Pretreatment Flow Cytometry of DNA Content in Adult Acute Leukemia

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Flow cytometric analyses of bone marrow cellular DNA content were performed in 115 adult patients with acute leukemia to assess whether and how proliferative activity relates to other disease and host parameters, to antileukemic effect of induction chemotherapy, and to patient prognosis. Eighty previously untreated patients had a smaller S-phase compartment than patients with morphologically normal bone marrow and than 35 patients studied in leukemic relapse. Among previously untreated patients, those with AML had a smaller S-phase compartment than patients with ALL. Hypodiploid leukemias had a higher G2M proportion than other cytogenetic categories, while lowest S and G2M phases were seen in patients with invaluable metaphases. Pretreatment S-phase compartment size correlated positively with degree of bone marrow blast cytoreduction during the first 8 days of induction treatment, but not with complete remission or with response duration, Magnitude of cytoreduction, however, correlated with complete remission, response duration, and survival. The pretreatment S-phase compartment size was a prognostic determinant only in patients with low cytoreduction. There was an inverse correlation between S-phase compartment size and number of courses to complete remission. Our study suggests that the percentage of pretreatment bone marrow cells in S phase is predictive for rate of cell kill and number of courses necessary for remission, but not for attainment of complete remission.

The study of cell kinetics has contributed greatly to the understanding of cellular proliferation and antineoplastic drug action in acute leukemia. In experimental leukemia, cytokinetic parameters are important determinants of the magnitude and efficacy of drug-induced cytotoxicity, because most useful drugs exert their major effect on actively cycling cells. In order for the effect of an antileukemic drug to be beneficial, however, relatively greater cell kill must be exerted on tumor cells than on host tissue, such as bone marrow.

Cell kinetic studies in human adult acute leukemia, employing radiolabeled thymidine, would indicate that leukemic cells have a longer generation time and longer G1 than normal myeloid precursor cells and, therefore, kinetically present unfavorable circumstances for treatment with cycle-active or phase-specific drugs. Among human neoplasia, however, acute leukemia is unique, as suggested by evidence in experimental systems and in human acute leukemia, showing interaction between normal and leukemic hematopoiesis with suppression of myeloid progenitor cell proliferation. This particular circumstance would tend to increase the relative toxic effects of cycle-active drugs on leukemic cells, while quiescent myeloid and erythroid elements remain relatively protected.

Assuming that normal myeloid cell growth is suppressed in patients with overt acute leukemia, the proliferative rate of leukemic blasts in predominantly leukemic bone marrow should be an important determinant for subsequent response to therapy with cycle-active agents. Studies of the pretreatment pulse tritiated thymidine labeling index have indeed shown prognostic significance of leukemic proliferative activity. Unfortunately, labeling index techniques are tedious and time-consuming. In addition, the prognostic value of such measurements has not been confirmed by all investigators studying adults and children.

The recent introduction of DNA flow cytometry has provided another means to obtain objective cytokinetic information on a large population of patients. This very precise and rapid method utilizes DNA specific and quantitative fluorochromes, such as mithramycin and ethidium bromide, to generate the instantaneous DNA content distribution of a cellular suspension, revealing the relative proportion of cells in all phases of the division cycle. The technique has proven easily adaptable to human bone marrow and solid tumors and is useful in assessing the cytokinetic effects of chemotherapeutic agents in vivo on cell-cycle progression.

Hillen et al. have analyzed the proliferative pattern of bone marrow from patients with and without acute leukemia, utilizing DNA flow cytometry. In normal bone marrow, the percentage of cells in S-phase and G2+M was found to be higher than corresponding values for patients with acute myeloblastic leukemia (AML). In the latter group, the prognostic significance was investigated, and with one exception, the...
ability to achieve complete remission with chemotherapy was predictable by simple analysis of the pretreatment DNA distribution. In view of these encouraging flow cytometric results, and of conflicting data with thymidine labeling techniques, we performed flow cytometric measurements of DNA content prior to induction chemotherapy, utilizing bone marrow cells from 115 adult patients with acute leukemia.

The purpose of the present analysis is to describe the proliferative characteristics of a large group of patients with acute leukemia, to compare these with morphologically normal bone marrow, and to determine whether observed variation in proliferative characteristics of leukemia correlate with commonly measured disease and patient-related parameters and with prognosis. Although clinical response is one parameter of cell kill and certainly the objective of any treatment, it is influenced by factors other than leukemic cell responsiveness, such as hemorrhage and infection. We have therefore also analyzed bone marrow blast cytoreduction during the first week of therapy to provide an in vivo measure of tumor cell kill following treatment. We have determined the relationship of this parameter to both pretreatment DNA distribution and to host response in terms of complete remission rate and duration.

MATERIALS AND METHODS

Patient Population

All patients with newly diagnosed and relapsing acute leukemia seen in the Department of Developmental Therapeutics at The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, between January 1976 and August 1977 were studied. Only patients with >70% leukemic cells in the bone marrow were considered, because DNA flow cytometry does not distinguish normal from leukemic cells with diploid DNA content. This also excludes patients with smoldering leukemia, in which cytotoxic chemotherapy is usually withheld until the time of frank leukemia or progressive bone marrow failure.

The diagnosis was made according to currently accepted F.A.B. criteria. Differential counts were performed on 500 cells and the cellularity of the clot section estimated. Cytogenetic analysis of the bone marrow was performed by routine culture methods. Bone marrow specimens obtained from 59 patients with morphologically normal bone marrow not having received prior chemotherapy were also subjected to flow cytometric analysis of DNA content.

Sample Processing

Samples were obtained immediately prior to initial therapy in newly diagnosed patients. All subjects in relapse were studied at least 21 days after previous chemotherapy had been discontinued and before starting the new regimen. From the posterior iliac crest, 1 ml of bone marrow was aspirated into a syringe containing 500 U of preservative-free heparin as anticoagulant. After Ficoll-Hypaque sedimentation (density, 1.076 g/ml, 1000g for 15 min at 4°C), interphase cells were washed with 0.9% NaCl, fixed in 70% ethanol in 0.9% NaCl, and stained with ethidium bromide and mithramycin. The initial staining was performed with 5 ml of

ethidium bromide 25 μg/ml (in 0.1 M Tris buffer) with 0.6% NaCl, (pH 7.4) for 10 min. Subsequently, 5 ml of mithramycin 50 μg/ml (containing 7.5 mM MgCl2) and 12.5% ethanol were added, resulting in final concentrations of 12.5 μg/ml of ethidium bromide and 25 μg/ml of mithramycin. RNase 0.1% in 0.3 M NaCl was added for 5 min at room temperature. Samples were measured in a Phywe ICP-11 pulse cytophotometer (Phywe Company, Göttingen, Germany). Routinely, more than 30,000 cells were measured and a 128-channel histogram generated. Distributions of DNA content were evaluated utilizing the model of Johnston et al. The coefficient of variation for the G1 compartment ranged from 0.5% to 7% (median 2.3%).

Antileukemic Therapy

Previously untreated patients with acute leukemia received several inductions regimens. All patients with acute nonlymphocytic leukemia ≤50 yr of age received induction chemotherapy with adriamycin, vincristine, cytosine arabinoside, and prednisone “Ad-OAP.” Remission was maintained with OAP. Patients over the age of 50 yr received the same or a similar treatment in which the anthracycline rubidazone was substituted for adriamycin “ROAP.” Patients with lymphocytic or undifferentiated leukemia received either Ad-OAP therapy or treatment with cyclophosphamide, adriamycin, vincristine, prednisone, and bleomycin, “CHOP-Bleo,” regardless of age. Patients in relapse received a variety of phase I, II, and III regimens.

Response Parameters

To appreciate the direct cytoxic effect of induction treatment on leukemic blast cells, without regard to host tissue factors, the speed of bone marrow blast cytoreduction was determined. Percent cytoreduction was defined as:

\[
\text{Percent blasts day } 0 - \frac{\text{Percent blasts day } 8}{\text{Percent blasts day } 0} \times 100
\]

All values were derived from bone marrow differentials. Negative values were reported as 0.

Complete remission was defined by the achievement for ≥30 days of a normocellular bone marrow with <5% blast cells <10% blasts + promyelocytes, and normal maturation of erythroid and myeloid progenitor elements. Peripheral blood absolute granulocyte count exceeding 1000/cumm, platelet count over 100,000/cumm, and hemoglobin above 10g/100 ml were also required. Length of remission was determined from the time of complete remission status until the first detection of relapse. Survival was determined from the time of flow cytometric study until death or last follow-up visit.

Statistical Methods

Analysis for differences in proliferative patterns between various populations were performed utilizing the Student’s t test. Response rates were compared utilizing a chi-square and linear trend analysis. Comparisons of duration of response and survival were made utilizing the Gehan modification of the Wilcoxon analysis in the case of two groups, and with the k sample test in the case of more than two groups. Comparisons of DNA flow cytometry and cytoreduction data were made utilizing linear regression analysis.

RESULTS

Patient Characteristics

During the period of study, flow cytometry of DNA content was performed on bone marrows of 115 adult patients with acute leukemia. Eighty patients—acute
myeloblastic leukemia (AML), 56; acute undifferentiated leukemia (AUL), 5; acute lymphoblastic leukemia (ALL), 15; and lymphoma leukemia (LL), 4—were studied at the onset of disease; while 35 patients—AML, 22; AUL, 3; ALL, 10—were evaluated during relapse. There was no difference in proliferative patterns related to subgroups of myeloid leukemia. Therefore, patients with myeloblastic, progranulocytic, monocytic, and myelomonocytic leukemia were combined and are hence designated AML.

For the 80 previously untreated patients, therapy utilized was Ad-OAP, 44 (55%); ROAP, 25 (31%); CHOP-Bleo, 8 (10%); rubidazone, 1 (1%); and no therapy, 2 (3%) due to death prior to the institution of antileukemic drugs.

In order to test the representativeness of this group of patients, the 80 previously untreated individuals were compared with the previous M.D. Anderson (MDAH) experience, a reference group of 325 patients treated between 1973 and 1977 with the Ad-OAP regimen. Comparing response data and factors found to be of major prognostic significance in the reference group, no difference was noted in age, leukemic morphology, infection status, serum LDH level, absolute blast count, or proportion with cytogenetic abnormality for the 80 patients undergoing cytokinetic analysis. Remission rates in both studies were comparable (62% reference group and 63% present group). The median duration of complete remission and survival were shorter in the present study, (31 and 37 wk, respectively) than in the reference group (51 and 43 wk), possibly reflecting superiority of the uniformly employed Ad-OAP regimen in the reference population compared to the various other treatments utilized in the present study.

**Relationship of DNA Flow Cytometry to Disease and Patient-Related Characteristics**

The pretreatment DNA distributions obtained in the leukemic patients were compared to 90 morphologically normal bone marrow aspirates from 59 patients. No significant difference between the $G_{10}$ and $G_{2}+M$ contents of leukemic versus normal populations or within leukemic subpopulations were noted. Significant differences emerged among different groups when the S-phase compartment was considered (Fig. 1). In both normal and in all leukemic subpopulations, there was considerable variation. Considering all previously untreated patients, the median S-phase compartment of 8% (mean 8% ± 1% standard error) for leukemic bone marrow was significantly lower ($p <0.05$) than that for morphologically normal bone marrow (median 13%; mean 16% ± 1%) and bone marrow from patients in leukemic relapse (median 11%; mean 12% ± 1%). Considering only untreated leukemic patients, significant differences ($p <0.05$) were noted between the S-phase compartment size of patients with AML (median 8%; mean 8% ± 1%) and ALL (median 10%; mean 12% ± 2%).

In the group of 80 previously untreated patients, we determined whether the variation in pretreatment proliferative characteristics was accounted for by disease or patient-related characteristics. Since all subcategories studied described populations of approximately normal distributions, the DNA distributions are henceforth expressed as mean ± standard error for ease of comparison.

Age, infection status, hemoglobin, platelet count, and granulocyte count were the patient-related characteristics analyzed. Although no relationship between infection status, hemoglobin, platelet count, or granulocyte count with DNA distribution was noted, there was a trend of decreasing S-phase compartment size with increasing age (<50 yr—9% ± 1%; 50-64 yr—8% ± 1%; ≥65 yr—7% ± 1%). This trend, however, disappeared when patients with acute lymphoblastic leukemia were removed from the group and only patients with myeloblastic disease were considered.

Analysis of disease-related characteristics showed no correlation between bone marrow DNA distribution and percent blasts in bone marrow, cellularity of

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**Fig. 1.** S-phase content determined by DNA flow cytometry for adult patients with morphologically normal bone marrow and with acute leukemia.
DNA CONTENT IN ACUTE LEUKEMIA

Table 1. Relationship of Karyotype to DNA Distribution and Response to Treatment in Previously Untreated Adult Acute Leukemia

<table>
<thead>
<tr>
<th>Cytogenetic Pattern</th>
<th>No. Patients</th>
<th>Percent G_{1,0}</th>
<th>Percent S</th>
<th>Percent G_{2+M}</th>
<th>No. CR (%)</th>
<th>Median Length CR (wk)</th>
<th>Median Length Survival (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diploid</td>
<td>35</td>
<td>88 (1)</td>
<td>9 (1)</td>
<td>3 (1)</td>
<td>23 (68)</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>Abnormal diploid</td>
<td>12</td>
<td>88 (2)</td>
<td>9 (1)</td>
<td>3 (1)</td>
<td>7 (58)</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>14</td>
<td>89 (2)</td>
<td>8 (2)</td>
<td>4 (1)</td>
<td>11 (79)</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>10</td>
<td>83 (2)</td>
<td>10 (2)</td>
<td>7 (1)</td>
<td>5 (60)</td>
<td>18+</td>
<td>9+</td>
</tr>
<tr>
<td>No metaphases</td>
<td>5</td>
<td>92 (2)</td>
<td>6 (1)</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>-</td>
<td>2+</td>
</tr>
</tbody>
</table>

*Mean.
†Standard error.
‡p < 0.05 when compared to combined normal diploid + abnormal diploid + hyperdiploid groups.
§p < 0.01.

bone marrow, fibrinogen values, and the presence or absence of Auer rods. When patients were divided according to the percentage of absolute bone marrow leukemic infiltrate\(^46\) (% blasts x % cellularity) to provide groups of equal size, a slightly lower mean S-phase compartment (7% ± 1%) was found for infiltrate values ≥70%, in contrast to infiltrates below this value (9% ± 1%). A higher mean S-phase compartment (10% ± 1%) was seen in patients with AML who had <1000 circulating blasts/cumm than for those with absolute blast counts of ≥1000/cumm (7% ± 1%) (p=0.07).

Cytogenetic analyses were available in 76 of the 80 previously untreated patients (Table 1). No difference in the proliferative characteristics of normal diploid, abnormal diploid, and hyperdiploid leukemia was seen. Patients with hypodiploid leukemia had significantly higher G_{2+M} compartments (p<0.01). Five patients had inevaluable karyotypes because of poor metaphase yield. This group had the lowest values for S and G_{2+M} compartment size and the highest G_{1,0} percentage of any subgroup, differing significantly from the remainder of the population (p<0.05). These observations held for the entire population and for patients with AML when considered separately. Interestingly, patients with inevaluable karyotypes had the worse prognosis of all subgroups, with none achieving remission.

Analysis of Proliferative Characteristics, Cytoreduction, and Treatment Response

In order to determine the relationship of S-phase compartment size to cytoreduction, patients were divided into three approximately equal sized groups, based on the percentage of cells in S phase (Table 2). Those patients with a higher S-phase compartment size had higher cytoreductive rates than patients with lower S-phase percentage (p<0.05). Utilizing the same groupings for S phase, however, the percentage of cells in DNA synthesis did not predict for complete remission, duration of remission, or survival.

To test whether bone marrow cytoreduction is indeed a measure of antileukemic drug efficacy, the relationship of this parameter to prognosis was also determined. Patients were divided into three groups of approximately equal size by percent cytoreduction (Table 3). Increasing remission rate with higher cytoreduction (p<0.04) was seen in all patients, but was most significant when those patients with AML were considered. Thus, patients with myeloblastic disease who experienced 90%-100% cytoreduction during the first week of therapy had a 92% complete remission rate, compared to 46% for those with <25% cytoreduction. A trend of increasing response duration and survival with higher degrees of cytoreduction was also seen, especially in AML.

In view of the positive relationship between S-phase compartment and cytoreduction, and between cytoreduction and response to therapy and lack of correlation between S-phase compartment with response was surprising. This could be explained by lack of sufficient correlation between all three factors. We therefore determined whether an interaction between cytoreduction and S-phase compartment as predictors for response existed. Previously untreated patients were divided into four approximately equal sized

Table 2. Relationship of S-Phase Compartment Size to Cytoreduction and Response to Treatment in Previously Untreated Adult Acute Leukemia

<table>
<thead>
<tr>
<th>Percent S</th>
<th>No. Patients</th>
<th>Percent Cytoreduction (SE)</th>
<th>No. CR (%)</th>
<th>Median Length CR (wk)</th>
<th>Median Survival (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7</td>
<td>30 (26)*</td>
<td>40 (10)</td>
<td>20 (67)</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>7-11</td>
<td>27 (22)</td>
<td>50 (9)</td>
<td>16 (64)</td>
<td>p &lt; .05</td>
<td>29 p &gt; 0.5</td>
</tr>
<tr>
<td>≥12</td>
<td>23 (19)</td>
<td>69 (9)</td>
<td>14 (61)</td>
<td>32</td>
<td>36 p &gt; 0.5</td>
</tr>
</tbody>
</table>

*Numbers in parenthesis in this column only indicate number of patients evaluable for cytoreduction, while numbers outside of parenthesis indicate patients evaluable for response.
groups with S-phase split at 9% and cytoreduction at 60% (Table 4). Consistent with the previous analysis, the response rate for patients with high cytoreduction (≥60%) was 20% greater than for patients with low cytoreduction (<60%), while S-phase compartment size was again not predictive for response. Patients with <60% cytoreduction, however, could be divided into two prognostic categories based on S-phase compartment size. Thus, although low cytoreduction indicates poor prognosis in general, a subgroup of these patients with high S-phase compartments (>9%) could be identified to have a better prognosis. On the other hand, occurrence of ≥60% cytoreduction indicates good prognosis, regardless of the percentage of cells undergoing DNA synthesis prior to therapy.

Because of the correlation between pretreatment S-phase compartment size and antileukemic effect as determined by cytoreduction, we determined whether there was also, for patients attaining complete remission, a relationship between number of courses to complete remission and S-phase compartment. The advantage of such an analysis is that only responders are considered. Therefore, factors such as infection and hemorrhage, which might account for treatment failure but which do not relate to antileukemic drug activity, are not analyzed. As seen in Table 5, there was an inverse relationship between the pretreatment S-phase properties and the number of courses to remission (p = 0.1). For example, patients with S <7% required an average of 1.65 courses to remission, with 45% entering CR after course 1, while those with ≥12% S-phase cells required 1.28 mean courses, with 71% in CR after initial therapy. Similarly, higher cytoreduction during the first week of therapy was also associated with greater ease of attaining remission.

Finally, a reanalysis was performed to characterize by pretreatment characteristics the four subgroups defined by high and low cytoreduction and S-phase compartment size (Table 6). Several patterns emerged. The low S-low cytoreduction group had the greatest percentage of karyotype abnormalities. In addition, all patients with absent metaphases on culture belonged to this group. Patients in this category had the lowest incidence of LDH >600. The low S-high cytoreduction group, with the exception of two patients, was exclusively AML. Patients in the high S-high cytoreduction category had the greatest incidence of ALL and of LDH >600. Similar, but less striking, was the incidence of ALL and elevated LDH in the low S-high cytoreduction group.

**DISCUSSION**

This study was undertaken to determine for adult acute leukemia whether bone marrow proliferation relates to other commonly measured disease and host-related parameters and to prognosis. We utilized the technique of DNA flow cytometry to gauge prolifera-

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**Table 3. Relationship of Bone Marrow Blast Percent Cytoreduction to Response in Previously Untreated Adult Acute Leukemia**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Percent Cytoreduction</th>
<th>No. Patients</th>
<th>No. CR (%)</th>
<th>Median Length CR (wk)</th>
<th>Median Survival (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>20</td>
<td>11 (55)</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>25-89</td>
<td>28</td>
<td>22 (78) p &lt; 0.04</td>
<td>27 p = 0.3</td>
<td>45 p = 0.2</td>
<td></td>
</tr>
<tr>
<td>≥90</td>
<td>19</td>
<td>16 (84)</td>
<td>36</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>13</td>
<td>6 (46) p &lt; 0.04</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>25-89</td>
<td>20</td>
<td>16 (75) p &lt; 0.04</td>
<td>30 p = 0.04</td>
<td>43 p = 0.08</td>
<td></td>
</tr>
<tr>
<td>≥90</td>
<td>12</td>
<td>11 (92)</td>
<td>40</td>
<td>26+</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4. Relationship of Complete Remission to S-Phase Compartment Size and to Percent Cytoreduction in Previously Untreated Adult Acute Leukemia**

<table>
<thead>
<tr>
<th>Cytoreduction</th>
<th>S ≥ 9% CR Rate (%)</th>
<th>S &lt; 9% CR Rate (%)</th>
<th>Total CR Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥60%</td>
<td>16/20(80)</td>
<td>13/15(87)</td>
<td>29/35(83)</td>
</tr>
<tr>
<td>&lt;60%</td>
<td>8/11(73)</td>
<td>12/21(57)</td>
<td>20/32(63)</td>
</tr>
<tr>
<td>Total* (67 patients)</td>
<td>24/31(71)</td>
<td>25/36(69)</td>
<td>49/67(73)</td>
</tr>
<tr>
<td>Total† (80 patients)</td>
<td>24/37(65)</td>
<td>26/43(60)</td>
<td>50/80(62)</td>
</tr>
</tbody>
</table>

*Total is for the 67 patients analyzable for both S-phase compartment size and percent cytoreduction.
†Total is for all 80 patients analyzable for S-phase compartment size.

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**Table 5. Relationship of Number of Courses to Remission to S-Phase Compartment Size and to Cytoreduction for Previously Untreated Adults With Acute Leukemia**

<table>
<thead>
<tr>
<th>Percent Cytoreduction</th>
<th>&lt;7 Courses</th>
<th>7-11 Courses</th>
<th>≥12 Courses</th>
<th>&lt;25 Courses</th>
<th>25-89 Courses</th>
<th>&gt;90 Courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number in CR</td>
<td>20</td>
<td>16</td>
<td>14</td>
<td>11</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>CR, No. (%)</td>
<td>9(45)</td>
<td>9(45)</td>
<td>10(71)</td>
<td>6(55)</td>
<td>11(50)</td>
<td>11(69)</td>
</tr>
<tr>
<td>1 course to CR</td>
<td>2 courses</td>
<td>9(45)</td>
<td>5(31)</td>
<td>4(29)</td>
<td>2(18)</td>
<td>10(45)</td>
</tr>
<tr>
<td>CR, No. (%)</td>
<td>9(45)</td>
<td>5(31)</td>
<td>4(29)</td>
<td>2(18)</td>
<td>10(45)</td>
<td>5(31)</td>
</tr>
<tr>
<td>3 courses to CR</td>
<td>Average no. of courses to CR</td>
<td>2(10)</td>
<td>2(13)</td>
<td>0(0)</td>
<td>3(27)</td>
<td>1(5)</td>
</tr>
<tr>
<td>CR, No. (%)</td>
<td>2(10)</td>
<td>2(13)</td>
<td>0(0)</td>
<td>3(27)</td>
<td>1(5)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Average no. of courses to CR</td>
<td>1.65</td>
<td>1.56</td>
<td>1.28</td>
<td>1.72</td>
<td>1.54</td>
<td>1.31</td>
</tr>
</tbody>
</table>
justified by reanalysis of the data utilizing only DNA CONTENT IN ACUTE LEUKEMIA our institution, since we began using cytosine arabinoside labeling to determine S-phase compartment size. In this determination, no significant change in the outcome of the results of this study was noted.

Table 6. Relationship of Pretreatment S-Phase Compartment and Antileukemic Effect of Therapy to Disease-Related Characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Low S% Low Cytoreduction</th>
<th>Low S% High Cytoreduction</th>
<th>High S% Low Cytoreduction</th>
<th>High S% High Cytoreduction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. AML (%)</td>
<td>21 (67)</td>
<td>15 (67)</td>
<td>11 (65)</td>
<td>20 (60)</td>
<td>67 (100)</td>
</tr>
<tr>
<td>No. ALL (%)</td>
<td>3 (14)</td>
<td>1 (7)</td>
<td>3 (27)</td>
<td>7 (35)</td>
<td>14 (21)</td>
</tr>
<tr>
<td>No. LDH &gt; 600 IU (%)</td>
<td>8 (38)</td>
<td>7 (47)</td>
<td>6 (55)</td>
<td>12 (60)</td>
<td>33 (49)</td>
</tr>
<tr>
<td>No. abnormal karyotype (%)</td>
<td>12 (57)</td>
<td>5 (33)</td>
<td>5 (45)</td>
<td>8 (40)</td>
<td>30 (45)</td>
</tr>
<tr>
<td>No. absent metaphases (%)</td>
<td>3 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (4)</td>
<td></td>
</tr>
</tbody>
</table>

* =< 9%.
† >= 9%.
‡ <= 60%.
§ >= 60%.

The use of this level of leukemic infiltration was similar chemotherapy combinations, results of this study are representative of adult patients with acute leukemia, when compared to newly diagnosed disease, is consistent with studies performed at our institution21 and by others utilizing tritiated thymidine or flow cytometry, although this has not been invariably observed. Within the group of previously untreated patients, the trend for higher S-phase fraction found in lymphoblastic leukemia may explain the greater sensitivity of this disorder to chemotherapeutic agents and the relative rapidity with which remission is achieved.

The 51% incidence of documented cytogenetic abnormalities (excluding patients with absent metaphases) is consistent with the incidence reported by other investigators. Studies by Trujillo et al. and by Rowley, have shown similar reduction in remission rates associated with cytogenetic aberrations, especially hypodiploidy. The low proliferative rate found in patients with absent metaphases may explain the lack of culturability for cytogenetic study and the poor response to cycle-active chemotherapeutic agents. The increased G2M in hypodiploid cases was not accompanied by significant mitotic index elevation (range 0.01-0.85, median 0.4). The combined increase in G2 and S phase suggests that the growth fraction in hypodiploid disease is increased.

One disadvantage of DNA flow cytometric analysis is its inability to distinguish normal from diploid leukemic cells. Thus, by design, only patients with ≥70% blast cells in the bone marrow were considered. The use of this level of leukemic infiltration was justified by reanalysis of the data utilizing only patients with bone marrow differential counts ≥90% leukemic blasts. In this determination, no significant change in the outcome of the results of this study was noted.

Further analysis of disease-related characteristics, using the combined criteria of S-phase compartment size and cytoreduction, showed that the patients low in both categories had the lowest serum LDH values, probably indicating minimal cell turnover. They also had the highest incidence of cytogenetic abnormalities with all patients having indeterminate cytogenetic examinations (inevaluative metaphases) in this group. This, and the poor response to treatment of these...
patients, suggests that they deserve further study to determine whether other treatment modalities could be more successful. Conversely, the high incidence of elevated serum LDH in patients with high cytoreduction, regardless of S-phase compartment size, suggests that LDH is an indicator of ongoing cell loss.

When considering response to treatment, the two endpoints—cytoreduction and complete remission—were utilized. The rate of cytoreduction was analyzed as a direct measure of drug antileukemic effect. A direct relationship was found between this parameter and complete remission rate, duration of remission, and survival. As expected, for patients achieving complete remission, an inverse correlation was found between this parameter and the number of courses to remission.

Although S-phase compartment size showed a direct relationship to cytoreduction, it did not relate to complete remission rate, remission duration, or survival. Several reasons for the inability of pretreatment S phase to predict clinical response are possible. First, as suggested by Rustum and Preisler,44 failure to achieve complete remission may be due to factors such as infection and hemorrhage, which are not related to failure of chemotherapy-induced cell kill. In support of this, an analysis of the nine patients in this study whose survival was less than 2 wk shows that for these early deaths a high incidence of preexisting infection (78%) and high median age (66 yr) was found. This is in contrast to the remaining 71 patients whose median age was 47 yr and preexisting infection rate 28% (p <0.05). In the nine patients no other distinguishing proliferative diagnostic or identifying characteristics were noted. In further support of this hypothesis, elimination of all treatment failures by consideration of only the number of courses necessary to achieve complete remission showed fewer courses to CR necessary in those patients with a higher S-phase compartment and cytoreductive rate. Thus, S-phase magnitude predicts both the rate of cytoreduction and the rate of achieving remission.

Another explanation for the inability of pretreatment S-phase compartment size to predict for response is the lack of total independence of this and cytoreduction as prognostic factors. Thus, in patients with high cytoreduction, the magnitude of this factor alone predicts CR. Conversely, for those with low cytoreduction, S-phase compartment becomes an important response prognosticator.

Since several parameters may interrelate to determine prognosis, stepwise logistic regression techniques, as described by Gehan et al. may be useful in predicting prognosis from cytokinetic and other pretreatment information.57

If S-phase compartment size is not the critical pretreatment cytokinetic prognosticator, what other cytokinetic factors should be considered? Since sizable quiescent cell populations are known to be present in acute leukemia,5,8,11,12,68-70 growth fraction may be a more critical prognostic determinant. Newer methods, such as rapid simultaneous multiparameter flow system analysis of RNA and DNA72 and the primer-available DNA-dependent DNA polymerase assay,72 are potentially applicable to a large population of patients and may provide a more sensitive prognostic and therapeutic measurement by directly quantitating growth fraction. Similarly, other measurements, such as tritiated cytosine arabinoside labeling when measured simultaneously with tritiated thymidine uptake, may show better prediction, as suggested by Burke et al.26 Serial cytokinetic measurements during therapy may also provide more sensitive prediction of response. Consistent with this are several studies in which the change in labeling index over a 2-day period during therapy was found to be a better prognosticator than a single pretreatment determination.20,26,37,33,68 In our study, the utilization of serial parameters obtained during treatment is suggested by the accuracy of percent cytoreduction in predicting response.

The technique of DNA flow cytometry is a rapid and accurate method for providing quantitative biologic information concerning neoplastic and normal cells. Limitations of the technique due to heterogeneous cell populations and resting cell fractions await the future use of cytochemical and flow cytometric techniques to distinguish the various cell compartments. Although the therapeutic relevance of cytokinetic measurements in acute leukemia remains unclear, the techniques provide an opportunity to rapidly monitor, on a prospective basis, changes in the proliferation of living cells. Such technology offers the possibility of eventually individualizing treatment for patients with acute leukemia, thereby increasing the curability of this disease.

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