Current concerns in haematology 2: Classification of acute leukaemia

B Bain, D Catovsky

Introduction

For the past century the classification of leukaemia has been predominantly clinical and morphological, supplemented in recent decades by the application of cytochemical techniques. During the past five to 10 years major advances in our knowledge of the nature of leukaemia consequent on the application of the techniques of immunology, cytogenetics, and molecular genetics, have taken place. Such new techniques have not only advanced knowledge but are now being applied in the diagnosis of individual patients. Information gained has led haematologists to review clinical and haematological features and to reinterpret them in the light of immunological and cytogenetic characteristics of the clone of leukaemic cells. Within the major categories of acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) many new entities have been recognised which differ in their biological features, including their prognoses. The recognition of such entities is important in using new forms of treatment which may benefit patients with leukaemias which can not be cured with current protocols.

The basis of the classification of acute leukaemia remains morphology and cytochemistry. Cases of acute leukaemia are most often classified according to predominant cell type as proposed by the FAB cooperative group.^{1,2} Information on immunophenotype and cytogenetic characteristics can be integrated with information derived from morphology and cytochemistry as is proposed in the MIC (Morphologic, Immunologic, and Cytogenetic) classifications of AML, ALL, and the myelodysplastic syndromes (MDS).³⁻⁵ An alternative approach to the morphological classification of acute leukaemia that emphasises the number of lineages involved and the degree of maturation of leukaemic cells has been proposed.⁶

Morphology and cytochemistry

The examination of both peripheral blood and bone marrow films is necessary for the diagnosis and classification of acute leukaemia. High quality staining of well spread films is of critical importance. The most useful cytochemical stains are either Sudan black B (SBB) or myeloperoxidase (MPO), together with a non-specific esterase stain such as α -naphthyl acetate esterase (ANAE). SBB is at least as sensitive as MPO in detecting myeloid differentiation and has the advantage that, unlike MPO, the reaction remains positive

when there has been delay in fixing the films.⁷ It has been suggested that SBB is less specific than MPO because positive reactions have been reported in ALL⁸ but we have not yet seen a positive SBB reaction in a well characterised case of ALL. The chloroacetate esterase reaction can also be used to identify myeloid cells. It is usually less sensitive than either MPO or SBB in detecting myeloid differentiation but can be useful, when combined with ANAE in a double esterase stain, for characterising leukaemia with both granulocytic and monocytic differentiation (AML M4). In identifying the monocyte lineage ANAE has a practical advantage over naphthol-AS acetate (NASA) or naphthol-ASD acetate (NASDA) esterase: it gives reactions which are weak or negative with the granulocyte lineage and the test does not therefore have to be performed with and without sodium fluoride to convey specificity. The periodic acid-Schiff (PAS) reaction is useful for supporting the diagnosis of ALL but its importance has declined as immunological markers have become more important for this purpose. PAS-block positivity, which is characteristic of ALL, correlates with the immunological phenotype, being more common in B-lineage cases. The presence of both vacuolated blasts and PAS positivity also correlates with reactivity for the common ALL antigen (CD10).⁹ PAS positivity can be seen in addition in monoblasts in AML M5 and in erythroblasts in AML M6; in these lineages the positive blocks appear on a background of diffuse or finely granular positivity in contrast to the clear background of ALL. Blasts of basophil lineage may also have strong PAS positivity. Like PAS staining, the acid phosphatase reaction has declined in importance with the availability of immunological markers. A strong focal acid phosphatase activity (or a strong focal ANAE activity), however, remains a useful indicator of T-lineage ALL. It should be noted, however, that a localised acid phosphatase and ANAE reaction is also a feature of rare forms of AML, M6, and M7.^{10,11}

Some prognostic information can be drawn from the morphology and cytochemistry of acute leukaemia. Among cases of ALL those with L3 morphology have a significantly worse prognosis, and in some studies L2 has shown a worse outcome than L1. In AML some differences in prognosis are seen among the different FAB categories. Cases of M5, M6, and M7 generally have a worse prognosis than those of M1-M4¹², and AML M0 (see

Department of
Haematology, St
Mary's Hospital
Medical School, Praed
Street, London W2
B Bain

Academic
Department of
Haematology and
Cytogenetics, The
Royal Marsden
Hospital, London
D Catovsky

Correspondence to:
Dr B Bain

Accepted for publication
5 April 1990

below) may be particularly bad.^{12,13} A poor prognosis is also associated with the presence of trilineage myelodysplasia¹⁴ and with PAS positive erythroblasts.⁶ Conversely, evidence of maturation of leukaemic cells, such as the presence of granules or Auer rods, sudanophilia and positive reactions for non-specific esterase, is associated with a more favourable prognosis.⁶

Immunological markers

Immunological techniques can be used to identify cytoplasmic and surface membrane antigens of leukaemic cells. For convenience and to increase sensitivity, an indirect two or three layer technique is usually used with the binding of the primary antibody (usually a monoclonal antibody of mouse hybridoma origin) being recognised by the application of a second and possibly third labelled antibody. The techniques most applicable to haematology are indirect immunofluorescence (IF), indirect immunoperoxidase (IP), and indirect immunoalkaline phosphatase (IAP) or the related indirect alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique. Techniques such as IF applied to living cells in suspension allow surface antigens to be detected. Techniques applied to fixed cells in blood or bone marrow films, or cytospin preparations permit the detection of surface antigens, cytoplasmic antigens such as μ chain or myeloperoxidase, and nuclear antigens such as terminal deoxynucleotidyl transferase (TdT). All the above methods are applicable to fixed cells. Indirect immunofluorescence on cells in suspension has the advantage of speed and, if a microtitre plate is used, economical use of reagents. There is an increasing tendency to use flow cytometers rather than fluorescence microscopy to read the results of IF. This permits the simultaneous detection of two or three antigens by the use of reagents directly labelled with different fluorochromes. Indirect immunofluorescence has the disadvantage that permanent preparations are not available, and the morphology of cells giving positive reactions cannot be easily determined. Conversely, techniques using fixed cells allow the morphological detail of positive cells to be recognised and provide permanent preparations. Because some antigens are expressed in the cytoplasm before being expressed on the cell surface, techniques with fixed cells can be more sensitive in detecting evidence of lineage commitment than techniques using living cells in suspension; this is relevant when looking for expression of CD3 (T-lineage), CD22 (B-lineage), or CD13 (myeloid lineage).

Immunological markers are important in the diagnosis of acute leukaemia for many reasons. They allow: (i) a presumptive diagnosis of ALL to be confirmed by demonstration of markers of either T or B lineage; (ii) some cases which give negative reactions with SBB, MPO, and non-specific esterase to be identified as myeloid; (iii) inappropriate antigen expression and bilineage and biphenotypic leukaemia to be

identified; and (iv) information which may be of prognostic importance, certainly in ALL, and possibly in AML to be gained.

The correct assignment of cases to the two major categories of ALL or AML is of practical importance as the drugs most likely to be effective in ALL differ from those most effective in AML, and as prophylaxis against central nervous system disease is essential in ALL and is generally not indicated in AML. Eighty to ninety per cent of cases of acute leukaemia can be correctly categorised as ALL or AML using morphology and cytochemistry alone. The addition of immunological markers allows roughly 98–99% of cases to be assigned correctly. Immunological markers are not essential in cases which can otherwise be positively identified as myeloid, but are indicated in those cases in which morphology and cytochemistry suggest ALL or do not give any clear evidence of lineage commitment. Most such cases will turn out to be ALL but a considerable minority are AML with the cells being either megakaryoblasts (AML M7), myeloblasts with minimal cytoplasmic maturation (AML M0), or blasts with the phenotype of an erythroid progenitor cell. The designation M0 (M zero) is useful for cases of AML which have fewer than 3% of cells positive with SBB or an MPO stain but which can be shown by more sensitive techniques, such as electron microscopy, to show myeloid differentiation.¹⁵ Ultrastructural morphology and cytochemistry can identify M7¹⁶ and M0¹⁵ AML and AML with an early erythroid phenotype,¹⁷ but immunological typing is more widely used and more easily applicable. AML M7 can be identified with monoclonal antibodies that detect platelet glycoprotein IIIa (CD61), platelet glycoproteins IIb or IIb/IIIa (CD41), and platelet glycoproteins IX and Ib (CD42). M0 AML can be identified with monoclonal antibodies of the CD13 and CD33 clusters.¹⁵ AML with an early erythroid phenotype can be identified with monoclonal antibodies against carbonic anhydrase.¹⁸ Erythroid cells can also be detected with monoclonal antibodies directed at the Gerbich blood group antigen (anti-Gero) or specific for glycophorin A. Also positive with erythroid cells but less specific are anti-transferrin antibodies (CD71) and antiplatelet glycoprotein IV (CD36), the latter being positive with early erythroid cells as well as with the megakaryocyte lineage.

In ALL immunological markers give information of relevance to prognosis. The worst prognosis is seen in the small minority of cases showing a mature B phenotype (surface membrane immunoglobulin positive); many such cases can also be identified morphologically because they fall into the FAB L3 category, but some B-ALL have L2 morphology and therefore can only be recognised by immunological techniques. Among other B-lineage cases a pre-B phenotype (cytoplasmic μ chain) was found in two studies to have a worse prognosis than other non-B, non-T lineage ALL^{19,20} but this was not observed in another study comparing the prognosis of pre-B ALL and common ALL.²¹

Among childhood cases of ALL T-lineage has generally been associated with a worse prognosis than B-lineage (excluding mature B) ALL, but this may not be true of adult cases in whom intensive multiagent chemotherapy has been used.²² In cases of T-lineage ALL various groups have reported an association between immunological phenotype and prognosis but findings have not been consistent. Crist *et al* reported that childhood cases with an early thymocyte phenotype (CD1a negative, membrane CD3 negative) had a lower remission rate than those with an intermediate (CD1a positive, mCD3 negative) or mature (CD1a negative, mCD3 positive) thymocyte phenotype, but there was no overall difference in survival.²³ Thiel *et al* found that adult cases whose cells failed to form rosettes with sheep red cells (pre-T ALL) had a worse prognosis than cases which were E-rosette positive, but they found no difference within the E-rosette positive group between those with an intermediate or mature phenotype.²⁴ Somewhat different observations have recently been made by other groups^{25,26} so that although immunophenotype is of prognostic importance in T-lineage ALL, it is not yet clear which markers are the most relevant.

Immunological phenotype may also be of some importance in AML. A lower complete remission rate has been associated with positivity for CD13,²⁷ CD14,²⁷ and CD34,^{28,29} and negativity for CD15,^{28,30} although the lower complete remission rate in CD13 positive cases was not confirmed in a second study.²⁹ Decreased survival has been associated with positivity for MY8²⁷ and CD17.³¹

Bilineage and biphenotypic leukaemias

The widespread application of immunological and molecular genetics techniques has led to the identification of many cases of acute leukaemia in which the leukaemic cells express markers of two lineages, either on a single population of cells or on two apparently separate populations. If factitious results such as those due to binding of antibodies to Fc receptors are excluded several explanations remain. (i) A marker may initially seem to be lineage specific when in fact it is lineage associated, but is expressed also in another lineage, albeit less frequently and sometimes less strongly. (ii) A leukaemic cell which seems to express inappropriate markers may correctly reproduce the phenotype of a normal counterpart which is rare and has therefore not been recognised. (iii) Malignant transformation may have occurred in a multipotent stem cell so that differentiation into cells of more than one lineage occurs. (iv) A leukaemic cell may show aberrant expression of one or more genes leading to cellular characteristics inappropriate to the lineage and not present on normal counterparts. A combination of the third and fourth mechanisms may occur with transformation of a multipotent stem cell being associated with abnormal differentiation so that hybrid cells are produced. Such a complex mechanism may be observed in blast crisis of

Ph-positive chronic myeloid leukaemia (CML) in which lymphoid, myeloid, and mixed transformations occur but hybrid cells such as basophil-mast cell hybrids can also be observed.

If large numbers of markers are applied to leukaemic cells an increasingly large proportion of patients are found to express markers of more than one lineage. The above mechanisms by which this may occur may differ in their clinical importance and it therefore seems desirable to distinguish between them by considering which markers—morphological, cytochemical, immunological, genetic or molecular—can be accepted as positive evidence of commitment to a lineage, and which are more likely to be lineage associated or the result of aberrant gene expression. Markers which are closely associated with the normal function of a lineage are generally accepted as firm evidence of differentiation to that lineage; such markers include the synthesis of immunoglobulin or cytoplasmic μ chain (B lineage), expression of CD3 and the T-cell receptor (α/β or γ/δ) (T lineage), and synthesis of myeloperoxidase and formation of peroxidase positive granules or Auer rods (granulocyte lineage). Other markers are strongly associated with a particular lineage but are not exclusive to it—for example, CD7 is characteristic of the T-lineage but is also expressed in some cases of AML.

When a case of acute leukaemia has two populations of cells expressing different markers the terms bilineage or mixed lineage leukaemia are used whereas the term biphenotypic leukaemia is now used when a single population of cells expresses the markers of two lineages. This distinction may be, to some extent, artificial as two populations of leukaemic cells differing in morphology, immunophenotype, or both, may nevertheless belong to a single clone. Examples of this phenomenon may be seen in patients with mixed lineage blastic crisis of Ph positive CML and in patients with the t(4;11) translocation who may have either a mixed lineage or biphenotypic leukaemia. The situation in which a bilineage or mixed leukaemia is actually biclonal is probably less common and could arise either by coincidence or because mutagenic influences on haemopoietic stem cells have led to two independent leukaemogenic events or because immune surveillance is defective. Biphenotypic leukaemia necessarily occurs at a single point in time, but in bilineage leukaemia the two populations of leukaemic cells may be present simultaneously (synchronous) or consecutively (metachronous). The latter is also designated lineage switch.

The recognition of bilineage and biphenotypic leukaemias is important for both practical and theoretical reasons. There are implications for treatment of the individual patient because treatment directed at a single lineage may be ineffective. The recognition of such cases also has the potential to increase our understanding of the nature of leukaemia. It is important, therefore, that cases are not assigned to these categories on the basis of single markers which

are lineage associated rather than lineage specific, and it is also desirable, although more difficult, to distinguish between aberrant gene expression and lineage commitment. Del Vecchio suggested that, using a panel of 10 well characterised monoclonal antibodies, the 18% of cases which expressed only a single "inappropriate" antigen should be regarded as showing ectopic expression of an antigen and only the 5.5% of cases which expressed two "inappropriate" antigens should be classified as biphenotypic.³² Mirro and Kitchingham proposed a scheme which is much more complex but also more satisfactory because it weights various characteristics, depending on their strength of association with a given lineage.³³ The expression of cytoplasmic μ chain and the T-cell receptor (TCR) is thus sufficient to identify a cell as lymphoid, but the expression of TdT or CD7 alone is not. Thorough investigation of cases with the application of clearly defined criteria will allow bilineage and biphenotypic leukaemias to be recognised and their clinical importance to be assessed. Whether expression of a single or even more than one "inappropriate" marker has implications for prognosis is not yet clear. Conflicting evidence has emerged from different studies with regard to the prognostic importance of TdT expression in AML and myeloid antigen expression in ALL.³³⁻³⁵

Cytogenetics

Cytogenetic studies in acute leukaemia yield important information from the point of view of aetiology, pathogenesis, prognosis and, potentially, treatment. Within the acute myeloid leukaemias abnormalities of chromosomes 5 and 7 (-5 , $5q-$, -7 , $7q-$) are associated with secondary leukaemia following exposure to cytotoxic chemotherapy and possibly also following exposure to environmental toxins such as benzene and various solvents and other chemicals. These abnormalities, together with others including $t(6;9)$, $inv(3)$, and complex karyotypic abnormalities, are associated with trilineage myelodysplasia, leukaemia arising in a multipotent stem cell, and poor prognosis. Conversely, certain chromosomal abnormalities are associated with de novo leukaemia, with a better prognosis, and with leukaemia which probably arises in a committed stem cell. Among these are $t(15;17)$, $inv(16)$, and probably $t(8;21)$. $t(15;17)$ is associated not only with acute hypergranular promyelocytic leukaemia (M3) but also with the variant form, M3V; the demonstration of $t(15;17)$ is useful in confirming M3V as this diagnosis can be difficult on morphological grounds. The karyotype associated with the most favourable prognosis is $inv(16)$ ^{12 36} which in most cases is found classified as M4Eo (acute myelomonocytic leukaemia with eosinophilia). An intermediate prognosis is associated with M2/ $t(8;21)$ and M3/ $t(15;17)$ leukaemias.^{12 36} There is more likelihood of achieving a cure with chemotherapy in patients with a favourable karyotype so that cytogenetic data are relevant in assessing whether a patient

with AML should be offered bone marrow transplantation.

In ALL certain translocations which are also associated with Burkitt's lymphoma— $t(8;14)$, $t(2;8)$ and $t(8;22)$ —are associated with a mature B phenotype, L3 morphology, and with a prognosis which has been poor in the past but which is improving with current intensive treatment protocols.³⁷ Recently ALL with this phenotype has been recognised in AIDS. Other unfavourable karyotypes include hypodiploidy and pseudodiploidy, particularly $t(9;22)$ with formation of the Philadelphia chromosome, $t(1;19)$, and $t(4;11)$. Conversely, hyperdiploidy with more than 55 chromosomes is associated with the most favourable prognosis.

As for unfavourable immunophenotypes, the poor prognostic importance of certain chromosomal abnormalities seems to have lessened with the wider use of intensive treatment³⁸ but cytogenetic data still remain relevant in choice of chemotherapy and in determining the appropriateness and timing of bone marrow transplantation.

Molecular genetics

Techniques of DNA analysis have shown the mechanisms operating in the pathogenesis of leukaemia, particularly in Ph-positive acute leukaemia and in Burkitt's related translocations. Such analysis has also proved a powerful tool in showing clonality and has been used, for example, to show that a lineage switch has occurred rather than a secondary leukaemia related to treatment.³⁹ Molecular genetic analysis has proved less useful than had been hoped in showing lineage commitment. Although rearrangement of the TCR β , γ , and δ genes occurs during T cell ontogeny, it also occurs in some B-lineage ALL and even in some cases of AML. Similarly, rearrangement of the immunoglobulin (Ig) heavy chain gene occurs in B cell ontogeny but also in some T-ALL and AML. Rearrangement of κ and λ genes does, however, seem to be specific for the B lineage. Transcription of non-rearranged immunoglobulin μ chain and TCR β genes sometimes occurs; these are truncated transcripts which may not be lineage specific.⁴⁰

In the myeloid lineage detection of MPO mRNA by hybridisation with cDNA has been suggested as a technique for showing myeloid differentiation. Some investigators have obtained positive results only in AML,⁴¹ but one group also obtained positive results in ALL⁴² so that the role of the technique cannot be regarded as established.

At this stage the techniques of molecular genetics are a powerful research tool but they have not yet affected patient management.

Relapse of leukaemia v secondary leukaemia

The thorough investigation of leukaemia in relapse and of suspected secondary leukaemia is of some importance in the management of individual patients, but it is of even more

importance in understanding leukaemogenesis and in determining treatment strategies for future patients. Apparent relapse should be reassessed by morphology, cytochemistry, cytogenetics and, if possible, DNA analysis in order to distinguish between relapse, with or without lineage switch, and a secondary leukaemia induced by treatment. The implication of relapse including lineage switch is that initial treatment was ineffective and may have selected for a subclone or a particular lineage, whereas the implication of a secondary leukaemia is that initial treatment has damaged DNA of residual normal stem cells and has been leukaemogenic.

When relapse of leukaemia occurs the morphological phenotype may show some changes. For example, L1 ALL may relapse as L2 ALL or M2, or M4 AML as M1 AML. Some change in immunological phenotype may also occur. Cytogenetic analysis usually shows an abnormal karyotype related to that present at diagnosis. Clonal evolution is common. A side-line with further karyotypic abnormality may have replaced the stem-line or may coexist with it.

When relapse is associated with a lineage switch the morphological and immunological phenotype have changed but any cytogenetic abnormality present is likely to be the same as or related to that which was present originally. Molecular genetic analysis of TCR β , γ , and δ and IgJ_H genes shows the same rearrangement and confirms that the two leukaemias belong to the one clone as in the case described by Scott *et al.*³⁹ Lineage switch may occur rapidly during induction chemotherapy in which case it is likely to indicate selection by chemotherapy. This phenomenon has been seen particularly in patients with the t(4;11) translocation and is an indication for change in treatment. Lineage switch may also occur at the first or subsequent relapse. Late lineage switch has commonly been from T lineage ALL to AML.

When a secondary leukaemia occurs as a result of chemotherapy administered for the primary malignancy the characteristic finding is of an AML which is often difficult to categorise morphologically with associated trilineage myelodysplasia and complex karyotypes and abnormalities of chromosomes 5 and 7 being common.^{43 44}

A group of 13 children with ALL was recently described in whom a recurrence of leukaemia seemed to be secondary AML rather than a relapse, but in whom the cytogenetic features differed from those usually seen in secondary AML.⁴⁵ The original ALL was either T- or B-lineage but with T-lineage over-represented. In nine of the 10 in whom cytogenetic analysis was performed a karyotypic abnormality was present which was not related to that present at diagnosis. In no case was there loss of material from chromosome 5 or 7 but in eight of the nine cases an abnormality with a breakpoint at 11q23 had been acquired. Further investigation is needed to assess the clinical importance of these findings as no DNA analysis was available and as abnormalities with an 11q23 breakpoint, including t(4;11), have been associated with

leukaemia arising in a multipotent stem cell—both biphenotypic leukaemia and bilineage leukaemia, synchronous and metachronous.

The investigation of apparent relapse of leukaemia after bone marrow transplantation is of particular importance. The necessity for caution in interpretation is highlighted by a recent report of a case in which bone marrow from a male was transplanted into a female. Relapse in donor cells was suspected when 20 mitoses in a bone marrow obtained at relapse were XY.⁴⁶ More detailed analysis using molecular genetics techniques showed that although all the cells in mitosis were of donor origin the relapse was in recipient cells which showed the same rearranged J_H gene as at diagnosis.

Conclusions

With our present state of knowledge the optimal management of individual patients with acute leukaemia requires that all cases be studied by morphology and cytochemistry and by immunological techniques. Ideally all cases should also have cytogenetic analysis. Other techniques are at present important in the advancement of knowledge but have not yet had a major impact on patient management. More detailed studies of individual patients at specialised centres with preservation of cells or of DNA for future study will be important in yielding more information on the clinical importance of the expression of various markers, the prevalence and relevance of bilineage and biphenotypic leukaemias and, above all, mechanisms of leukaemogenesis and of disease evolution and refractoriness to treatment.

- 1 Bennett JM, Catovsky D, Daniel MT, *et al.* Proposals for the classification of the acute leukaemias. *Br J Haematol* 1976;33:451–8.
- 2 Bennett JM, Catovsky D, Daniel MT, *et al.* Proposed revised criteria for the classification of acute myeloid leukemia. *Ann Intern Med* 1985;103:620–9.
- 3 First MIC cooperative study group. Morphologic, immunologic, and cytogenetic (MIC) working classification of acute lymphoblastic leukaemias. *Cancer Genet Cytogenet* 1986;23:189–97.
- 4 Second MIC cooperative study group. Morphologic, immunologic, and cytogenetic (MIC) working classification of acute myeloid leukaemias. *Br J Haematol* 1988; 68:487–94.
- 5 Third MIC cooperative study group. Recommendations for a morphologic, immunologic and cytogenetic (MIC) working classification of the primary and therapy-related myelodysplastic disorders. *Cancer Genet Cytogenet* 1988;32:1–10.
- 6 Hayhoe FGJ. Classification of acute leukaemias. *Blood Rev* 1988;2:186–93.
- 7 Hayhoe FGJ, Quaglino D. *Haematological cytochemistry*. Second edn. Edinburgh: Churchill Livingstone, 1988.
- 8 Tricot G, Broekaert-Van Orshoven A, Van Hoof A, Verwilghen RL. Sudan Black B positivity in acute lymphoblastic leukaemia. *Br J Haematol* 1982;51:615–21.
- 9 Lilleyman JS, Hann IM, Stevens RF, Richards SM, Eden OB. Blast vacuoles in childhood lymphoblastic leukaemia. *Br J Haematol* 1988;70:183–6.
- 10 de Oliveira MP, Gregory C, Matutes E, Parreira A, Catovsky D. Cytochemical profile of megakaryoblastic leukaemia: a study with cytochemical method, monoclonal antibodies and ultrastructural cytochemistry. *J Clin Pathol* 1987;40:663–9.
- 11 De Oliveira MP, Matutes E, Catovsky D. The cytochemistry, membrane markers and ultrastructure of megakaryoblastic (M7) and erythro (M6) leukaemias. In: Scott CS, Horwood E, Eds. *Leukaemia cytochemistry: principles and practice*. Chichester: John Wiley, 1989:137–54.
- 12 Schiffer CA, Lee EJ, Tomiyasu T, Wiernik PH, Testa JR.

- Prognostic impact of abnormalities in patients with de novo acute nonlymphocytic leukemia. *Blood* 1989;73:263-70.
- 13 Lee EJ, Pollak A, Leavitt RD, Testa JA, Schiffer CA. Minimally differentiated acute nonlymphocytic leukemia: a distinct entity. *Blood* 1987;70:1400-6.
 - 14 Brito-Babapulle F, Catovsky D, Galton DAG. Clinical and laboratory features of *de novo* acute myeloid leukaemia with trilineage myeloid dysplasia. *Br J Haematol* 1987;66:445-50.
 - 15 Matutes E, de Oliveira MP, Foroni L, Morilla R, Catovsky D. The role of ultrastructural cytochemistry and monoclonal antibodies in clarifying the nature of undifferentiated blasts in acute leukaemia. *Br J Haematol* 1988;69:205-11.
 - 16 Breton-Gorius J, Reyes F, Vernant JP, Tulliez M, Dreyfus B. The blast crisis of chronic granulocytic leukaemia: megakaryoblastic nature of the cells revealed by the presence of platelet peroxidase—a cytochemical ultrastructural study. *Br J Haematol* 1978;39:295-303.
 - 17 Reiffers J, Bernard P, Larrue J, et al. Acute erythroblastic leukemia presenting as undifferentiated leukemia: a report of two cases with ultrastructural features. *Leuk Res* 1985;9:413-20.
 - 18 Villeval JL, Cramer F, Lemoine A, et al. Phenotype of early erythroblastic leukemias. *Blood* 1986;68:1167-74.
 - 19 Crist W, Boyett J, Roper M, et al. Pre-B leukemia responds poorly to treatment: a pediatric oncology group study. *Blood* 1984;63:407-14.
 - 20 Crist W, Boyett J, Jackson J, et al. Prognostic importance of the pre-B-cell immunophenotype and other presenting features in B-lineage childhood acute lymphoblastic leukaemia: a pediatric oncology group study. *Blood* 1989;74:1252-9.
 - 21 Lilleyman JS, Hinchcliffe RF. Pre-B and 'common' lymphoblastic leukaemia of childhood compared. *Br J Haematol* 1989;71:227-31.
 - 22 Hoelzer D, Thiel E, Löffler H, et al. Prognostic factors in a multicentric study for treatment of acute lymphoblastic leukemia in adults. *Blood* 1988;71:123-31.
 - 23 Crist WM, Shuster JJ, Falletta J, et al. Clinical features and outcome in childhood T-cell leukemia-lymphoma according to the stage of thymocyte differentiation: a pediatric oncology group study. *Blood* 1988;72:1891-7.
 - 24 Thiel E, Kranz BR, Raghavachar A, et al. Prethymic phenotype and genotype of pre-T(CD7+/ER-)-cell leukemia and its clinical significance within adult acute lymphoblastic leukemia. *Blood* 1989;73:1247-58.
 - 25 Shuster JJ, Falletta JM, Pullen J, et al. Prognostic factors in childhood T-cell acute lymphoblastic leukemia: a pediatric oncology group study. *Blood* 1990;75:166-73.
 - 26 Pui C-H, Behm FG, Singh B, et al. Heterogeneity of presenting features and their relation to treatment outcomes in 120 children with T-cell acute lymphoblastic leukemia. *Blood* 1990;75:174-9.
 - 27 Griffin JR, Davis R, Nelson DA, et al. Use of surface marker analysis to predict outcome of adult myeloblastic leukemia. *Blood* 1986;68:1232-41.
 - 28 Civin CI, Vaughan WP, Strauss LC, Schwartz JF, Karp JE, Burke PJ. Diagnostic and prognostic utility of cell surface markers in acute non-lymphocytic leukemia (ANLL). *Exp Hematol* 1983;11(Suppl 14):152a.
 - 29 Borowitz MJ, Gockerman JP, Moor JO, et al. Clinicopathological and cytogenetic features of CD34 (My10)-positive acute nonlymphocytic leukemia. *Am J Clin Pathol* 1989;91:265-70.
 - 30 Holowiecki J, Lutz D, Krzemien S, et al. CD-15 antigen detected by the VIM-D5 monoclonal antibody for prediction of ability to achieve complete remission in acute nonlymphocytic leukemia. *Acta Haematol* 1986;76:16-19.
 - 31 Merle-Beral H, Duc LNC, Leblond V, et al. Diagnostic and prognostic significance of myelomonocytic cell surface antigens in acute myeloid leukaemia. *Br J Haematol* 1989;73:323-30.
 - 32 Del Vecchio L, Schiavone EM, Ferrara F, et al. Immunodiagnosis of acute leukemia displaying ectopic antigens: proposals for a classification of promiscuous phenotypes. *Am J Hematol* 1989;31:173-80.
 - 33 Mirro J, Kitchingman GR. The morphology, cytochemistry, molecular characteristics and clinical significance of acute mixed-lineage leukaemia. In: Scott CS, Horwood E, eds. *Leukaemia cytochemistry: principles and practice*. Chichester: John Wiley, 1989:153-79.
 - 34 Sobol RE, Mick R, Royston I, et al. Clinical importance of myeloid antigen expression in adult acute lymphoblastic leukaemia. *N Engl J Med* 1987;316:1111-17.
 - 35 Pui C-H, Behm FG, Singh B, et al. Myeloid-associated antigen expression lacks prognostic value in childhood acute lymphoblastic leukemia. *Blood* 1990;75:198-202.
 - 36 Fenaux P, Preudhomme C, Lai JL, Morel P, Beuscart R, Bauters F. Cytogenetics and prognostic value in *de novo* myeloid leukaemia: a report on 283 cases. *Br J Haematol* 1989;73:61-7.
 - 37 Bloomfield CD, Secker-Walker LM, Goldman AT, et al. Six-year follow-up of the clinical significance of karyotype in acute lymphoblastic leukaemia. *Cancer Genet Cytogenet* 1989;40:171-85.
 - 38 Fletcher JA, Kimball VM, Lynch E, et al. Prognostic implications of cytogenetic studies in an intensively treated group of children with acute lymphoblastic leukemia. *Blood* 1989;74:2130-5.
 - 39 Scott CS, Vulliamy T, Catovsky D, Matutes E, Norfolk DR. DNA genotypic conservation during phenotypic switch from T-cell acute lymphoblastic leukaemia to acute myeloblastic leukaemia. *Leukaemia and Lymphoma* 1989;1:21-8.
 - 40 Hara J, Yumara-Yagi K, Tawa A, et al. Molecular analysis of acute undifferentiated leukemia: two distinct subgroups at the DNA and RNA level. *Blood* 1989;74:1738-46.
 - 41 Zaki SR, Austin GE, Swan D, Srinivasan A, Ragab AH, Chan WC. Human myeloperoxidase gene expression in acute leukemia. *Blood* 1989;74:2096-102.
 - 42 Ferrari S, Mariano MT, Tagliafico E, et al. Myeloperoxidase gene expression in blast cells with a lymphoid phenotype in cases of acute lymphoblastic leukemia. *Blood* 1988;72:873-6.
 - 43 Fourth international workshop on chromosomes in leukemia. Secondary leukemia associated with neoplasia; treated and untreated. *Cancer Genet Cytogenet* 1984;11:319-21.
 - 44 Groupe Française de cytogénétique hématologique. Chromosome analysis of 63 cases of secondary nonlymphoid blood disorders: a cooperative study. *Cancer Genet Cytogenet* 1984;12:95-104.
 - 45 Pui C-H, Behm FG, Raimondi SC, et al. Secondary acute myeloid leukemia in children treated for acute lymphoid leukemia. *N Engl J Med* 1989;321:136-42.
 - 46 Nave T, Kiyoi H, Yamanaka K, Naito K, Yamada K. A case of cALL relapse after allogeneic BMT; recurrence of recipient cell origin, initially determined as being that of donor cell origin by sex chromosome analysis. *Br J Haematol* 1989;73:420-2.