Prognostic Importance of Structural Chromosomal Abnormalities in Children With Hyperdiploid (>50 Chromosomes) Acute Lymphoblastic Leukemia


Approximately one fourth of children with newly diagnosed acute lymphoblastic leukemia (ALL) have hyperdiploid (>50 chromosomes) blasts and a relatively favorable prognosis. Nonetheless, a substantial proportion of these patients fail therapy. We studied 138 children (70 male, 68 female) with hyperdiploid >50 ALL to assess initial clinical and cytogenetic features that might predict treatment failure. In 85 of these cases (62%), structural chromosomal abnormalities were also present; clinical and laboratory features in this group did not differ from those of the 53 cases with only numeric abnormalities. However, of the 28 failures seen at a median follow-up of 4 years, 22 occurred in cases with structural chromosomal abnormalities (P = .03 by Breslow test). In a multivariate analysis, only the presence of structural chromosomal abnormalities and male gender were independently associated with treatment failure. Structural chromosomal abnormalities in cases of ALL with greater than 50 chromosomes may define a biologically different form of leukemia characterized by increased likelihood of drug resistance.

T HE IMPORTANT prognostic implications of chromosome number (ploidy) in childhood acute lymphoblastic leukemia (ALL) have been confirmed by several groups.2-10 Among the major karyotypic subgroups of ALL, those cases characterized by hyperdiploid >50 chromosomes have the most favorable prognosis.11-18 Hyperdiploid >50 is significantly more frequent in early pre-B ALL than are other karyotypic subgroups and is associated with other established favorable prognostic features, including lower leukocyte count, lower serum lactic dehydrogenase levels, white race, and ages between 2 and 10 years.11,18,19 Despite this generally favorable constellation of prognostic variables, one third to one fourth of these patients relapse with current therapy for reasons that are largely unknown.4,5,7,9 In this study of 138 cases of childhood hyperdiploid >50 ALL, we demonstrate that patients with concurrent structural chromosomal abnormalities have a less favorable prognosis than those with only numeric abnormalities.

MATERIALS AND METHODS

From August 1980 to July 1988, 695 children with newly diagnosed ALL were admitted consecutively to St Jude Children's Research Hospital. Chromosomal analysis of bone marrow leukemia cells was performed in 582 patients, 519 of whom had successful G-banding of the chromosomes. The present study was restricted to the 138 cases with hyperdiploidy >50 who had banded leukemia-cell chromosomes; detailed cytogenetics for approximately one fourth of these cases have been reported previously.1,21 These children were enrolled in two Total Therapy studies (X and XI) after informed, written consent was obtained. The investigations were approved by the institution's clinical trials review committee.

Cytogenetic analysis. Bone marrow samples were processed according to the method of Williams et al,22 and metaphase preparations were G-banded by a modification of the trypsin method of Seabright.7 The International System for Human Cytogenetic Nomenclature (1985) was used to classify chromosomal abnormalities11; definition of an abnormal stemline was that proposed by the Second International Workshop on Chromosomes in Leukemia.19 Cases with hyperdiploidy >50 were then subclassified into those with only numeric abnormalities (gain of whole chromosomes) and those with additional structural chromosomal abnormalities (translocations, deletions, duplications, isochromosomes, inversions, and unclassifiable markers).

DNA content determination. Leukemic marrow samples were stained with a DNA-specific dye, propidium iodide, and were analyzed by flow cytometry, as previously described.20 The DNA index (ratio of DNA content in leukemic G0/G1 cells vs normal diploid G0/G1 cells) was determined. This measure correlates closely with chromosome number; hence leukemic cells with a DNA index of 1.0 have approximately 46 chromosomes, and those with a DNA index of 1.16 have approximately 53 chromosomes.

Statistical analysis. Differences in the distribution of clinical and biological features between cases with numeric only vs structural plus numeric abnormalities were tested by the two-tailed Fisher exact test or the Pearson chi-square test. Time to failure (defined as the interval between remission and relapse or death due to any cause) was estimated by the Kaplan-Meier procedure20; differences were analyzed by the Breslow test, which tends to be sensitive to differences evident early in time.21 Patients who did not enter complete remission were assigned a failure time of zero. The influence of potentially significant prognostic factors on time to failure for the overall group with hyperdiploidy >50 was estimated with the Cox proportional hazards model,20 which permits comparison of treatment outcome for subsets of patients while simultaneously adjusting for the effect of other factors (covariates). A stepwise selection procedure was used to determine the most significant factors related to time to failure. Any factor with a P value less than 0.10 at each step of the analysis was selected to the multivariate model. The factors tested included structural chromosomal abnormalities, leukocyte count, hemoglobin level, age, race, sex, French-American-British (FAB) classification, DNA index, liver and spleen size, and treatment efficacy.
RESULTS

The 138 children (70 male, 68 female) with hyperdiploid >50 ALL ranged in age from 0.9 to 17.4 years (median, 3.6 years). Although cytogenetic data were available for 13 patients under 1 year of age, only one had hyperdiploidy >50. Presenting features of the patients with hyperdiploid >50 ALL are summarized in Table 1. All but 11 cases were tested for blast-cell immunophenotype; 81% had early pre-B ALL, 16% pre-B, and 3% T-cell. The morphological subtypes of blast cells were FAB L1 in 123 patients and L2 in 13; two cases were not classifiable. Leukocyte counts ranged from 0.8 to 332 x 10^9/L (median, 6.6 x 10^9/L); platelet counts from 0 to 831 x 10^9/L (median, 46 x 10^9/L); and hemoglobin levels from 1 to 14.3 g/dL (median, 7.6 g/dL). Other clinical findings were massive hepatosplenomegaly (liver or spleen edge palpable more than 5 cm below the costal margins) in 56 cases, CNS leukemia in six, and mediastinal mass in three.

The distribution of modal chromosome numbers of the primary leukemic cell lines revealed a peak at 55 chromosomes (Fig 1). Structural chromosomal abnormalities in addition to the gain of whole chromosomes were found in 85 cases (62%). These abnormalities comprised translocations (26), duplications (25), deletions (15), isochromosomes (9), inversions (4), ring chromosome (1), and unclassifiable markers (41). Many cases had more than one abnormality. The distribution of modal chromosome numbers and the presenting clinical and laboratory features did not differ between the 53 cases with only numeric abnormalities and the 85 cases with both numeric and structural abnormalities (Fig 1 and Table 1).

At a median follow-up of 4 years, 28 patients (18 boys and ten girls) with hyperdiploid >50 ALL had failed treatment: 18 patients experienced hematologic relapse and 4 extramedullary relapse; 2 were induction failures; 2 died; and 2 showed lineage shift to acute myeloid leukemia. These patients were ages 2.1 to 16.6 years (median, 3.8) at diagnosis. Of the 26 cases tested for immunophenotype, 19 had early pre-B, five pre-B, and two T-cell ALL. Morphol-
ogy was FAB L1 in 23 cases and L2 in four; one case was not classifiable. Initial leukocyte counts ranged from 0.8 to 332 \times 10^9/L (median, 9.7 \times 10^9/L), platelet counts from 0 to 445 \times 10^9/L (median, 31.5 \times 10^9/L) and hemoglobin levels from 3.2 to 13 g/dL (median, 8.1 g/dL). Massive hepatosplenomegaly was noted in 11 patients, initial CNS leukemia in three, and mediastinal mass in one. The distribution of modal chromosomal numbers of primary leukemic stemlines in these 28 patients was similar to that of the overall group with hyperdiploidy \geq 50 (Fig 1). Structural chromosomal abnormalities were found in 22 of these 28 patients and included deletions (7 cases), translocations (4), isochromosome 17q (4), duplications (5), and unclassifiable markers (12).

Among the presenting features analyzed, two were associated with early treatment failure: presence of structural chromosomal abnormalities \( (P = .03, \text{Fig 2}) \) and male sex \( (P = .07). \) In the stepwise Cox regression analysis, both of these features retained independent prognostic significance (Table 2).

When compared with 321 cases in other ploidy groups with structural chromosomal abnormalities, the treatment result was significantly better for the hyperdiploid \( \geq 50 \) cases with only numeric abnormalities \( (P = .001) \) but not for those with additional structural abnormalities \( (P = .15). \)

**DISCUSSION**

The presence of structural chromosomal abnormalities was associated with early treatment failure in a group of 138 patients with hyperdiploidy \( \geq 50 \) ALL. The data suggest that 80% of cases with numeric chromosomal abnormalities only \( v 60% \) of those with concurrent structural abnormalities will be long-term survivors. Structural chromosomal abnormalities in general are associated with a poor treatment outcome in this study; additional studies are needed to determine whether any specific structural rearrangements confer a particularly adverse prognosis. We have previously suggested isochromosome 17q as an adverse cytogenetic feature in hyperdiploid \( \geq 50 \) childhood ALL.\(^\text{11}\) Four of the six cases with isochromosome 17q in this study have failed treatment. Male gender also had a significant adverse influence on treatment outcome in hyperdiploid \( \geq 50 \) ALL. This finding is not unexpected, as male gender has been shown to be a poor prognostic factor in other studies of childhood ALL.\(^\text{25}\)

Four patients with leukemic-cell chromosome number above 65 were included in this study. Although cytogenetic patterns of these four cases (near-tetraploidy in three) differed from those with chromosome numbers between 51 and 65, the rarity of their occurrence precluded meaningful analysis of clinical significance. In a reanalysis excluding these patients (data not shown), the presence of structural chromosomal abnormalities remained an important adverse prognostic feature \( (P = .036, \text{Brelow test}). \)

Table 2. Analysis of Risk Factors for Treatment Failure Among Patients With Hyperdiploidy \( \geq 50 \) ALL

<table>
<thead>
<tr>
<th>Feature</th>
<th>Category</th>
<th>Values</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural abnormality</td>
<td>Absent</td>
<td>Present</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Total XI</td>
<td>Total X</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td>DNA index</td>
<td>( \geq 1.16 )</td>
<td>( &lt; 1.16 )</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level (g/dL)</td>
<td>( &lt; 8 )</td>
<td>( \geq 8 )</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Leukocyte count (( \times 10^9/L ))</td>
<td>( \leq 10 )</td>
<td>( &gt; 10 )</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>Black</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>FAB classification</td>
<td>L1</td>
<td>L2</td>
<td>0.41</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>2 to 9</td>
<td>Others</td>
<td>0.80</td>
<td></td>
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<tr>
<td>Spleen size (cm)</td>
<td>( &lt; 5 )</td>
<td>( \geq 5 )</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Liver size (cm)</td>
<td>( &lt; 5 )</td>
<td>( \geq 5 )</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

*From the likelihood ratio test.  
†Comparison of time to failure, with use of the Cox proportional hazard model, between patients with favorable v unfavorable features, without adjustment for the effects of other variables.  
‡As above, except that stepwise regression analysis was used to identify the best predictors of treatment failure, taking into account the competing effects of all covariates entered into the Cox model. The hazard ratio gives the estimated proportional increase in the risk of treatment failure at any given time for a patient in the unfavorable category relative to one in the favorable category.
some 6 with improved prognosis among children with hyperdiploid ALL. We were not able to demonstrate such an association in our cases with hyperdiploidy >50 (data not shown). However, in the study by Jackson et al., cases with hyperdiploidy 47-50 were included in the analysis. In our experience, an added chromosome 6 was found in only one of 36 cases with hyperdiploidy 47-50 but in 120 of 138 cases with hyperdiploidy >50 (P < .0001). Thus the good prognosis reported for cases with extra chromosome 6 could be explained by their association with hyperdiploidy >50, which is related to a better treatment outcome than is seen with hyperdiploidy 47-50.1,4,6-10

Past efforts to demonstrate structural abnormalities in cases of hyperdiploid ALL have met with limited success because of technical difficulty in obtaining well-banded preparations.4,5,12,23 With marked improvements in cytogenetic techniques, it has been possible to identify almost all chromosomes in cases with hyperdiploidy >50.24 Among the hyperdiploid >50 cases reported here, structural chromosomal abnormalities were found in two thirds, a proportion far exceeding that recognized previously. This technical advance undoubtedly contributed to our ability to identify the subset of hyperdiploid >50 cases with prognostic relevance. In this regard it is noteworthy that hyperdiploid >50 cases with and without structural chromosomal abnormalities have similar presenting features and would not otherwise be distinguished from each other.

Flow cytometric analysis of cellular DNA is a powerful tool for identifying hyperdiploidy and has definite advantages over karyotyping.20,25 It is automated and much more rapid; results can be obtained from virtually all cases; and measurements are not affected by the proliferative rate and mitotic index of the cell population. Thus the hyperdiploid stemline may be identified in some cases with inadequate cytogenetic studies. However, this technique does not identify the specific chromosomes gained or lost, nor does it detect structural rearrangements with important prognostic implications. Therefore determination of cellular DNA content and karyotypic analysis should be regarded as complementary studies in the evaluation of patients with ALL.

The mechanism by which hyperdiploidy influences response to therapy remains unclear. It has been proposed that hyperdiploid cells are more sensitive to treatment with cell phase-specific drugs because they have a longer duration of S-phase.26 Others have suggested that hyperdiploid cells have the capacity to differentiate into nonproliferating cells that are less aggressive and more responsive to corticosteroid treatment.27 The presence of structural chromosomal abnormalities in hyperdiploid cases may reflect an increased rate of somatic cell mutation and thus correlate with an increased likelihood of development of drug resistance.

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CH Pui, SC Raimondi, RK Dodge, GK Rivera, LA Fuchs, M Abromowitch, AT Look, WL Furman, WM Crist and DL Williams