The Role of Prognostic Features in the Treatment of Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common cancer in children and is among the most curable of the pediatric malignancies. Many clinical, biological, genetic, and molecular features have been identified as having prognostic significance in the outcome of patients with ALL. The standard features are age and WBC at diagnosis, with infants (less than one year), adolescents (greater than nine years), and children with WBC above 50,000/µl being at higher risk. Certain chromosomal abnormalities are also strong predictors; in particular, the Philadelphia chromosome and MLL gene rearrangements (especially in infants) are adverse features, while TEL-AML1 is favorable. It is important to note, however, that even the most important known predictors explain only a small proportion of the variability in outcome. These features are currently used to tailor the intensity of treatment so that the toxicity of treatment can be minimized and cure rates can continue to improve. This article reviews time-honored prognostic features, recent advances, and future directions in this field. The Oncologist 2000;5:321-328

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood, and dramatic advances in its treatment over the past three decades have changed what was essentially a universally fatal disease to one that is now cured nearly 80% of the time. As pediatric oncologists have become more successful at treating ALL, much of the clinical research efforts have focused on stratifying patients into various risk groups based on known prognostic features, so that patients with lower-risk disease can be treated less intensively and the late effects and toxicities of treatment minimized, while patients with a higher risk of treatment failure can be targeted for more aggressive or different types of therapies.

Prognostic features play a critical role in directing therapy for ALL, and as scientific and treatment advances are made, this area of investigation changes rapidly. For example, at one time the immunophenotype of the lymphoblast (the most common being B-precursor, followed by T-cell, and finally mature B-cell) was a highly significant prognostic feature. In contrast to acute myelogenous leukemia, the blasts of ALL have no unique morphologic or cytochemical features (except for the rare mature B cell with its distinct French-American-British classification [FAB] L3 morphology). Therefore, immunophenotyping has traditionally been used in the diagnostic evaluation to determine the leukemia cell lineage. The T-cell and mature B-cell immunophenotypes were once associated with a very poor prognosis, but with the evolution of intensive chemotherapy and changes in treatment strategies, their prognoses are now almost equivalent to that of B-precursor ALL [1-3].

In addition, advances in genetics have led to the discovery of new prognostic features, such as the TEL-AML1 gene. The TEL-AML1 fusion gene, a result of the translocation t(12;21), is usually not detected by standard cytogenetic analysis. However, through molecular techniques it has been found to be the most common cytogenetic abnormality in B-lineage ALL [4], present in one-fourth of cases, and its presence appears to be a strong, independent predictor of a favorable prognosis [5, 6]. This article will review the prognostic features commonly used to determine treatment strategies for childhood ALL (Table 1) and will also discuss recent
advances in the field which are likely to change our approaches in the near future.

**PROGNOSTIC FEATURES CURRENTLY USED TO DIRECT THERAPY IN ALL**

There is considerable variation in risk classification of ALL used by the major clinical trials groups treating pediatric cancer in the US. Since mature B-cell ALL (FAB L3) is treated quite differently from B-precursor and T-cell ALL with a lymphoma-based approach, it is not part of the ALL risk classification schemas. For the most part, these systems for the treatment of B-precursor ALL (and sometimes T-cell ALL) are based on age and WBC at diagnosis, prognostic features which have consistently been found to be important [7, 8]. Table 2 shows the relationship between age, WBC, and prognosis of children treated on Pediatric Oncology Group (POG) and Children’s Cancer Group (CCG) regimens as of 1993. However, nonuniformity in the definitions of treatment-related risk groups have made it difficult to compare the results of various clinical trials [10]. Because of this, a workshop was held in 1993 by the Cancer Therapy Evaluation Program of the National Cancer Institute to attempt to create a uniform approach to risk classification in order to increase the efficiency of clinical research. The participating investigators reached a consensus on the basis of available data that patients with B-precursor ALL aged one to nine years and with WBC less than 50,000/µl at diagnosis (which represent two-thirds of patients) are at lower risk than other patients. Table 3 shows the four-year event-free survival (EFS) for POG (ALinC-14) and CCG (-100 and -1800 series) protocols by multiple age and WBC categories. *Note: Outcomes are for all patients in that age/WBC count category, regardless of treatment received. (Reproduced from [10]).

## Table 1. Prognostic factors in childhood ALL

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Favorable</th>
<th>Unfavorable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≥1 and ≤9 years</td>
<td>&lt;1 or &gt;9 years</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>WBC count at diagnosis</td>
<td>&lt;50,000/mm³</td>
<td>≥50,000/mm³</td>
</tr>
<tr>
<td>DNA index</td>
<td>&gt;1.16</td>
<td>≤1.16</td>
</tr>
<tr>
<td>Chromosome number per leukemic cell</td>
<td>&gt;50</td>
<td>&lt;45, especially 24-28</td>
</tr>
<tr>
<td>Induction response to prednisone on day 8</td>
<td>No peripheral blasts</td>
<td>Peripheral blasts</td>
</tr>
<tr>
<td>CNS status</td>
<td>CNS 1</td>
<td>CNS 2 or 3</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Trisomies 4 and 10</td>
<td>t(4;11), t(9;22)</td>
</tr>
<tr>
<td>Molecular genetics</td>
<td>TEL-AML1</td>
<td>MLL gene rearrangements</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>Precursor B</td>
<td>T cell, mature B cell</td>
</tr>
</tbody>
</table>

## Table 2. Outcome (four-year EFS) for children with B-precursor ALL treated by POG (ALinC-14) and CCG (-100 and -1800 series) protocols by multiple age and WBC categories

<table>
<thead>
<tr>
<th>WBC count (µl)</th>
<th>Age (years)</th>
<th>1.00-2.99</th>
<th>3.00-5.99</th>
<th>6.00-9.99</th>
<th>≥10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10,000</td>
<td>4-year EFS (%)</td>
<td>82.9</td>
<td>84.7</td>
<td>82.0</td>
<td>69.6</td>
</tr>
<tr>
<td>n of patients treated</td>
<td>490</td>
<td>937</td>
<td>437</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td>% B-precursor patients</td>
<td>10.7</td>
<td>20.5</td>
<td>9.6</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>10,000-49,999</td>
<td>4-year EFS (%)</td>
<td>74.6</td>
<td>74.5</td>
<td>80.2</td>
<td>59.2</td>
</tr>
<tr>
<td>n of patients treated</td>
<td>436</td>
<td>608</td>
<td>205</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>% B-precursor patients</td>
<td>9.5</td>
<td>13.3</td>
<td>4.5</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>≥50,000</td>
<td>4-year EFS (%)</td>
<td>68.3</td>
<td>73.9</td>
<td>47.5</td>
<td>41.1</td>
</tr>
<tr>
<td>n of patients treated</td>
<td>278</td>
<td>280</td>
<td>122</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>% B-precursor patients</td>
<td>6.1</td>
<td>6.1</td>
<td>2.7</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Outcomes are for all patients in that age/WBC count category, regardless of treatment received. (Reproduced from [10]).

## Table 3. Uniform age and WBC criteria for B-precursor ALL standard- and high-risk cohorts adopted at the CTEP/NCI workshop

<table>
<thead>
<tr>
<th>Risk</th>
<th>Definition</th>
<th>4-year EFS (%)*</th>
<th>% of B-precursor patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>WBC count &lt;50,000/µl and age 1.00-9.99 years</td>
<td>80.3</td>
<td>68</td>
</tr>
<tr>
<td>High</td>
<td>WBC count ≥50,000/µl or age ≥10.00 years</td>
<td>63.9</td>
<td>32</td>
</tr>
</tbody>
</table>

*EFS and percentages of patients are derived from data listed in Table 3. (Reproduced from [10]).
Although age and WBC at diagnosis continue to remain important independent prognostic factors, much of the variation in prognosis for patients based on the above factors can be accounted for by other features, such as immunophenotype and genetic subgroups among different age and WBC groups. For example, T-cell ALL patients are generally older than B-precursor ALL patients, partially explaining the “high risk” status of older patients [11]. Similarly, the very poor prognostic feature of Philadelphia chromosome positivity (Ph+) occurs more often in older patients, tends to present a prognostic feature of Philadelphia chromosome positivity (Ph+) occurs more often in older patients, tends to present a higher WBC, and accounts for much of the difference in prognosis between adult and childhood ALL [12, 13]. Although a study by the CCG found a similar outcome for young adolescents (aged 10 to 15 years) compared to older adolescents (aged 16 to 21 years) [14], there are data indicating that as many as three-fourths of older adolescents are not treated on cooperative group trials [15]. Thus, decreased access to clinical trials, as well as poor compliance with treatment in adolescents, may also partially account for a poorer outcome in the older age group. With respect to the adverse outcome of infants, a large percentage of infants have a t(4;11) with rearrangement of the MLL gene on chromosome band 11q23 which conveys a poor prognosis [16]. Infants also tend to present with a high leukocyte count and have an increased frequency of central nervous system (CNS) leukemia.

In addition to the time-honored features of age and WBC, the presence of extramedullary disease (CNS or overt testicular involvement) is a factor used to determine the intensity of treatment. CNS disease is defined as more than five white blood cells per ml of spinal fluid (in a nonbloody sample) which are blasts morphologically (referred to as “CNS 3”). There is an intermediate state, “CNS 2,” in which there are fewer than five cells per ml but blasts are detectable by cytocentrifugation, a procedure that concentrates the leukemic cells and increases diagnostic sensitivity. In “CNS 1,” there is no evidence of CNS involvement (fewer than five cells per mm3 and no blasts). Overt CNS leukemia is more common in T-cell ALL than B-precursor ALL [17], occurs in fewer than 5% of children at diagnosis, and is generally predictive of a poor treatment outcome [18]. Overt testicular involvement at diagnosis is even less common. A series from St. Jude reported the incidence to be 1.9% of boys [19], although occult testicular disease has been found by biopsy in as many as 25% of newly diagnosed boys [20]. As with CNS disease, overt testicular involvement is associated with poorer survival (38% versus 58% in the St. Jude series); however, these patients had a higher frequency of other adverse prognostic features as well.

In previous POG trials, male gender has consistently been an adverse prognostic feature [21, 22]. Recent data showed 38% lower five-year EFS for boys over age 10 or with an intermediate WBC (between 10,000 and 50,000/µl) than for girls, and boys fared worse than girls in all age subsets analyzed [21]. The most recent CCG trials, however, have not found gender to be a significant variable [3], and this discrepancy exemplifies the fact that prognostic variables are often treatment-dependent and change over time with treatment advances and differing approaches.

Most of the prognostic features currently used to determine the intensity of therapy are clinical or biologic and can be determined at the time of diagnosis. However, the CCG and the German BFM (Berlin-Frankfurt-Munster) group have used early response to therapy, a treatment-related feature, to assign risk and guide therapy. Slow early response to induction has been defined in several ways: greater than 1,000 blasts/microliter in peripheral blood following one week of prednisone [23], the presence of any circulating blasts following one week of multiagent induction [24], or greater than 25% blasts in the marrow on day 7 of induction [25]. All of these findings have been found to be important predictors of an adverse outcome, although intensified therapy appears to abrogate the prognostic significance of a slow early response [26]. Slow clearance of peripheral blasts has also been found to be an adverse prognostic feature in T-cell ALL and Ph+ ALL [22, 27, 28].

**GENETIC FEATURES**

There are numerous genetic features that have been found to be important prognostically and are commonly used to plan treatment for patients with B-precursor ALL. One such feature is DNA index, which was found to be the strongest predictor of treatment outcome when compared to age, WBC, sex, and immunophenotype in a POG study [29]. The DNA index, also referred to as ploidy, is the ratio of DNA content of leukemic cells to that of normal cells and can be measured by flow cytometry. Hyperdiploidy, defined as a DNA index greater than 1.16 (which correlates with more than 50 chromosomes per leukemic cell), is present in about 20% of patients over one year of age and is associated with a very favorable prognosis regardless of age and presenting WBC (90% four-year EFS in POG series). Although the DNA index has an independent influence on prognosis [30], it is closely correlated with other genetic features. Specifically, genetically unfavorable chromosomal abnormalities (such as t(9;22) and t(1;19)) occur less frequently in hyperdiploid ALL [31], while the favorable finding of trisomies of chromosomes 4 and 10 is correlated with hyperdiploidy [32]. An advantage of using the DNA index in risk assignment is that it is readily obtainable and nearly always successful, in contrast to cytogenetic studies which are not routinely available for all patients and are sometimes not technically feasible [29].
Along the same lines, hypodiploidy has been repeatedly found to be an adverse prognostic feature [33, 34]. A recent CCG study reported a worse outcome for patients with fewer than 45 chromosomes [33], and in particular patients with near-haploidy (24 to 28 chromosomes) have had very poor outcomes in studies from the CCG, St. Jude Children’s Research Hospital, and the United Kingdom [33, 35, 36].

Finally, several chromosomal abnormalities are very important predictors of outcome in B-precursor ALL. Many of these can be detected using standard cytogenetic analysis, while some more recently discovered structural changes are diagnosed through other techniques, such as fluorescence in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR), which can be performed on samples that are inadequate for cytogenetic analysis. These molecular techniques are quicker and both more sensitive and specific than karyotyping.

The best characterized chromosomal translocation in ALL is the Ph chromosome, designated t(9;22), which moves the ABL protooncogene from chromosome 9 into the BCR gene on chromosome 22, forming a BCR-ABL fusion gene. This translocation is present in 3%-5% of childhood ALL cases, 25% of adult ALL cases, and 95% of chronic myelogenous leukemia cases [37]. Although there may be a subset of patients with Ph+ ALL and low WBC at diagnosis who can be cured with chemotherapy [38, 39], for the most part this group of patients with ALL has an extremely poor prognosis with conventional chemotherapy, even when it is intensified [40].

In general this cytogenetic finding is considered an indication for allogeneic bone marrow transplantation in first remission. Whereas many other cytogenetic findings have lost prognostic significance with improvements and intensification of therapy, the Ph chromosome has maintained its adverse prognostic implications [41].

The most common translocation found by standard cytogenetics in ALL is the t(1;19) translocation, which represents 5%-6% of all cases [42]. This rearrangement fuses a portion of the E2A gene on chromosome 19 with part of PBX1 on chromosome 1 to form a hybrid protein which acts as a transcription factor. This translocation generally confers “high risk” status in classification schemata. Earlier treatment regimens found t(1;19) to be an adverse prognostic feature [43]; however, unlike the Ph chromosome, it has lost its prognostic significance with intensification [44]. A recent detailed cytogenetic study of the t(1;19) found that patients with a balanced t(1;19) are less likely to have hyperdiploidy than patients with an unbalanced der(19)t(1;19) [45]. In addition, the prognosis for children in the CCG trials with an unbalanced translocation was equivalent to those without a t(1;19), while the balanced translocation remained an adverse feature.

As mentioned previously, infants often have a translocation involving chromosome band 11q23 (usually t(4;11)), which frequently disrupts the MLL gene. Molecular techniques to detect MLL rearrangements include Southern blot analysis and RT-PCR, and have demonstrated this change in 68% to 81% of infant ALL cases, many of which do not have cytogenetically detectable 11q23 abnormalities [46, 47]. Infants with MLL rearrangements have a very poor prognosis despite intensification, whereas infants who lack this genetic abnormality have an intermediate prognosis and are more readily treated on risk-directed protocols [16, 46]. It has also been suggested that older children with MLL abnormalities have a poor prognosis [48].

In addition to the adverse prognostic cytogenetic abnormalities described above, there are abnormalities that have been found to be associated with a favorable prognosis. One such feature that is sometimes used to place patients into a lower-risk group is that of trisomies of chromosomes 4 and 10. In a POG study, the simultaneous presence of both trisomies 4 and 10 was associated with a very favorable prognosis (96.6% four-year EFS in hyperdiploid patients) regardless of DNA index, age, and leukocyte count [32]. As mentioned previously, another favorable cytogenetic feature is the recently described TEL-AML1 fusion which results from translocation of chromosomes 12 and 21 (t(12;21)(p12;q22)). This is currently being evaluated prospectively in ongoing clinical trials.

**Future Directions**

With the advance in molecular diagnostic techniques, investigators have discovered changes at the DNA level that help to further subclassify ALL beyond immunophenotype, even beyond karyotype, and will continue to further refine therapeutic approaches. One exciting area of leukemia research that utilizes these techniques is the study of minimal residual disease (MRD). The term MRD in leukemia refers to leukemic cells that are still present despite clinical remission. Most patients have approximately $10^3$ leukemic cells at diagnosis, and remission is defined as fewer than 5% blasts in the bone marrow, at which time patients may still have as many as $10^{10}$ leukemic cells remaining [49].

There are many different strategies to detect lower levels of disease than can be resolved through morphologic examination of the bone marrow. Techniques can be focused on the detection of cells with karyotypic abnormalities (through FISH or standard cytogenetics); these approaches are not particularly sensitive and in general can only detect leukemia if it is present in about 1 of 100 cells. A highly sensitive technique uses PCR to detect genetic abnormalities, which can find one leukemia cell in between $10^3$ and $10^6$ normal cells. In addition, flow cytometry to detect abnormal immunophenotypes...
is very sensitive [49]. One can also look for antigen-receptor gene rearrangements by PCR, which takes advantage of the fact that lymphoid cells have unique rearrangements of the immunoglobulin and T-cell receptors; these can be detected to identify a monoclonal population. This last technique is the most widely used and applicable for MRD studies in ALL patients. Targets can be identified in about 95% of patients [50], whereas a much lower percentage of patients will have karyotypic abnormalities to be used as targets.

Although the clinical relevance of the presence and amount of MRD at various time points during and after therapy is still being worked out, several studies indicate that MRD in childhood ALL is an important independent prognostic feature that can help to identify patients at high and low risk of relapse [50, 51]. This is particularly intriguing from a therapeutic standpoint when we consider that there are data to suggest that augmentation of therapy for children with a slow response to therapy can improve outcome [52].

Another exciting area of research which may prove to be important in further refining therapy for patients with ALL is the field of pharmacokinetics. It has been recognized that there is wide variability in systemic clearance of antineoplastic drugs (by as much as a factor of 3 to 10) [53], as well as in the absorption of orally administered chemotherapy agents such as 6-mercaptopurine [54]. Studies have shown significant relationships between systemic drug exposure to both methotrexate [55] and 6-mercaptopurine [56] and outcome in childhood ALL. In addition, a recent study showed improved outcome in children with ALL who received individualized postremission doses of chemotherapy based on rates of clearance, compared to children receiving conventionally dosed drugs calculated on the basis of body-surface area [57]. Table 4 outlines known pharmacologic and pharmacokinetic predictors of outcome.

It is possible that the prognosis for ALL can be further improved by customizing chemotherapy regimens beyond groups of patients with genetically and clinically similar subtypes of ALL to the level of the individual patient.

**CONCLUSION**

Despite the large number of known prognostic features, the most important predictors of poor outcome (such as Ph+ and hyperleukocytosis) occur in a small proportion of patients and therefore explain only a fraction of the variability in outcome for patients with ALL [58]. It is interesting to note that many features which at one time carried substantial prognostic significance have lost their weight with improvements in therapy; this attests to the success of the risk classification system and supports the need for further investigation in this rapidly moving field. At the same time, the constantly emerging new data also pose a great challenge for clinical researchers to determine how to best incorporate the data into ongoing clinical trials.

We continue to need better predictors in order to further refine therapy and choose populations needing novel therapy, at the same time avoiding overtreating patients likely to do well. Perhaps the solutions to this problem lie in the continuing advancement of molecular diagnostic techniques, detection and quantitation of MRD, and individualized therapy based on pharmacokinetic investigations. Indeed, it is possible to imagine a time when the classic distinctions based on age and WBC will no longer be relevant with the further identification of the changes at the DNA level that lead to leukemogenesis, and treatments will be based solely on the genetic subtype of ALL or a more direct measure of the biological properties of the leukemic cell. Also, duration of therapy could theoretically be adapted to the individual patient as techniques for quantifying minimal disease improve and its clinical significance becomes better understood.

### Table 4. Pharmacologic determinants of outcome in ALL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relationship to outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic exposure to methotrexate during post-remission chemotherapy</td>
<td>Individualized dosing based on clearance improved outcome in B-lineage ALL</td>
<td>[57]</td>
</tr>
<tr>
<td>Systemic exposure to 6-mercaptopurine during continuation</td>
<td>Relapsed patients had lower mean area under the concentration-time curve than patients in remission</td>
<td>[56]</td>
</tr>
<tr>
<td>Dose intensity of 6-mercaptopurine during continuation</td>
<td>Increased dose intensity associated with improved outcome</td>
<td>[59]</td>
</tr>
<tr>
<td>Thiopurine methyltransferase (TPMT) phenotype (enzyme for mercaptopurine metabolism)</td>
<td>TPMT defective phenotype associated with increased toxicity Improved outcome in patients with TPMT defective phenotypes</td>
<td>[60] [59]</td>
</tr>
<tr>
<td>Use of dexamethasone versus prednisone during post-remission chemotherapy</td>
<td>Improved outcome when prednisone replaced with dexamethasone, with lower risk of systemic and CNS relapse</td>
<td>[61, 62]</td>
</tr>
</tbody>
</table>
REFERENCES


6. Shurtleff SA, Buijs A, Behm FG et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia 1995;9:1985-1989.


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