Clinical Significance of CD34 Expression in Childhood Acute Lymphoblastic Leukemia

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The CD34 antigen was detected on ≤10% of the blast cells in 235 (70%) of 335 cases of newly diagnosed childhood acute lymphoblastic leukemia (ALL) treated in two consecutive chemotherapy trials. By immunophenotype, the distribution of positive cases favored early pre-B ALL (83%; n = 180) followed by pre-B ALL (61%; n = 88) and then T-cell ALL (48%; n = 61) (P < .001). Among the B-lineage cases, CD34 expression was significantly associated with favorable presenting features: age 1 to 10 years, white race, absence of central nervous system (CNS) leukemia, low serum lactate dehydrogenase level, CD10 expression, and leukemic cell hyperdiploidy (≥50 chromosomes or DNA index ≥1.16). Event-free survival was clearly superior for patients with CD34+ leukemia (P = .01), with an estimated 83% ± 6% (SE) of the cohort remaining free of adverse events at 5 years post diagnosis, as compared to 63% ± 10% of the group without this feature. Multivariate analysis showed that the prognostic influence of the antigen was independent of age, leukocyte count, and other well-recognized factors, suggesting that it would add discriminatory power to current systems of risk assignment.

Findings in T-cell ALL were the reverse: CD34 expression showed positive correlations with initial CNS leukemia and CD10 negativity but not with any good-risk presenting characteristics. Log-rank analysis indicated no adverse effect on treatment outcome by CD34 antigen expression, although additional patients will need to be studied to obtain a definitive answer. The opposed clinical associations of CD34 expression in B- and T-lineage ALL may reflect fundamental biologic differences between these leukemia species.

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CD34, a 110- to 115-Kd transmembrane sialoglycoprotein, is expressed by early hematopoietic (myeloid and lymphoid) progenitor cells,1,4 endothelial cells,5,7 murine embryonic fibroblasts,8 and bone marrow (BM) stromal cells and their precursors.9 Found on 1% to 5% of normal BM cells,1,3,10 the CD34+ population contains virtually all of the hematopoietic colony-forming cells,1,4,11 Levels of CD34 expression are highest on the most immature hematopoietic progenitors and decrease progressively with cell maturation.1,11 Parallelling the hierarchy of CD34 expression on normal hematopoietic cells, a large percentage of acute leukemias are CD34+, whereas the chronic leukemias, which involve more mature cell types, are uniformly CD34−.1,2,10,14

The clinical significance of CD34 expression has been studied extensively in acute myeloid leukemia (AML), where the antigen is found on leukemic cells in 40% to 60% of both adult1,5-22 and childhood23-24 cases. In adult AML, CD34 expression has been correlated with a higher frequency of French-American-British (FAB) M1 or M2 morphology, secondary leukemia and karyotypic abnormalities involving chromosome 5 or 7, and with a lower remission-induction rate.10,15,22 Although CD34 expression was negatively correlated with age younger than 2 years at diagnosis and FAB M4/M5 morphology,23 it had no prognostic impact in two childhood AML studies.21,22 Evaluation of the clinical relevance of CD34 expression in acute lymphoblastic leukemia (ALL) is limited to a Pediatric Oncology Group (POG) study of B-lineage cases in children older than 1 year,25 in which CD34 expression was associated with hyperdiploidy greater than 50 chromosomes, a lower frequency of initial central nervous system (CNS) leukemia, and a favorable prognosis. Our intent in this study is to establish the prevalence of CD34 expression in childhood ALL overall and to relate its presence to initial clinical and biologic features and treatment outcome.

MATERIALS AND METHODS

From November 1986 to December 1991, 360 consecutive children with newly diagnosed ALL were admitted to St Jude Children's Research Hospital. Three hundred thirty-five of the patients (93%) had complete leukemia immunophenotyping, including evaluation of CD34 expression. All patients were enrolled in two successive total therapy studies (XI and XII).16,26 In both trials, induction treatment consisted of prednisone, vincristine, daunorubicin, asparaginase, teniposide, and cytarabine. On completion of consolidation therapy with high-dose methotrexate, patients in study XI26 received continuation treatment with four pairs of drugs: etoposide plus cyclophosphamide, 6-mercaptopurine plus methotrexate, teniposide plus cytarabine, and prednisone plus vincristine, whereas those in study XII27 received 6-mercaptopurine and methotrexate with pulses of high-dose methotrexate or teniposide plus cytarabine. At the time of analysis, the median follow-up in study XI was 5.3 years (range, 4.3 to 6.3 years), while that in study XII was 2.7 years (range, 1.2 to 4.3 years). Both trials were approved by the St Jude institutional review board and the National Cancer Institute. Signed informed consent was obtained from the participating patients and their guardians.

Morphologic and cytochemical studies. BM cells were stained by standard methods, including the use of Wright-Giemsa, periodic acid-Schiff, myeloperoxidase, Sudan black B, naphthol AS-D chloroacetate esterase, and α-naphthyl butyrate esterase. The diagnosis of ALL was based on morphologic and cytochemical criteria of the FAB Working Group.28 Thus, by definition, all patients had...
less than 3% blast cells positive for myeloperoxidase and Sudan black B (myeloid pattern), and less than 20% positive for α-naphthyl butyrate esterase (myeloid pattern); none of the cells contained Auer rods.

**Immunophenotyping.** BM cells were separated on a Ficoll-sodium metrizoate gradient (Pharmaca, Uppsala, Sweden; Nycomed, Oslo, Norway); only samples containing greater than 90% blasts were assayed. Cell-surface antigens were detected by a standard indirect immunofluorescence assay using a panel of monoclonal antibodies (MoAbs) representative of cluster groups (CD) described at the International Workshops on Human Leukocyte Differentiation Antigens. They included HLA-DR, CD1a (Leu 6), CD2 (Leu 5b), CD3 (Leu 4), CD4 (Leu 3a), CD5 (Leu 1), CD7 (Leu 9), CD8 (Leu 2a), CD10 (J5), CD11b (Mo1), CD13 (MY7), CD14 (MY4 and Leu M3), CD15 (MY1), CD19 (Leu 12), CD20 (Leu 16), CD21 (anti-CD2), CD22 (Leu 14), CD33 (MY9), CD34 (HPCA-1/ MY10), CD36 (5F1), CD41 (α-gpIIb/IIIa), and CD45 (HLe-1/2D1). In all experiments, isotypically matched murine myeloma Ig and anti-α, microglobulin were used as negative and positive controls, respectively, at the same protein concentrations as the test antibodies. Blast cells were also tested for surface (slg) and cytoplasmic (clg) Iggs with goat antihuman μ (Southern Biotechnology, Birmingham, AL). An indirect immunofluorescence technique was used to detect nuclear terminal deoxynucleotidyl transferase (TdT).

Fluorescence activity was analyzed with an EPICS C flow cytometer equipped with a 5-watt coherent laser (Coulter, Hialeah, FL) for MoAb studies or with a Zeiss epifluorescent microscope (Zeiss, Oberkochen, Germany) for clg, slg, and TdT analyses. Histograms of fluorescence intensity were based on a linear scale. The percentage of positive cells was determined by enumerating the events in which fluorescence intensity was greater than the isotype-matched negative control. Results were considered positive if ≥20% of the cells reacted with a particular MoAb or if ≤10% were positive for CD34, clg, and TdT. The results of CD34 assays in B-lineage ALL were corrected for contaminating normal T cells in the test sample by the formula [ %CD34/(100–%CD3)]. Because all samples of T-lineage ALL contained greater than 90% blasts, no correction for contaminating normal hematopoietic cells was made. Depending on the pattern of reactivity, lymphoblasts were classified as T (CD7+ plus CD5+ or CD2+), early pre-B (CD19+, CD22+, HLA-DR+, CD7+, CD5+, clg+, slg+, and CD10+)/pre-B (clg+), or B (slg+). Non–T-cell cases for which expression of clg was not tested were designated “B-cell lineage”, and presumably included cases of early pre-B and pre-B ALL.

**Cytogenetic and DNA flow cytometric studies.** Bone marrow samples were prepared by the method of Williams et al. Meta-phases were G-banded by treatment with trypsin and studied with Wright stain; chromosomal abnormalities were classified according to the International System for Human Cytogenetic Nomenclature. Leukemic marrow samples were also stained with propidium iodide and analyzed for cellular DNA content by flow cytometry, as previously described. Results were expressed as a DNA index (ratio of DNA content in leukemic v normal G0/G1 cells), a measure that correlates closely with chromosome number. Differences in the distribution of clinical and biologic features among subgroups of patients were analyzed by the two-tailed Fisher exact test. Life-table estimates of event-free survival (EFS) were derived by the method of Kaplan and Meier and compared with the log-rank test. Early death or failure to enter remission was considered an event at zero time. The EFS experience of patients in study XII was not analyzed separately because of the inadequate follow-up of this group (median, 2.7 years). The influence of potential prognostic factors on EFS was estimated with the Cox proportional hazard model. The variables tested included age, leukocyte count, sex, race, liver and spleen size, CNS leukemia, mediastinal mass, FAB type, leukemic cell ploidy and DNA index, chromosomal translocation, CD10 expression, and serum lactate dehydrogenase level. Each factor was first tested as a single regressor variable in the Cox model (univariate analysis). A stepwise multivariate regression approach was then used to identify the most important predictor variables with respect to EFS. A P value of ≤.10, after adjustment for the effects of other variables, was required for retention in the model. The relative risk of failure was calculated with the coefficient and standard error from the Cox analysis; the P value was from the likelihood-ratio test. All analyses reflect follow-up observations through January 22, 1993.

**RESULTS**

CD34 antigen was expressed on ≥10% of blast cells in 235 (70%) of the 335 cases tested. The frequency of CD34 expression was highest (83%) in the 180 early pre-B cases, intermediate (61%) in the 89 pre-B cases, and lowest (46%) in the 61 T-cell cases (P<.001). Within the T-cell group, there was no clear indication of loss of the antigen with advancing thymocyte maturity: early stage (CD1+/CD3−), 9 of 13 (69%) CD34+; intermediate stage (CD1+/CD3−), 3 of 17 (18%), and late stage (CD3+), 16 of 31 (52%).

Among B-lineage cases, expression of the antigen was significantly associated with several favorable presenting features: age of 1 to 10 years, white race, absence of CNS leukemia, low serum lactate dehydrogenase level, leukemic cell CD10 expression, hyperdiploidy greater than 50 chromosomes, and DNA index ≥1.16 (Table 1). No significant correlation was found between CD34 antigen expression

| Table 1. Presenting Features According to CD34 Expression in Children With B-Lineage or T-Cell ALL |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|
| Feature                          | CD34+ (n = 67) | CD34+ (n = 207) | P Value         |
| Age (yr)                         | 8               | 9               | <.01            |
| ≤1                              | 51              | 173             | .17             |
| 1-10                            | 16              | 34              | .20             |
| Race                            | 56              | 195             | <.01            |
| White                           | 11              | 12              | .33             |
| CNS leukemia present            | 58              | 199             | <.01            |
| Absent                          | 9               | 8               | <.01            |
| Serum LDH (U/L)                 | 19              | 100             | <.01            |
| ≤400                            | 44              | 103             | .84             |
| ≥400                            | 28              | 24              | .84             |
| CD10 expression                 | 60              | 200             | <.01            |
| Positive                        | 7               | 7               | .02             |
| Negative                        | 11              | 8               | .89             |
| Chromosome ploidy               | 54              | 117             | .32             |
| ≥50                             | 10              | 67              | <.01            |
| ≤1.16                           | 1.16            | 32              | .35             |

Data missing in some categories. Abbreviation: LDH, lactate dehydrogenase.
and sex, FAB type, chromosomal translocation, and liver and spleen size. By contrast, in T-cell ALL, CD34 antigen expression was associated with the presence of CNS leukemia and a lack of CD10 expression, both unfavorable prognostic factors. It did not correlate with other presenting characteristics.

In B-lineage ALL, CD34+ cases were less likely than CD34- cases to have a pre-B phenotype (clg+) but were more likely to express CD22 (P < .01, Table 2); CD34+ cases also had a higher frequency of myeloid-associated antigen (CD33) positivity. In T-cell ALL, there was a negative association between CD34 and CD1 expression; CD34- cases were more likely to express CD13, a myeloid-associated marker. CD34 expression was not significantly related to expression of other lymphoid- or myeloid-associated antigens in either B-lineage or T-cell ALL.

CD34 expression was a favorable prognostic sign in B-lineage ALL (Fig 1A). For the 88 CD34+ cases treated in study XI, the 5-year EFS estimate (±SE) was 83% ± 6%, contrasted with 63% ± 10% for the 41 CD34- cases (P = .01). The lack of CD34 expression in these B-lineage cases showed independent adverse prognostic influence in a multivariate analysis after adjustment for competing covariates (relative risk of failure, 2.2; 95% confidence interval, 1.0 to 4.8; P = .06). Among T-cell cases treated in study XI, the 5-year EFS estimate was 42% ± 23% for the 12 CD34+ cases and 55% ± 15% for the 22 CD34- cases (Fig 2A, P = .5). The above findings were essentially unchanged when survival analyses included patients from study XII (Figs 1B and 2B).

**DISCUSSION**

In this study of 335 children with newly diagnosed ALL, 235 (70%) had blast cells that expressed the CD34 surface antigen. A threshold of 10% was selected to identify positive cases because it marked the nadir of the distribution curve for percentages of positive cells overall. This criterion also yielded the most significant differences between presenting clinical or laboratory features and treatment outcome. Others have used the same rationale in choosing the cut point to define CD34 positivity.17,21,35

**Table 2. Antigen Expression (%) According to CD34 Reactivity in Children With B-Lineage or T-Cell ALL**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CD34+</th>
<th>CD34-</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>CD10*</td>
<td>90</td>
<td>97</td>
<td>.02</td>
</tr>
<tr>
<td>CD19*</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>48</td>
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<td>.83</td>
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<tr>
<td>clg*</td>
<td>53</td>
<td>27</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>CD33*</td>
<td>2</td>
<td>9</td>
<td>.04</td>
</tr>
</tbody>
</table>

Data missing in some categories; eg, clg not determined in five cases.

**Fig 1.** Comparison of EFS durations according to CD34 expression in patients with B-lineage ALL treated in St Jude Study XI (A) or in studies XI and XII (B). Significantly worse treatment results are evident for CD34+ cases. The bars represent one SE of the mean.

**Fig 2.** Comparison of EFS duration according to CD34 expression in patients with T-cell ALL treated in St Jude Study XI (A) or in studies XI and XII (B). Outcome was not affected by the presence of the marker. The bars represent one SE of the mean.
In the earlier Pediatric Oncology Group series, the CD34 antigen was detected on at least 10% of the blast cells from 74% of children over 1 year of age with newly diagnosed B-lineage ALL, as compared with 77% of those in the present study. As in our analysis, the presence of this marker was correlated positively with CD22 expression but negatively with cfl (pre-B phenotype). By contrast, we did not find any association between CD34 and CD20 expression: 42% of the CD34+ cases expressed the CD20 antigen, a proportion not significantly different from the 49% of CD34+ cases. In normal B-lymphoid cell differentiation, the CD34 molecule is expressed on very early progenitor cells, whereas CD20 and CD22 appear during the intermediate stage of development. The simultaneous expression of CD34 and an antigen that normally appears later in differentiation, such as CD20 or CD22, is common in B-lineage leukemia and has been termed ‘asynchronous expression’. Indeed, coexpression of CD34 and CD22 was found in 70% of the cases in our study and in 61% and 52% of cases in previous series. Using fluorescence-activated cell sorting and magnetic bead separation, Re et al detected CD34 mRNA only in the subpopulation of leukemic blast cells that lacked surface expression of CD20 and CD22. They suggested that the asynchronous expression of these molecules results from the persistence of CD34 on the cell surface rather than from simultaneous synthesis of CD34 and the late-stage differentiation antigen. Regardless of the mechanism of asynchronous expression, Hurwitz et al recently identified a rare subpopulation of normal marrow B-lymphoid cells that expressed both CD34 and CD22. Among the CD34+ cells in normal BM, they recognized 2.6% to 9.4% as CD22+ and 1.8% to 15.3% as CD20+. Thus, acute leukemias coexpressing CD34 and CD22 may represent the transformed counterparts of normal B-lymphoid cells with this unusual phenotype.

Of particular interest is the association between CD34 antigen expression in B-lineage childhood ALL and the presence of favorable presenting features: age between 1 to 10 years, white race, absence of CNS leukemia, low serum lactate dehydrogenase level, leukemic cell CD10 expression, and DNA index £ 1.16 (hyperdiploidy). Borowitz et al reported similar correlations, although they were unable to show a significant relation between CD34 expression and age at presentation or CD10 expression. Perhaps more important is the finding, in both studies, of independent favorable prognostic significance for CD34 expression in B-lineage ALL, despite the use of entirely different treatments. This suggests that the CD34 marker would be a worthwhile addition to the panels of features currently used to recognize patients who are candidates for relatively nontoxic therapies.

Little information has been available on the prevalence of CD34 antigen in T-cell ALL. Failure to detect the antigen in small series of cases suggested that its expression by malignant T lymphoblasts was either rare or absent. We report a relatively high frequency of CD34 expression in this ALL phenotype (46%), although it does not exceed the figure for B-lineage cases. The lack of negative correlation of CD34 expression with advancing developmental stage of T-cell ALL in this study suggests that the antigen may be under aberrant genetic control or perhaps functions in processes other than lymphopoiesis. In contrast to the findings in B-lineage ALL, CD34 expression was associated with unfavorable presenting features in T-cell ALL: lack of CD10 expression and initial CNS leukemia. The diametrically opposed relationships of CD34 expression in the T- and B-cell derived acute leukemias may reflect fundamental differences in pathophysiology. The apparent adverse prognostic influence of CD34 positivity in T-cell ALL did not achieve statistical significance in our analysis; however, the patient sample was too small for definitive comparison.

In summary, we have shown prognostic significance for CD34 expression in childhood B-lineage ALL and noted a suggestive association with poor-risk features in T-cell ALL. Although CD34 is thought to play a role in adhesive interactions, its precise function remains unknown. The availability of a cloned and sequenced human CD34 cDNA should open the way for productive studies of CD34 on normal as well as leukemic cells.

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