Pretreatment Cytokinetics in Acute Myelogenous Leukemia

Age-related Prognostic Implications

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Abstract

To determine the clinical and biologic relevance of cellular kinetics in leukemia, DNA flow cytometric analysis was performed on bone marrow biopsy specimens from 148 previously untreated adult patients with acute myelogenous leukemia. The proportion of cells in synthesis, second growth, and mitosis (S + G_2M) ranged from 4% to 33% with a median of 14%. The overall incidence of complete remission was not affected by the pretreatment cell cycle distribution. As in earlier studies, there was a marked decline in remission rate with advancing age from 73% for patients age ≤50 yr to 50% for those >50 ($P < 0.01$). Although not affecting remission induction overall, an increasing proportion of cells in S + G_2M phase was favorable in patients under the age of 50 yr, but was associated with a progressive decline in remission rate in older patients ($P = 0.01$). This age-related divergent effect of cell cycle kinetics on initial response to therapy was confined to the less favorable subgroup of patients with karyotypic abnormalities, whereas patients with normal diploid cytogenetics had a consistently higher response rate regardless of proliferative activity. A positive correlation was also observed between percent of S + G_2M cells and the proportion of diploid metaphases in young patients, contrasting with a negative correlation in the older age group. Our observations strongly suggest that the well-recognized prognostic effect of age on remission induction is not entirely host-mediated, but is at least partly an expression of disease-intrinsic differences between young and older patients.

Introduction

Recent advances in combination chemotherapy and supportive care have significantly improved the prognosis of acute myelogenous leukemia (AML)[1] in adults. However, ~30% of patients still fail primary induction therapy, and of those achieving complete remission, 80% will relapse within 2 yr (1–3). Identification of prognostic factors affecting response and remission duration has been useful in conducting risk-directed therapy (4, 5).

Several host and tumor characteristics have already been found to influence the outcome of adult patients with AML (4, 5). Poor tolerance to chemotherapy has been proposed as the reason for a high failure rate among older patients; however, differences in disease characteristics have not been fully investigated. Cytogenetic abnormalities in AML have been demonstrated to be nonrandom and seem to identify subgroups of patients with unique clinical features and prognoses (6–8).[2] Because of the implications of cytokinetic parameters for drug- and radiation-induced cell kill in experimental systems (9–18), similar research has also been conducted in human tumors, including leukemia. These studies have demonstrated that leukemic cells do not necessarily proliferate faster than their normal hematopoietic counterparts (19–24). A low growth fraction and long cycle times seem to constitute a cytokinetic sanctuary against tumor cell kill from chemotherapy. The ultimate growth advantage of leukemic cells over normal hematopoietic cells may result from inhibition of residual normal hematopoiesis by leukemic cells and a lower rate of cell loss in tumors than in normal cells (25, 26).

A number of clinical investigations have addressed the prognostic implications of pretreatment cytokinetics in acute leukemia (27–30). The notion that a high pretreatment S-phase compartment size affects remission favorably (31–34) and exerts an adverse effect upon remission duration (27, 31, 35) has been contradicted by other reports (29, 36–38). These discrepancies may be attributable to multiple factors, an important one being the use of bone marrow aspirates contaminated to a variable degree with peripheral blood (39, 40). Other reasons relate to relatively small numbers of patients, heterogeneous population groups resulting in different remission rates and durations, and the lack of detailed analysis in relation to other established prognostic parameters.

We evaluated the clinical significance of pretreatment cellular kinetics in adult patients with AML. The proportion of cells in synthesis, second growth, and mitosis (S + G_2M) phase was determined by flow cytometry on bone marrow biopsy specimens of previously untreated patients. This cytokinetic feature was analyzed in relationship to other important prognostic variables, such as age and cytogenetics, in an attempt to understand better the biology of leukemia and to relate the well-known clinical heterogeneity to an objective and quantitative feature of tumor cell proliferation.

Methods

Pretreatment cytokinetic studies were carried out on bone marrow biopsy material from 148 of 179 successive previously untreated adult patients with AML. Treatment consisted of combination chemotherapy

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1. *Abbreviations used in this paper:* AML, acute myelogous leukemia; CR, complete remission; S + G_2M, synthesis, second growth, and mitosis (phase).

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that used vincristine, cytosine arabinoside, and prednisone with either doxorubicin or amsidine. Patients had therapy planned according to a prognostic factor model that determined the probability of response; once the patients were in remission, the factor model also determined the probability of remaining in complete remission for 1 yr (5, 41).

Although cytometric analysis was conducted on both DNA and RNA content at different times during the course of the patient's disease, our study analyzes the pretreatment cytokinetics only. Marrow biopsy samples were obtained from the posterior iliac crest under local anesthesia and placed into RPMI 1640 culture medium containing 5,000 U/ml of heparin. The specimens were mechanically minced, then subjected to shaking and repeated syringing to obtain single-cell suspensions (40). Monodispersed cells were stained for DNA and RNA with the metachromatic dye acridine orange (42). Cytometric analysis was performed with an ICP-22 flow cytometer (Ortho Diagnostics, Westwood, MA) (43). Determination of the $S + G_2M$ compartment size involved gating along the $G_{1/0}$-S boundary from low to high RNA content values (43).

Cytogenetic studies included Giemsa-banding in all patients. At least 25 metaphases were required for evaluation. For the purpose of correlations, aneuploidy was defined as the presence of any numeric or structural cytogenetic abnormality.

To satisfy the criteria of complete remission (CR), patients were required to have <5% blasts in the bone marrow, a normal marrow maturation, and normal blood parameters, including a hemoglobin > 12 g/dl, a platelet count of >100,000/μl and a granulocyte count of >1,500/μl (4). Distributions of cytokinetic measurements in different age and cytogenetic groups were compared by the paired t test. The χ^2 test was utilized to determine statistical differences in response rate in patient groups. The homogeneity of the effect of cytokinetics on CR rates across age subgroups involved the test of interactions of variables described by Fleiss (44), categorizing patients according to age (≤50 vs. >50 yr) and $S + G_2M$ percentage values (0–15% vs. >15%). Correlation coefficients were calculated to assess the linear relationship between several pairs of pretreatment characteristics. Curves for remission duration and survival were calculated by the method of Kaplan and Meier (45), and statistical differences between curves were tested by the generalized Wilcoxon test (45).

Results

The overall CR rate to combination chemotherapy was 63% for the 179 patients treated and 67% for the 148 patients with available cytokinetic information. Age was an important prognostic variable for response: while there was no appreciable change in CR rate by decade up to 50 yr, a significant decrease from >70 to 50% was noted upon transition to the sixth decade (Table I; Fig. 1). Cytogenetics also affected initial response: a higher CR rate was noted in patients with a diploid karyotype than in those with karyotype abnormalities (76% vs. 53%; $P < 0.01$).

Table I. Incidence of Complete Remission in Relationship to Age, Cytogenetics, and Cytokinetics

Parameter	No. of patients	Percentage of complete remission	P value
	n	%	
Age (in yr)			
≤50	99	73	<0.01
<50	80	50	
Cytogenetic category			
Diploid	75	76	<0.01
Aneuploid	87	53	
$S + G_2M$ (%)			
≤5	6	50	
6–10	35	77	
11–15	47	64	>0.25
16–20	38	74	
>20	22	55	

The percentage of cells in the $S + G_2M$ compartment ranged from 4 to 33%, with a median value of 14%. There was no significant difference in CR rate for different levels of $S + G_2M$ percentage in the overall patient population (Table I, Fig. 2). Pursuing the possibility that the previously noted significant drop in CR rate above the age of 50 yr was determined by disease rather than host factors, the prognostic implications of pretreatment cytokinetics were reexamined as a function of age: an increase in $S + G_2M$ percentage was favorable for patients ≤ 50 yr, but it was detrimental for the older age group ($P < 0.01$) (Fig. 3). To evaluate the effect of cytokinetic parameters independent of age and cytogenetics, $S + G_2M$ percent was analyzed in relation to CR rate separately for each age and karyotypic subcategory (Fig. 3). Divergence was found only in the aneuploid category, whereas there were only minor fluctuations in CR rate with increasing $S + G_2M$ percentage among patients with diploid karyotypes.

To clarify the biological basis for the age- and karyotype-

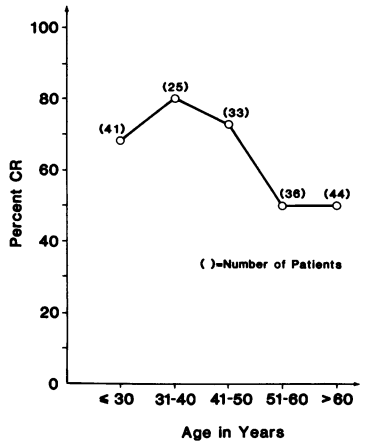

Figure 1. Relation between CR rate and age.

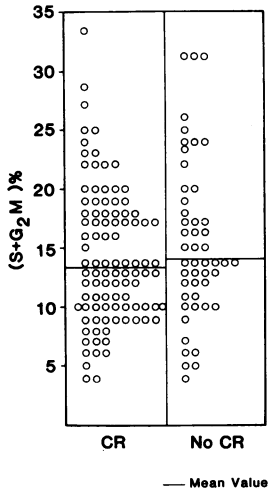

Figure 2. Relation between CR rate and $S + G_2M$ percentage.

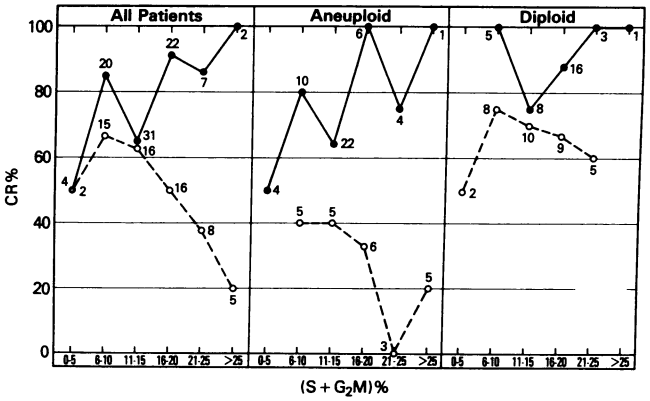

Figure 3. Relation between CR rate and cytokinetics within each age and cytogenetic category. Overall, an increasing S + G_2M percentage translated into an increased CR rate in young patients and a decreased CR rate in old patients (*left*). This prognostic divergence was important only within the aneuploid cytogenetic category (*middle*) while having no significant effect in patients with diploid cytogenetics (*right*). ●, ≤50; ○, >50.

dependent prognostic implications of pretreatment cytokinetics, the relationship between age, cytogenetics, and cytokinetics was further investigated. There was no difference in mean S + G_2M percentage in patients <50 yr of age compared to older patients (13.5 vs. 14.5%). Divergent relationships were observed in young and old patients between cytokinetics and ploidy state as well as percentage of aneuploid metaphases. Thus, compared to diploid cytogenetics, aneuploid karyotypes were associated with lower values of S + G_2M percent in young patients and with higher values of S + G_2M percent in old patients, respectively ($P = 0.02$) (Fig. 4). Similarly, a negative correlation ($r = -0.22$) existed between the proportion of aneuploid metaphases and S + G_2M percentage in young

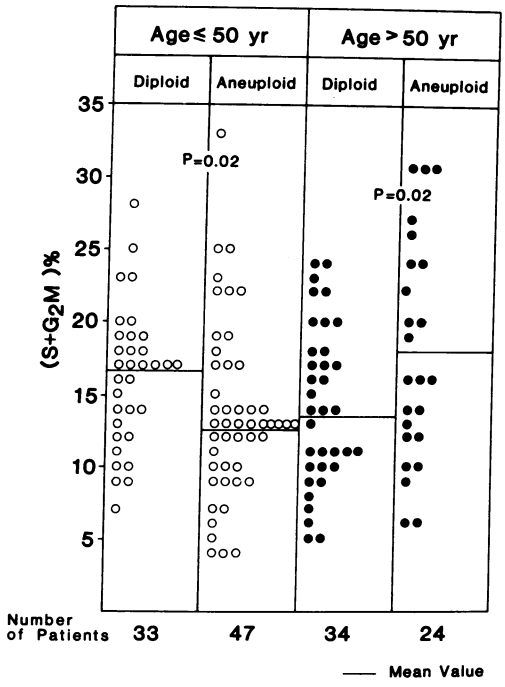

Figure 4. Relation between ploidy state and S + G_2M percentage in young and old patients.

Table II. Relationship of Pretreatment Cytokinetics to Complete Remission Duration and Survival

Pretreatment S + G_2M percent	No. of patients	Median remission duration	Median survival
	n	*wk*	*wk*
<12	48	77	79
12–20	78	60 $P = 0.3$	66 $P = 0.05$
>20	22	42	41

patients, contrasting with a positive correlation ($r = +0.26$) of similar magnitude in older patients.

Pretreatment cytokinetics not only affected remission induction but also had an effect upon the subsequent disease course. Patients with lower pretreatment S + G_2M percentage had longer remission duration and survival times compared to patients with higher values (Table II; Fig. 5).

The heterogeneity of AML in young and old patients was also investigated at the cytogenetic level by looking at the relation of specific karyotypic abnormalities (balanced translocations, numeric abnormalities) to age and cytokinetics (Table III). Compared to the elderly group, young patients had a higher incidence of inversion of chromosome 16 and of balanced translocations (26 vs. 7%; $P < 0.01$), and a lower incidence of diploid karyotypes (38 vs. 55%; $P = 0.07$). The presence of abnormalities involving inversion of chromosome 16 or a balanced translocation between chromosomes 8 and 21 carried a good prognosis with a corresponding CR rate of 95% compared to CR rates of 79% for patients with diploid cytogenetics and 47% for those with other karyotypic abnormalities ($P < 0.001$). Significantly lower S + G_2M percent values were also observed for patients with balanced translocations compared to other cytogenetic categories (mean 11.6 vs. 15.8%; $P < 0.001$).

Patterns of treatment failures (death during induction therapy versus resistant disease) were analyzed within various subgroups (Table IV). Two patients achieving a partial remission are excluded from the analysis. 17 (36%) of the remaining 46 patients who failed to achieve a remission had resistant disease. No significant difference of occurrence of resistant disease was noted by age, S + G_2M values or cytogenetic category, except a trend of a lower incidence in patients with balanced translocations compared to other subgroups ($P = 0.09$).

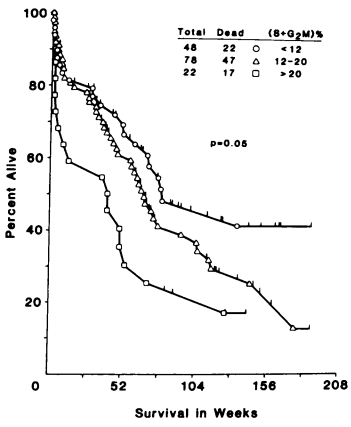

Figure 5. Survival by pretreatment S + G_2M percentage in adult patients with AML.

Table III. Incidence of Specific Cytogenetic Abnormalities in Young and Old Patients and Their Relation to Complete Remission Rate and Cytokinetics

Specific cytogenetic abnormality	No. of patients	Incidence by age			Percentage of complete remission	Mean S + G_2M percent (range)
		Total	≤50 yr	>50 yr		
	n	%	%	%	%	%
Inversion 16	9	6	9	2	100	14.8 (9–25)
Translocation 8; 21	8	5	7	3	87.5	10.1 (4–13)
Translocation 15; 17	10	7	10	2	40	9.7 (5–15)
Diploid karyotype	67	45	38	55	78	15.3 (5–25)
−5; −7; 5q-; 7q- (with or without additional abnormalities)	12	8	7	10	42	18.3 (9–33)
Other cytogenetic abnormalities	32	22	22	21	50	16.8 (7–31)
Insufficient metaphases	10	7	6	8	70	10.9 (6–18)

Discussion

In this analysis, pretreatment cytokinetic studies provided important biologic and clinical information on adult AML. Cytokinetic measurements of bone marrow biopsy material demonstrated significantly lower S + G_2M values during active AML compared to morphologically normal marrow (43). This observation is consistent with earlier autoradiographic studies revealing a lower growth fraction or longer generation times in acute leukemia when compared to normal granulopoiesis (19–25). Contrary to previous, often contradictory, reports on the prognostic implications of cell kinetics in AML, our study was conducted on a large patient population treated at the same institution using bone marrow biopsy material exclusively. It is the first study to investigate correlations between cytokinetics and other important prognostic determinants in adults with AML, thus allowing comparisons within each category.

As in previous studies, both age and cytogenetics affected remission induction, with a significantly lower CR rate occurring in patients over 50 yr of age and those with aneuploid karyotypes (4, 6–8 and manuscript submitted for publication). No correlation, however, was found between increasing S + G_2M percentage and remission when the overall population was considered. Data pooling could mask significant but opposite relationships among various subcategories. Thus, when age was entered as a variable, younger patients showed increasing, and older patients decreasing response rates with increasing S + G_2M percentage. The divergent prognostic effect of pretreatment kinetics in different age groups may, therefore, explain the conflicting results reported to date. This finding, as well as the opposite cytogenetic and cytokinetic correlations in young and old patients, strongly supports the notion that AML in young and old patients may be two biologically distinct disease entities. Thus, the well-recognized adverse prognostic effects of advancing age may be disease- rather than host-related.

Table IV. Patterns of Failures (46 Patients)

Category	No. of failures	Percent with failure from resistant disease
Age (in yr)		
≤50	19	47
>50	27	30
S + G_2M percent		
≤15	26	35
>15	20	40
Karyotype		
Balanced translocations	7	0
Diploid karyotype	14	43
−5; −7; 5q-; 7q- (with or without other abnormalities)	7	57
Other abnormalities	15	33
Insufficient metaphases	3	66

In view of the inverse relationship between S + G_2M percentage and the percentage of aneuploid metaphases in young patients, high values of S + G_2M percentage may be a reflection of a lower proportion of leukemic cells and a higher proportion of residual normal hematopoietic cells. Such a condition would favor a higher incidence of complete remission. Different biologic assumptions, however, seem to pertain to the older patient population. A high S + G_2M compartment size associated with a higher proportion of aneuploid metaphases may reflect a higher leukemic burden and, in cytokinetic terms, may result from a prolonged S-phase transit time associated with a decreased sensitivity to chemotherapy (13, 14).

Once in remission, the prognostic direction of pretreatment cytokinetics in AML, at least for the younger age group, seems to be reversed. This observation is consistent with several literature reports in acute leukemia and in other diseases, including lymphoma (46) and myeloma (47, 48). The paradox of opposite prognostic implications of pretreatment cytokinetics for remission induction and duration suggests that the duration of disease control is not so much determined by the efficacy of maintenance chemotherapy to produce continued reduction in tumor mass, but by the original tumor growth characteristics, with delayed relapse when the proliferative activity is low. This interpretation is supported by clinical investigations suggesting that prolonged conventional maintenance therapy may not improve remission duration (49, 50). Therefore, the value of more intensive therapy in remission is currently being addressed.

In summary, the age-associated divergence in the biologic relationship between cytogenetics and cytokinetics and in their prognostic implications for adults with AML support a disease- rather than host-related basis for the well-recognized difference in the clinical course of young and old patients with AML.

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