Prognostic Importance of Chromosome Number in 136 Untreated Children With Acute Lymphoblastic Leukemia

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Leukemia cell karyotypes were determined at diagnosis for 136 of 159 consecutive patients with acute lymphoblastic leukemia (ALL) who were followed for up to 35 mo. Ninety patients (67%) had abnormal karyotypes. Five chromosome categories were designated, based on the distribution of modal numbers: hyperdiploid (>50) (n = 41), hyperdiploid 47-50 (n = 18), pseudodiploid (n = 28), normal (n = 46), and hypodiploid (n = 3). Treatment response was assessed for the categories in terms of time to failure (induction failure, first relapse, or death). Children in the hyperdiploid category had the best responses to treatment, with only 2 failures, and those in the pseudodiploid category had the poorest (p < 0.001). The remaining 3 chromosome categories had intermediate responses and formed a third prognostic group. This same influence of chromosome number on time to failure was evident within the 2 clinical prognostic groups: high risk, signified by a leukocyte count >100 x 10^9/liter, meningeal leukemia, mediastinal mass, or the presence of blasts that formed rosettes with sheep erythrocytes at 37°C, and standard risk, indicated by the absence of these features. The influence of chromosome number on time to failure was also the same within the historically favorable prognostic group that had common ALL. Results of a multivariate analysis indicated that chromosome number was the strongest single predictor of outcome (p < 0.001) and was the only variable that added significant prognostic information to leukocyte count (p < 0.001). The combination of chromosome number and leukocyte count should more clearly distinguish patients with ALL at low or high risk of relapse.

The relationship of chromosome abnormalities to prognosis in acute lymphoblastic leukemia (ALL) has been debated for a number of years. Among selected patients, a poor prognosis has been associated with specific abnormalities in leukemia cells: the Philadelphia chromosome (Ph1-positive ALL), the 14q+ marker (B-cell ALL), and low modal numbers (near-haploid ALL). However, early studies of large groups of unselected patients failed to disclose the clinical significance of specific abnormalities and patterns, or the correlation between karyotype and treatment outcome. More recently, Secker-Walker et al. and Swansbury et al. have presented evidence that chromosome number is an independent prognostic indicator in ALL. Likewise, the Third International Workshop on Chromosomes in Leukemia found that chromosome number and also specific karyotypic aberrations were significant prognostic indicators.

We report the relationship of the karyotype chromosomal number at diagnosis to treatment outcome for 136 children and adolescents with ALL admitted to St. Jude Children’s Research Hospital and followed for up to 35 mo. The results clearly demonstrated that chromosome number is a stronger predictor of treatment outcome than any other prognostic factor evaluated in this study.

MATERIALS AND METHODS

Patients and Treatment

From July 1, 1978 to July 31, 1980 a total of 159 patients with ALL were admitted to this center. Their diagnosis was based on malignant cell cytology and special staining characteristics. Cytogenetic analysis of leukemia cells was attempted for all 159 patients, with successful studies obtained for 136. 53 were entered in Total-Therapy Study IX and 83 in Total-Therapy Study X (unpublished, protocols available on request).

All patients in study IX received induction therapy with prednisone, vincristine, and daunomycin. One-third received asparaginase as a fourth agent, and another third received an intensified phase of treatment with cytosine arabinoside (ara-C) and asparaginase. All patients received central nervous system (CNS) prophylaxis with 2400 rad cranial radiation and intrathecal methotrexate (i.t. MTX). Continuation therapy for all patients was weekly MTX and daily 6-mercaptopurine (6-MP). Patients at standard or high risk of relapse were given additional therapies distributed evenly among the three treatment groups. High-risk features included a leukocyte count >100 x 10^9/liter, mediastinal mass, CNS leukemia, or blasts that formed rosettes with sheep erythrocytes (E) at 37°C. Patients at standard risk of relapse were those without high-risk features.

In study X, patients at high risk of relapse were initially treated with the podophyllium compound VM-26 in combination with ara-C, followed by prednisone, vincristine, and asparaginase. Continuation therapy consisted of alternating courses of 6-MP + MTX and VM-26 + ara-C. Standard-risk patients were randomized into two groups after remission induction with prednisone, vincristine, and asparaginase. One group received high-dose MTX and i.t. MTX for CNS prophylaxis, with 6-MP and MTX for continuation therapy;
the other received 1800 rad cranial radiation plus i.t. MTX, followed by sequential courses of 6MP + MTX, cyclophosphamide + Adriamycin, and VM-26 + ara-C. Unless disease recurred, therapy continued for 2.5 yr for all patients.

**Chromosome Analysis**

For chromosome preparations, we used a direct bone marrow technique that included several modifications (unpublished) of standard procedures. These modifications include: (1) the use of media 1640 plus 30% fetal calf serum as both the carrying vehicle and the vehicle for colchicine treatment; (2) only 0.1 ml of packed cells or less processed per centrifuge tube; (3) 25 min as the total time for exposure to colchicine; (4) careful control of time of exposure of cells to the hypotonic solution; (5) use of an edging-flaming method of slide preparations; and (6) use of a G-banded technique modified from Seabright. For 26 marrow samples that had a low percentage of mitotic cells and an identified leukemia stem line, a 24-hr unstimulated culture of lymphoblasts was also used to supplement the bone marrow. For each case, 10-30 (mean 20) metaphases were studied by direct microscopy to determine the stem lines and modal karyotype. Karyotypes were prepared from photographic prints to confirm the analysis.

Banding allowed successful identification of most or all chromosomes in 92 (68%) of the 136 cases. For the remaining 44 cases, we determined the modal number, the Denver groups, any marker chromosomes, and the karyotype category.

The definition of an abnormal stem line proposed by the International Workshop on Chromosomes in Leukemia was used. In our study, a case was designated as abnormal if it had an abnormal clone, regardless of the proportion of normal metaphases. Normal cells were assumed to be present in most leukemic marrows, even though they may not have been evident microscopically. We confirmed this assumption by simultaneous flow cytometric analysis of the DNA content in marrow cells.

In three unusual cases that had both pseudodiploid and hyperdiploid blasts, the predominant line (hyperdiploid in all three) was used for classification. By definition, a pseudodiploid karyotype is one that contains 46 chromosomes with one or more structural abnormalities, the gain–loss of whole chromosomes, or both types of abnormalities.

**Determination of Other Cell Features**

Marrow samples were simultaneously assessed for cytological features (including FAB classification), special staining characteristics, and surface marker features. The special stains included periodic acid Schiff, Sudan black, peroxidase, and specific/nonspecific esterases. Lymphoblasts were classified as common ALL (cALL), thymus (T), bone-marrow-derived (B), or undifferentiated (U) cells, based on the presence or absence of E-rosette formation, surface immunoglobulin, and reactivity with rabbit heteroantisera against T, cALL, and Ia antigens.

**Statistical Analysis**

Significant associations of prognostic features to chromosome number were determined by one-way analysis of variance for the factors age and leukocyte count, and by the generalization of Fisher's exact test for discrete variables such as sex and race.

We then assessed the influence of chromosome number on treatment responses, measured as the time to failure (failure being a lack of response to induction therapy, a relapse during first complete remission, or death). The time to failure for patients grouped according to the chromosome categories was estimated by means of Kaplan-Meier life-table curves, and significant differences were determined by the chi-square test.

We also compared the chromosome categories with time to failure in patients at standard and high risks of relapse and in patients subgrouped according to cell surface markers.

Because of the possible correlation of chromosome number with known prognostic factors, the influence of these factors on treatment outcome was determined by Cox regression analysis. Log leukocyte
RESULTS

Chromosome Categories

The distribution of chromosome modal numbers for all 136 patients with successful studies suggested a bimodal curve with a major peak at 46, a small peak at 55, and a hiatus at 50 (Fig. 1). The hyperdiploid group (n = 59) was divided accordingly: those with more than 50 chromosomes (n = 41) and those with 47–50 chromosomes (n = 18). (In this study, the term hyperdiploid indicates any case having more than 46 chromosomes. No attempt was made to distinguish near triploid and near tetraploid numbers.) The 47–50 group contained 4 patients with Down’s syndrome; 2 of the 4 had chromosome abnormalities in addition to trisomy 21. In the hyperdiploid >50 cases, the added chromosomes most often formed trisomies, and in each case, the number of whole chromosomes gained (or lost) exceeded the number of structural abnormalities. The other three groups were pseudodiploid—the most frequent single mode for the abnormal cases—(n = 28), normal (n = 46), and hypodiploid (n = 3).

Chromosome Categories and Prognostic Features

Comparison of the initial clinical and laboratory features of patients in the five chromosome categories (Table 1) indicated a significant relationship between chromosome number and seven features: age, sex, race, mediastinal mass, E-rosette status, leukocyte count, and risk classification (p < 0.05). The >50 group had the most favorable features (lower mean leukocyte count and fewer patients with mediastinal mass, CNS leukemia, or E-rosette-positive blasts), whereas the pseudodiploid group had the least favorable. However, with the exception of the two B-cell (L-3) cases, which were pseudodiploid, none of the features were specific for any chromosome category. Although hyperdiploidy characterized the group at standard risk of relapse, 15 karyotypes were pseudodiploid. Similarly, although many patients at high risk of relapse were pseudodiploid, some were hyperdiploid.

Chromosome Categories and Time to Failure

When the times to failure for patients with normal (n = 46) versus abnormal (n = 90) karyotypes were studied, there was no significant difference (p = 0.74,
Fig. 2. Estimated time-to-failure curves for patients with normal and abnormal karyotypes. The curves of these two groups were not significantly different. About one-third of the patients in each group failed.

Fig. 3. Estimated time-to-failure curves for four major chromosome categories. The >50 group and the pseudodiploid group had the lowest and highest failure rates, respectively. The pseudodiploid curve begins with a lower proportion of patients, indicating more induction failures. The intermediate curves of the 47–50 and normal groups were not significantly different and could be combined to form an intermediate prognostic group (see text). The three hypodiploid cases were also added to the combined group, even though this group’s eventual classification is uncertain.
pseudodiploid curves were again significantly different ($p < 0.001$). Even at the outset of treatment, the differences among the chromosome categories are apparent, as shown by the proportions of induction failures. Fifty-three percent of the induction failures occurred in the pseudodiploid group.

Analysis of individual patient response disclosed that the two failures in the >50 group were among the 3 patients with multiple abnormal chromosome lines. One of the two patients who failed had a 53-chromosome line and a small pseudodiploid line that appeared to be independent clones. The other who failed had a predominant line containing 92 chromosomes, which was an exact doubling of a pseudodiploid stemline. The third patient with multiple abnormal lines had apparently unrelated pseudodiploid and 56-chromosome lines and has not failed.

An extremely important finding was the absence of induction failures and deaths among the >50 group. Equally important, in addition to the large number of induction failures, half of the deaths occurred in the small pseudodiploid group. Another main finding was that the therapeutic responses of patients with apparently normal karyotypes were not as good as one might expect; a third of the induction failures, first relapses, and deaths were in this group.

**Chromosome Categories, Prognostic Subgroups, and Time to Failure**

The influence of chromosome number on therapeutic responses was assessed for patients at standard and high risks of relapse (see Materials and Methods). In both clinical groups (Fig. 4, A and B), the time-to-failure curves for the chromosome categories were very similar to those shown in Fig. 3. Studies were also done for 94 patients with cALL-antigen-positive blasts—a subgroup with a good prognosis.31 Again, the time-to-failure curves corresponded with those in Fig. 3: only 1 of 33 patients failed in the >50 group, whereas 12 of 16 failed in the pseudodiploid group and 9 of 45 failed in the combined group ($p < 0.001$). Only 23 patients had T-cell ALL and only 10 had U-cell ALL, perhaps accounting for the lack of a significant difference between their time-to-failure curves. There were only two B-cell cases; both were pseudodiploid and both patients died of disease.

**Translocations in Pseudodiploid Cases and Survival Duration**

Certain translocations carry a very poor prognosis.9 Since 10 (36%) of the 28 pseudodiploid karyotypes had identifiable translocations, we compared the outcomes of pseudodiploid patients with and without translocations. Over a 44-mo period, 80% of those with translocations failed (median survival, 14 mo; range, 1–40 mo), compared to 61% without translocations (median survival, 27.5 mo; range, 1–42 mo). Different translocation categories were not included in our time-to-failure analysis because the numbers were too small; however, two patients with B-cell leukemia, a 46 stem line, and the t(8;14) abnormality failed and died at 3 (induction failure) and 5 mo after diagnosis. All four patients with the Philadelphia chromosome and a 46 stem line failed; three died at 7, 10, and 25 mo, and the fourth is still living at 40 mo. Another patient with the Philadelphia chromosome and 47 and 49 chromosome lines survived for 1 mo (induction failure). The translocation to chromosome 9 or another chromosome could be established in only 3 of the 5 cases with 22q-.- No case with t(4;11) was found in this study.

Five other cases had translocations that were all different and did not match any previously recognized category. Survival times were 7, 18 (induction failure), 33+, 23+, and 24+ mo. All were pseudodiploid
Table 2. Cox Regression Analysis of Time-to-Failure as a Function of Prognostic Variables

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>2 x Log Likelihood Ratio for the Prognostic Variable*</th>
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<tbody>
<tr>
<td></td>
<td>2 x Log Likelihood Ratio</td>
</tr>
<tr>
<td>Log leukocyte count</td>
<td>22.64</td>
</tr>
<tr>
<td>Chromosome number, 5 categories†</td>
<td>33.81</td>
</tr>
<tr>
<td>Chromosome number, 3 categories‡</td>
<td>33.62</td>
</tr>
<tr>
<td>Relapse risk (standard/high)‡</td>
<td>12.97</td>
</tr>
<tr>
<td>Mediastinal mass</td>
<td>7.46</td>
</tr>
<tr>
<td>Age (&lt;2/2–5/&gt;5 yr)</td>
<td>8.04</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5.38</td>
</tr>
<tr>
<td>Race (white/nonwhite)</td>
<td>5.45</td>
</tr>
<tr>
<td>Cell markers (cALL/T/B/U)</td>
<td>7.68</td>
</tr>
<tr>
<td>FAB morphology (L-1/L-2)</td>
<td>1.43</td>
</tr>
<tr>
<td>E-rosette status (+/-)</td>
<td>0.95</td>
</tr>
<tr>
<td>CNS leukemia</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*For single variables, 2 x log likelihood ratio had to exceed 3.84–9.49 (depending on the number of categories) to be significant at the 0.05 level.

†Hyperdiploid >50, pseudodiploid, hyperdiploid 47–50, normal, and hypodiploid; the last three groups were combined into one in the second analysis.

‡High risk designated the presence of one or more of these features: leukocyte count >100 x 10^9/liter, mediastinal mass, CNS leukemia, or E-rosette-positive blasts. Standard risk designates the absence of these features.

except the last case, in which there were 47 chromosomes.

**Multivariate Analysis**

When all variables were analyzed as single factors with the Cox regression model, chromosome number (considered as 5 or 3 categories) and log leukocyte count were the strongest indicators of outcome (Table 2, both p < 0.001). Each of the remaining factors, except FAB morphology, E-rosette status, and CNS leukemia, had a significant but lesser influence on treatment response. The lack of significant effects for E-rosette status and CNS leukemia may have been due to the low numbers of patients who were positive. When log leukocyte count was the first variable in the model, chromosome number was the only other variable that added significant prognostic information.

**Other Analyses**

Our 136 patients were subjects of successive treatment studies of different chemotherapy regimens. However, the proportions of failures within our study population when separated by treatment group were not significantly different. Thus, it was justifiable to combine these treatment groups into one to assess the influence of chromosome number on treatment outcome.

Of the 159 patients entered in this study, 23 had chromosome preparations that could not be successfully assessed because of an inadequate amount of marrow or an insufficient number of metaphases. The clinical features and time to failure for these 23 patients did not differ significantly from those of the 136 patients who were successfully studied. Thus, the study sample was not inadvertently biased by difficulties in obtaining the marrow aspirates.

All studies presented in terms of time to failure were also analyzed in terms of length of first complete remission and length of survival. In these additional studies, the patterns of response by chromosome categories were similar to those presented here.

**DISCUSSION**

This study provides definitive evidence that chromosome number is directly related to treatment outcome in childhood ALL. Our findings confirm the earlier work of Secker-Walker et al.7 for a smaller group of patients and closely agree with a preliminary report of Swansbury et al.8 Our results also agree with the portions of data presented by the Third International Workshop on Chromosomes in Leukemia, which relate to prognosis and modal number in children with ALL. We offer new information on the importance of chromosome number relative to currently recognized prognostic variables and to clinical subgroups at standard and high risk of relapse.

Our decision to separate the hyperdiploid group at the 50-chromosome mark was somewhat arbitrary. However, a natural division at that point may exist because there appeared to be a bimodal distribution of
chromosome numbers and because the treatment responses of the >50 and 47–50 groups were different. Since the >50 group had the best therapeutic response overall, one would expect that these cases would be concentrated in the clinical subgroups historically associated with a good prognosis: those at standard risk of relapse and those with cALL.\textsuperscript{21} The results presented in Table 1 support this expectation.

The reason for the markedly different treatment responses between the hyperdiploid >50 and pseudodiploid groups is unknown. It may be pertinent that in each case within the >50 group, most abnormal chromosomes were intact additions; morphological abnormalities, when present, formed a less prominent part of the total karyotype, as compared with pseudodiploid cases. The possibility that large amounts of added chromosomal material adversely affect hyperdiploid cell function should be considered. A second distinguishing feature is the uniform lack of a normal complement of chromosomes in pseudodiploid cells, whereas cells with more than 50 chromosomes may contain a normal complement in addition to various numerical and structural abnormalities. These differences could contribute to the relative lack of aggressiveness shown by hyperdiploid >50 ALL versus pseudodiploid ALL (lower initial white counts and lower frequency of high-risk features, Table 1). A survival advantage of pseudodiploid cells over hyperdiploid >50 cells, in the face of equivalent chemotherapy, is suggested by the outcome of one of our cases in which the child had blasts of both types at diagnosis (predominantly hyperdiploid), but only pseudodiploid blasts at relapse. Thus, in cases with multiple abnormal chromosome lines, the lines may respond independently to chemotherapy, and the predominant line at diagnosis may not carry the most prognostic weight.

The Third International Workshop on Chromosomes in Leukemia\textsuperscript{8} has associated translocations with a poor prognosis in A.L.L. Many cases with the Philadelphia chromosome, a poor prognostic feature, have been pseudodiploid at diagnosis.\textsuperscript{1} Similarly, B-cell leukemia has been associated with a pseudodiploid karyotype in many cases, a structural abnormality, and a poor prognosis.\textsuperscript{22} Likewise, the (t4;11) abnormality has been associated with a poor prognosis and had a pseudodiploid karyotype in many cases.\textsuperscript{9} Our studies indicate that both the pseudodiploid pattern and translocations have a poor prognosis; however, when these factors are separated, the prognosis of the translocation group may be worse.

Yunis et al.,\textsuperscript{12} using high resolution banding, recently identified chromosome abnormalities in 100\% of their cases of acute nonlymphocytic leukemia. Consistently abnormal findings may also characterize ALL once a large series is studied by synchronized high-resolution techniques. Thus, some of those cases judged to be normal in our ALL series may have had extremely small morphological abnormalities or gene mutations undetectable by standard techniques. That our normal group had an intermediate prognosis suggests that these structural abnormalities, if present, may be functionally different from those in our pseudodiploid group, which had the poorest prognosis.

Leukocyte count is generally regarded as the best predictor of treatment outcome in A.L.L.\textsuperscript{24} The array of phenotypes that distinguish lymphoid cell subgroups has helped to increase our knowledge of normal versus malignant lymphoid differentiation, but has not sharpened prognostic capability to the degree that one would like. Any measure that could more accurately indicate the outcome of the disease would be important, particularly in modifying treatment for patients most likely to relapse. We present evidence that, as a prognostic factor, chromosome number is as good or better than leukocyte count, even within the clinical subgroup at high risk of relapse. The use of these two factors in concert may permit a sharper delineation of prognostic groups and therefore be of value in the development of future clinical trials.

**ACKNOWLEDGMENT**

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