Numerous biochemical and immunologic markers of hematopoietic cells have been described that serve to delineate subclasses of lymphoid and myeloid leukemias. Identifying markers and classification schemes that convey clinically useful information with regard to prognosis and therapeutic responsiveness of disease is a difficult, but important task. Markers of leukemic cells are particularly valuable if they identify patients at diagnosis who will not respond to conventional therapy or if they can be measured during remission to predict relapse of disease before it is clinically obvious. We have focused our attention on one particular biochemical marker, terminal deoxynucleotidyl transferase or TdT (reviewed by Bollum). It has proved valuable in distinguishing acute lymphoid and myeloid leukemias. Because the approaches to therapy of lymphoid and myeloid leukemias differ, measurements of TdT do convey clinically useful information in differential diagnosis. There is also the possibility that TdT activity in blasts at the time of diagnosis of ALL conveys prognostic information because there is marked variation in activity from patient to patient that is not accounted for by variation in the relative percentage of leukemic blasts. Finally, TdT activity in ALL blasts is generally elevated at both diagnosis and relapse of disease. It seemed probable that quantitative variation in TdT activity in cells from peripheral blood and bone marrow during remission of TdT ALL might convey prognostic information with regard to relapse. We have measured TdT activity serially in cells from the bone marrow and peripheral blood of 164 children during all phases of treatment of ALL. Our data have permitted an assessment of the value of quantitative measurements of TdT activity in the clinical management of ALL. As an ancillary study, we also examined other factors as prognostic indicators in childhood ALL.

Materials and Methods

Patients

Mononuclear cells from the peripheral blood and bone marrow were examined in 169 children at the time of initial diagnosis of acute lymphoblastic leukemia and serially thereafter. The protocol for obtaining specimens was approved by the Human Studies Committee of the participating institutions. Diagnosis was based on morphological examination of leukemic blasts stained with Wright-Giemsa, periodic acid-Schiff, peroxidase, and Sudan black. Five children were excluded from our analyses, unless otherwise indicated, because their blasts contained surface immunoglobulin and they were not considered to have typical ALL. The remaining 164
children were considered to have typical childhood ALL (T and non-T, non-B marked) and constituted the primary study population. An additional 37 patients were studied serially for evidence of relapse, but blasts were not available for biochemical study at diagnosis. These children were excluded from analyses of prognostic factors at diagnosis, but were included in analyses of TdT activities during remission and at disease relapse. Patients were entered on study from January 1973 through December 1978. Follow-up data were obtained through September 1979. Children were diagnosed and treated in 5 different institutions. Patients in whom one or more of the following factors were present were considered high risk: age less than 2 yr or greater than 10 yr, mediastinal mass, peripheral leukocyte count greater than 50,000/μl, and central nervous system leukemia. Induction therapy consisted of vincristine, l-asparaginase, and prednisone in all patients. All patients received central nervous system prophylaxis consisting of cranial irradiation and/or intrathecal methotrexate. Multiagent maintenance chemotherapy was continued for 3 yr or until relapse occurred. Average and high-risk patients received more intensive maintenance regimens. There were changes in the treatment protocols during the 5 yr patients were entered on the study.

Data recorded at diagnosis for subsequent analysis of prognostic factors included: institution where treatment was given, treatment group, age, sex, race, peripheral leukocyte count, presence or absence of a mediastinal mass on chest x-ray, presence or absence of surface immunoglobulin on blasts, percent of blasts forming rosettes with sheep erythrocytes and TdT activity in mononuclear cells from bone marrow and peripheral blood. Follow-up data included: whether remission was achieved, duration of complete remission (time to relapse at any site), whether a second remission was achieved, survival, and TdT measurements on sequential specimens of bone marrow and peripheral blood.

Methods

Procedures for the isolation of cells, assay of TdT activity, measurement of E-rosettes, and detection of surface immunoglobulin have been described in detail.15 Peripheral blood and bone marrow were obtained from patients, mononuclear cells were isolated on Ficoll-Hypaque gradients, and surface markers were measured at the participating institutions. Cells with 3 or more adherent sheep erythrocytes were considered positive for E-rosette formation. More than 50% of blasts were required to form rosettes at 4°C to classify the cell as E+. Surface immunoglobulin was detected by direct immunofluorescence with fluorescein-conjugated antisera. An aliquot of mononuclear cells was frozen at −80°C and shipped to Dr. Coleman's laboratory for measurement of TdT activity. Activities are expressed in units per 10^6 nucleated cells, where 1 unit equals 1 nmole of dGTP polymerized onto oligo d(pA)₉₆ per hour. Data relating to markers and prognosis were not analyzed until the completion of the study and no therapeutic decisions were based on measurements of markers.

Data were encoded during the study, but statistical analyses were not carried out until entry of patients had ceased. Plots of the duration of remission and length of survival were produced using Kaplan-Meier product limit estimates.14 These survival and remission curves were tested for equality by Gehan's generalized Wilcoxon statistic for right-censored data.15 Baseline variables such as leukocyte count, age, and TdT activities were compared among institutions by one-way analyses of variance. The p values apply to the test for simultaneous equality of means of all groups, and a significant p value suggests that the mean of at least one group is different from the others. The Cox regression model for right-censored data was used to assess potential prognostic factors regarding duration of remission or length of survival.14 A stepwise version of this model was employed and the p values corresponding to variables reflect only the presence of some (even slight) relationship to remission or survival. They do not measure the strength of the relationship or its predictive value. The generalized likelihood ratios, λ, were used to estimate the order of importance of variables and to define their sequence of entry into the Cox regression. Factors prognostic of achievement or nonachievement of remission were determined by a stepwise version of the logistic regression model of Walker and Duncan.17 Interpretations of p values are similar to those described for the Cox method.

RESULTS

Characteristics of Patients

Analyses of variance showed no statistically significant differences (p > 0.1) among characteristics of patients in the various institutions. Consequently, data have been pooled across institutions for most analyses. The mean age of the 164 children entered on study was 7.6 ± 5.1 mo (6 yr, 4 mo), range 3 mo to 16 yr. Ninety-six percent of the patients were white. Complete remission of leukemia was obtained in 97% (159/164) of patients. Forty percent of the patients relapsed while on study. The median duration of complete remission has not been reached, but will be longer than 1300 days.

The surface membrane phenotype could be determined on 147 of the 164 patients. Of these, 8.8% (13/147) were E-SmIg− and 91.2% (134/147) were E-SmIg+. Surface marker phenotype was indeterminate on 10.4% (17/164). Of the 22 patients who had either an E− marker or a mediastinal mass (M), 6 were E− M+, 7 were E− M−, and 3 were M− E-indeterminate. Multiparameter analyses of blasts now in use define leukemic phenotypes more precisely than we have done.10,11 Under present circumstances, non-T, non-B ALL is defined by multiple markers as ALL+, la−, TdT+, HuTLA+, E−, SmIg−; putative thymocyte (Thy) leukemias are defined at HuTLA−, E+, TdT+, ALL−, le−, SmIg−. Measurement of E, TdT, and SmIg, as we have done, will misclassify approximately 28% of Thy-ALL as non-T, non-B ALL10 because of variant phenotypes of Thy-ALL that are E+, but have other distinctive thymocyte markers. By definition there is no E− variant of non-T, non-B ALL, so this type of leukemia will not be misclassified in our study.

The mean TdT activity in cells from bone marrow was 105 ± 150 U/10^6 cells and was similar in each institution. The large standard deviation reflects the wide range of individual values. A significant portion of the variation is caused by a relatively small number of patients with extremely high activities. The distribution of TdT activities in cells from the peripheral blood and bone marrow is illustrated in Fig. 1. One-hundred and two of the 164 patients had TdT activity measured in both types of cells at the time of diagnosis of ALL.
The remainder of the patients had TdT activity measured in either bone marrow or peripheral blood, but not both. TdT activities in bone marrow and peripheral blood do not follow a Gaussian distribution, but show a skew to the right (Fig. 1A) with a tail of high values. If we exclude the high outliers by setting the upper limit of values at 120 U/10⁸ cells, the distribution is as shown in Fig. 1B. There was a significant correlation between the TdT activities in peripheral blood and bone marrow when all pairs of measurements were considered (Fig. 1A, r = 0.52, p < 0.001), but this estimation of correlation is spuriously high because of the presence of pairs of unusually high values in a few patients. When high outliers were eliminated (Fig. 1B), the correlation coefficient dropped to an insignificant value (r = 0.05, p = 0.35). The mean TdT activity in bone marrow was 105 ± 150 U/10⁸ cells (n = 135), if no values were excluded, but 36 ± 28 U/10⁸ (n = 96) if values greater than 120 U/10⁸ cells were excluded. TdT activities in peripheral blood were similar. Eleven percent of patients (11/102) were diagnosed as ALL but had TdT activities of less than 10 U/10⁸ cells in both bone marrow and peripheral blood (Fig. 1). TdT activities in cells from bone marrow and peripheral blood of children without malignancies are 5.9 ± 19 (n = 198) and 0.5 ± 0.7 (n = 51) U/10⁸ cells, respectively. If we exclude values of TdT greater than 120 U, then the average value in
bone marrow from children with ALL is 6 times the average normal value. However, there is considerable overlap between values observed in children with and without ALL.

The activities of TdT in bone marrow and peripheral blood were not correlated with the age of the patient at diagnosis. The activity of TdT in cells from the peripheral blood of 12 patients with mediastinal mass was 25 ± 34 U/10^6 cells compared to 72 ± 184 U in 117 patients without a mediastinal mass (*p* = 0.38). For cells from bone marrow, these values were 71 ± 115 U in 10 patients with a mass compared to 108 ± 152 U in 125 patients without a mass (*p* = 0.46).

The activity of TdT in leukemic cells from children diagnosed as ALL has a wide range of values (Fig. 1). Because TdT activity is generally higher than normal in such children, there was the possibility that particularly low values (<10 U/10^6 cells) were caused by technical problems with the collection of cells, difficulties with the enzymatic assay, or the existence of a subtype of ALL with distinctive clinical characteristics. We examined these possibilities in 7 patients with the lowest TdT activities. In all cases, the number of cells assayed, the percentage of blasts in the specimen, and the length of time specimens were frozen were similar to the group as a whole. Five of the 7 patients were less than 3 yr old. At least 3 had L₂ rather than the typical L₁ morphotype childhood ALL. One of the 7 did not achieve a complete remission and the 6 who did achieve a remission subsequently relapsed. Two of the patients had a mediastinal mass, but their blasts were E. In two cases TdT activities were measured at diagnosis and relapse. Cells from one patient contained 6.7 U TdT/10^6 cells at diagnosis, but none at relapse. Each specimen contained approximately 80% blasts. In contrast, cells from the other patient did not contain TdT activity at diagnosis, but had 44 U/10^6 cells at relapse. We concluded that patients who had T or non-T, non-B ALL by morphological and surface marker criteria, but did not have elevated TdT activity in their blasts, might have a prognostically unfavorable type of disease. However, the number of cases is too small to reach definitive conclusions.

During the 6 yr patients were accessioned into our study, we identified 5 patients whose blasts were SmIg⁺ (B-marked ALL). These patients were excluded from subsequent analyses. Differences between these patients and the patients with typical ALL, but low TdT, were immediately apparent. In 5 of 5 cases of B-marked ALL, TdT activities in bone marrow cells were less than 0.5 U/10^6 cells. Blasts from 4 of 5 patients had L₁, and 1 of 5 had L₂ morphology. Four of the 5 patients with B-marked ALL failed to achieve a complete remission and their median survival was approximately 2 mo. They are older than average (range 6–15 yr), rather than younger as in low TdT ALL.

**Analysis of Prognostic Factors at Diagnosis**

Five of the 164 children (3%) did not achieve a complete remission of their ALL. Characteristics of these children are listed in Table 1. One of these patients is an infant (2350) who had low TdT activity in the leukemic cells. This patient and patient 2055 would be considered high risk because their WBC was greater than 50,000/μl. Otherwise, the children who failed to achieve a remission did not appear to share a common characteristic or to differ from the population of children treated for leukemia.

We measured the effect of a number of variables at diagnosis on the prognosis of children with ALL (Table 2). Our primary aim was to test TdT as a prognostic indicator by comparing its utility to other factors reported to be of prognostic significance. Variables included institution where care was delivered, treatment group, mediastinal mass, the E surface marker, age, white blood count, and TdT activities in cells from bone marrow and peripheral blood. Age, white blood count, and TdT activities were treated as continuous variables. *p* Values and likelihood ratios both permit a listing of variables in order of relative importance. Two

### Table 1. Characteristics of Patients Who Failed to Achieve a Complete Remission

<table>
<thead>
<tr>
<th>Patient</th>
<th>yr</th>
<th>Age</th>
<th>WBC (K/μl)</th>
<th>Mediastinal Mass</th>
<th>Surface Markers</th>
<th>TdT Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E SmIg</td>
<td>BM (U/10^6 Cells)</td>
</tr>
<tr>
<td>2017</td>
<td>10</td>
<td>08</td>
<td>2,600</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
</tr>
<tr>
<td>2055</td>
<td>02</td>
<td>03</td>
<td>89,500</td>
<td>—</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>2145</td>
<td>08</td>
<td>00</td>
<td>12,900</td>
<td>—</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>2248</td>
<td>11</td>
<td>00</td>
<td>7,000</td>
<td>—</td>
<td>—</td>
<td>9.3</td>
</tr>
<tr>
<td>2350</td>
<td>01</td>
<td>01</td>
<td>56,400</td>
<td>—</td>
<td>—</td>
<td>0 (96)†</td>
</tr>
</tbody>
</table>

*ID, indeterminate.†The percentage of blasts is indicated in parentheses.
PROGNOSTIC SIGNIFICANCE OF TdT

Table 2. Analysis of Prognostic Factors at Diagnosis*

<table>
<thead>
<tr>
<th>Short Duration of First Remission</th>
<th>Poor Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using terminal transferase activity in cells from peripheral blood (n = 124)</td>
<td>Using terminal transferase activity in cells from bone marrow (n = 131)</td>
</tr>
<tr>
<td>1. Mediastinal mass (p &lt; 0.01, λ = 0.02)</td>
<td>1. Mediastinal mass (p &lt; 0.01, λ = 0.02)</td>
</tr>
<tr>
<td>2. Treatment group (p &lt; 0.01, λ = 0.03)</td>
<td>2. Treatment group (p = 0.03, λ = 0.10)</td>
</tr>
<tr>
<td>1. Mediastinal mass (p &lt; 0.01, λ = 0.01)</td>
<td>1. Mediastinal mass (p &lt; 0.01, λ = 0.005)</td>
</tr>
<tr>
<td>2. Treatment group (p &lt; 0.06, λ = 0.15)</td>
<td>3. Absent sheep cell receptor (p = 0.02, λ = 0.06)</td>
</tr>
<tr>
<td>4. Institution (p = 0.04, λ = 0.14)</td>
<td></td>
</tr>
</tbody>
</table>

*Risk factors selected for analysis in the Cox regression model included: TdT activity, treatment group, institution, presence or absence of a mediastinal mass, present or absence of sheep erythrocyte receptor, age, and peripheral nucleated cell count. Variables significantly affecting prognosis are listed in order of importance, as defined by the general likelihood ratio, λ. Each successive variable must add something after the previous ones are taken into account.

Separate analyses were done initially, one using TdT activity in peripheral blood cells at diagnosis and another using TdT activity in bone marrow cells at diagnosis. The analyses were separated because many children had either one or the other, but not both at diagnosis. Because of the large number of patients analyzed, variables may have minor, but statistically significant effects on prognosis. These effects may be so small that they are not clinically significant.

Factors associated with failure to achieve a complete remission could not be analyzed in this fashion because only five children were in this subpopulation. The characteristics of these children are simply listed in Table 1. We examined factors that predict a short duration of first complete remission (Table 2). Of the factors tested, the presence of a mediastinal mass and treatment according to protocol 2 were associated with a short remission. When the group as a whole is considered, there is no general relationship between TdT activity and duration of remission. However, the Cox regression model is not designed to detect very small subpopulations of patients such as the group with very low TdT activities and a poor prognosis previously discussed. It is noteworthy that age and the presence of sheep cell receptors (E⁺) were not associated with a short duration of remission, when the effect of mediastinal mass was factored out.

We next examined factors that predict poor survival. These are listed in order of importance in Table 2. The presence of a mediastinal mass was most predictive. In the subgroup of patients who had TdT activity measured in bone marrow, treatment group, absent sheep cell receptor, and institution also affected survival, although the effects appeared to be weak. TdT activities in cell isolated from peripheral blood and bone marrow were not predictive for the group as a whole.

When the data were reanalyzed by coding TdT activity as a dichotomous variable, high or low, rather than a quantitative variable, slightly different results were obtained. The analysis was repeated twice, each with a different cutpoint between high and low TdT. TdT activity was considered high if it was >10 and then >20 U/10⁸ cells in bone marrow. The two analyses gave the same results. Low TdT activity at diagnosis was associated with a longer duration of complete remission than high TdT activity (p = 0.05). However, TdT activity was less important than mediastinal mass, treatment group, and absent sheep cell receptor, so the effect of TdT was very weak. TdT activity at diagnosis did not affect survival, when analyzed in this fashion. High and low TdT activities were not associated with high and low percentages of blasts in specimens. The percentage of blasts ranged from 70% to 100% in both groups of patients.¹²

To be absolutely certain that level of activity of TdT in blasts did not affect prognosis, we analyzed the survival of patients whose blasts contained high levels of TdT activity in comparison to patients whose blasts contained low levels of TdT activity. High level of TdT activity was defined as greater than 20 U/10⁸ cells from bone marrow or greater than 10 U/10⁸ mononuclear cells from peripheral blood. These ranges were originally chosen to distinguish activities characteristic of ALL from those typical of acute myelogenous leukemia in our laboratory.² We excluded children with E⁺ lymphoblasts. The survival of the 138 children in the E⁺ SmIg⁺ group is shown in Fig. 2. The closed and open circles identify children with low and high activities of TdT, respectively. There were 27 children in the low TdT group (median survival 1094 days) and 111 children in the high TdT group (median survival 1529 days). The difference in survival was not significant (p = 0.15 generalized Wilcoxon, p = 0.17 generalized Savage).

We compared the distribution of TdT activities in patients who were dead at the end of our study with the distribution in patients who were alive. Activities in peripheral blood were plotted separately from those in
bone marrow. There was no clustering of dead patients at any particular range of TdT activities in either bone marrow or peripheral blood (data not shown).

**Prediction of Relapse**

TdT activity is increased in cells from the peripheral blood and bone marrow of most children at the time of diagnosis of ALL. We examined whether quantitative measurements of TdT on cells from patients in remission are useful in predicting relapse of the leukemia. To illustrate the problems encountered, Fig. 3A shows TdT activities in cells from peripheral blood of patient 2024 during a period of 1600 days following the diagnosis of ALL. At the time of initial diagnosis, TdT activity in peripheral blood was less than 2 U/10⁶ nucleated cells, although it was elevated to 45 U/10⁶ cells in bone marrow. During the first year of therapy, the activity in peripheral blood was as high as 69 U/10⁶ nucleated cells. This high value was not associated with the appearance of an increased number of morphologically identifiable blasts and treatment of the patient was in the standard way according to protocol. During the 5 yr following initial diagnosis, except for the high values obtained on 2 occasions during the first year, the TdT activities in peripheral blood remained less than 2 U. The patient eventually relapsed in a testis. At the time of relapse, the TdT value in peripheral blood was less than 0.1 U/10⁶ mononuclear cells.

Figure 3B shows TdT activity in mononuclear cells from the bone marrow during therapy of patient 2020. TdT activity at diagnosis was 150 U/10⁶ cells. During the next several years, this value fluctuated and reached activities as high as 40 U/10⁶ cells, but without morphological relapse in bone marrow. Testicular relapse occurred on approximately day 1200. At this time, the bone marrow activity was 17 U/10⁶ cells, a value within the range typical of this patient. Bone marrow relapse with TdT activity of approximately 300 U/10⁶ cells was documented on day 1400. This had not been predicted by assays of TdT activity in bone marrow obtained less than 2 mo before relapse.

Statistical approaches to the question of whether increases in TdT activity occur before frank relapse are difficult to design. If significant increases in TdT activity did occur, then we could predict relapse of the leukemia and make some type of therapeutic intervention. A rough estimate of the potential usefulness of TdT measurements in predicting relapse can be made by examining the increases actually seen at the time of obvious clinical relapse. Unless significant increases in TdT activity (i.e., a noticeable jump when compared to
baseline) can be demonstrated to occur at relapse, it would be hopeless to try to predict relapse by these measurements. For this assessment, we calculated standardized Z scores defined as:

\[ Z \text{ score for relapse} = \frac{\text{TdT at relapse} - \text{Average TdT in remission}}{\text{Standard deviation of average TdT in remission}} \]

The Z score for relapse measures how many standard deviations from the mean of previous observations the one at first relapse is. The Z score applies to measurements made either in cells from peripheral blood or bone marrow. The method requires at least 2 measurements of TdT while a patient is in remission to establish a mean and standard deviation. It does not assume that the remission represents a disease-free state. One measurement is required at relapse. Table 3 presents the activity of TdT in bone marrow at diagnosis and at relapse, mean and standard deviation of TdT during remission, and Z scores for 10 patients with ALL. TdT activity during remission is presented (Table 3, footnote) for an additional randomly selected 5 patients who have not relapsed to permit comparison with the children who did develop recurrent disease. Patient 2020 (Table 3) for example had 10 measurements of TdT activity made in cells from bone marrow during remission. The mean activity \( \pm \) standard deviation was 12.1 \( \pm \) 11.6 U/10⁶ mononuclear cells. TdT activities at diagnosis and relapse had been 150 and 271 U/10⁶ cells, respectively. The Z score for relapse was 22, indicating that the activity of TdT in cells from bone marrow at the time of relapse was 22 standard deviations above the mean activity seen during remission. Obviously, this represents a noticeable jump in TdT activity at relapse. In most patients with extremedullary relapse, however, TdT activity was very low in mononuclear cells from bone marrow. In such cases (e.g., 2409 and 2561), the Z score is negative or very low, indicating that the TdT activity at relapse was not significantly different from values seen while the patient was in a complete remission. Similar data were derived from assay of mononuclear cells from peripheral blood (Table 4). Patient 2280 and 13 measurements of TdT activity in cells from peripheral blood during remission. TdT activity was 2.3 \( \pm \) 5.6 during remission and 39 U/10⁶ cells at relapse. The Z score for relapse was 6.5, indicating that TdT activity at relapse was well above the mean activity detected during remission. We calculated Z scores for relapse using measurements made on cells from the peripheral blood of 11 patients and the bone marrow of 20 patients. Not all are presented in Tables 3 and 4. Z was greater than 3 in 7 of 11 patients with measurements in peripheral blood and in 13 of the 20 patients with measurements in bone marrow. In other words, in 64% of patients having repeated measurements of TdT activity in mononuclear cells from peripheral blood or bone marrow, the activity of TdT at relapse is at least 3 standard deviations above the mean value during remission. Conversely, in 36% of patients, TdT activity at relapse is within 3 standard deviations of the mean. These general conclusions also hold when derived in a different way. At the time of morphological relapse in bone marrow, 65% of patients had TdT activity in marrow greater than 20 U/10⁶ cells and 50% had TdT

<table>
<thead>
<tr>
<th>Patient</th>
<th>At Diagnosis</th>
<th>At Relapse</th>
<th>Mean in Remission†</th>
<th>SD of Mean in Remission†</th>
<th>n</th>
<th>Z Score for Relapse</th>
<th>Site of Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td>150</td>
<td>271</td>
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<td>11.6</td>
<td>10</td>
<td>22</td>
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</tr>
<tr>
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<td>158</td>
<td>247</td>
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<td>0.3</td>
<td>2</td>
<td>850</td>
<td>BM</td>
</tr>
<tr>
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<td>5.4</td>
<td>17</td>
<td>0.2</td>
<td>0.5</td>
<td>4</td>
<td>36</td>
<td>BM</td>
</tr>
<tr>
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<td>695</td>
<td>5.4</td>
<td>1.6</td>
<td>1.3</td>
<td>6</td>
<td>2.9</td>
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</tr>
<tr>
<td>2231‡</td>
<td>—</td>
<td>4.0</td>
<td>8.4</td>
<td>10.5</td>
<td>2</td>
<td>-0.4</td>
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</tr>
<tr>
<td>2309</td>
<td>30</td>
<td>7.4</td>
<td>1.1</td>
<td>1.0</td>
<td>3</td>
<td>6.7</td>
<td>Testis</td>
</tr>
<tr>
<td>2409‡</td>
<td>—</td>
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<td>0.8</td>
<td>1.1</td>
<td>2</td>
<td>-0.7</td>
<td>Testis</td>
</tr>
<tr>
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<td>2.0</td>
<td>2.4</td>
<td>4</td>
<td>43</td>
<td>BM</td>
</tr>
<tr>
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<td>0.7</td>
<td>1.4</td>
<td>4</td>
<td>0.8</td>
<td>CNS</td>
</tr>
<tr>
<td>2707</td>
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<td>6.6</td>
<td>19</td>
<td>22</td>
<td>2</td>
<td>-0.6</td>
<td>BM</td>
</tr>
</tbody>
</table>

*Presentation of data is restricted to patients having at least one measurement of TdT activity in bone marrow made within 45 days of relapse, in addition to measurements made at relapse. Relapse is defined as recurrent leukemia at any site, e.g., bone marrow (BM), testis, or central nervous system (CNS).
†For purposes of comparison, 5 patients who had not relapsed at greater than 5 yr after complete remission had the following mean TdT values in bone marrow cells while in remission: 2.7 \( \pm \) 2.5, \( n = 5 \); 11.1 \( \pm \) 7.3, \( n = 9 \); 5.3 \( \pm \) 4.6, \( n = 4 \); 0.4 \( \pm \) 0.5, \( n = 4 \); 1.2 \( \pm \) 1.1, \( n = 3 \).
‡Patients 2231 and 2409 were not seen at the time of initial diagnosis for measurement of TdT activity. However, they were studied during remission and relapse and are included in this table.
activity in peripheral blood greater than 10 U/10^8 cells. The remainder of patients did not have TdT activities elevated to these values, despite frank hematologic relapse.

The extensive data presented in Tables 3 and 4 illustrate the wide fluctuations in TdT activity characteristically seen in mononuclear cells from patients with ALL. These occur during all phases of therapy, including times when children are off chemotherapy during remission. Therefore, a single value of TdT activity cannot indicate the activity of the underlying ALL because TdT activity fluctuates widely through the course of the disease.

TdT activities in cells from the bone marrow and peripheral blood of 5 patients who have not relapsed are presented in the footnotes to Tables 3 and 4. In each case, the patient has been followed without relapse for greater than 5 yr from diagnosis, including at least 2 yr off chemotherapy. Mean TdT activities in cells from peripheral blood ranged from 0.2 to 1.4 U/10^8 cells and in bone marrow from 0.4 to 11.1 U/10^8 cells. We compared mean TdT activities during remission in patients who did and did not relapse using the Mann-Whitney rank sum test. There was no significant difference (p > 0.05) between TdT activities in mononuclear cells from patients who did relapse in comparison to those who did not.

We measured TdT activities in cells from several patients at both diagnosis and relapse. Sets of measurements are presented for 8 randomly selected patients in Table 3 (bone marrow) and 7 patients in Table 4 (peripheral blood). Activities of TdT at diagnosis and relapse were not correlated.

**DISCUSSION**

The major aim of our study was to assess whether quantitative variation in TdT activity in mononuclear cells from bone marrow and peripheral blood conveys prognostic information in childhood acute lymphoblastic leukemia. TdT activity was measured prospectively and repeatedly from time of diagnosis in 164 children with T and non-T, non-B marked ALL. The duration of follow-up ranged from 1 to 6 yr. Ninety-seven percent of the children achieved a complete remission of their disease and 40% relapse during the study. TdT activity was elevated to >10 U/10^8 cells from peripheral blood and bone marrow in 89% of patients with ALL. TdT activities in cells from bone marrow and peripheral blood of children without malignancies are 5.9 ± 19 (n = 198, median 2.6) and 0.5 ± 0.7 (n = 51, median 1.0) U/10^8 cells, respectively. TdT activities at diagnosis of ALL ranged from less than 1.0 to greater than 1000 U/10^8 cells. For the group of 164 patients there was no correlation between TdT activity and age, duration of complete remission, or survival. However, among the small subgroup of 7 patients with the lowest TdT activities, 5 were less than 3 yr old. All of these patients relapsed during the study. They may have a biologically distinct type of disease. These patients differed from 5 patients with B-marked ALL who also have very low TdT activities in their blasts. Patients with B-marked ALL were older, had L2 or L1 blast morphology, and either failed to achieve a complete remission or relapsed within a few months of diagnosis.

A second major aim of our study was to assess the value of quantitative measurements of TdT activity in cells from bone marrow or peripheral blood in predicting relapse of disease. At the time of frank morphological relapse at any site, including testis and central nervous system, TdT activity was elevated at least 3 standard deviations above the mean value during remission in 64% of patients (Tables 3 and 4). While TdT activity was increased in the bone marrow of most patients with bone marrow relapse (e.g., Table 3, patients 2020, 2032, and 2482), it was rarely elevated
in the bone marrow of patients with isolated testicular or central nervous system relapse (e.g., Table 3, patients 2231, 2309, 2409, and 2561). At the time of morphological relapse in bone marrow, the percentage of blasts was not usually as high as it had been at diagnosis. This may account for some of the low TdT activities seen in marrow at relapse. However, in a previous paper we were unable to demonstrate a close relationship between TdT activity and percentage of lymphoblasts in a specimen. There appears to be variation in the TdT content of blasts from patient to patient and even from cell to cell within the same patient. Wide fluctuations in TdT activity were characteristically seen in mononuclear cells from patients with ALL at all stages of their disease. During remission, patients who would eventually relapse did not have statistically significant increases in TdT activity in cells from their peripheral blood or bone marrow. The wide fluctuations in TdT activity made interpretation of individual values difficult with regard to circulation of leukemic blasts and activity of the underlying ALL. Fluctuations of TdT enzymatic activity in leukemia during remission have been noted by others. Froehlich et al. and Bradstock et al. used immunofluorescence to detect TdT antigen in cells from leukemic patients before and during therapy. They observed wide fluctuations in the relative numbers of cells containing TdT. The first group of investigators studied cells from the peripheral blood of 15 patients with ALL or lymphoblastic lymphoma. They documented an increase in the number of TdT-containing cells several weeks prior to clinical relapse, although the authors did not determine whether these were leukemic cells or normal lymphoid precursors. The second group studied cells from the bone marrow of a large number of patients with ALL and were not able to correlate changes in number of TdT-containing cells with the activity of the ALL.

TdT is found in a subset of normal lymphoid cells within the immunologic system. The role of these cells is not known, but they appear to increase and decrease in response to a variety of stimuli. For example, in rat bone marrow, normal TdT-containing cells virtually disappear in response to pharmacologic doses of corticosteroids and then comprise approximately 10% of the cells in the regenerating marrow. This type of lymphoid cell has been demonstrated to circulate under certain conditions, particularly as part of normal cellular traffic among bone marrow, thymus, and spleen. It seems possible to detect small numbers of TdT-containing cells and to classify them as leukemic or normal by using multiple cell markers. This procedure should permit a direct measurement of the circulation of each type of cell under various circumstances in individual patients, although it is somewhat tedious.

Simple measurement of TdT by either biochemical or immunofluorescence methods is helpful in the differential diagnosis of the leukemias. However, repeated measurements of TdT activity or antigen alone in cells from the peripheral blood or bone marrow is not yet of proved value in the management of childhood ALL. Relapse cannot be confidently predicted from such measurements alone because both normal and leukemic cells may circulate and contain TdT. The numbers of normal cells containing TdT are known to increase and decrease dramatically in response to unknown signals. These fluctuations can easily be confused with changes in activity of the underlying leukemia and lead to unwarranted changes in therapy, so that techniques for detecting residual leukemic cells must be carefully validated by comparative studies of normal and leukemic patients before acceptance in clinical practice.

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Prognostic significance of terminal transferase activity in childhood acute lymphoblastic leukemia: a prospective analysis of 164 patients

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