LEUKAEMIA DIAGNOSIS

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a Blackwell Publishing company
Blackwell Science, Inc., 350 Main Street, Malden, Massachusetts 02148-5018, USA
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Blackwell Science Asia Pty Ltd, 550 Swanston Street, Carlton South Victoria 3053, Australia
Blackwell Wissenschafts Verlag, Kurfürstendamm 57, 10707 Berlin, Germany

First published 1990
(Published by Gower Medical Publishing)
Reprinted 1993 (by Wolfe Publishing)
Second edition 1999
Third edition 2003

Library of Congress Cataloging-in-Publication Data
Bain, Barbara J.
Leukaemia diagnosis/Barbara J. Bain.—3rd ed.
p. ; cm.
Includes bibliographical references and index.
ISBN 1-4051-0661-1 (hardback : alk. paper)
1. Leukemia—Diagnosis.
[DNLM: 1. Leukemia—classification. 2. Leukemia—diagnosis. WH 15 B162L 2003]
I. Title.
RC643 .B35 2003
616.99'419075—dc21
2002155879

ISBN 1-4051-0661-1

A catalogue record for this title is available from the British Library

Set in 9/11½pt Meridien by Graphicraft Limited, Hong Kong
Printed and bound in India by Thomson Press (India)

Commissioning Editor: Maria Khan
Managing Editor: Elizabeth Callaghan
Production Editor: Rebecca Huxley
Production Controller: Kate Charman

For further information on Blackwell Publishing, visit our website:
http://www.blackwellpublishing.com

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PREFACE

Leukaemias are a very heterogeneous group of diseases, which differ from each other in aetiology, pathogenesis, prognosis and responsiveness to treatment. Accurate diagnosis and classification are necessary for the identification of specific biological entities and underpin scientific advances in this field. The detailed characterization of haematological neoplasms is also essential for the optimal management of individual patients. Many systems for the classification of leukaemia have been proposed. Between 1976 and 1999, a collaborative group of French, American and British haematologists (the FAB group) proposed a number of classifications, which became widely accepted throughout the world. In the case of the acute leukaemias and the related myelodysplastic syndromes, the FAB classifications also provided the morphological basis for more complex classifications such as the morphologic–immunologic–cytogenetic (MIC) classification and the MIC-M classification, which also incorporates molecular genetic analysis. A quarter of a century after the first FAB proposals, a WHO expert group proposed a further system for the classification of leukaemia and lymphoma incorporating aetiology, cytology and the results of cytogenetic analysis. In this book I have sought to illustrate and explain how laboratory techniques are used for the diagnosis and classification of leukaemia.

I have sought to discuss leukaemia diagnosis and classification in a way that will be helpful to trainee haematologists and to laboratory scientists in haematology and related disciplines. However, I have also tried to provide a useful reference source and teaching aid for those who already have expertise in this field. In addition, I hope that cytogeneticists and molecular geneticists will find that this book enhances their understanding of the relationship of their discipline to the diagnosis, classification and monitoring of leukaemia and related disorders.

Acknowledgements. I should like to express my gratitude to various members of the FAB group for their useful advice. In particular I should I like to thank Professor David Galton and Professor Daniel Catovsky, who have given me a great deal of help, but at the same time have left me free to express my own opinions. Professor Galton read the entire manuscript of the first edition and, by debating many difficult points with me, gave me the benefit of his many years of experience. Professor Catovsky also discussed problem areas and kindly permitted me to photograph blood and bone marrow films from many of his patients. My thanks are also due to many others who helped by lending material for photography, including members of the United Kingdom Cancer Cytogenetics Study Group.

Barbara J. Bain 2002

ABBREVIATIONS

AA	all metaphases abnormal (description of a karyotype)
aCML	atypical chronic myeloid leukaemia (a category in WHO classifications)
AL	acute leukaemia
ALIP	abnormal localization of immature precursors
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
AN	a mixture of normal and abnormal metaphases (description of a karyotype)
ANAE	α-naphthyl acetate esterase (a cytochemical stain)
ANBE	α-naphthyl butyrate esterase (a cytochemical stain)
ATLL	adult T-cell leukaemia/lymphoma
ATRA	all-*trans*-retinoic acid
BCSH	British Committee for Standards in Haematology
BFU-E	burst-forming unit—erythroid
BM	bone marrow
CAE	chloroacetate esterase (a cytochemical stain)
c	cytoplasmic or, in cytogenetic terminology, constitutional
CD	cluster of differentiation
CFU-E	colony-forming unit—erythroid
CFU-G	colony-forming unit—granulocyte
CFU-GM	colony-forming unit—granulocyte, macrophage
CFU-Mega	colony-forming unit—megakaryocyte
CGL	chronic granulocytic leukaemia
cIg	cytoplasmic immunoglobulin
CLL	chronic lymphocytic leukaemia
CLL/PL	chronic lymphocytic leukaemia, mixed cell type (with prolymphocytoid cells)
CML	chronic myeloid leukaemia
CMML	chronic myelomonocytic leukaemia (a category in the FAB and WHO classifications)
EGIL	European Group for the Immunological Characterization of Leukemias
ERFC	E-rosette-forming cells
FAB	French–American–British classification
FISH	fluorescent *in situ* hybridization
G-CSF	granulocyte colony-stimulating factor
H & E	haematoxylin and eosin (a stain)
Hb	haemoglobin concentration
HCL	hairy cell leukaemia
HLA-DR	histocompatibility antigens
Ig	immunoglobulin
JCML	juvenile chronic myeloid leukaemia
JMML	juvenile myelomonocytic leukaemia (a category in WHO classifications)
LGLL	large granular lymphocyte leukaemia
M0-M7	categories of acute myeloid leukaemia in the FAB classification
MAC	morphology–antibody–chromosomes (technique)
McAb	monoclonal antibody
MDS	myelodysplastic syndrome/s
MDS-U	myelodysplastic syndrome, unclassified (a category in WHO classifications)
MGG	May–Grünwald–Giemsa (a stain)
MIC	morphologic–immunologic–cytogenetic (classification)
MIC–M	morphological–immunological–cytogenetic–molecular genetic (classification)

MPO	myeloperoxidase
mRNA	messenger RNA
NAP	neutrophil alkaline phosphatase
NASA	naphthol AS acetate esterase (a cytochemical stain)
NASDA	naphthol AS-D acetate esterase (a cytochemical stain)
NCI	National Cancer Institute
NK	natural killer
NN	all metaphases normal (description of a karyotype)
NSE	non-specific esterase (a cytochemical stain)
PAS	periodic acid–Schiff (a cytochemical stain)
PB	peripheral blood
PcAb	polyclonal antibody
PCR	polymerase chain reaction
PLL	prolymphocytic leukaemia
PPO	platelet peroxidase
RA	refractory anaemia (a category in the FAB and WHO classifications)
RAEB	refractory anaemia with excess of blasts (a category in the FAB classification)
RAEB-1	refractory anaemia with excess of blasts, 1 (a category in the WHO classification)
RAEB-2	refractory anaemia with excess of blasts, 2 (a category in the WHO classification)
RAEB-T	refractory anaemia with excess of blasts in transformation (a category in the FAB classification)
RARS	refractory anaemia with ring sideroblasts (a category in the FAB and WHO classifications)
RCMD	refractory cytopenia with multilineage dysplasia (a category in the WHO classification)
RCMD-RS	refractory cytopenia with multilineage dysplasia and ring sideroblasts (a category in the WHO classification)
RQ-PCR	real time quantitative polymerase chain reaction
RT-PCR	reverse transcriptase PCR
SB	Southern blot
SBB	Sudan black B (a cytochemical stain)
SLVL	splenic lymphoma with villous lymphocytes
Sm	surface membrane (of a cell)
SmIg	surface membrane immunoglobulin
TCR	T-cell receptor
TdT	terminal deoxynucleotidyl transferase
TF	transcription factor
TRAP	tartrate-resistant acid phosphatase
WHO	World Health Organization

CHAPTER 1

ACUTE LEUKAEMIA
Cytology, Cytochemistry and the FAB Classification

The nature of leukaemia

Leukaemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which form a clone of leukaemic cells. Often there is a series of genetic alterations rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of tumour suppressor genes. The cell in which the leukaemic transformation occurs may be a lymphoid precursor, a myeloid precursor or a pluripotent stem cell capable of differentiating into both myeloid and lymphoid cells. Myeloid leukaemias can arise in a lineage-restricted cell or in a multipotent stem cell capable of differentiating into cells of erythroid, granulocytic, monocytic and megakaryocytic lineages.

Genetic alterations leading to leukaemic transformation often result from major alterations in the chromosomes of a cell, which can be detected by microscopic examination of cells in mitosis. Other changes are at a submicroscopic level but can be recognized by analysis of DNA or RNA.

Leukaemias are broadly divided into: (i) acute leukaemias, which, if untreated, lead to death in weeks or months; and (ii) chronic leukaemias, which, if untreated, lead to death in months or years. They are further divided into lymphoid, myeloid and biphenotypic leukaemias, the latter showing both lymphoid and myeloid differentiation. Acute leukaemias are characterized by a defect in maturation, leading to an imbalance between proliferation and maturation; since cells of the leukaemic clone continue to proliferate without maturing to end cells and dying there is continued expansion of the leukaemic clone and immature cells predominate. Chronic leukaemias are characterized by an expanded pool of proliferating cells that retain their capacity to differentiate to end cells.

The clinical manifestations of the leukaemias are due, directly or indirectly, to the proliferation of leukaemic cells and their infiltration into normal tissues. Increased cell proliferation has metabolic consequences and infiltrating cells also disturb tissue function. Anaemia, neutropenia and thrombocytopenia are important consequences of infiltration of the bone marrow, which in turn can lead to infection and haemorrhage.

Lymphoid leukaemias need to be distinguished from lymphomas, which are also neoplastic proliferations of cells of lymphoid origin. Although there is some overlap between the two categories, leukaemias generally have their predominant manifestations in the blood and the bone marrow whilst lymphomas have their predominant manifestations in lymph nodes and other lymphoid organs.

The classification of acute leukaemia

The purpose of any pathological classification is to bring together cases that have fundamental similarities and that are likely to share features of causation, pathogenesis and natural history. Acute leukaemia comprises a heterogeneous group of conditions that differ in aetiology, pathogenesis and prognosis. The heterogeneity is reduced if cases of acute leukaemia are divided into acute myeloid leukaemia (AML) (in North America often designated

'acute non-lymphoblastic leukaemia'), acute lymphoblastic leukaemia (ALL) and acute biphenotypic leukaemia; even then, however, considerable heterogeneity remains within each of the groups. The recognition of homogeneous groups of biologically similar cases is important as it permits an improved understanding of the leukaemic process and increases the likelihood of causative factors being recognized. Since such subgroups may differ from each other in the cell lineage affected and in their natural history and their prognosis following treatment, their recognition permits the development of a more selective therapeutic approach with a resultant overall improvement in the prognosis of acute leukaemia.

Although the best criteria for categorizing a case of acute leukaemia as myeloid or lymphoid may be disputed, the importance of such categorization is beyond doubt. Not only does the natural history differ but the best current modes of treatment are still sufficiently different for an incorrect categorization to adversely affect prognosis. Assigning patients to subtypes of acute myeloid or acute lymphoblastic leukaemia is becoming increasingly important as the benefits of more selective treatment are identified. Similarly, the suspected poor prognosis of biphenotypic acute leukaemia suggests that the identification of such cases may lead to a different therapeutic approach and an improved outcome. Cases of acute leukaemia can be classified on the basis of morphology, cytochemistry, immunophenotype, cytogenetic abnormality, molecular genetic abnormality, or by combinations of these characteristics. Morphology and cytochemistry will be discussed in this chapter and other diagnostic techniques in Chapter 2. The cytochemical stains most often employed are summarized in Table 1.1 [1, 2].

Patients may be assigned to the same or different subgroups depending on the characteristics studied and the criteria selected for separating subgroups. All classifications necessarily have an element of arbitrariness, particularly since they need to incorporate cut-off points for continuous variables such as the

Table 1.1 Cytochemical stains of use in the diagnosis and classification of acute leukaemia [1, 2].

Cytochemical stain	Specificity
Myeloperoxidase	Stains primary and secondary granules of cells of neutrophil lineage, eosinophil granules (granules appear solid), granules of monocytes, Auer rods; granules of normal mature basophils do not stain
Sudan black B	Stains primary and secondary granules of cells of neutrophil lineage, eosinophil granules (granules appear to have a solid core), granules of monocytes, Auer rods; basophil granules are usually negative but sometimes show metachromatic staining (red/purple)
Naphthol AS-D chloroacetate esterase ('specific' esterase)	Stains neutrophil and mast cell granules; Auer rods are usually negative except in AML associated with t(15;17) and t(8;21)
α-naphthyl acetate esterase ('non-specific' esterase)	Monocytes and macrophages, megakaryocytes and platelets, most T lymphocytes and some T lymphoblasts (focal)
α-naphthyl butyrate esterase ('non-specific' esterase)	Monocytes and macrophages, variable staining of T lymphocytes
Periodic acid–Schiff*	Neutrophil lineage (granular, increasing with maturation), leukaemic promyelocytes (diffuse cytoplasmic), eosinophil cytoplasm but not granules, basophil cytoplasm (blocks), monocytes (diffuse plus granules), megakaryocytes and platelets (diffuse plus granules), some T and B lymphocytes, many leukaemic blast cells (blocks, B more than T)
Acid phosphatase*	Neutrophils, most T lymphocytes, T lymphoblasts (focal), variable staining of eosinophils, monocytes and platelets, strong staining of macrophages, plasma cells and megakaryocytes and some leukaemic megakaryoblasts
Toluidine blue	Basophil and mast cell granules
Perls' stain	Haemosiderin in erythroblasts, macrophages and, occasionally, plasma cells

*These cytochemical stains are largely redundant if immunophenotyping is available.

percentage of cells falling into a defined morphological category, positivity for a certain cytochemical reaction, or the presence of a certain immunological marker. An ideal classification of acute leukaemia must be biologically relevant. If it is to be useful to the clinical haematologist, as well as to the research scientist, it should also be readily reproducible and easily and widely applicable. Rapid categorization should be possible so that therapeutic decisions can be based on the classification. The classification should be widely acceptable and should change as little as possible over time so that valid comparisons can be made between different groups of patients. Ideal classifications of acute leukaemia do not yet exist, although many have been proposed.

The development of the French–American–British (FAB) classification of acute leukaemia by a collaborating group of French, American and British haematologists [3–7] was a major advance in leukaemia classification, permitting a uniform classification of these diseases over two decades. It appears likely that the WHO classification, published in its definitive form in 2001 [8], will gradually take the place of the FAB classification. However, since application of the WHO classification requires knowledge of the results of cytogenetic analysis it appears equally likely that haematologists will make an initial diagnosis in FAB terms, pending the availability of results of cytogenetic or molecular genetic analysis. It is important that FAB designations (which have a precise, carefully defined meaning) are not applied to WHO categories for which the diagnostic criteria differ. For maximum clarity, all publications relating to acute leukaemia and the myelodysplastic syndromes (MDS) should state which classification is being used and should adhere strictly to the criteria of the relevant classification.

The FAB group both established diagnostic criteria for acute leukaemia and proposed a system of classification. There is usually no difficulty in recognizing that a patient with ALL is suffering from acute leukaemia, although arbitrary criteria are necessary to distinguish ALL from the closely related lymphoblastic lymphomas. In the case of AML, more difficulty can arise because of the necessity to distinguish between acute leukaemia and MDS. The latter term indicates a group of related conditions, characterized by an acquired intrinsic defect in the maturation of myeloid cells, which has been designated myelodysplasia or dysmyelopoiesis. MDS is a clonal, neoplastic disorder, which is closely related to, and in some patients precedes, acute leukaemia. In other patients MDS persists unchanged for many years or leads to death from the complications of bone marrow failure without the development of acute leukaemia; it is therefore justifiable to regard the myelodysplastic syndromes as diseases in their own right rather than merely as preludes to acute leukaemia. As the prognosis of MDS is generally better than that of acute leukaemia, and because therapeutic implications differ, it is necessary to make a distinction between acute leukaemia (with or without coexisting myelodysplasia or a preceding MDS) and cases of MDS in which acute leukaemia has not supervened. The FAB group proposed criteria for making the distinction between acute leukaemia and MDS, and for further categorizing these two groups of disorders. The distinction between AML and MDS will be discussed in this chapter and the further categorization of MDS in Chapter 3.

The FAB classification

The FAB classification of acute leukaemia was first published in 1976 and was subsequently expanded, modified and clarified [3–7]. It deals with both diagnosis and classification.

Diagnosing acute leukaemia

The diagnosis of acute leukaemia usually starts from a clinical suspicion. It is uncommon for this diagnosis to be incidental, resulting from the performance of a blood count for a quite different reason. Clinical features leading to suspicion of acute leukaemia include pallor, fever consequent on infection, pharyngitis, petechiae and other haemorrhagic manifestations, bone pain, hepatomegaly, splenomegaly, lymphadenopathy, gum hypertrophy and skin infiltration. A suspicion of acute leukaemia generally leads to a blood count being performed and, if this shows a relevant abnormality, to a bone marrow aspiration. The diagnosis then rests on an assessment of the peripheral blood and bone marrow.

The FAB classification requires that peripheral blood and bone marrow films be examined and that differential counts be performed on both. In the case of the bone marrow, a 500-cell differential count is required. Acute leukaemia is diagnosed if:

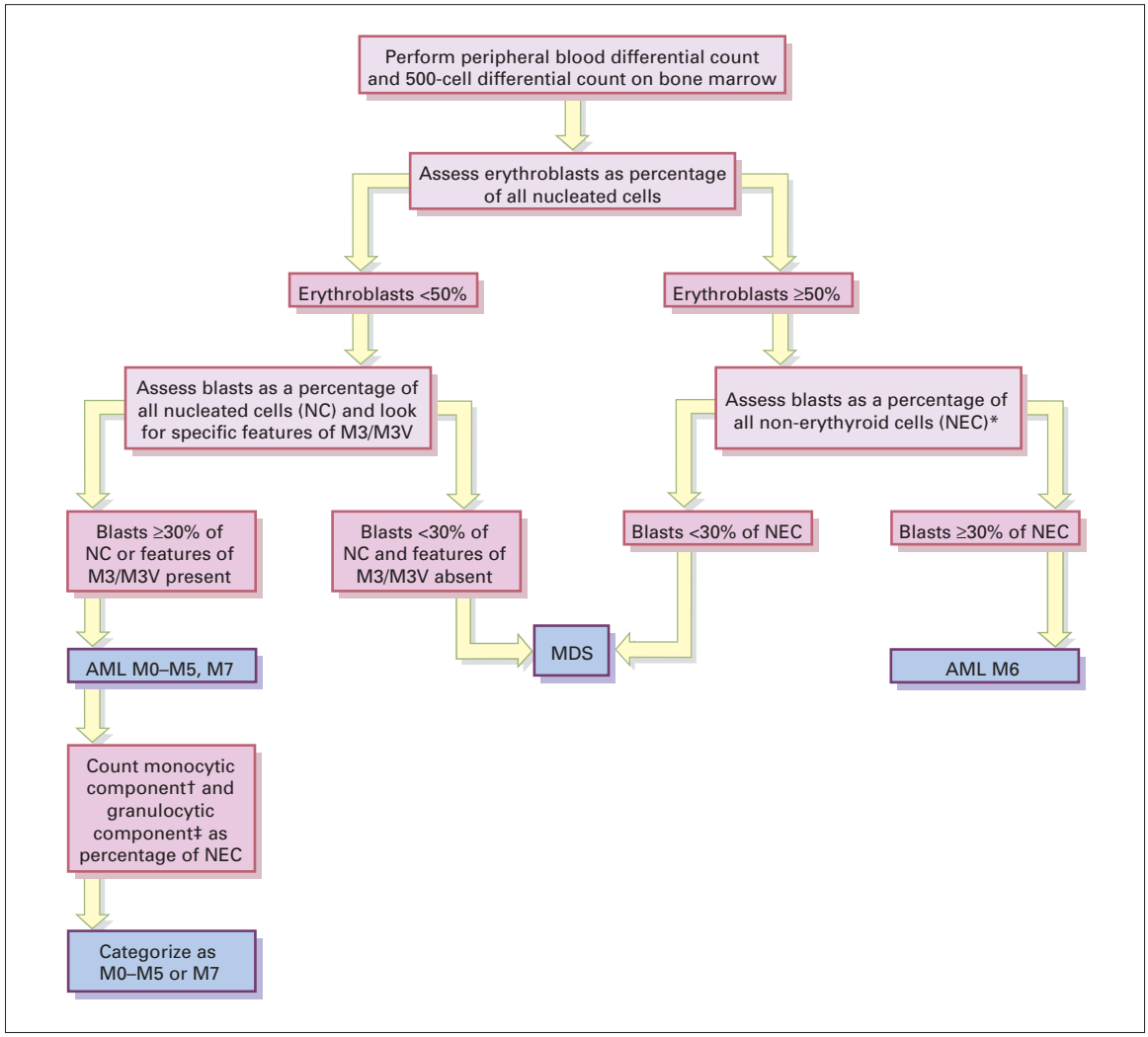


Fig. 1.1 A procedure for diagnosing acute myeloid leukaemia (AML) and for distinguishing it from the myelodysplastic syndromes [6]. *Excludes also lymphocytes, plasma cells, mast cells and macrophages. †Monoblasts to monocytes. ‡Myeloblasts to polymorphonuclear leucocytes.

1 at least 30%* of the total nucleated cells in the bone marrow are blast cells; or
2 if the bone marrow shows erythroid predominance (erythroblasts ≥50% of total nucleated cells) and at least 30% of non-erythroid cells are blast cells (lymphocytes, plasma cells and macrophages also being excluded from the differential count of non-erythroid cells); or
3 if the characteristic morphological features of acute promyelocytic leukaemia (see page 16) are present (Fig. 1.1).

Cases of ALL will be diagnosed on the first criterion since erythroid hyperplasia does not occur in this condition, but the diagnosis of all cases of AML requires application also of the second and third criteria. The bone marrow in acute leukaemia is usually hypercellular, or at least normocellular, but this is not necessarily so since some cases meet

*It should be noted that the criterion of at least 30% blast cells has been altered, in the WHO classification, to at least 20% blast cells (see page 127).

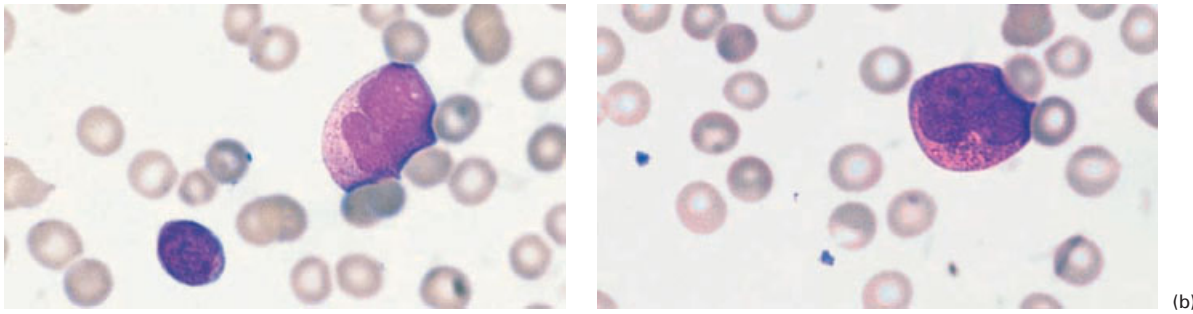


Fig. 1.2 The peripheral blood (PB) film of a patient with AML showing: (a) a type II blast with scanty azurophilic granules; (b) a promyelocyte with more numerous granules and a Golgi zone in the indentation of the nucleus. May–Grünwald–Giemsa (MGG) × 870.

the above criteria when the bone marrow is hypocellular.

Defining a blast cell

The enumeration of blasts in the bone marrow is crucial in the diagnosis of acute leukaemia and the definition of a blast cell is therefore important. Whether immature myeloid cells containing small numbers of granules are classified as blasts is a matter of convention. The FAB group chose to classify such cells as myeloblasts rather than promyelocytes. They recognized two types of myeloblast [9]. Type I blasts lack granules and have uncondensed chromatin, a high nucleocytoplasmic ratio and usually prominent nucleoli. Type II blasts resemble type I blasts except for the presence of a few azurophilic granules and a somewhat lower nucleocytoplasmic ratio. Cells are categorized as promyelocytes rather than type II blasts when they develop an eccentric nucleus, a Golgi zone, chromatin condensation (but with the retention of a nucleolus), numerous granules and a lower nucleocytoplasmic ratio. The cytoplasm, except in the pale Golgi zone, remains basophilic. Cells that have few or no granules, but that show the other characteristics of promyelocytes, are regarded as hypogranular or agranular promyelocytes rather than as blasts. Examples of cells classified as type II myeloblasts and promyelocytes, respectively, are shown in Figs 1.2 and 1.3. The great majority of lymphoblasts lack granules and are therefore type I blasts; they resemble myeloblasts but are often

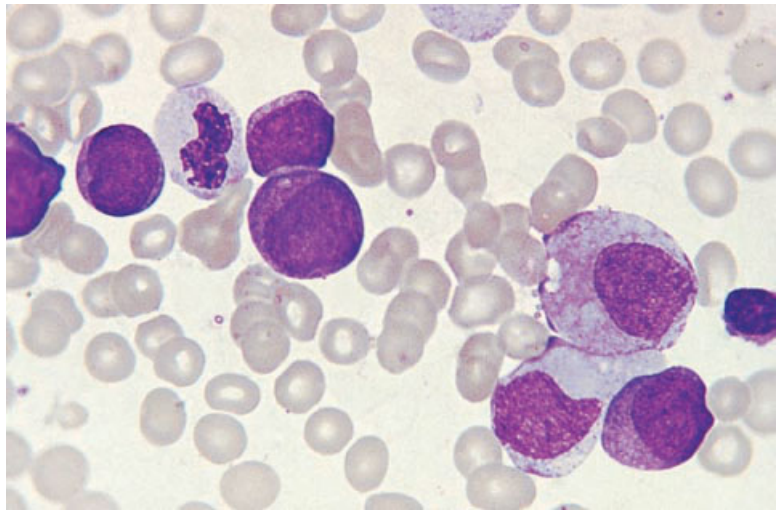


Fig. 1.3 Bone marrow (BM) of a patient with AML (M2/t(8;21)) showing a cell that lacks granules but nevertheless would be classified as a promyelocyte rather than a blast because of its low nucleocytoplasmic ratio; defective granulation of a myelocyte and a neutrophil is also apparent. Type I and type II blasts are also present. MGG × 870.

smaller with scanty cytoplasm and may show some chromatin condensation (see Table 1.11, p. 43).

Distinguishing between AML and ALL

The diagnosis of acute leukaemia requires that bone marrow blast cells (type I plus type II) constitute at least 30% either of total nucleated cells or of non-erythroid cells. The further classification of acute leukaemia as AML or ALL is of critical importance. When the FAB classification was first proposed, tests to confirm the nature of lymphoblasts were not widely available. The group therefore defined as AML cases in which at least 3% of the blasts gave positive reactions for myeloperoxidase (MPO) or with Sudan black B (SBB). Cases that appeared to be non-myeloid were classed as 'lymphoblastic'. The existence of cases of AML in which fewer than 3% of blasts gave cytochemical reactions appropriate for myeloblasts was not established at this stage, and no such category was provided in the initial FAB classification. In the 1980s and 1990s the wider availability and application of immunological markers for B- and T-lineage lymphoblasts, supplemented by ultrastructural cytochemistry and the application of molecular biological techniques to demonstrate rearrangements of immunoglobulin and T-cell receptor genes, demonstrated that the majority of cases previously classified as 'lymphoblastic' were genuinely lymphoblastic but that a minority were myeloblastic with the blast cells showing only minimal evidence of myeloid differentiation.† These latter cases were designated M0 AML [7]. It should be noted that SBB is more sensitive than MPO in the detection of myeloid differentiation and more cases will be categorized as M1 rather than M0 if it is used [10].

Correct assignment of patients to the categories of AML and ALL is very important for prognosis and choice of therapy. Appropriate tests to make this distinction must therefore be employed. Despite the advances in immunophenotyping, cytochemical reactions remain useful in the diagnosis of AML [11]. The FAB group recommended the use of MPO, SBB and non-specific esterase (NSE) stains. If cytochemical reactions for myeloid cells are negative, a presumptive diagnosis of ALL must be confirmed by immunophenotyping. When immunophenotyping is available the acid phosphatase reaction and the periodic acid–Schiff (PAS) reaction (the latter identifying a variety of carbohydrates including glycogen) are no longer indicated for the diagnosis of ALL. When cytochemical reactions indicative of myeloid differentiation and immunophenotyping for lymphoid antigens are both negative, immunophenotyping to demonstrate myeloid antigens and thus identify cases of M0 AML is necessary. It should be noted that when individuals with an inherited MPO deficiency develop AML, leukaemic cells will give negative reactions for both MPO and SBB.

The incidence of acute leukaemia

AML has a low incidence in childhood, less than 1 case per 100 000/year. Among adults the incidence rises increasingly rapidly with age, from approximately 1/100 000/year in the fourth decade to approximately 10/100 000/year in those over 70 years. AML is commoner in males than in females. ALL is most common in childhood, although cases occur at all ages. In children up to the age of 15 years the overall incidence is of the order of 2.5–3.5/100 000/year; the disease is more common in males than in females. ALL has also been observed to be more common in white people than in black people, but this appears to be related to environmental factors rather than being a genetic difference since the difference disappears with an alteration in socioeconomic circumstances.

The classification of AML

Once criteria for the diagnosis of AML have been met and cases have been correctly assigned to the broad categories of myeloid or lymphoid, further classification can be carried out. The FAB group suggested that this be based on a peripheral blood differential count and a 500-cell bone marrow differential count, supplemented when necessary by cytochemistry, studies of lysozyme concentration in serum or urine, and immunophenotyping; with the greater availability of immunophenotyping, measurement of lysozyme concentration is no longer in current use. Broadly speaking, AML is categorized as acute

†In discussing the FAB classification I have used the terms 'differentiation' and 'maturation' in the sense in which they were used by the FAB group, that is with differentiation referring to an alteration in gene expression that commits a multipotent stem cell to one pathway or lineage rather than another, and maturation indicating the subsequent changes within this cell and its progeny as they mature towards end cells of the lineage.

Table 1.2 Criteria for the diagnosis of acute myeloid leukaemia of M0 category (acute myeloid leukaemia with minimal evidence of myeloid differentiation).

Blasts ≥30% of bone marrow nucleated cells
Blasts ≥30% of bone marrow non-erythroid cells*
<3% of blasts positive for Sudan black B or for myeloperoxidase by light microscopy
Blasts demonstrated to be myeloblasts by immunological markers or by ultrastructural cytochemistry

*Exclude also lymphocytes, plasma cells, macrophages and mast cells from the count.

myeloblastic leukaemia without (M1) and with (M2) maturation, acute hypergranular promyelocytic leukaemia and its variant (M3 and M3V), acute myelomonocytic leukaemia (M4), acute monoblastic (M5a) and monocytic (M5b) leukaemia, acute erythroleukaemia (M6) and acute megakaryoblastic leukaemia (M7). M0 is AML without maturation and with minimal evidence of myeloid differentiation. In addition to the above categories there are several very rare types of AML, which are not included in the FAB classification. These include mast cell leukaemia and Langerhans' cell leukaemia. In addition, the diagnosis of hypoplastic AML requires consideration. Transient abnormal myelopoiesis of Down's syndrome may also be regarded as a variant of AML.

AML with minimal evidence of myeloid differentiation—M0 AML

The FAB criteria for the diagnosis of M0 AML are shown in Table 1.2 and morphological and immunocytochemical features are illustrated in Figs 1.4 and 1.5. The blasts in M0 AML usually resemble M1 myeloblasts or L2 lymphoblasts (see page 45) but in a minority of cases they resemble the monoblasts of M5 AML. Associated dysplastic features in erythroid and megakaryocyte lineages may provide indirect evidence that a leukaemia is myeloid, not lymphoid. Dysplastic features are present in up to a quarter of cases. Definite evidence of myeloid differentiation may be provided by:

1 demonstration of ultrastructural features of cells of granulocytic lineage, e.g. characteristic basophil granules (Table 1.3) [12–17];

2 demonstration of MPO activity by ultrastructural cytochemistry (Table 1.4) [13, 18, 19] (Fig. 1.6);

3 demonstration of MPO protein by immunocytochemistry with an anti-MPO monoclonal antibody;

4 demonstration of other antigens characteristic of myeloid cells by the use of monoclonal antibodies such as CD13, CD14, CD15, CD33, CD64, CD65 and CD117 (but without expression of platelet-specific antigens, which would lead to the case being categorized as AML M7).

Although not included in the criteria suggested by the FAB group, the demonstration of messenger RNA (mRNA) for MPO could also be taken as evidence of myeloid differentiation [20], but its expression may not be restricted to myeloid cells [21].

Immunophenotyping is now widely used for identifying cases of M0 AML and as a consequence ultrastructural examination and ultrastructural cytochemistry are rarely used. However, these techniques remain useful for the identification of immature cells of basophil, mast cell and eosinophil lineage. Immunophenotyping shows that the most specific lymphoid markers—cytoplasmic CD3, cytoplasmic CD79a and cytoplasmic CD22—are not expressed in M0 AML but there may be expression of less specific lymphoid-associated antigens such as CD2, CD4, CD7, CD10 and CD19, in addition to CD34, HLA-DR and terminal deoxynucleotidyl transferase (TdT).

M0 AML is associated with adverse cytogenetic abnormalities and poor prognosis [22, 23]. The molecular genetic abnormalities recognized include a high incidence of mutations of the *AML1* gene, most of which are biallelic [24].

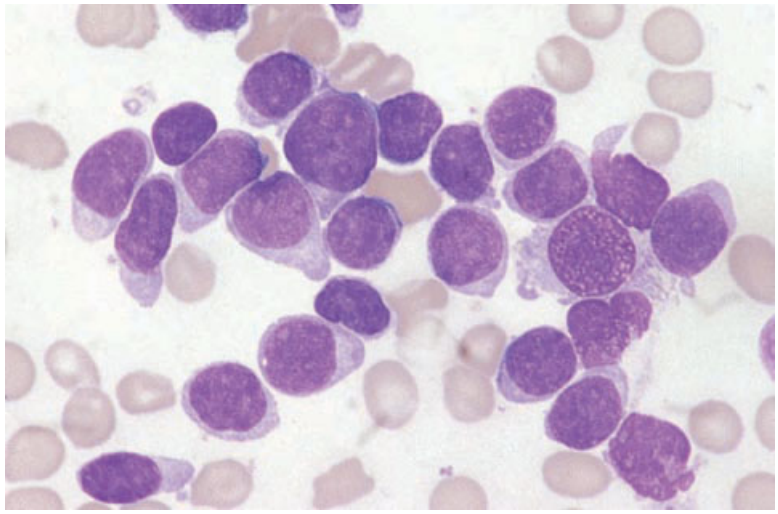
Cytochemical reactions in M0 AML

By definition fewer than 3% of blasts are positive for MPO, SBB and CAE since a greater degree of positivity would lead to the case being classified as M1 AML. Similarly, blast cells do not show NSE activity, since positivity would lead to the case being classified as M5 AML. Maturing myeloid cells may show peroxidase deficiency or aberrant positivity for both chloroacetate and non-specific esterase [25].

AML without maturation—M1 AML

The criteria for diagnosis of M1 AML are shown in

(a)



(b)

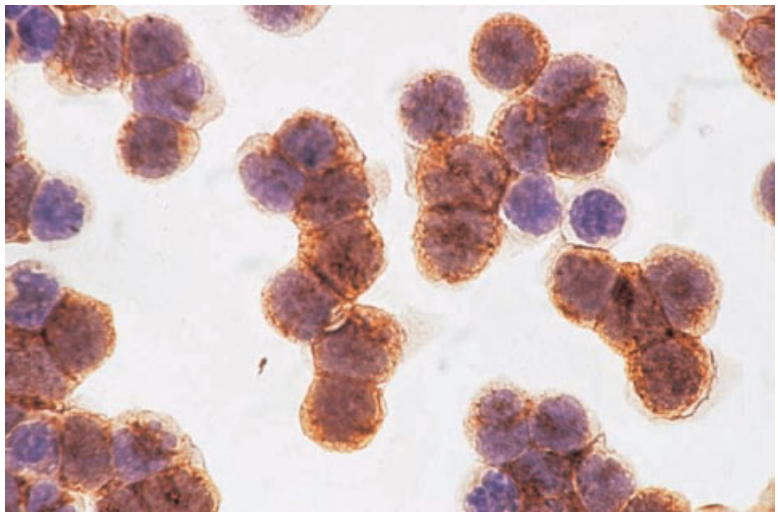


Fig. 1.4 PB and BM preparations from a patient with M0 AML: (a) BM film stained by MGG showing agranular blasts; (b) immunoperoxidase reaction of PB cells with a CD13 monoclonal antibody (McAb) showing many strongly positive blasts; the blasts were also positive for CD34, HLA-DR and terminal deoxynucleotidyl transferase (TdT). × 870.

Table 1.5 and the cytological features are illustrated in Figs 1.7–1.10. M1 blasts are usually medium to large in size with a variable nucleocytoplasmic ratio, a round or oval nucleus, one or more nucleoli, which range from inconspicuous to prominent, and cytoplasm that sometimes contains Auer rods, a few granules or some vacuoles. Auer rods are crystalline cytoplasmic structures derived from primary granules either just after their formation in the cisternae of the Golgi apparatus or by coalescence of granules within autophagic vacuoles. Auer rods may thus be seen as cytoplasmic inclusions or, less often, within a cytoplasmic vacuole. In children, the presence of Auer rods has been found to be associated with a better prognosis [26]. In M1 AML the blasts are predominantly type I blasts. In some cases the blasts are indistinguishable from L2 or even L1 lymphoblasts (see page 43).

M1 is arbitrarily separated from M2 AML by the requirement that no more than 10% of non-erythroid cells in the bone marrow belong to the maturing granulocytic component (promyelocytes to neutrophils).

The M1 category accounts for 15–20% of AML.

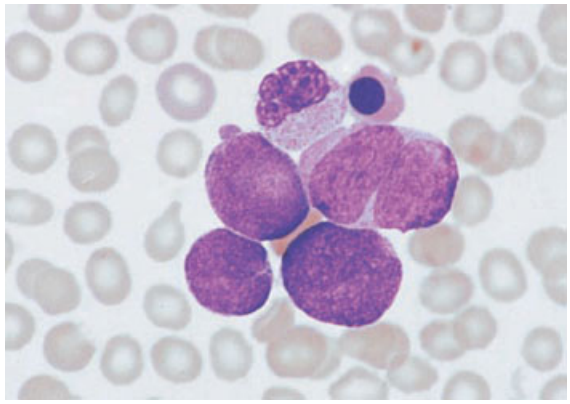


Fig. 1.5 BM film of a patient with M0 AML showing agranular pleomorphic blasts with a high nucleocytoplasmic ratio; the presence of a neutrophil with a nucleus of abnormal shape suggests the correct diagnosis. MGG × 870.

Cytochemical reactions in M1 AML

By definition M1 AML has a minimum of 3% of blasts that are positive for MPO or SBB. Hayhoe and Quaglino [2] found that the SBB reaction is a more sensitive marker of early granulocyte precursors than MPO. M1 blasts are usually positive for CAE, although this marker is usually less sensitive than either MPO or SBB in the detection of neutrophilic differentiation. Myeloblasts give a weak or negative reaction for a number of esterases that are more characteristic of the monocyte lineage, and that are collectively referred to as non-specific esterases. In the case of α-naphthyl acetate esterase (ANAE) and α-naphthyl butyrate esterase (ANBE) the reaction is usually negative, whereas in the cases of naphthol AS-D acetate esterase (NASDA) there is usually a weak fluoride-resistant reaction. Myeloblasts show

Table 1.3 Ultrastructural characteristics distinguishing blast cells and other immature leukaemic cells from each other [12, 13].

Myeloblasts of neutrophil lineage
Small, medium or large granules; sometimes Auer rods, which may be homogeneous or be composed of longitudinal tubules or dense material with a periodic substructure [14]

Promyelocytes of promyelocytic leukaemia
In hypergranular promyelocytic leukaemia the cytoplasm is packed with granules ranging from 120 to 1000 nm in diameter [15, 16]; in the variant form of hypergranular promyelocytic leukaemia the granules are much smaller, ranging from 100 to 400 nm, with some cells being packed with granules and other being agranular. Auer rods in promyelocytic leukaemia differ from those in M1 and M2 AML; they are composed of hexagonal structures and have a different periodicity from other Auer rods [16]; microfibrils and stellate configurations of rough endoplasmic reticulum are also characteristic of M3 AML, particularly M3 variant [17]

Myeloblasts of eosinophil lineage
Granules tend to be larger than those of neutrophil series; homogeneous in early cells, in later cells having a crystalline core set in a matrix; sometimes there is asynchrony with granules lacking a central core, despite a mature nucleus; Auer rods similar to those of the neutrophil lineage may be present [14]

*Myeloblasts of basophil or mast cell lineage**
Basophil granules may be any of three types: (i) large electron-dense granules composed of coarse particles; (ii) pale granules composed of fine particles; (iii) θ (theta) granules, which are small granules containing pale flocculent material and bisected by a membrane [13]. Mast cell precursors sometimes have granules showing the scrolled or whorled pattern that is characteristic of normal mast cells

Monoblasts and promonocytes
Monoblasts are larger than myeloblasts and cytoplasm may be vacuolated. Granules are smaller and less numerous

Megakaryoblasts
More mature megakaryoblasts show α granules, bull's eye granules and platelet demarcation membranes

Early erythroid precursors
Immature cells can be identified as erythroid when they contain aggregates of ferritin molecules or iron-laden mitochondria or when there is rhopheocytosis (invagination of the surface membrane in association with extracellular ferritin molecules)

*Sometimes in myeloid leukaemias and myeloproliferative disorders there are cells containing a mixture of granules of basophil and mast cell type.

Table 1.4 Ultrastructural cytochemistry in the identification of blast cells and other immature cells of different myeloid lineages.

Myeloblasts of neutrophil lineage Myeloperoxidase (MPO) activity in endoplasmic reticulum, perinuclear space, Golgi zone, granules and Auer rods (if present); detected by standard technique for MPO and by platelet peroxidase (PPO) techniques (reviewed in [13])
Myeloblasts of eosinophil lineage MPO-positive granules and Auer rods (if present) detected by MPO and PPO techniques
Myeloblasts of basophil or mast cell lineage Granules may be peroxidase positive or negative; endoplasmic reticulum, perinuclear space and Golgi zone are rarely positive; more cases are positive by PPO technique than MPO technique
Promyelocytes of acute promyelocytic leukaemia MPO-positivity is seen in granules, Auer rods, perinuclear space and some rough endoplasmic reticulum profiles [17]; strong lysozyme activity of granules and Auer rods is seen in M3 AML whereas in M3 variant AML activity varies from weak to moderately strong [17]
Monoblasts and promonocytes The first granule to appear in a monoblast is a small, peripheral acid phosphatase-positive granule [18]. MPO activity appears initially in the perinuclear envelope, Golgi apparatus and endoplasmic reticulum. Subsequently, mainly at the promonocyte stage, there are small MPO-positive granules. A PPO technique is more sensitive in the detection of peroxidase-positive granules than an MPO technique. Non-specific esterase activity can also be demonstrated cytochemically
Megakaryoblasts PPO activity in endoplasmic reticulum and perinuclear space only [13, 19]
Proerythroblasts PPO-like activity may be present in the Golgi zone

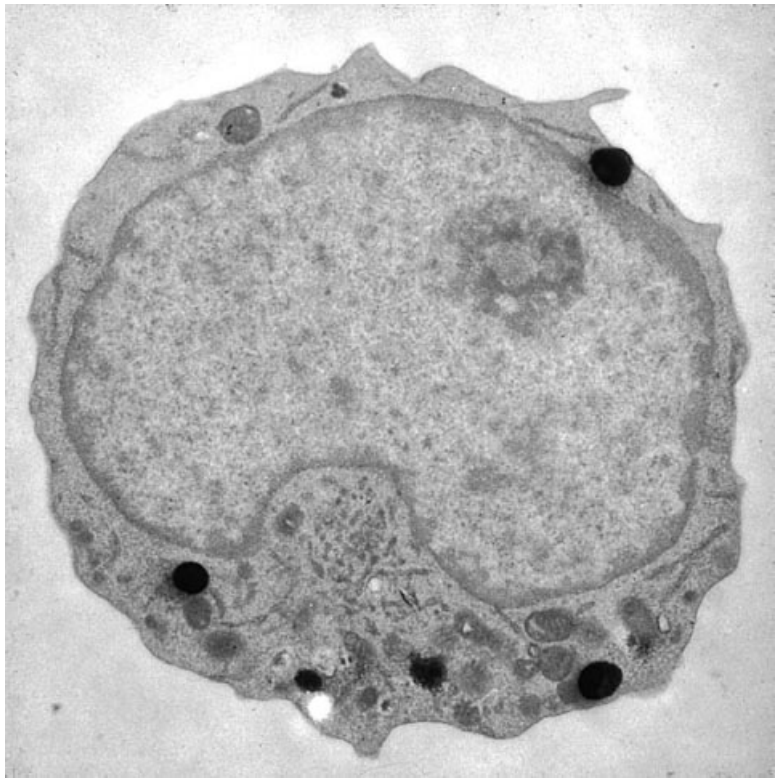


Fig. 1.6 Ultrastructural cytochemistry showing peroxidase-positive granules in a myeloblast (with thanks to Professor D Catovsky, London).

Table 1.5 Criteria for the diagnosis of acute myeloid leukaemia of M1 category (acute myeloid leukaemia without maturation).

Blasts ≥30% of bone marrow cells
Blasts ≥90% of bone marrow non-erythroid cells*
≥3% of blasts positive for peroxidase or Sudan black B
Bone marrow maturing monocytic component (promonocytes to monocytes) ≤10% of non-erythroid cells
Bone marrow maturing granulocytic component (promyelocytes to polymorphonuclear leucocytes) ≤10% of non-erythroid cells

*Exclude also lymphocytes, plasma cells, macrophages and mast cells from the count.

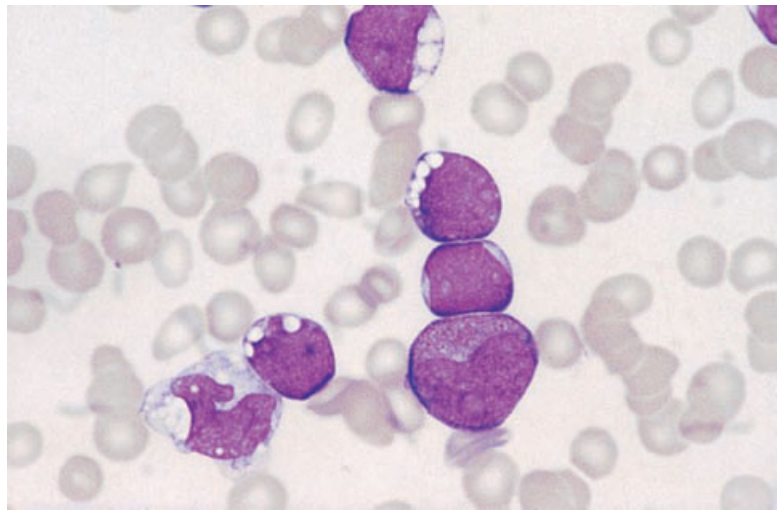


Fig. 1.7 PB film of a patient with M1 AML showing type I and type II blasts, some of which are heavily vacuolated, and a promyelocyte. MGG × 870.

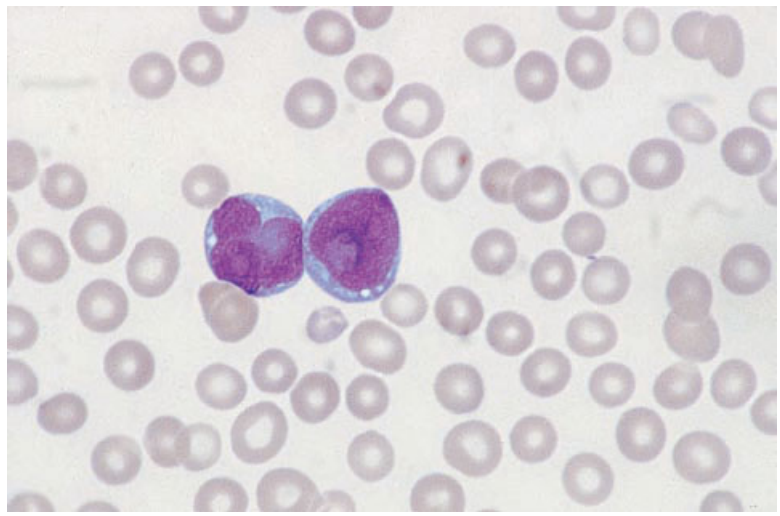


Fig. 1.8 PB film of a patient with M1 AML showing type I blasts with cytoplasmic vacuolation and nuclear lobulation. MGG × 870.

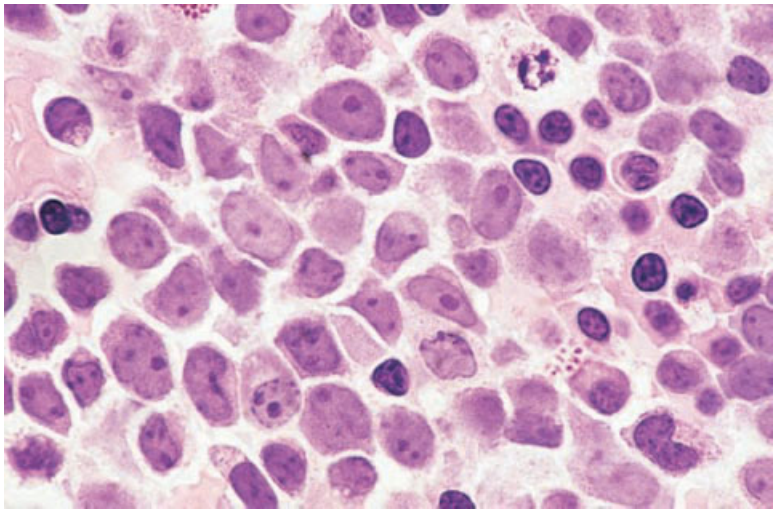
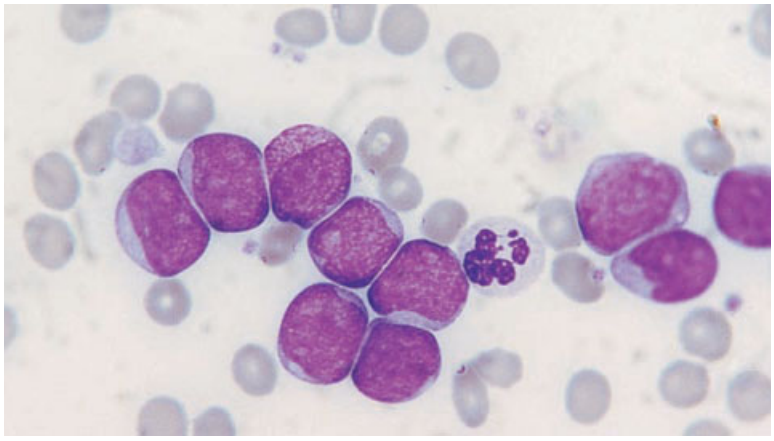


Fig. 1.9 Histological section of a trephine biopsy of a patient with M1 AML. The majority of cells present are blasts with a high nucleocytoplasmic ratio and prominent nucleoli; there are also some erythroblasts. Plastic embedded, haematoxylin and eosin (H & E) × 870.

(a)



(b)

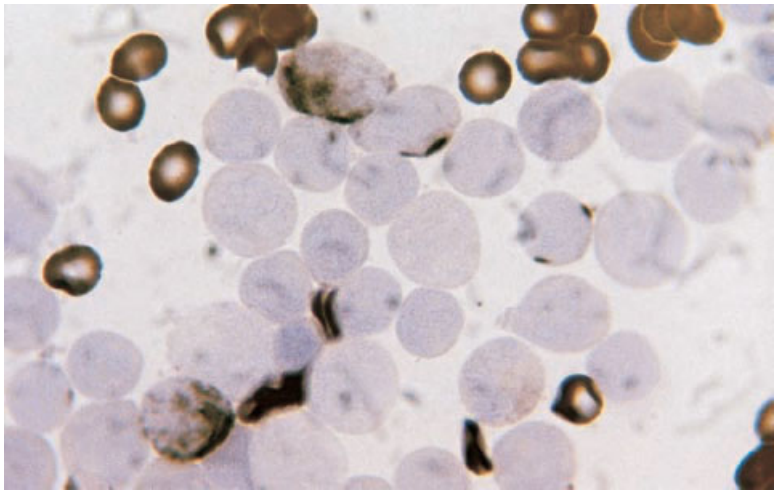
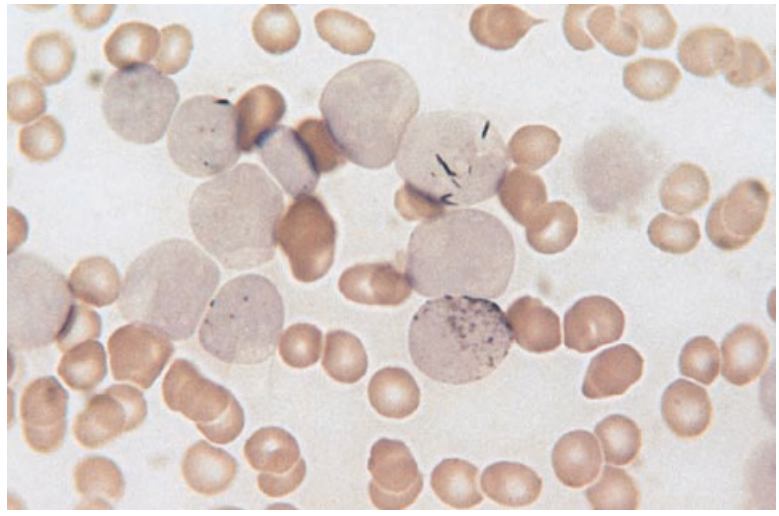
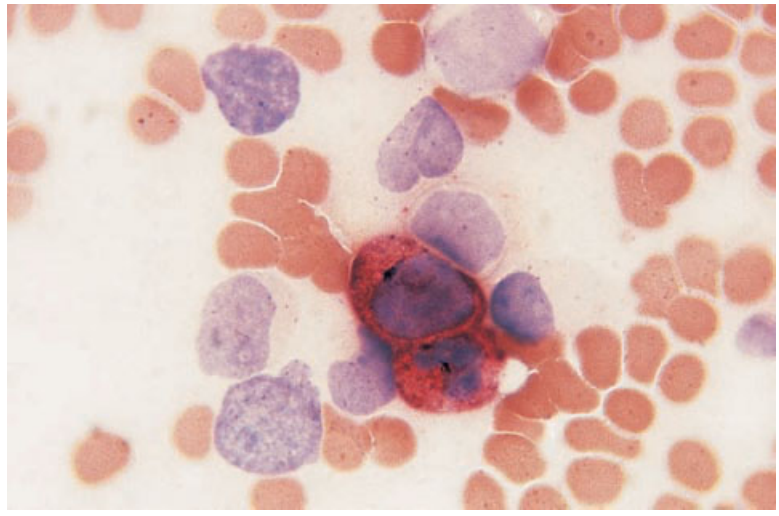


Fig. 1.10 Cytochemical reactions in a patient with M1 AML: (a) MGG-stained PB film showing largely type I blasts which in this patient are morphologically similar to lymphoblasts. One leukaemic cell is heavily granulated and would therefore be classified as a promyelocyte; this cell and the presence of a hypogranular neutrophil suggest that the correct diagnosis is M1 AML. MGG × 870. (b) myeloperoxidase (MPO) stain of BM showing two leukaemic cells with peroxidase-positive granules and two with Auer rods. × 870.



(c)



(d)

Fig. 1.10 (*Continued*) (c) Sudan black B (SBB) stain of BM showing some blasts with Auer rods and some with granules. × 870. (d) Chloroacetate esterase (CAE) stain of BM showing a positive neutrophil and a positive blast; other blasts present are negative. × 870.

diffuse acid phosphatase activity, which varies from weak to strong. The PAS reaction is usually negative, but may show a weak diffuse reaction with superimposed fine granular positivity.

Auer rods give positive reactions for MPO and SBB and occasionally weak PAS reactions. The reaction with CAE is usually weak or negative [2] except in M2 AML associated with t(8;21) (see page 78) in which Auer rods are often positive with CAE [1]. Although Auer rods are often detectable on a Romanowsky stain, they are more readily detectable on an MPO or SBB stain and larger numbers are apparent. Sometimes they are detectable only with cytochemical stains. Typical cytochemical stains in a case of M1 AML are shown in Fig. 1.10.

AML with maturation—M2 AML

The criteria for the diagnosis of M2 AML are shown in Table 1.6. In this context cells included in the maturing granulocytic category are promyelocytes, myelocytes, metamyelocytes and granulocytes, and also cells that differ cytologically from normal promyelocytes but that are too heavily granulated to be classified as blasts. Typical cytological and cytochemical features in M2 AML are shown in Figs 1.11–1.13.

Blasts ≥30% of bone marrow cells
Blasts 30–89% of bone marrow non-erythroid cells
Bone marrow maturing granulocytic component (promyelocytes to polymorphonuclear leucocytes) >10% of non-erythroid cells
Bone marrow monocytic component (monoblasts to monocytes) <20% of non-erythroid cells and other criteria for M4 not met

Table 1.6 Criteria for the diagnosis of acute myeloid leukaemia of M2 category (acute myeloid leukaemia with maturation).

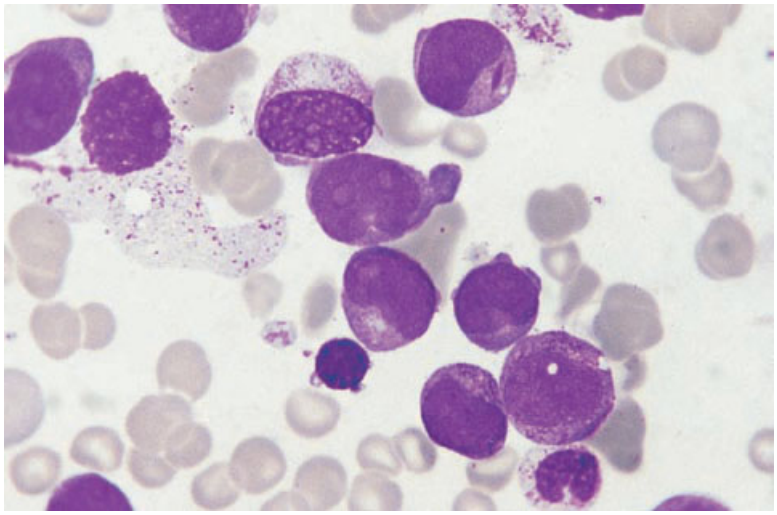


Fig. 1.11 BM film of a patient with M2 AML showing blasts (one of which contains an Auer rod), promyelocytes and a neutrophil. Note the very variable granulation. MGG × 870.

In contrast to M1 AML, blasts are often predominantly type II. Auer rods may be present. In children, Auer rods have been associated with a better prognosis [26], probably because of an association between Auer rods and t(8;21) (see page 78). Dysplastic features, such as hypo- or hypergranularity or abnormalities of nuclear shape are common in the differentiating granulocytic component of M2 AML. Maturation of myeloblasts to promyelocytes occurs in both M2 and M3 AML, and promyelocytes are prominent in some cases of M2 AML. Such cases are distinguished from M3 AML by the lack of the specific features of the latter condition (see below). M2 AML is distinguished from M4 AML by the monocytic component in the bone marrow being less than 20% of non-erythroid cells and by the lack of other evidence of significant monocytic differentiation. In most cases of M2 AML, maturation is along the neutrophil pathway but eosinophilic or basophilic maturation occurs in a minority. Such cases may be designated M2Eo or M2Baso. Other morphologically distinctive categories within M2, associated with specific cytogenetic abnormalities, are recognized (see Chapter 2).

The M2 subtype accounts for about 30% of cases of AML.

Cytochemical reactions in M2 AML

The cytochemical reactions in M2 AML are the same as those in M1 AML, but generally reactions are stronger and a higher percentage of cells are positive for MPO and SBB. CAE is more often positive in M2 than in M1 AML and reactions are stronger. Auer rods show the same staining characteristics as in M1 AML but are more numerous. When leukaemic myeloblasts undergo maturation, as in M2 AML, there may be a population of neutrophils, presumably derived from leukaemic blasts, which lack SBB and MPO activity. This may be demonstrated cytochemically or by means of an automated differential counter based on the peroxidase reaction, which shows a low mean peroxidase score and an abnormally placed neutrophil cluster. The neutrophil cluster

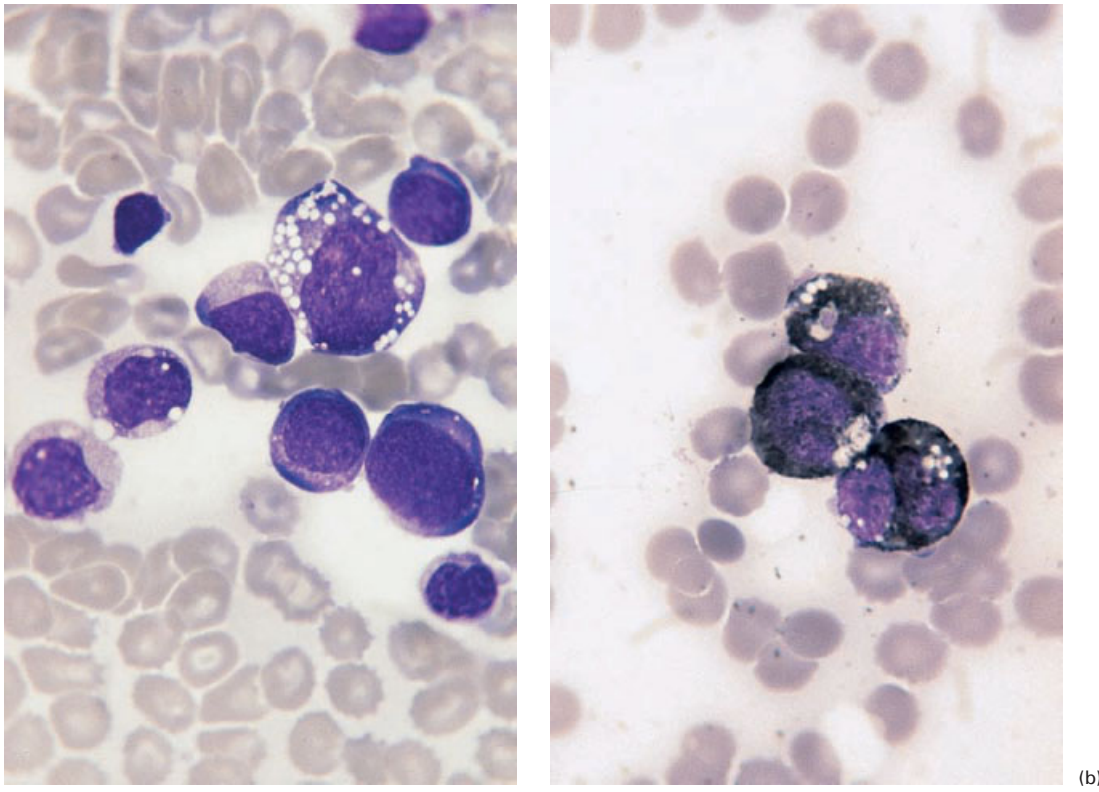


Fig. 1.12 BM film of a patient with M2 AML stained by (a) MGG and (b) SBB. In this patient both blasts and maturing cells were heavily vacuolated. × 870.

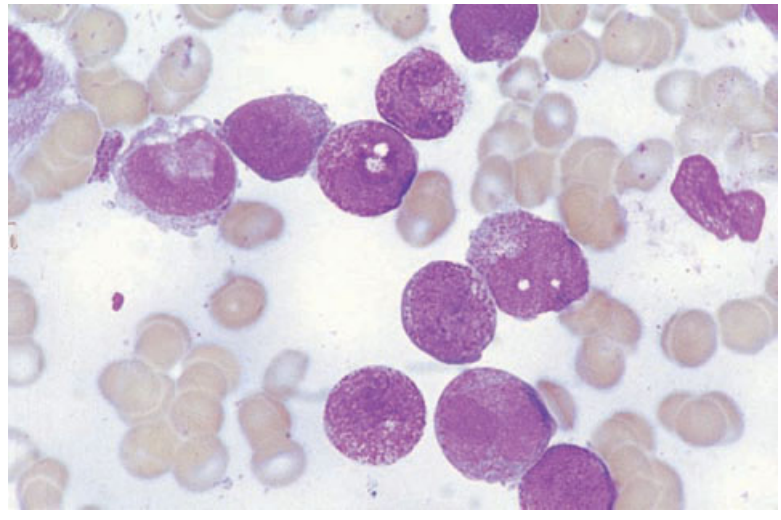


Fig. 1.13 BM film of a patient with M2 AML showing unusually heavy granulation of neutrophils and precursors (courtesy of Dr D. Swirsky, Leeds). MGG × 870.