Independent prognostic significance of day 21 cytogenetic findings in newly-diagnosed acute myeloid leukemia or refractory anemia with excess blasts

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Background and Objectives. We investigated whether cytogenetic findings (CG) on day 21 (D21) of the first course of chemotherapy predicted subsequent outcome in patients who presented with CG abnormalities.

Design and Methods. D21 CG analysis was performed in 197 patients.

Results. Nineteen percent of the patients had exclusively abnormal metaphases (*AA*), 31% had only normal metaphases (*NN*), 39% had normal and abnormal metaphases (*AN*), and 11% had insufficient metaphases (*0/0*) on D21. A complete response was achieved in 79% of patients with NN, 60% with AN, 27% of those with AA, and 32% of those with 0/0.

Interpretations and Conclusions. Disease-free survival in CR was longest in patients who had ≥1 normal metaphase on D21, with this finding being independent of D21 marrow and initial CG results. D21 CG can be used in therapeutic decision-making.

Key words: AML, day 21 cytogenetics.

Algorithms predicting outcome in patients with untreated acute myeloid leukemia (AML) are typically based on information collected prior to beginning therapy. These algorithms explain only a minor part of the variability in outcome among different patients.[1] It appears logical that data collected at defined times after therapy has begun might reduce this variability. Given the prognostic significance of pre-treatment cytogenetic results,[2-4] we assessed whether cytogenetic findings on day 21 of the first course of therapy are a useful predictor of subsequent outcome.

Design and Methods

Four hundred and forty-one patients with newly diagnosed AML (>20% blasts, and excluding patients with

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acute prolymphocytic leukemia) and 62 with refractory anemia with excess blasts (RAEB) received treatment at the MDACC from 11/1/95-12/31/98. Conventional methods indicated that 180 (36%) patients had a normal karyotype (≥ 10 normal metaphases in the absence of an abnormal clone as defined below) and 24 (4.8%) had inv(16) or t(8;21); the 299 (59%) patients with other cytogenetic abnormalities are the basis of this report; the inv(16) or t(8;21) patients were excluded because of their generally favorable outcomes. Two-hundred and seventy-two of the 299 (91%) were alive on day 21 (D21) and 197 of the 272 (72%) had a sample sent for D21 cytogenetic analysis. The outcome of patients was unrelated to whether a sample was sent.

The median age of the 197 patients was 61 years. Overall, 50% had abnormalities of chromosomes 5 and/or 7 (*-5/-7* poor prognosis) and the remainder had intermediate prognosis abnormalities;[3] 119 had an antecedent hematologic disorder. Patients primarily received combinations of ara-C with fludarabine, idarubicin, or cyclophosphamide and topotecan.[5]

Although, pre-treatment, ≥ 2 metaphases carrying the same pseudo- or hyperdiploid aberration, or ≥ 3 metaphases with the same hypodiploid aberration, defined the presence of *an abnormality*, an abnormality was said to be present on D21 provided ≥ 1 cell showed the same aberration present initially. We defined 4 groups according to D21 cytogenetic findings: only abnormal (≥ 1) metaphases on day 21 (*AA*), only normal metaphases (≥ 1) (*NN*), both abnormal and normal metaphases (*AN*) and no metaphases (*0/0*).

We monitored two outcomes: complete response (CR), defined using standard criteria, and time to relapse or death in CR (disease-free survival) in patients who entered CR. The Cox model[6] was used to assess the prognostic significance of D21 cytogenetic findings after accounting for the information already provided by age, pre-treatment cytogenetics (-5/-7 vs. other), AA or AN pre-treatment, and marrow findings on D21 (too few cells to count vs. other). Model criticism was carried out as described by Thall and Estey.[7]

Results

At least one analyzable metaphase was obtained from 175 of the 197 patients studied (89%). Even 70% of the 40 patients whose marrow had too few cells to establish a differential count could be categorized as having NN, AA, or AN. The mean number of metaphases exam-

Table 1. Day 21 cytogenetics by pre-treatment cytogenetic group.

Cytogenetic group	0/0	AA	NN	AN	Total
-5,-7[a]	13 (13%)	21 (21%)	27 (27%)	38 (38%)	99
+8	4 (14%)	3 (10%)	8 (28%)	14 (48%)	29
11q	–	1 (7%)	8 (57%)	5 (36%)	14
Abnormal, but not –5/-7, +8,or 11q	5 (9%)	12 (22%)	18 (33%)	20 (36%)	55
Total	22	37	61	77	197
-5/-7	13 (13%)	21 (21%)	27 (27%)	38 (38%)	99
Other abnormal	9 (9%)	16 (16%)	34 (35%)	39 (40%)	98

[a]Refers to monosomies, and/or deletions of the long arms of chromosomes 5 and/or 7.

Table 2. Relation between day 21 cytogenetics and % blasts on day 21.

% blasts day 21	0/0	Cytogenetic category			Total
		AA	NN	AN	
TFTC	12 patients (30% of 40 patients with TFTC)	8 (20%)	11 (28%)	9 (23%)	40
0-9	4 (5%)	12 (13%)	38 (43%)	35 (39%)	89
10-29	4 (9%)	9 (21%)	11 (26%)	19 (44%)	43
≥30	2 (8%)	8 (32%)	1 (4%)	14 (56%)	25
Total	22	37	61	77	197

Table 3. CR rate by day 21 cytogenetic results.

D21 cyto	Patients	CRs (rate, exact 95% confidence interval)
0/0	22	7 (32%;13-55%)
AA	37	10 (27%;14-44%)
NN	61	48 (79%;66-88%)
AN	77	46 (60%;48-71%)

ined on day 21 was 17. Only 6 patients (3%) had ≤ 3 evaluable metaphases (1 patient 1 metaphase, 2 patients 2 metaphases, 3 patients 3 metaphases).

Thirty-nine percent of the 197 patients had AN, 31% had NN, and 19% had AA on day 21, with the remaining 11% having 0/0. Cytogenetic group had little influence on whether the patient was considered 0/0, NN, AA, or AN (Table 1); in particular, the percentage of patients in these 4 groups was similar in the –5/-7 and *other abnormal* groups.

Predictably, patients with residual abnormal metaphases (AA or AN) were more likely to have residual blasts on D21 and vice versa (Table 2). Also as expected, 0/0 was most frequent in patients whose marrow had too few cells to count (TFTC) on D21 (Table 2).

CR was observed in 79% of patients with NN but in only 27% of those with AA (Table 3). The AN group had a 60% CR rate. If the insufficient metaphase (0/0) group was an average of the NN, AA, and AN groups, the expected CR rate would be 55%, the average CR rate in these groups. In fact, the CR rate in the 0/0 (32%) group was reminiscent of the AA group (27%). DFS in CR was longest in the NN group (Figure 1), while survival dated from day 21 was longest in the NN group, intermediate in the AN group and shortest in the AA and 0/0 groups.

A multivariate analysis was done to see whether knowledge of D21 cytogenetics added any information to that provided by the D21 blast count or the other covariates noted in Methods. We regarded the D21 blast count as either TFTC (unfavorable) or other (favorable) because preliminary analysis indicated that this was the grouping with the most prognostic significance for DFS *once CR had occurred* (TFTC vs not TFTC p=0.001, 1-9% vs ≥ 10% excluding TFTC p=0.90). The fitted Cox model indicated that D21 cytogenetic status was an independent predictor of DFS in CR (Table 4). After accounting for pre-treatment cytogenetic status and D21 blast count, patients without normal metaphases on D21 had a 2.7-fold higher risk of relapse or death in CR per unit time than did patients with at least one normal metaphase on D21. This relative risk was essentially equivalent to that associated with the presence –5/–7 prior to treatment. The relative risk associated with the presence of at least 1 abnormal metaphase was lower (1.61, Table 4).

Discussion

The prognostic importance of pretreatment cytogenetics is unchallenged.[2-4] Although it seems highly plausible that changes in cytogenetic status as treatment progresses might also be predictive, less is known about this possibility. Freireich *et al.*[8] and Bloomfield (personal communication) have noted that the small proportion (<10%) of patients who have residual abnormal metaphases at CR have shorter remissions than patients who have only normal metaphases at this time. The current paper extends this work in two ways. First, it demonstrates the independent prognostic value of cytogenetics obtained in the post-treatment period (Table 4). Second, it shows that cytogenetic findings are relevant on D21 of course 1, i.e. before the great majority of patients are known to be in CR and thus at a time when there is much greater variability in cytogenetic status (for example, only 31% of
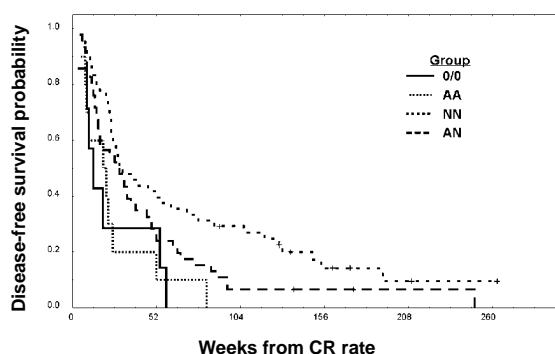
Figure 1. Kaplan-Meier plot showing probability of DFS in patients achieving CR in the 4 groups. NN vs AN *p*-value=0.054; NN vs AN+AA+0/0 *p*=0.007; AA vs 0/0 *p*=0.849 (log-rank test). The NN group comprised 48 patients, the AN group 46, the AA group 10, and the 0/0 group 7. All patients in the latter 2 groups have had an event, while 7 in the NN group and 2 in the AN remain alive in first CR.

Table 4. Independent predictors of DFS in CR.

Variable	Relative Risk	p value
-5/-7	2.78[a]	< 0.0001
0 normal metaphases on D21 (i.e. AA or 0/0 groups)	2.70[b]	0.0004
At least 1 abnormal metaphase on D21 (i.e. AA or AN groups)	1.61[c]	0.0293
Acellular marrow on D21(TFTC)	2.19[d]	0.0051

[a]versus other cytogenetic abnormalities; [b]versus at least 1 normal metaphase on day 21; [c]versus 0 abnormal metaphases on day 21; [d]versus patients with countable cells on day 21.

patients have entirely normal metaphases). Such earlier knowledge allows treatment to be changed when more benefit might result from such a change.

Our data suggest that 89% of patients, and even 70% of those with hypocellular (TFTC) marrows, can be categorized as having either NN, AA, or AN on D21. Although the criterion, i.e. at least 1 analyzable metaphase, we used to place patients into one of these groups is less stringent than that required prior to treatment, our criterion seems reasonable, at least for AA or AN given that, in all such cases as determined on D21, the abnormality detected had also been present at diagnosis. The validity of cat-

egorizing patients as NN if only a single (normal) metaphase was present on D21 rests on empiricism, i.e. is such a classification of predictive value? Our results suggest the answer is *yes*. We admit that the ≥1 abnormal metaphase or ≥1 normal metaphase categorization is arbitrary and that categorizations based on the number of abnormal or normal metaphases are, in principle, preferable. Our attempts to construct such a system foundered in the face of small sample sizes.

Even the information that no metaphases can be found to analyze may be prognostically useful. In particular, the 0/0 group appeared to have a distinctive (and poor) prognosis that was not merely an average of that seen in the NN, AA, or AN groups. Such averaging of course would be expected if the 0/0 finding was a technical artifact.

We conclude that D21 cytogenetic results are an independent predictor of outcome in AML. Thus, they can be used in making treatment decisions: in particular, patients with NN should be regarded as distinct (relative risk 0.37). At M.D. Anderson, cytogenetic results are known within 3 days. Our data suggest that attempts might be made to make this rapid turnaround time more common.

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Pre-publication Report & Outcomes of Peer Review

Contributions

MK collected day 21 cytogenetic data, did preliminary analyses,and wrote the paper S-C C performed the multivariate analyses and critically reviewed the article. JC, MA, FG, S O'B, and HK helped with data interpretation,contributed revisions to the article, and managed a large number of patients whose data contributed to the publication. EE suggested looking at cytogenetics on day 21, performed multivariate analyses, and helped revise the paper. Each author has given final approval to the paper as submitted.

Primary responsibility for the paper and for Tables 1-4 and Figure 1; MK.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by Dr. Claudia Schoch, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Schoch and the Editors. Manuscript received May 2, 2003; accepted June 4, 2003.

In the following paragraphs, Dr. Schoch summarizes the peer-review process and its outcomes.

What is already known on this topic

Several pretherapeutic parameters such as cytogenetics, age and white blood cell count have been established as prognostic markers in AML and MDS. Recently studies focused on therapy-dependent parameters such as early response (assessed by day 16 blasts on the basis of cytomorphology) or minimal residual disease (based on data obtained using quantitative PCR or flow cytometry) and showed that these are also of prognostic impact.

What this study adds

This study introduces a new approach using cytogenetics performed at day 21 to predict outcome in AML and MDS. Although the data need to be confirmed in a larger cohort of patients before they can be used in making treatment decisions, the study nicely shows that cytogenetic analysis is feasible at day 21 after chemotherapy and adds therapy-dependent prognostic information in AML and MDS with aberrant karyotype at diagnosis.