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Advances In The Classification Of Acute Leukemia In Children

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Recent therapeutic advances have led to an improved outlook for children with acute lymphoblastic leukemia (ALL), the most common form of leukemia in children (Table 1). The development of effective combination chemotherapy regimens for both induction and maintenance of remission, the specific treatment of the central nervous system leukemic sanctuary early in remission and improvements in supportive care have resulted in a current disease-free, complete remission rate of greater than three years for approximately 50% of children with ALL.[1]

Further refinements or improvements in therapy will occur as the result of one or more of several different approaches. First, further investigation into the etiology of leukemia could lead to identification of the cause of this illness and ultimately to the means to prevent it. Second, improved treatment of all groups of acute leukemia might be accomplished by the development of new chemotherapeutic agents or new adjuvant measures such as immunotherapy. Such measures are being tested in ongoing clinical trials. Third, improvement in characterization and classification of subtypes of acute leukemia will lead to recognition of those subtypes which are very responsive to current therapeutic modalities and identification of types resistant to current treatment for which new forms of therapy will need to be developed.

An example of the latter has been the subclassification of ALL in children according to cell surface markers. It had previously been recognized that children with ALL who had clinical features such as age less than 2 years or greater than 10 years, over 50,000/mm^3 circulating lymphoblasts in the peripheral blood or a mediastinal mass tended to have a worse response to therapy than children who did not have these parameters.[2] After T-lymphocytes were identified by their ability to form spontaneous rosettes with sheep red blood cells (E-rosettes) and B-lymphocytes by the presence of surface immunoglobulin (SIg) or by the formation of rosettes with antibody coated sheep red blood cells (EAC rosettes), the malignant lymphoblasts from children with ALL were studied by these techniques.[3,4,5] The majority of children with ALL have lymphoblasts that are predominantly "null cell"; i.e. they have neither T or B surface markers detectable by the methods described above (Table II). Approximately 25% of children with ALL will have the majority of their blast cells form E-rosettes (Figure 1) which indicates that they have T-cell disease and a very small percentage have B-cell leukemia. The clinical importance of these observations is that children with "null cell" disease have a much more favorable response to current therapy regimens as compared to those who have either T-cell or B-cell surface markers.[4] Children with T-cell leukemia also tend to have the clinical features such as a mediastinal mass and elevated white count that are associated with a poor prognosis. It is thus reasonable to treat children with "null cell" ALL with current regimens that are effective in the majority of patients and to direct newer forms of therapy to those children whose ALL is less responsive as determined by clinical presentation and/or on the basis of detectable cell surface markers.

TABLE 1

Type of Leukemia	Percentage of Cases
1. Acute lymphoblastic	80
2. Acute myelogenous	15
3. Acute monoblastic	2
4. Chronic myelogenous	3
5. Chronic lymphocytic	virtually none

Legend: Classification of leukemia in children less than 18 years of age by standard morphological and histochemical criteria.

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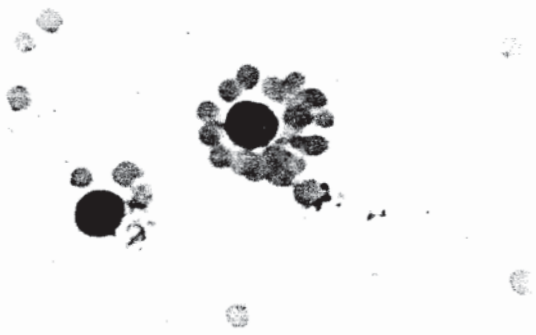

FIGURE 1
T-cell acute lymphoblastic leukemia. The malignant blasts form spontaneous rosettes with sheep red blood cells (E-rosettes).

Another new approach is to classify ALL according to the presence or absence of a biochemical marker. The enzyme terminal deoxynucleotidyl transferase (terminal transferase) is a DNA polymerase which is normally present in the thymus[6] and at lower levels in normal bone marrow.[7] Levels of terminal transferase are not measurable by current methods in normal peripheral blood lymphocytes.[7] However, approximately 95% of children with acute lymphoblastic leukemia have markedly elevated levels of terminal transferase in their circulating blast cells and in the bone marrow during initial presentation and relapse of their disease.[8,9] Elevated levels of terminal transferase are rare in children with acute myelogenous or monoblastic leukemia.

TABLE II
CLASSIFICATION OF CHILDHOOD ALL BY CELL SURFACE MARKERS

Type of Leukemia	Percentage of Cases
1. Null cell (no surface markers)	75
2. T-cell	20-25
3. B-cell	0-5

Terminal transferase has also been found in about 30% of cases of chronic myeologenous leukemia (CML) in blastic crisis.[10] This suggests that CML is a pluripotent disease that may have a lymphoblastic component. Also patients with CML who have detectable terminal transferase are more responsive to chemotherapeutic agents such as vincristine and prednisone which are effective against lymphoblastic malignancies. The assay of terminal transferase is thus useful for both identification of active lymphoblastic malignancies and also for possibly selecting those leukemias which would be responsive to treatment with vincristine and prednisone.

Prednisone responsiveness of ALL has also been determined by the direct measurement of steroid receptors within leukemic cells.[11] Those children with ALL who have a high level of glucocorticoid receptors within their leukemic blast cells have a better response to treatment as compared to those patients who have low levels of glucocorticoid receptors. This biochemical technique also appears to be very promising in its ability to define subclasses of ALL which would be more responsive to therapy.

Newer cytogenetic techniques such as Giemsa banding have also been applied to the study of acute leukemia. Although the banding of bone marrow chromosomes from patients with acute leukemia still poses some technical problems,[12] progress has been made in the area of chromosomal abnormalities associated with acute leukemia. Approximately 50% of patients with acute leukemia have a detectable karyotypic abnormality.[13,14] Although a wide variety of different chromosomal patterns have been noted, the chromosomal changes appear to be non-random in certain specific instances. The classical non-random abnormality is the presence of the Philadelphia chromosome in patients with the adult form of chronic myelogenous leukemia. Recently it has been observed that blasts in acute promyelocytic leukemia have a translocation of the long arms of chromosome 17 to chromosome 15 and also that in acute myelogenous leukemia in adults trisomy 8 is frequently present in the leukemic cell population.[14] Therefore, just as the Philadelphia chromosome has assisted in the classification of chronic myelogenous leukemia, chromosomal changes can also identify certain sub-groups of acute leukemia.

Cytogenetic studies have also provided evidence of the clonal nature of acute leukemia in that the same chromosomal abnormality which is present at the time of initial presentation disappears during remission and reappears when there is relapse of the disease. Studies of chromosomal changes in second malignancies which occur after treatment of the original malignancy will also help to define those malignancies which are new as compared to those which are evolutions of the original cancer. In addition, information as to the effects of cancer treatment with chemotherapy or radiation therapy on specific chromosomes will also be obtained.

Another intriguing speculation as to the role of cytogenetic abnormalities in acute leukemia has been the preliminary observation that the response to therapy in adults with acute myelogenous leukemia is worse in that group of patients who have the greatest percentage of detectable cytogenetically abnormal cells in their bone marrow.[13] Further studies will be required to define the usefulness of cytogenetic determinations in determining the potential response to treatment in children with acute leukemia.

A rare form of acute leukemia, acute monoblastic leukemia, has been better defined by its characteristic histochemical staining pattern.[15] The cytochemical staining patterns of acute lymphoblastic leukemia, acute myelogenous leukemia and acute monoblastic leukemia are outlined in Table III. Use of this cytochemical profile has led to a more accurate classification of acute leukemia.[16] The Periodic Acid Schiff (PAS) reaction may be weakly positive in some cases of acute myelogenous or acute monoblastic leukemia, however acute lympho-

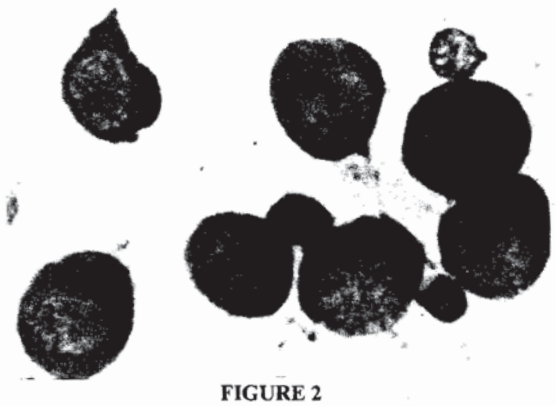

FIGURE 2

Acute monoblastic leukemia. The blast cells show markedly positive staining with alpha naphthol esterase. This stain is a deep red-orange when positive.

TABLE III

CYTOCHEMICAL STAINING OF ACUTE LEUKEMIA

Stain	Lymphoblastic	Myelogenous	Monoblastic
1. PAS	+	±	±
2. Sudan Black B/Peroxidase	-	+	-
3. Specific esterase (chloroacetate esterase)	-	+	-
4. Non-specific esterase (α naphthol butyrate esterase)	-	−	+

blastic leukemia is characterized by its marked block positivity in the PAS stain along with negative Sudan Black and esterase stains. The pattern of reaction in acute myelogenous leukemia is markedly positive staining with Sudan Black B, peroxidase and chloroacetate (specific) esterase, while the α naphthol butyrate esterase is weakly positive or negative. Due to FDA restrictions on a carcinogenic reagent (benzidine) used in the peroxidase test, we no longer use this stain as identical information can be obtained from the pattern of staining with Sudan Black B.

Blast cells from acute monoblastic leukemia stained with Wright's stain tend to appear relatively uniform in size with diameter of 18 to 25 microns and have abundant blue-gray cytoplasm. Fine azurophilic granulation may be present in the cytoplasm. These blasts are very undifferentiated in appearance and have been confused with both myeloblasts and lymphoblasts. The characteristic cytochemical staining is a strongly positive reaction to α naphthol butyrate (non-specific) esterase in acute monoblastic leukemia (Figure 2 while in contrast to AML the Sudan Black stain tends to be weakly positive or negative. The identification and recognition of acute monoblastic leukemia is important as patients with this disorder tend to have a different response to therapy compared to children with ALL or AML.

Myeloid precursor cells, stimulated by colony stimulating factor (CSF), will form in-vitro colonies (CFU-C) in semisolid media such as agar or methylcellulose. This technique has been applied to the study of bone marrow cells in acute leukemia by several investigators.[17,18,19]

TABLE IV

BONE MARROW TESTS IN THE CLASSIFICATION OF ACUTE LEUKEMIA IN CHILDREN

I. Standard Techniques
  1) Histochemistry
    a) Wright's stain
    b) Special stains (PAS, Sudan Black, esterase)
  2) Surface markers in ALL (E-rosette, EAC rosette)

II. Investigational Techniques
  1) Terminal transferase
  2) Steroid receptors
  3) Cytogenetics
  4) In-vitro colony growth

Technical differences in the culture systems make comparisons between the results of different laboratories very difficult, but one recent study of acute myelogenous leukemia in adults utilizing an agar culture system has defined three groups of AML on the basis of cell growth and colony formation in the in-vitro system. Those patients who had an extensive in-vitro proliferation of colonies and large aggregates had a much worse response to the chemotherapeutic regimen used as compared to patients whose in-vitro colony formation was less extensive.[19] Additional investigations are necessary, including an evaluation of this technique in children, to confirm these findings. The evidence from in-vitro culture experiments such as the above and from studies of the cellular kinetics of acute myelogenous leukemia indicate that AML is not a homogenous entity and that there are certain subtypes of AML which have differing responses to therapy.

SUMMARY:

A bone marrow examination remains necessary for the establishment of the diagnosis of acute leukemia. Advances in morphology and histochemical staining of the leukemic blast cells within the marrow has been helpful in categorizing acute leukemia, particularly in defining the rare type of leukemia now recognized as acute monoblastic leukemia. However the ability to define specific subgroups such as T-cell ALL within the major categories of acute leukemia by morphological and histochemical criteria alone remains limited. Recent investigations into the functional aspects utilizing cell surface markers, biochemical markers and cell culture techniques have been developed as supplements to standard morphology (Table IV). The determination of cell surface markers in children with acute lymphoblastic leukemia to define T-cell disease should be considered standard procedure. Other techniques remain investigational, and two of the authors (JJH and FLM) are continuing to pursue the prognostic significance of terminal transferase determinations in children with leukemia. Cytogenetic analysis of the blast cell population has also provided further information about subgroups of acute leukemia. These new techniques discussed in this review have been useful in defining subgroups of children with ALL who are more responsive to current therapy, and have provided suggestive evidence that acute myelogenous leukemia can also be divided into subgroups with different therapeutic responses.

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