Relationship of Megakaryocyte Size at Diagnosis to Chemotherapeutic Response in Children With Acute Nonlymphocytic Leukemia

By Carl W. Jackson and Gary V. Dahl

Small megakaryocytes are frequently seen in patients with acute nonlymphocytic leukemia (ANLL). In this study, median megakaryocyte diameters were determined in marrow biopsy specimens of 32 children at diagnosis of ANLL and related to platelet count and chemotherapeutic response. The association between median megakaryocyte size and time-to-failure was striking. Seven of 9 patients with median megakaryocyte diameters ≥20 μm remain in continuous complete remission for more than 3 yr, whereas 20 of 23 patients with smaller median megakaryocyte diameters failed therapy within 15 mo (p = 0.002). By Cox-regression analysis, megakaryocyte size had independent prognostic value (p < 0.001), surpassing that of spleen size, the only other feature having significant association with time-to-failure. Megakaryocyte size at diagnosis may be useful for predicting the likelihood of prolonged complete remission in ANLL.

ABNORMALITIES of megakaryocytes, including the presence of small forms, have been reported in adults with acute nonlymphocytic leukemia (ANLL). In a study of marrows from 132 adults with myelomonocytic leukemia, Saarni and Linman reported that megakaryocytes were atypical in 62% of cases. The megakaryocytes varied greatly in size with small atypical forms being particularly prominent. In a quantitative study of megakaryocytes in 12 cases of adult ANLL, Cowan observed that the average megakaryocyte volume was decreased in 8 cases and increased in one. The present work was prompted by results of a recent study of childhood ANLL at this institution in which platelet count at diagnosis was correlated with response to induction chemotherapy. In this study, we have examined whether megakaryocyte diameter in marrow biopsies at diagnosis is related to platelet count at diagnosis and responsiveness to chemotherapy.

MATERIALS AND METHODS

Marrow biopsy specimens were obtained from 49 of the first 59 children with ANLL entered in a recently completed clinical trial at this center. The criteria for diagnosis and details of treatment are reported elsewhere. Briefly, induction chemotherapy consisted of 2–5 weekly cycles of vincristine-daunomycin (day 1), followed by sequential cytosine arabinoside and 6-azauridine (days 4–7). Continuation chemotherapy was monthly vincristine-adriamycin-cyclophosphamide, weekly cytosine arabinoside, and daily 6-mercaptopurine.

Marrow biopsies were obtained at diagnosis from the posterior iliac crest by use of a Jamshidi biopsy needle. The marrow core was fixed in Zenker's fixative, decalcified with decalcifying solution (American Scientific Products, McGaw Park, IL), sectioned at 5 μm, and stained with hematoxylin and eosin. Megakaryocytes were identified by their characteristically larger size, lobulated nuclei with thick, clumped, deeply basophilic stained, and usually abundant acidophilic cytoplasm. They were easily distinguished from multinucleated osteoblasts, which were occasionally seen. At least one complete section from each biopsy sample was scanned for megakaryocytes at a magnification of 500×. Where possible, an additional section that had been taken at least 50 μm deeper in the biopsy specimen was examined when the number of megakaryocytes in the first section was <10. The diameter of megakaryocytes was measured with an eyepiece micrometer at a magnification of 1250× and expressed as the square root of the product of two measurements made at right angles. These determinations were made without the observer having knowledge of any of the clinical or laboratory features of the patients. The diameter of megakaryocytes ranged from 10 to 39 μm.

Of the 49 specimens examined, 35 had adequate marrow for evaluation. From 4 to 73 megakaryocytes were identified in the samples and ≥7 megakaryocytes were measured for 30 of the 35 patients. The median megakaryocyte diameter was determined for each patient. Three of these 35 patients died before completing induction therapy, so their measurements were not included in the analysis. The 32 patients whose megakaryocyte diameters were analyzed appear representative of the larger study group with respect to presenting features (Table 1) and to treatment response: 9 of 27 (33%) who achieved complete remission remain free of disease compared to the total of 20 of 68 (29%).

For most analyses, a median megakaryocyte diameter of 20 μm was used to divide the patients into groups. This division was based on determinations by Harker and Cowan that the mean megakaryocyte diameter in biopsy sections of normal adult marrow was 20 μm.

To verify that the median diameters derived from the small numbers of megakaryocytes in some specimens adequately represented true median diameters, we compared medians obtained from the first 4 and the first 7 megakaryocytes in each case with those derived from the total megakaryocyte population. The results were quite similar. In only one case each was there a difference that would cause a patient to be moved from the >20 μm median megakaryocyte diameter group to the ≤20 μm group.

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Supported in part by Public Health Service Grants CA21765, CA20180, and CA15956 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by ALSAC.

Submitted April 19, 1982; accepted November 9, 1982.

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0006-4971/83/6105-0007$01.00/0

Blood, Vol. 61, No. 5 (May), 1983: pp. 867–870
obtained from both patients and their parents, or the patients only if they were over 17 yr old.

The relationship between megakaryocyte size and treatment outcome was examined by comparing remission rates among different groups of patients based on megakaryocyte diameter categories. The relationship was assessed using Kaplan-Meier estimates and the log-rank test.

Treatment failure was defined as the inability to achieve remission within 3-5 wk of induction therapy or when relapse occurred during first complete remission. The relationship between median megakaryocyte diameter and treatment outcome was determined using the proportional hazards regression model of Cox.

Table 1. Presenting Characteristics of Study Group (n = 32)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Category</th>
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<tbody>
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<tr>
<td></td>
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<tr>
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<td></td>
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<td></td>
<td>12-17</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
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</tr>
<tr>
<td>Leukocyte count (10^3/cu mm)</td>
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</tr>
<tr>
<td></td>
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<td>Spleen size (cm)</td>
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RESULTS

Figure 1 shows the median megakaryocyte diameter, platelet count at diagnosis, and treatment outcome for each patient. Median megakaryocyte diameter was below the reported normal mean in 22 of 32 patients, confirming earlier reports that small megakaryocytes are a frequent finding in ANLL. Platelet count was less than 100,000/cu mm in 24 of 32 patients. If ANLL uniformly affected megakaryocytopoiesis and platelet survival, one would expect to find a correlation between low platelet count and megakaryocyte size. However, such an association was not seen.

When possible associations between these two features and treatment outcome were examined, platelet count had no apparent relation to overall treatment outcome, although the five induction failures had low platelet counts, consistent with the lower remission induction rate observed in patients with low platelet counts in our larger study. In contrast, the relationship between megakaryocyte size and treatment results was striking. Seven of 9 patients who remain in continuous complete remission had median megakaryocyte diameters >20 µm (median diameter for these 9 patients was 22 µm), whereas none of the 5 who were induction failures and only 2 of the 18 who relapsed after achieving remission had median diameters >20 µm (median diameter for these 23 patients was 18 µm).

The relationship between diameter and time-to-failure is shown in Fig. 2. Of the 9 patients with median megakaryocyte diameters >20 µm, 7 have been in continuous complete remission for more than 36 mo. In contrast, of the 23 patients with initial median megakaryocyte diameters ≤20 µm, only 2 remain in remission. These failure rates are significantly different at the p = 0.002 level.

There was no correlation between median megakaryocyte diameter and the number of megakaryocytes measured, indicating that differences in median megakaryocyte diameter among the treatment response groups were not related to sample size. The range of megakaryocyte diameters within individual samples was similar in the two groups of patients. Of the 32 biopsy specimens, 27 contained megakaryocytes both smaller and larger than 20 µm. Megakaryocytes >20 µm were present in 19 of the 23 samples having median megakaryocyte diameters ≤20 µm; hence, some normal-sized megakaryocytes were being formed in most patients, even though smaller megakaryocytes were dominant.

We also have examined whether megakaryocyte size correlated with other clinical and laboratory features at diagnosis. There was no correlation between median megakaryocyte diameter and the amount of marrow replacement or 3H-thymidine labeling index of marrow blasts at diagnosis. Furthermore, there was no correlation between median megakaryocyte diameter and the

The investigations were approved by the Clinical Trials Committee of St. Jude Children's Research Hospital; informed consent was obtained from both patients and their parents, or the patients only if they were over 17 yr old.
**MEGA KARYOCYTE SIZE AND PROGNOSIS IN ANLL**

**Fig. 2.** Kaplan-Meier analysis of time-to-failure for patients with median megakaryocyte diameters >20 μm versus those with median diameters ≤20 μm. Failure is defined as failure to achieve remission (5 patients) or relapse after attaining remission (18 patients). Open circles with arrows designate patients who remain in continuous complete remission. Each closed circle represents the time at which a patient failed therapy. The difference is significant at the p = 0.002 level.

white blood cell count. Three of the 7 patients with median megakaryocyte diameters >20 μm who remain disease-free had white blood cell counts >50,000/cu mm at diagnosis.

Finally, using the proportional hazards regression model of Cox,17 we assessed the individual prognostic value of 18 clinical and laboratory features: age, sex, morphological (FAB) classification, WBC, platelet count, spleen size, cycles of induction therapy, and others.14 Besides median megakaryocyte diameter (p < 0.001 by the likelihood ratio test), only spleen size (<5 and ≥5 cm) was significantly related to time-to-failure (p < 0.034). Sex and number of cycles of induction therapy were the next most significant variables (p = 0.053 and 0.080, respectively) and were used in combination analyses with spleen size and median megakaryocyte size. Median megakaryocyte diameter added significant prognostic information to the most significant combination of the other variables (p = 0.002), indicating that median megakaryocyte diameter had independent prognostic significance.

**DISCUSSION**

Megakaryocyte size is one indicator of abnormalities in megakaryocytopoiesis and platelet kinetics.18 Small abnormal megakaryocytes have been reported in patients with ANLL as well as chronic myelogenous leukemia and refractory anemia.1-13 In this study, a median megakaryocyte diameter of ≤20 μm was found in 23 of 32 patients (72%). The median diameter for the group that failed therapy was 18 μm compared to 22 μm for the group continuing in complete remission. If one calculates median megakaryocyte volume, assuming a spherical cell shape for these two groups, then the actual size differences become more apparent.

The median volume for the treatment failures is 3052 cu μm compared with 5572 cu μm for patients remaining in complete remission, an approximately twofold disparity.

The lack of correlation between megakaryocyte size and platelet count at diagnosis agrees with results of others2 and is not surprising, since in adults with ANLL, megakaryocytopoiesis may be ineffective and platelet survival shortened to various degrees.2

The basis of the relationship between megakaryocyte size and response to therapy is unknown, but could reside in the level of differentiation of the affected progenitor cell. Since megakaryocytes undergo nuclear replication without cytoplasmic division, their size is related to DNA content,19 and hence, the number of nuclear replications they have undergone from the diploid precursor stage. Thus, a small median megakaryocyte size could reflect a disturbance of nuclear replication7 not present in the megakaryocyte populations with a larger median size. We speculate that the megakaryocyte populations containing mostly small cells arise from pluripotential leukemic precursors, whereas in the other cases, malignant transformation occurs at a later stage of differentiation, sparing the megakaryocyte line. Since the proportion of cycling normal hematopoietic progenitors varies depending on the level of differentiation (CFU-S < CFU-GM),20 one would predict that leukemias arising from more primitive hematopoietic cells would be less responsive to cycle-dependent therapy, as used in this study.

An important aspect of this observation is its implication for planning of treatment protocols. With modern chemotherapy, about one-third of patients will remain in complete remission for at least 2 yr.14,21,22 Nevertheless, some groups have proposed or have
begun bone marrow transplantation as alternate therapy once remission has been induced.\textsuperscript{23} If megakaryocyte size proves a reliable prognostic factor, patients with normal-sized megakaryocytes could be identified early and entered in treatment programs\textsuperscript{14} lacking the toxicity of more intensive chemotherapy or bone marrow transplantation.

ACKNOWLEDGMENT

We wish to thank John Gilbert for his editorial assistance and Dennis Givens for assistance with statistical analysis.

REFERENCES

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