Nonpositive Terminal Deoxynucleotidyl Transferase in Pediatric Precursor B-Lymphoblastic Leukemia

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Key Words: Terminal deoxynucleotidyl transferase; Acute lymphoblastic leukemia; Immunophenotype; MLL

Abstract

Terminal deoxynucleotidyl transferase (TdT) is a unique intranuclear DNA polymerase that catalyzes the template-independent addition of deoxynucleotides to the 3’-hydroxyl terminus of oligonucleotide primers,1 causing insertion of N regions during immunoglobulin and T-cell receptor (TCR) gene rearrangements at the DJ and VDJ junction sites.2-4 This mechanism of junctional diversity is essential to the development of an adult-type immunoglobulin and TCR repertoire.4 The expression of TdT is restricted to lymphoid precursors. It is a useful marker in distinguishing acute lymphoblastic leukemia (ALL) from mature lymphoid neoplasms. Although TdT– T-cell ALL has been reported in the literature rarely, the frequency and significance of TdT-nonpositive (TdTnp) B-cell ALL have not been examined extensively. We reviewed the immunophenotypes of 186 new cases of pediatric B-cell ALL and found 5 TdTnp cases (2.7%). They showed significantly higher frequencies of a WBC count of more than 50,000/µL (>50.0 × 109/L), CD10–, CD34–, and MLL gene rearrangement compared with those in TdT+ cases (3/5 [60%] vs 27/181 [14.9%], P = .03; 3/5 [60%] vs 11/181 [6.1%], P = .003; 4/5 [80%] vs 24/179 [13.4%], P = .002; 3/5 [60%] vs 9/181 [5.0%], P = .0019; respectively). These results indicate that nonpositive TdT does not rule out a diagnosis of ALL and suggest that TdTnp B-cell ALL might be associated with CD10– and CD34– disease, a high WBC count, and MLL gene rearrangement.

Terminal deoxynucleotidyl transferase (TdT) is a unique intranuclear DNA polymerase that catalyzes the template-independent addition of deoxynucleotides to the 3’-hydroxyl terminus of oligonucleotide primers,1 causing insertion of N regions during immunoglobulin and T-cell receptor (TCR) gene rearrangements at the DJ and VDJ junction sites.2-4 This mechanism of junctional diversity is essential to the development of an adult-type immunoglobulin and TCR repertoire.4 The expression of TdT is restricted to lymphoid precursors. It is a useful marker in distinguishing acute lymphoblastic leukemia (ALL) from mature lymphoid neoplasms. Although TdT– T-cell ALL has been reported in the literature rarely, the frequency and significance of TdT-nonpositive (TdTnp) B-cell ALL have not been examined extensively. We report a series of 5 pediatric cases of TdTnp B-cell ALL. The morphologic, immunophenotypic, and genetic features are reviewed and analyzed to better characterize this unusual neoplasm.

Materials and Methods

Cases

A retrospective search of the hematopathology archives of the Children’s Hospital, Denver, CO, for cases during the period January 1996 to December 2002 yielded 5 cases of
TdT<sup>®</sup> B-cell ALL in the 186 newly diagnosed B-cell ALL cases. The diagnosis of TdT<sup>®</sup> B-cell ALL was made based on clinical manifestations, blastic morphologic features (French-American-British Classification, L1 or L2 morphologic features), B-cell phenotype with absence of surface light chain expression by immunophenotyping, and genotype.

**Pathologic Examination**

The peripheral blood smears and bone marrow aspirate smears were stained with standard Wright stain. The bone marrow biopsy specimens were evaluated in B-5–fixed, paraffin-embedded, and H&E-stained sections. Immunohistochemical staining for κ (dilution 1:3,000; DAKO, Carpenteria, CA) and λ (dilution 1:3,000; DAKO) was performed in TdT<sup>®</sup> cases in which the κ and λ light chains were not evaluated by flow cytometric analysis. TdT immunohistochemical staining (dilution 1:10; Supertechs, Bethesda, MD) was performed in all cases to confirm TdT<sup>®</sup> by flow cytometric analysis.

**Flow Cytometric Analysis**

Immunophenotyping was performed on fresh bone marrow aspirate or peripheral blood samples by using directly conjugated fluorescent monoclonal antibodies to CD2, CD7, CD10, CD19, CD20, CD13, CD33, CD34, and HLA-DR (dilution 1:6 [for all]; Beckman Coulter, Miami, FL); CD45 (dilution 1:11; Beckman Coulter); and TdT, κ, and λ (dilution 1:11 [for all]; DAKO). The cell suspension was analyzed using a 3-color Coulter EpicsXL flow cytometer (Beckman Coulter). CD45 was used as a gating parameter. With a 4 log immunofluorescence scale, bright expression is defined so that the median of antigen-positive immunofluorescence is equal to or greater than 1 log more than the negative control median with no overlap with the negative control; dim expression is defined so that the median of antigen-positive immunofluorescence is less than 1 log more than the negative control median with no significant overlap with the negative control. Expression of an immunologic marker in fewer than 10% of lymphoblasts was considered nonpositive or negative, and at least 10% of lymphoblasts expressing an antigen for the neoplasm was considered positive. The time from specimen collection to TdT ranged from 2 hours to 29.5 hours.

**Pathologic Findings**

Wright-stained preparations of bone marrow aspirate showed L1 morphologic features of lymphoblasts in all cases. Replacement of the bone marrow space by a uniform population of lymphoblasts was noted in H&E-stained bone marrow sections.

**Immunophenotypic Findings**

The results of immunophenotypic characterization by flow cytometric analysis are summarized in **Table 1**. All MLL locus have a fused red-green signal. If MLL rearrangement occurs, the probe becomes separated and is seen as 2 independent signals in metaphase and interphase cells.

**Molecular Analysis**

RNA extraction and reverse transcriptase–polymerase chain reaction (RT-PCR) analysis were performed using AF-4/MLL, bcr/abl, E2A/PBX1, and TEL/AML1 primers to detect t(4;11)(q21;q23), t(9;22)(q34;q11.2), t(1;19)(q23;p13.3), and t(12;21)(p13;q22) as described previously. DNA extraction and PCR analysis for the detection of clonal gene rearrangement of the immunoglobulin heavy chain (IγH) gene were performed as described previously. Clonality was characterized by the presence of a distinct spike.

**Statistical Analysis**

The significance of differences in the female/male ratio and frequencies of a high WBC count (>50,000/µL, >50.0 ×10<sup>9</sup>/L); central nervous system (CNS) involvement; absent expression of CD10, CD34, and CD20; and MLL gene rearrangement between TdT<sup>®</sup> cases and TdT<sup>+</sup> cases was estimated by using the Fisher exact test. The significance of the difference in the time from sample collection to TdT processing for flow cytometric analysis between 2 groups was analyzed by using the t test.

**Results**

**Clinical Characteristics of Patients**

The clinical characteristics of TdT<sup>®</sup> B-cell ALL cases are summarized in **Table 1**. These 5 cases included 1 boy and 4 girls ranging in age from 3 months to 7 years at diagnosis. Of 5 cases, 3 (60%) had a high WBC count, and 2 cases had CNS involvement at diagnosis. No patients experienced relapse during the course of the disease. Two patients underwent cord blood transplantation. One patient died of infection at 8 months. The remaining 4 patients were in remission with 2 to 3 years of follow-up.

**Pathologic Findings**

Wright-stained preparations of bone marrow aspirate showed L1 morphologic features of lymphoblasts in all cases. Replacement of the bone marrow space by a uniform population of lymphoblasts was noted in H&E-stained bone marrow sections.
cases were TdT<sup>np</sup>CD19+κ−λ−. Image 2. TdT<sup>np</sup> results by flow cytometric analysis were confirmed by immunohistochemical staining in all cases. Of 5 cases, 3 (60%) were CD10−, 4 (80%) were CD34−, and 2 (40%) were CD20−. The mean time between specimen collection and TdT processing for flow cytometric analysis was 9 hours (range, 2.0–18.0 hours; n = 5; SD = 8.22) in TdT<sup>np</sup> cases and 11.46 hours (range, 2.0–29.5 hours; n = 132; SD = 8.26) in TdT<sup>+</sup> cases. There was no significant difference between the 2 groups (P = .514; t test).

**Cytogenetic and Molecular Genetic Findings**

The results of karyotype, FISH, and RT-PCR studies are shown in Table 3. Of 5 cases, 3 (60%) had the MLL gene

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**Table 1**

**Clinical Characteristics of Patients With TdT-Nonpositive B-Cell ALL.**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age</th>
<th>FAB Morphologic Classification</th>
<th>WBC Count, /µL (× 10⁹/L)</th>
<th>CSF Findings</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/7 y</td>
<td>L1</td>
<td>35,500 (35.5)</td>
<td>Negative</td>
<td>Alive, 3 y</td>
</tr>
<tr>
<td>2/M/5 y</td>
<td>L1</td>
<td>57,200 (57.2)</td>
<td>Atypical</td>
<td>Alive, 3 y</td>
</tr>
<tr>
<td>3/F/1 y</td>
<td>L1</td>
<td>125,000 (125.0)</td>
<td>Positive</td>
<td>Alive, 3 y</td>
</tr>
<tr>
<td>4/F/3 mo</td>
<td>L1</td>
<td>91,000 (91.0)</td>
<td>Positive</td>
<td>CBT, 4 mo; died of infection, 8 mo</td>
</tr>
<tr>
<td>5/F/1 y</td>
<td>L1</td>
<td>27,200 (27.2)</td>
<td>Negative</td>
<td>CBT, 4 mo; alive, 2 y</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; CBT, cord blood transplantation; CSF, cerebrospinal fluid; FAB, French-American-British classification; TdT, terminal deoxynucleotidyl transferase.
rearrangement, and 1 case (20%) had the E2A/PBX1. No bcr/abl or TEL/AML1 translocations were seen in TdT<sup>+</sup> cases. Clonal gene rearrangement of IgH was detected in all cases except case 4, in which the DNA was insufficient for analysis.

**Comparison of TdT-Nonpositive and TdT+ Cases**

A comparison of the common clinicopathologic features between TdT<sup>+</sup> and TdT<sup>-</sup> cases is given in Table 4. Children with TdT<sup>-</sup> disease were younger than those with TdT+ disease; a greater proportion of girls had TdT<sup>-</sup> disease than in the TdT+ group; there was a greater percentage of CNS involvement in the TdT<sup>-</sup> cases than in the TdT+ cases; and a greater percentage of TdT+ cases were CD20<sup>-</sup>. However, there was no statistical difference in the female/male ratio, CNS involvement, and CD20<sup>-</sup> between the 2 groups. A greater percentage of TdT<sup>-</sup> cases had a high WBC count, and the difference was statistically significant. Furthermore, a greater percentage of the TdT<sup>-</sup> cases than TdT+ cases were CD10<sup>-</sup> and CD34<sup>-</sup>, and the differences were statistically significant (Fisher exact test). A greater proportion of TdT<sup>-</sup> cases than TdT+ cases had the MLL gene rearrangement, and the difference was statistically significant.

**Discussion**

TdT<sup>-</sup> B-cell ALL cases represent 2.7% (5/186) of the pediatric B-cell ALL population in our study. These patients typically were girls. Morphologically, the tumor cells displayed a lymphoblastic appearance (French-American-British Classification, L1). Immunophenotypically, they manifested dim to intermediate CD45 expression and an absence of surface light chain expression characteristic of true lymphoblasts. Genetically, most (4/5 [80%]) had chromosomal translocations characteristic of B-cell ALL (3 cases with MLL gene rearrangement and 1 case with E2A/PBX1). While 1 child (case 4), an infant with leukemia, died of infection 4 months after cord blood transplantation, the remaining children (4/5 [80%]) remained in remission.

Taken together, the clinical, pathologic, immunophenotypic, and genetic features of these 5 cases support the diagnosis of TdT<sup>+</sup> B-cell ALL. Faber et al<sup>8</sup> found 3 cases of TdT– ALL in approximately 200 ALL cases in their study, and all cases were of T-cell lineage. Therefore, they proposed that TdT– ALL might be restricted to T-cell ALL. Our data expanded their findings and established the presence and characteristics of TdT<sup>-</sup> B-cell ALL.

In B-lymphoid precursors, TdT expression is limited to the early stages of maturation (pro-B and pre-B), during which IgH gene rearrangement occurs. TdT expression is promptly down-regulated following the onset of IgH (µ) synthesis in the cell and is, therefore, absent in all postprecur sor (peripheral or mature) B-cells. In some T-cell ALL cases in the study by Faber et al<sup>8</sup> showed both T- and B-cell gene rearrangement despite the lack of TdT expression.
These findings raise questions about whether TdT is absolutely necessary for gene rearrangement of IgH and TCR. A possible explanation is that TdT was expressed at a very low level that was undetectable using current immunologic methods but might be detectable at the level of messenger RNA expression or more sensitive immunohasys.17 Alternatively, the TdT protein might degrade more rapidly in vivo following IgH or TCR gene rearrangement in these cases than in the usual situation. Further studies are needed to address these issues. Finally, a prolonged interval between specimen collection and TdT processing might cause TdT protein degradation in vitro, resulting in false TdT results. However, this possibility was unlikely in our study. The mean time from specimen collection to TdT processing for flow cytometric analysis between TdT− cases and TdT+ cases was similar, 9 hours vs 11.46 hours, and statistical analysis did not show a significant difference.

Various clinical and laboratory findings at the time of diagnosis have been correlated with prognosis in ALL. Certain cytogenetic alterations (such as MLL gene rearrangement, E2A gene rearrangement, and bcr/abl translocation), a high WBC count, organ infiltration, and CD10 negativity are known indications of a poor prognosis.18-23 Our study showed that cases with TdT− B-cell ALL had significantly higher frequencies of a high WBC count, CD10 negativity, and MLL gene rearrangement than TdT+ cases (Table 4). However, the role of TdT as an independent prognostic indicator in ALL cannot be determined owing to the limited number of TdT− B-cell ALL cases in our study. In addition, although CNS involvement in TdT− cases seemed to be more frequent than that in TdT+ cases, statistical analysis did not demonstrate a significant difference. Larger series, long-term follow-up, and multivariate analysis are needed to further evaluate these issues.

The potential link between MLL gene rearrangement and TdT− aberrant expression in B-cell ALL is uncertain at present. It has been reported that ALL with MLL gene rearrangement is characterized by coexpression of myeloid- and lymphoid-associated antigens on leukemic blasts.24,25 Paietta et al26 also found that the intensity of TdT staining was considerably weaker in myeloid-positive ALL than in myeloid-negative ALL by flow cytometric analysis. Similarly, we also noted that the intensity of TdT was dim in TdT+ cases with MLL gene rearrangement by flow cytometric analysis (data not shown), in addition to TdT+ cases. Because of the unique association of MLL gene rearrangement with the intriguingly mixed lineage phenotype of leukemic cells, it is tempting to speculate that the wild-type MLL gene has an important role early in the development of the hematopoietic system, and the disruption of the MLL gene by chromosomal translocations in human leukemia will result in dyssynchronization of blood cell development, down-regulation of TdT expression, and/or up-regulation of myeloid marker expression in lymphoblasts. Absence of CD34 expression was correlated with TdT− in our study (P = .002), also suggesting the developmental dyssynchronization in malignant lymphoblastic transformation.

A pro-B immunophenotype (CD19+CD10−) is known to be associated with ALL in children younger than 1 year.23,27-29 In our study, there were 3 cases (1, 4, and 5) with a pro-B immunophenotype. Among them, only case 4 was an infant at the time of diagnosis. No correlation between TdT− and infant leukemia was evident.

The main differential diagnostic consideration with TdT− B-cell ALL is Burkitt leukemia, which clinically requires different therapeutic strategies. Burkitt leukemia represents a lymphoid population of more mature B cells and usually shows distinct morphologic features with a deeply basophilic and vacuolated cytoplasm. Immunophenotypically, it differs from lymphoblasts by expressing surface immunoglobulins and a growth fraction of 100% with Ki-67 in addition to the absence of TdT and CD34 expression. Genetically, it has a translocation involving c-myc, the molecular hallmark of Burkitt leukemia. Therefore, integrating morphologic, immunophenotypic, and genetic findings is mandatory to distinguish the 2 entities.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Comparison of TdT-Nonpositive and TdT-Positive ALL Cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpositive</td>
<td>Positive</td>
</tr>
<tr>
<td>No. of patients</td>
<td>5</td>
</tr>
<tr>
<td>Median age (y)</td>
<td>1</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>4:1</td>
</tr>
<tr>
<td>WBC count &gt;50,000/µL (&gt;50.0 x 10⁹/L)</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>Central nervous system involvement</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>CD10−</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>CD34−</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>CD20−</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>MLL gene rearrangement</td>
<td>3/5 (60)</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase.
* Data are given as number not positive or positive/total number tested (percentage) unless otherwise indicated.
Our data demonstrate that the frequency of TdT<sup>+</sup> B-cell ALL in pediatric patients is about 3%. It seems that TdT<sup>+</sup> B-cell ALL is associated with a high WBC count, CD10 and CD34 negativity, and MLL gene rearrangement. The absence of positive TdT expression as indicated by flow cytometric or immunohistochemical analysis does not rule out the diagnosis of lymphoblastic leukemia.

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References


