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*Recent Advances in the Cytobiology of Leukemias**

DEFINITION OF ACUTE BIPHENOTYPIC LEUKEMIA

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ABSTRACT

Background and Objective. A minority of acute leukemias have features characteristic of both the myeloid and lymphoid lineages and for this reason are designated mixed-lineage, hybrid or biphenotypic acute leukemias (BAL). There have been difficulties in establishing whether BAL represents a distinct clinico-biological entity due to a lack of objective criteria for distinguishing BAL from acute myeloid leukemias (AML) or acute lymphoblastic leukemias (ALL) with aberrant expression of a marker from another lineage. In this work we analyze diagnostic criteria for BAL.

Methods. We describe the features of 26 patients (19 adults and 7 children) with BAL diagnosed at the Royal Marsden Hospital. BAL was defined according to a scoring system devised by our group and the *European Group for the Immunological Classification of Leukemia* (EGIL). This system is based on the number and degree of specificity of the markers (lymphoid and myeloid) expressed by the blasts.

Results. According to the FAB criteria, BAL may present as "ALL" or as one of the "AML" subtypes, often M1. It is not infrequent to identify two distinct blast populations: one of small size resembling lymphoblasts and the other larger. The most common immunophenotype is coexpression of B-lymphoid and myeloid markers and less frequently, T-lymphoid and myeloid markers. Cases with a B and T lymphoid phenotype or with trilineage differentiation are rare. BAL has a high incidence of clonal chromosomal abnormalities, the most common being the t(9;22)(q34;q11) (Ph chromosome) and structural abnormalities involving 11q23. Data are emerging that BAL has a negative prognosis in both children and adults and this may be related to the underlying chromosome abnormalities.

Interpretation and Conclusions. In summary, BAL is an uncommon type of leukemia which probably arises from a multipotent progenitor cell and carries a poor prognosis. Although there are no uniform criteria about whether to treat these patients as ALL or AML, it is likely that an intensive approach with high-dose therapy followed by bone marrow transplantation will be required to eradicate the disease permanently.
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Key words: leukemia, immunophenotype, stem cell

Most cases of acute leukemia can be classified as myeloid or lymphoid by standard light microscopy morphology and cytochemistry and a comprehensive panel of immunological markers which detect antigens in the membrane or cytoplasm of myeloid and B and T lymphoid cells. There are, however, some cases (around 5%) that remain difficult to classify with this technique. This is due to the co-expression of several myeloid and lymphoid antigens in the same cells. These cases have been designated biphenotypic or mixed-lineage acute leukemias. Because there are no widely accepted criteria for defining such leukemias, there are difficulties in establishing whether they represent a distinct clinical and biological entity. The criteria for defining acute biphenotypic leukemias formulated by our group, and the *European Group for the Immunological Classification of Leukemias* (EGIL) proposals[1-4] will be described and the morphological, cytochemical, immunological and cytogenetic features of cases studied at the Royal Marsden Hospital will be presented.

Criteria for defining biphenotypic acute leukemia and description of a proposed scoring system

Biphenotypic acute leukemias have been described in the literature as mixed lineage or hybrid acute leukemia, myeloid antigen-positive acute lymphoblastic leukemia (My+ ALL) and lymphoid antigen-positive acute myeloid leukemia (Ly+ AML). It is likely that the cases referred to as My+ALL and Ly+ ALL include a miscellaneous group

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that encompasses true biphenotypic leukemias as well as others which are ALL or AML with atypical expression of a single marker from another lineage. Cases described as phenotypic switch probably represent examples of biphenotypic acute leukemias originating from a primitive stem cell with the potential to differentiate along the lymphoid or myeloid lineage, with the pathway taken being determined by the therapy used.

We have proposed a scoring system aimed at distinguishing cases of *bona fide* biphenotypic acute leukemia from those with aberrant expression of a marker from another lineage, eg. Ly+AML and My+ALL. This system is based on the number and degree of specificity of the markers (lymphoid and myeloid) expressed by the leukemic cells.[2] Table 1 shows the markers regarded as most specific as follows: i) *for the B-lymphoid lineage,* CD79a (mb-1) detects a transmembrane protein linked to the immunoglobulin which constitutes part of the receptor for antigen recognition in B lymphocytes, cytoplasmic immunoglobulin and CD22; ii) *for the T-lymphoid lineage*, CD3 linked to the T cell receptor complex and expressed in the cytoplasm early in T-cell development; iii) *for the myeloid lineage*, myeloperoxidase (MPO) detected either by conventional cytochemistry or with a monoclonal antibody against the α-chain of MPO and the proenzyme form. According to this scoring system, a case is considered biphenotypic when point values are greater than two for the myeloid and one of the lymphoid lineages. Cases involving both lymphoid lineages (B and T) are very rare; cases with markers for the three lineages (triphenotypic) are also rare, but we have documented one such case. It is likely that in the future other markers with a high degree of lineage specificity will be incorporated into the scoring system, for instance markers recognizing the α/β and γ/δ chains of the T cell receptor for the T-lymphoid lineage or CD117 (c-kit), which seems to be specific for myeloid cells. Some of these markers are currently being evaluated by the EGIL group.

Table 1. Scoring system for the definition of acute biphenotypic leukemias. Biphenotypic acute leukemia is established when the score from two separate lineages is greater than 2.

Scoring points	*Lineages*		
	B-lymphoid	*T-lymphoid*	*Myeloid*
2	CD79a (mb-1) CD22 cyt IgM	CD3	MPO*
1	CD19 CD10	CD2 CD5	CD13 CD33
0.5	TdT	TdT CD7	CD14, CD15 CD11b,CD11c

** MPO demonstrated by cytochemical or immunological methods.*

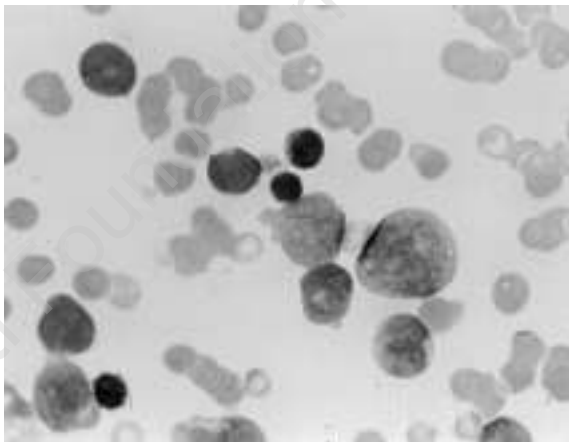

Figure 1. Blast cells from a patient with acute biphenotypic leukemia.

Morphology and cytochemistry

In one third of the cases, the blasts resemble lymphoblasts (L1 or L2 morphology). Cytochemical staining with Sudan black B (SBB), MPO and α-naphthyl esterase (ANAE) are negative (< 3%+ blasts). The remaining cases can be classified as AML on the basis of standard morphology and cytochemistry because they show more than 3% blasts positive with SBB or MPO, or display a pattern of ANAE activity typical of monoblasts (diffuse, strong and sensitive to NaF). According to FAB criteria most of the latter cases are either M1 or M2 or, rarely, M4 and M5. We have not yet seen a case with biphenotypic features which corresponds to the M3, M6 or M7 subtypes. It is not unusual to identify two distinct blast populations in the same patient, one of small size with a high nucleus/cytoplasm ratio resembling lymphoblasts and the other larger with more abundant cytoplasm with or without granulation (Figure 1). Despite these distinct morphological features, there is nearly always a clear overlap in the expression of lymphoid and myeloid markers in both types of cells.

Immunological markers

Diagnosis of biphenotypic acute leukemia is based on immunophenotyping. According to the lymphoid and myeloid markers expressed by the blasts, four groups can be distinguished. The most common are those in which the blasts coexpress myeloid and B-lymphoid, less often T-lymphoid antigens. Trilineage differentiation with expression of B, T and myeloid markers is rare and coexistence

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of blasts expressing only B and T cell markers is very uncommon. Most cases are terminal deoxynucleotidyl transferase (TdT) positive and express early hemopoietic markers such as CD34 and class-II HLA-DR determinants.

In cases of biphenotypic acute leukemia classified as ALL by light microscopy morphology and cytochemistry (SBB–, MPO–), MPO activity can be demonstrated using sensitive techniques on unfixed cells at the ultrastructural level[3] or with the anti-MPO monoclonal antibody.[5] These findings support the myeloid commitment of the blasts. The fact that MPO activity cannot be detected by light microscopy cytochemistry is related to the small amount of this enzyme, which is only seen by electron microscopy in very small granules and in cell membranes. Alternatively, it is possible that the blasts contain the enzyme as a proenzyme form, which nevertheless is detectable with the monoclonal antibody.

In cases of biphenotypic acute leukemia presenting with morphological and cytochemical features of AML, there is a high incidence (c.50%) of rearrangement of the Ig-heavy chain gene and/or T cell receptor chain genes, further confirming the lymphoid commitment of the blasts at a genomic level (simultaneously with the myeloid features)[2]

Cytogenetics

There is no single chromosomal abnormality which is uniquely associated with biphenotypic acute leukemia. Our own data and those of others demonstrate that structural chromosome abnormalities are frequent and that there is a high incidence of the Philadelphia chromosome t(9;22),[6] seen in one third of the cases, rearrangements involving 11q23 and complex cytogenetic abnormalities.[7]

Clinical features

Biphenotypic acute leukemias may affect adults or children, particularly infants under 2 years old. They may present as de novo or, rarely, they become apparent during a relapse following anti-AML or ALL therapy. The WBC is often high and most cases have a varying proportion of circulating blasts.[8] There are no uniform criteria about whether to treat these cases as ALL or AML when they are diagnosed only by standard morphology and cytochemistry, or whether to use an approach which combines drugs that are effective for ALL and AML,[9] followed by bone marrow or mobilized peripheral stem cell transplantation in complete remission.[10] Extensive data on response to therapy and clinical outcome are not available; however, our impression based on cases treated at the RMH and from single cases reported in the literature is that of a poor outcome in both children and adults. This may be related to the underlying chromosome abnormality.

Conclusions

In summary, biphenotypic acute leukemia is an uncommon type of leukemia which probably arises in a multipotent progenitor cell with the capability of differentiating along both myeloid and lymphoid lineages. This is supported by: i) immunological, cytochemical and molecular involvement of genes and/or protein products present in lymphoid and myeloid cells; ii) cytogenetic findings such as the presence of the Philadelphia chromosome or the MLL gene at 11q23, and iii) the documented phenomenon of *in vivo* and *in vitro* phenotypic switch in some of the cases. Data are emerging that biphenotypic acute leukemia has a poor prognosis and thus is likely to require a more intensive treatment approach to achieve long-term complete remissions, probably obtainable only with high-dose therapy followed by an allo- or autograft, which may eradicate the disease permanently.

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