Prognostic Significance of Additional Cytogenetic Abnormalities at Diagnosis of Philadelphia Chromosome-positive Chronic Granulocytic Leukemia

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Of 661 patients with Philadelphia chromosome (Ph)-positive, nonblastic chronic granulocytic leukemia, 58 had cytogenetic abnormalities in addition to the Ph at the time of diagnosis. Twenty patients had reduplication of the Ph in one or more metaphases. Twenty-one patients had reduplication of the Ph in one or more metaphases. Eleven patients had two or more hypodiploid metaphases as their only numerical abnormality. The remaining six patients had a variety of abnormalities. Many patients had more than one type of abnormality. Survival of patients in different subgroups was similar, but these 58 patients had a shorter course than the 603 patients without additional cytogenetic abnormalities \( P < .02 \).

Survival curves for the two populations did not diverge until the 2-year point, after which the annual death rate among patients with additional cytogenetic abnormalities was approximately 40% higher than that of patients without such abnormalities. The two populations had similar relative risk values according to a hazard ratio formula previously described by the International CGL Prognosis Study Group. Thus, they would have been expected to have essentially identical survival curves. We conclude that the presence of additional cytogenetic abnormalities at the time of diagnosis constitutes an independently significant prognostic feature with an unusually delayed influence on survival.

I T IS OFTEN STATED that the presence of additional karyotypic abnormalities in Philadelphia chromosome (Ph)-positive chronic granulocytic leukemia (CGL) is an ominous sign, but the precise prognostic implication of such findings is by no means clear. Additional cytogenetic abnormalities are detected in 70% to 80% of patients with blastic disease. This results in a statistical association with early death. However, these abnormalities often follow disease transformation and thus may not have predictive value. Some authorities make a distinction between the presence of additional cytogenetic abnormalities at diagnosis and their appearance later. Detection of a new abnormality during the course of CGL is viewed as evidence of impending blast transformation, whereas a similar finding at the time of diagnosis is considered to have little or no prognostic significance. This apparent paradox has not yet been resolved.

To properly evaluate the prognostic import of additional karyotypic abnormalities, these must be examined in conjunction with other disease features that may have prognostic significance in a patient population selected for cytogenetic study without bias. Patients registered in the International CGL Prognosis Study who underwent cytogenetic examination as part of their initial workup constitute such a population. A review of these patients, whose relative risk was estimated on the basis of other disease parameters, permitted the evaluation of additional cytogenetic abnormalities as an independent prognostic criterion at the time of diagnosis. We found that such abnormalities were associated with higher risk but that there was a significant delay before an increase in the mortality rate was evident.

MATERIALS AND METHODS

Patients. The computer file of the International CGL Prognosis Study was searched for Ph-positive patients who had been reported as having additional cytogenetic abnormalities at diagnosis. Questionnaires regarding these cases were sent to the responsible investigators at the cooperating institutions. These requested detailed information regarding the original cytogenetic findings. Patients who met our criteria for blastic disease at the time of initial study and had not been included in analyses of prognostic features were excluded. Adequate information was received from seven institutions: Duke University Medical Center, Roswell Park Memorial Institute, Memorial Sloan-Kettering Cancer Center, University of Bologna, University of Barcelona, University of Ulm, and Finsen Institute. These cytogenetic examinations were performed during the period 1962 to 1985. The majority of patients were studied before 1977, thereby providing a median follow-up of 10 years.

Classification of additional cytogenetic abnormalities. In about half of the cases, the initial cytogenetic examination had been performed before the adoption at the cooperating centers of modern banding techniques. Therefore, chromosomes involved in abnormalities were often not identified precisely, and some subtle structural changes were probably not recognized. Because of this, the following simplified classification of cytogenetic abnormalities was adopted: (a) reduplication of the Ph in one or more metaphases; (b) hyperdiploidy in one or more metaphases; (c) hypodiploidy, if present in at least two metaphases (a few cases were excluded because the preparation was described as of poor quality by the cytogeneticist, and chromosome loss appeared to be random); and (d) other. This category included structural abnormalities, additional translocations, and the presence of many chromosome breaks. In many cases, more than one of the aforementioned abnormalities was recorded.

Male patients reported as having a missing Y chromosome and...
one patient with the Ph resulting from a complex translocation with no other abnormalities were excluded since these abnormalities do not constitute unfavorable prognostic signs.  

Because of differing goals at individual member institutions at different time periods, in addition to technical problems with some specimens, the number of metaphases examined in these cases varied considerably. In almost one fifth of the cases, fewer than ten metaphases were examined, whereas in slightly over one fifth, 40 or more were scored. The median number examined was 20.

**Prognostic evaluation.** Patients with cytogenetic abnormalities in addition to the Ph were compared with those from the same seven institutions whose registry forms indicated that additional cytogenetic abnormalities had not been detected. This required exclusion of patients whose forms did not include either a “yes” or a “no” answer to the question regarding additional karyotypic abnormalities. The relative risk of each patient was calculated from the hazard ratio formula previously described by our group, with variables representing age, spleen size, platelet count, and the percentage of circulating blasts.

**Data processing.** The techniques of data collection and processing used by our group have been described previously. Survival estimates were obtained by the Kaplan-Meier product-limit method. Two patients who underwent allogeneic bone marrow transplantation used by our group have been described previously. Survival estimates were obtained by the Kaplan-Meier product-limit method. In almost one fifth of the cases, fewer than ten metaphases were examined, whereas in slightly over one fifth, 40 or more metaphases were examined, whereas in slightly over one fifth, 40 or more were scored. The median number examined was 20.

**RESULTS**

The study population consisted of 661 nonblastic patients. Of these, 58 (8.8%) were identified as having karyotypic abnormalities in addition to the Ph (other than missing Y or complex translocations) at the time of diagnosis (Table 1). This group consisted of 30 males and 28 females ranging in age from 11 to 85 years. The age distribution was quite similar to that of the 603 patients without additional cytogenetic abnormalities. There was a small relative excess of females in the group with additional cytogenetic abnormalities. There was a small relative excess of females in the group with additional cytogenetic abnormalities; this was not statistically significant ($P = .3$). At the last follow-up, nine patients (16%) were alive, 4 (7%) had died of causes unrelated to leukemia, and the remainder had died of leukemia 2 months to 11 years after diagnosis.

The distribution of relative risk values among the patients with additional karyotypic abnormalities was very similar to that among the 603 controls, and the medians were 1.00 and 0.95, respectively. These values indicate that the median patients in both of these groups had quantitative estimates of risk indistinguishable from that of the representative “average” patients in the population from which this hazard ratio formula was derived.

Of the 58 patients with additional abnormalities, 20 had reduplication of the Ph. In four cases, this was seen in only a single metaphase, and in 16, two or more metaphases contained extra Ph chromosomes. One patient had two metaphases containing three Ph chromosomes, and in one case, each of four metaphases examined contained five copies of the Ph! Nine of these 20 patients exhibited hyperdiploidy in addition to reduplication of the Ph, usually with extra C group chromosomes. No patient had hypodiploidy in association with double Ph. Reduplication of the Ph was usually seen in only a minority of cells examined, but in five cases, this abnormality was present in most or all metaphases. The course of these five patients did not appear to differ from that of the other 15; the earliest death was at 16 months, and their median survival was 31 months.

Of the remaining 38 patients, 21 exhibited hyperdiploidy, in a single metaphase and 13 in two or more metaphases. Five of these 21 patients with hyperdiploidy also had two or more hypodiploid metaphases.

Eleven patients had two or more hypodiploid metaphases as their only numerical abnormality. One of these patients also had an additional translocation, t(4;13), in all metaphases.

The remaining six patients had a variety of abnormalities including structural changes, frequent chromosome breaks, a second translocation in all metaphases, t(3;17), and “aneuploidy” as the only information furnished.

Nine of the 58 patients had only a single abnormal metaphase recorded. In two cases, this was a double Ph and in seven, a hyperdiploid metaphase. The survival of these patients did not differ significantly from that of the 49 patients who exhibited at least two abnormal metaphases ($P = .09$).

To determine whether there were significant differences in the prognostic import of the different types of abnormalities described earlier, we compared the survival of the three major groups (Fig 1). The 20 patients with reduplication of the Ph, the 21 with a single Ph but one or more hypodiploid

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**Table 1. Characteristics of Patients With and Without Additional Cytogenetic Abnormalities**

<table>
<thead>
<tr>
<th>Feature</th>
<th>No (n = 603)</th>
<th>Yes (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>355 (59%)</td>
<td>30 (52%)</td>
</tr>
<tr>
<td>F</td>
<td>248 (41%)</td>
<td>28 (48%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5-85</td>
<td>11-85</td>
</tr>
<tr>
<td>Median</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>Relative risk*</td>
<td>0.41-10.3</td>
<td>0.43-6.03</td>
</tr>
<tr>
<td>Median</td>
<td>0.95</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*According to a hazard ratio formula based on age, spleen size, platelet count, and percentage of circulating blasts.*

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![Fig 1. Survival of 20 patients with reduplication of the Ph. 21 patients with a single Ph and hyperdiploidy, and 11 patients with hypodiploidy.](image-url)
metaphases, and the 11 with two or more hypodiploid metaphases all had similar survival curves.

To rule out artifacts attributable to differences in the source of the patients and in cytogenetic practice at different institutions, we examined the survival of patients with additional cytogenetic abnormalities according to the reporting institution. No significant differences were seen among the four centers contributing at least ten patients or between any of these and the pooled group of nine patients from the other three centers.

Treatment of these patients varied considerably. The majority had received conventional single-agent chemotherapy, most often with busulfan or hydroxyurea. However, a large minority had received more intensive therapy ranging from periodic courses of two-drug combinations administered on an outpatient basis to a very aggressive multiantigen protocol requiring hospitalization. There was no difference in survival between these two categories of patients ($P = .9$).

Finally, we examined survival according to the percentage of metaphases with additional cytogenetic abnormalities. These figures ranged from 2% (eg, one metaphase with a double Ph of 50 examined) to 100% (eg, each of 25 metaphases showing the loss of chromosome 20 and an additional translocation). There was a slight trend toward longer survival in association with fewer additional cytogenetic abnormalities, but this was clearly not significant. This is seen in Fig 2, which plots survival against percentage of metaphases with additional cytogenetic abnormalities for the 45 patients who died of leukemia.

Since no significant survival differences were detected among the various subgroups of patients with additional cytogenetic abnormalities, these were pooled for comparison with the 603 patients without additional karyotypic abnormalities. This is shown in Fig 3. The patients with additional cytogenetic abnormalities had a significantly poorer survival rate than those with only the Ph chromosome ($P < .02$). However, the survival curves for these two populations did not begin to diverge until after the second year of the disease. After this point, the annual death rate among the patients with additional cytogenetic abnormalities was approximately 40% higher than that among the patients without such abnormalities. Because of the delay in increased mortality among the patients with additional abnormalities, the difference in median survival between the two groups was only 1 year. At the 25th percentile, however, the difference in survival was more than 2 years.

**DISCUSSION**

We recorded additional karyotypic abnormalities at the time of diagnosis in 9% of nonblastic Ph-positive patients. This is very similar to the frequency reported by Kantarjian et al. Both we and the latter group may have missed a few patients with subtle structural abnormalities who were studied before adoption of banding techniques. Adjustment for such cases would only raise the frequency slightly. On the other hand, the true incidence of additional abnormalities must be somewhat higher because routine cytogenetic examination will often miss findings present in only a small proportion of cells. Obviously, the percentage of patients found to have additional cytogenetic abnormalities will vary with the number of metaphases examined, and it is possible to calculate the probability that the presence of an additional abnormality will be detected under various conditions. If most cells contain additional abnormalities, only a few metaphases need be examined to establish their presence. On the other hand, if only 2% of cells contain an additional abnormality, examination of 20 metaphases would probably fail to detect it, and 150 metaphases would have to be examined to achieve a 95% likelihood of finding the abnormality. A study by Sonta and Sandberg provides an example of these quantitative relationships. These authors compared the findings on routine cytogenetic examination of 20 to 50 cells with those of examination of 110 to 500 metaphases. In these paired studies, findings present in 6% or more of the cells, as defined by examination of 200 or more metaphases, were almost always detected by routine examination. However, findings present in 1% to 4% of cells were usually missed.

Most other authors have reported cytogenetic findings among patients "in the chronic phase" without segregating findings at the time of diagnosis from those later in the course of the disease. Such reports cite a higher frequency of
additional abnormalities (other than a missing Y or complex translocations) than we or Kantarjian et al found; these are described in 15% to 25% of cases.\textsuperscript{2,10-13} These larger figures probably reflect both (a) the increased likelihood of finding an abnormality if multiple examinations are performed (as was the case in many of the reported studies) and (b) the development of new karyotypic abnormalities during the course of the disease. It seems reasonable to estimate that the frequency of additional cytogenetic abnormalities at diagnosis, detectable by examination of 20 to 50 metaphases with modern banding techniques, is somewhat lower than these figures but higher than those that we and Kantarjian et al recorded—perhaps 15%.

Insofar as we could determine, none of the additional abnormalities defined by our simplified classification had a prognostic import significantly different from any other (Fig 1). This experience is similar to that of others who have reported cytogenetic studies in CGL. Of the various abnormalities in addition to the Ph that have been described, only i(17q) appears to have a worse implication than the others. This isochromosome is generally accepted as a marker of blastic disease,\textsuperscript{1,3,11,12} although it is occasionally seen during the chronic stage.\textsuperscript{12,13} We did not identify an i(17q) in any of the 58 patients with additional abnormalities.

We did not have enough information in many cases to justify classification of the additional abnormalities as clonal or random. By the customary definition of clonality (the same abnormal chromosome present in at least two cells; in hypodiploidy, the same chromosome missing from at least three cells), 80% of the patients with double Ph had clonal abnormalities, and obviously, the nine cases in which only a single metaphase contained an additional abnormality were nonclonal. Survival in these groups did not differ from that of any of the other subgroups examined. Almost all of the abnormalities found in this study would be classified as "simple" in contrast to the complex mixture of multiple abnormalities and often multiple subclones that is typical of the blastic stage of CGL.

It was surprising not to find a significant correlation between the percentage of cells with additional cytogenetic abnormalities and survival (Fig 2). One might expect, since the presence of additional abnormalities was an unfavorable finding, that there would be quantitative relationship between the frequency of such abnormalities and survival, similar to what we had recorded for percentage of blasts or the platelet count.\textsuperscript{4} However, it would be premature to conclude that no such relationship exists. We saw no effect on survival during the first 2 years after diagnosis (Fig 3). Fourteen of these patients died during the first 2 years and another eight between 2 and 2.4 years. Thus, our failure to see the expected correlation with survival may be due simply to the fact that we did not have enough patients to provide an answer to this question.

The delay in the unfavorable effect on survival and the fact that our patients did not present with the classic cytogenetic pattern of blastic disease suggest that the abnormalities recorded in this study represented evidence of genetic instability of the leukemic clone rather than early signs of disease transformation. This view is supported by anecdotal observations in some of these cases that the original abnormalities were not seen in later cytogenetic examinations or were replaced by different abnormalities. The alternative hypothesis, that we were recording the early presence of the terminal, lethal subclone, seems less attractive. This would require a second hypothesis to explain the rather long latent period before a higher death rate was recorded. Furthermore, although a significant minority of our patients exhibited more than one abnormality, these were usually in different cells (consistent with genetic instability) and did not resemble the complex abnormalities of blastic disease.

The relative risk values for the patients with and without additional cytogenetic abnormalities, as calculated from a formula using a combination of four prognostic parameters, were very similar (Table I). This simplifies the interpretation of Fig 3. The significantly poorer survival of the patients with additional abnormalities in the face of essentially identical expected survival on the basis of other prognostic discriminants indicates that these cytogenetic abnormalities constitute an independent prognostic criterion. This confirms a conclusion reached previously from multivariate regression analyses by our group\textsuperscript{5} and by Kantarjian et al.\textsuperscript{8} Furthermore, unlike many other parameters of CGL, the presence of additional cytogenetic abnormalities exhibits little or no correlation with other features of the disease.\textsuperscript{6}

The survival pattern for the patients with additional abnormalities would introduce some degree of inaccuracy in conventional Cox model analysis because of the change in the relationship to the reference group of patients between the first 2 years and the subsequent years. An assumption intrinsic to the model is that an individual patient's risk, relative to the "average" for the entire population, is constant over time. However, the advantages of adding an additional, independent prognostic feature to the model should outweigh a small decrease in precision.

REFERENCES

6. Sandberg AA: Chromosomes and causation of human cancer and leukemia: XL. The Ph\textsuperscript{1} and other translocations in CML. Cancer 46:2221, 1980
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