dl with a low reticulocyte count) as the primary symptom with variable dyserythropoiesis alone or accompanied by dysgranulo- and megakaryopoiesis (Table 13-5). Granulocytopenia or thrombocytopenia, or both, may be minimal or absent. Blast cells usually are not present in the peripheral blood (less than 1 per-

cent) and fewer than 5 percent in the bone marrow. Ringed sideroblasts should be less than 15 percent. This subtype represents 25 to 30 percent of the MDS group, with a median survival of 40 to 50 months and a 10 percent rate of progression to AML.¹²¹

Refractory Anemia With Ringed Sideroblasts

The morphologic features of RARS are similar to those of RA, except that there are more than 15 percent ringed sideroblasts in the bone marrow. Dysmorphic red cells and macrocytosis are present in the peripheral blood (Plate 13-27). Cases with more than 15 percent ringed sideroblasts and more than 5 percent marrow blasts should be considered RAEB. Dysgranulopoiesis and dysmegakaryopoiesis are absent in pure RARS. In the dysplastic form of sideroblastic anemia, iron overload is associated with marrow dysplasia; those cases are associated with leukemic progression and shorter overall survival.

Refractory Anemia With Excess of Blasts

Conspicuous changes in all three lineages are present, associated with variable cytopenia affecting two or more of the hematopoietic lines. Dysgranulopoiesis is more pronounced than in the preceding groups. Occasional blasts may be present in the peripheral blood but are always less than 5 percent. Blasts (type I + II + III) range from greater than 5 percent to less than 20 percent in the bone marrow (Plate 13-27). The presence of ALIP in the bone marrow biopsy has been reported to be an adverse prognostic factor. RAEB accounts for up to 20 percent of MDS, with a median survival of 7 to 17 months and a 28 to 44 percent likelihood of leukemic transformation.^{121,140}

Chronic Myelomonocytic Leukemia

The criteria for the diagnosis of chronic myelomonocytic leukemia (CMML) is the presence of peripheral absolute monocytosis higher than $1 \times 10^9/L$ associated with a marrow proliferation of monocytes. For most cases, the peripheral blood and bone marrow smears of patients fulfil all of the classical criteria for MDS (variable degrees of trilineage dysplasia) and show the identical chromosome abnormalities (Plate 13-28). However, the inclusion of CMML into MDS was long debated. One point of criticism is a very wide survival range of these patients (11 to greater than 60 months). However, the percentage of bone marrow blasts and peripheral blood monocytosis stratifies the prognosis of patients with CMML.¹⁴¹ It can be helpful to distinguish between (1) CMML with less than 5 percent marrow blasts with few or no dysplastic features; (2) CMML associated with 5 to 20 percent marrow blasts and clear dysplastic features; and (3) CMML with 20 to 30 percent marrow blasts (CMML in transformation). A second point of criticism is the failure of this classification to make a clear distinction between CMML and the

Table 13-5. Myelodysplastic Syndrome

Refractory anemia (RA)
Refractory anemia with sideroblasts (RA-S)
Refractory anemia with excess of blasts (RAEB)
Chronic myelomonocytic leukemia (CMML)
Refractory anemia with excess of blasts in transformation (RAEB-T)

chronic myeloproliferative syndromes, particularly from Philadelphia-negative CML. In a study of the Groupe Français de Cytogénétique Hématologique,¹⁴² rearrangements within the major breakpoint cluster region (m-bcr) were analyzed in 35 Philadelphia-negative CML, 55 chronic phase CML, and 100 CMML patients. Philadelphia-negative, bcr-positive patients have the identical clinical disease as Philadelphia-positive, bcr-positive cases. By contrast, bcr-negative patients resemble CMML patients in terms of frequency and type of cytogenetic abnormalities and myelodysplastic features. This group proposed that bcr-negative CML can probably be termed "atypical CML" (aCML). Recently, the FAB Cooperative Group¹⁴³ analyzed a series of cases that included Philadelphia-positive/bcr-positive and Philadelphia-negative/bcr-positive CGL, CMML, and cases that were Philadelphia-negative/bcr-negative but that resembled CML (aCML). They have developed a statistical model confirming that CML, aCML, and CMML can be distinguished from each other by employing five quantitative parameters (WBC count, percentage immature granulocytes, percentage monocytes and percentage basophils in peripheral blood, and percentage erythroid precursors in bone marrow) and one qualitative one (granulocytic dysplasia). The application of these FAB proposals should enable investigators to confirm the uniqueness of aCML or whether it is a quantitative and/or qualitative variant of CMML.

Refractory Anemia With Excess Blasts in Transformation

Diagnosis of refractory anemia with excess blasts in transformation (RAEB-t) is based on a percentage of bone marrow blasts (type I + II) of 21 to 30 percent. The presence of type III blasts can shift the diagnosis to a worse subtype.³⁸ These blasts show the same morphologic abnormalities seen in RAEB. Cases with more than 5 percent of blasts in the peripheral blood and patients with less than 20 percent bone marrow blasts, but having Auer rods in the blasts, are included in the RAEB-t group. The application of the proposals for RAEB-t have indicated a strikingly shorter median survival (5 months) than is observed in the other MDS subtypes with a high transformation to acute leukemia (60 percent). However, several groups of investigators suggested that patients under 50 years of age with or without Auer rods and features of RAEB-t respond well to standard AML chemotherapy. This observation is confirmed by our own experience.¹³³ Therefore, it is reasonable to modify the FAB proposals. Patients with RAEB-t under the age 50 years should be reclassified as RAEB-1/FAB M2 and should be offered combination chemotherapy.

Hypocellular MDS

The hypocellular form of MDS was not included in the proposals for the classification of MDS by the FAB Cooperative Group. However, several reports have dealt with hypocellular forms of MDS.¹⁴⁴⁻¹⁴⁶ Bone marrow

cellularity is the critical determinant to recognize hypocellular MDS. According to most investigators, a case of MDS is considered hypocellular when the bone marrow exhibits cellularity of less than 30 percent or less than 40 percent. According to our experience,¹⁰⁸ hypocellularity is a more common feature of MDS than AML, even after age adjustment based on normal biopsy. To recognize the clinical and morphologic features of these patients, we would consider bone marrow cellularity of 30 percent the upper limit, to include such cases in hypocellular MDS.

Hypocellular MDS accounts for less than 10 to 15 percent of all cases of MDS. As a result of hypocellularity, specimens from these patients can be diagnostically challenging, because exclusion of aplastic anemia or hypocellular AML may be difficult. The diagnostic criteria for hypocellular MDS must be met for FAB criteria. Most hypocellular MDS cases fall into the categories of RA and RAEB. Although the prognostic significance of hypocellular MDS has been addressed in a number of studies, consensus has not been reached on this issue. Some investigators have reported that patients with hypocellular MDS transform to AML less frequently and enjoy a longer survival than do normocellular or hypercellular MDS cases.¹⁴⁴ Other reports state that no statistically significant difference occurs between the two groups with regard to leukemic transformation and survival.¹⁴⁵ Our experience¹⁴⁷ confirms the latter.

Myelodysplastic Syndromes in Children

MDS has been described in young adults and in children.^{148,149} The frequency of these cases may account for 1 to 3 percent of all childhood malignancies, but it appears to be increasing with the description of t-MDS in children. MDS can be diagnosed in children using the same criteria used in adults. Although some investigators concluded that children with MDS might benefit from more aggressive therapy, in general the survival rate was poor and transformation to AML is more frequent than in adults.

Therapy-Related MDS and AML

AML, most often presenting as MDS, has become one of the most feared long-term complications of cancer therapy. The risk of therapy-related t-MDS and t-AML, the clinical features of the disease, the characteristic chromosomal aberrations, and the poor results of intensive antileukemic chemotherapy are known.¹⁵⁰⁻¹⁵² In contrast to primary MDS, most cases of t-MDS are difficult to classify according to FAB criteria. At diagnosis, the marrow may be of variable cellularity. A significant minority of cases (25 to 30 percent) demonstrate hypocellularity, and an additional 25 to 50 percent show significantly increased fibrosis. Consequently, the aspirated marrow cells may not be representative of the malignant process. It is in this situation that the bone marrow biopsy becomes of extreme importance in establishing a diagnosis.¹⁵³ Most cases diagnosed as AML

or progressing to AML from MDS have FAB types M1, M2, and M4 (Table 13-4). However, classification of t-AML according to FAB criteria can be difficult, as most of these leukemias demonstrate trilineage involvement and appear to bridge several subtypes. Moreover, the presence of Auer rods and MPO or of nonspecific esterase positivity is uncommon among t-AML cases compared to de novo AML. Clonal chromosomal abnormalities are found in 76 to 97% of cases of t-MDS and t-AML. Losses of part of or all chromosomes 5 and 7 have been considered the characteristic findings. Recently, Pedersen-Bjergaard et al.¹⁵⁴ have reviewed their 115 consecutive patients with t-MDS or t-AML over the past 16 years. These investigators have reported that in patients treated primarily with alkylating agents, the risk of t-MDS and t-AML increased by 1 percent per year from 2 to at least 8 years after initiation of treatment. In most of these cases, the disease presented as t-MDS with loss of a whole chromosome 5 or 7, or various parts of their long arms, and the leukemias were of FAB subtypes M1, M2, or M4. By contrast, in patients treated with drugs targeting DNA-topoisomerase II combined with drugs reacting directly with DNA, the risk of leukemia increased much more steeply from only 1 year after start of therapy. These cases often presented with overt leukemia of FAB subtypes M4 or M5 with balanced translocations to chromosome bands 11q23 and 21q22. These latter cases resemble de novo AML and are much more likely to achieve remission with standard chemotherapy.

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208 *Neoplastic Diseases of the Blood*

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