Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: The Cancer and Leukemia Group B experience

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Abstract. The Cancer and Leukemia Group B has performed central review of karyotypes submitted by institutional cytogenetics laboratories from patients with acute myeloid (AML) and acute lymphoblastic (ALL) leukemia since 1986. We assessed the role of central karyotype review in maintaining accurate, high quality cytogenetic data for clinical and translational studies using two criteria: the proportion of karyotypes rejected (i.e. inadequate), and, among accepted (i.e. adequate) cases, the proportion of karyotypes whose interpretation was changed on central karyotype review. We compared the first four years during which central karyotype review was performed with a recent 4-year period and found that the proportion of rejected samples decreased significantly for both AML and ALL. However, during the latter period, central karyotype reviews still found 8% of AML and 16% of ALL karyotypes inadequate. Among adequate cases, the karyotype was revised in 26% of both AML and ALL samples. Some revisions resulted in changing the patients' assignment to particular World Health Organization diagnostic categories and/or moving patients from one prognostic group to another. Overall, when both data on rejection rates and data on karyotype revisions made in accepted cases were considered together, 32% of AML and 38% of ALL samples submitted were either rejected or revised on central karyotype review during the recent 4-year period. These data underscore the necessity of continued central karyotype review in multi-institutional cooperative group studies.

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Introduction

Cytogenetic findings have become an integral part of diagnosis, prognostication and therapeutic stratification of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Such recurrent chromosome abnormalities and their molecular equivalents as t(8;21)(q22;q22)/*RUNX1-RUNX1T1*, inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFB-MYH11*, t(15;17)(q22;q12-21)/*PML-RARA*, and balanced abnormalities involving band 11q23 and the *MLL* gene are now included in the World Health Organization (WHO) classification of AML, and together with morphology, immunophenotype and clinical features are being used to define distinct disease entities (1). Notably, the role of cytogenetics will be substantially increased in the 2008 revision of the WHO classification (2).

Moreover, pretreatment cytogenetic findings constitute one of the most important prognostic factors in both AML (3-10) and ALL (7,11-13), are used to determine post-remission therapy (14-17), and are important for molecular genetic studies investigating mutations (18-29) and changes

in expression of specific genes (30-32), as well as gene- (33-37) and microRNA-expression (38,39) profiles in patients with acute leukemias. Importantly, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology specify that cytogenetic analysis is an obligatory part of the diagnostic workup in AML (40). Likewise, cytogenetic analysis is one of the minimum laboratory requirements for the diagnosis of AML according to the 'Guidelines on the Management of Acute Myeloid Leukaemia in Adults' issued by the British Committee for Standards in Haematology (41). Consequently, the Cancer and Leukemia Group B (CALGB) now mandates cytogenetic analyses for adult AML and ALL front-line treatment trials and many correlative studies. It is therefore imperative that cytogenetic data are accurate.

Despite continuous improvements in cytogenetic methodology, not all cytogenetic studies of bone marrow (BM) or blood from patients with acute leukemia are successful. In addition to a fraction of cases in which BM or blood cultures yield no mitotic cells, some cytogenetic preparations from leukemic samples are of suboptimal quality. This can make correct interpretation of karyotypes difficult. It has long been recognized that metaphase chromosomes of leukemic cells (especially, but not exclusively, from ALL patients) often have poor morphology with indistinct banding (42). Furthermore, a remarkable heterogeneity of chromosome findings in acute leukemias, with over two hundred recognized recurrent aberrations, many of which are relatively rare having been reported in <10 cases worldwide (43-45), underscores the necessity for cytogeneticists analyzing patients with AML and ALL to have vast experience in leukemia cytogenetics.

Therefore, CALGB pioneered the central review of karyotypes, which has been performed biannually since 1986 (46). In the current study, we evaluated the role of central karyotype review in providing accurate cytogenetic data. Our analysis shows an improvement in the quality of submitted karyotypes over time but also confirms the continued need for performing central karyotype review in the multi-institutional cooperative group setting to obtain the best possible data for clinical and translational studies.

Patients and methods

Cytogenetic analyses of diagnostic (n=5259) and relapse (n=909) samples from patients with AML and ALL enrolled onto a prospective cytogenetic companion study, CALGB 8461 (5), were performed in multiple, currently 33, CALGB-approved institutional cytogenetic laboratories. Written IRB-approved informed consent was obtained from all patients. For each specimen, two karyotypes and metaphase spreads from each clone were submitted with the data on processing methods to the CALGB Cytogenetic Data Management Center. If applicable, images of interphase and/or metaphase cells subjected to fluorescence *in situ* hybridization (FISH) were also submitted. All cases underwent biannual central karyotype review performed by the CALGB Karyotype Review Committee consisting of ten expert cancer cytogeneticists. At central karyotype review sessions, every karyotype, metaphase spread, FISH image, and processing and interpretive data were reviewed by two cytogeneticists. In some cases, usually those with more complex chromosome abnormalities and/or with suboptimal banding quality, other reviewers also rendered their opinion. Once consensus was reached, each submission was judged as either acceptable with adequate banding quality, acceptable with borderline banding quality, or inadequate and consequently rejected. Reasons for rejection included poor banding quality that makes unequivocal karyotype interpretation impossible, and, only in cases with a normal karyotype, analysis of <20 metaphase cells from a marrow sample cultured for 24-48 h or analysis of blood only (5). Since the aim of this study was to assess the role of central karyotype review, our analyses did not include 202 AML and 125 ALL cases for whom cytogenetic analysis yielded no metaphase cells.

In addition to data on rejection rates collected routinely at each central karyotype review, for the purpose of this study, we prospectively collected detailed information on the reasons for revisions made by central karyotype review in the submitted karyotypes that were accepted or borderline accepted during eight recent central karyotype review sessions. The reasons for revision were divided into the following categories: i) major errors in karyotype interpretation, such as failure of the submitting laboratory to recognize a clonal abnormality, identification of an abnormality found on central karyotype review not to be present, and incorrect interpretation of an abnormality; ii) the need for refinement of breakpoint assignment in structural abnormalities properly recognized by the submitting laboratory, iii) misidentified or upside-down chromosomes, and iv) incorrect use of the ISCN (1995) nomenclature (47). In this study, we excluded samples analyzed cytogenetically during complete remission, because these samples differ from pretreatment and relapse samples in that they rarely contain leukemic cells and are usually karyotypically normal (48). The rejection rates between the first and the recent 4-year periods (Table I) have been compared using the Fisher's exact test. All analyses were performed by the CALGB Statistical Center.

Results and Discussion

Table I shows rejection rates and reasons for rejection for AML and ALL specimens that underwent central karyotype review during the entire period between 1986 and 2006. Overall, 12% of 4991 AML and 23% of 1177 ALL karyotypes submitted were rejected. The most common reason for rejection was inadequate banding quality, which accounted for rejection of 53% (325 of 612) of AML and 46% (123 of 270) of ALL inadequate cases. When we compared rejection rates between the first four years during which central karyotype review was performed and the recent 4-year period, we found a significant improvement in the quality of submitted specimens in both AML ($P<0.0001$) and ALL ($P<0.001$). This was mainly due to the significant decrease in the numbers of karyotypes with inadequate banding quality (AML, $P<0.0001$ and ALL, $P=0.02$). Nevertheless, the recent central karyotype reviews still found 8% of AML and 16% of ALL samples inadequate.

Another indication of the quality improvement over time was an increase in the proportion of abnormal karyotypes pretreatment, from 52% in the first 4 years to 57% in the recent 4 years ($P=0.05$) in AML and from 59 to 75% in ALL

Table I. Rejection rates and reasons for rejection by central karyotype review among AML and ALL cases enrolled onto CALGB 8461.

Disease	Reason for rejection	All samples	First 4 years	Recent 4 years	P-value[a]
AML	No. of samples reviewed	4991	934	1126	
	No. (%) of samples rejected	612 (12)[b]	196 (21)[b]	90 (8)[b]	<0.0001
	Inadequate banding (%)	325 (53)	149 (76)	26 (29)	<0.0001
	Normal BM karyotype and <20 cells (%)[c]	168 (27)	20 (10)	40 (44)	<0.0001
	Normal karyotype in blood only (%)[d]	115 (19)	26 (13)	23 (26)	0.02
ALL	No. of samples reviewed	1177	222	128	
	No. (%) of samples rejected	270 (23)	74 (33)	20 (16)	<0.001
	Inadequate banding (%)	123 (46)	49 (66)	7 (35)	0.02
	Normal BM karyotype and <20 cells (%)[c]	78 (29)	13 (18)	9 (45)	0.02
	Normal karyotype in blood only (%)[d]	69 (26)	12 (16)	4 (20)	0.74

[a]Comparisons of the rejection rates between the first 4 years and the recent 4 years of central karyotype reviews were calculated using Fisher's exact test. [b]Samples from 4 AML cases, including 1 reviewed during the first 4 year and 1 during the recent 4 year periods, were rejected due to the inability of the submitting laboratory to provide sufficient documentation for suspected abnormalities. [c]BM denotes bone marrow. [d]Cytogenetic analysis of bone marrow was either not performed or was unsuccessful.

Table II. Changes in karyotype interpretation made in accepted AML and ALL cases during 8 recent central karyotype review sessions.

Changes in karyotype interpretation made by central karyotype review	Total n=1138	AML n=1031	ALL n=107
No. (%) of samples revised[a]	300 (26)	272 (26)	28 (26)
Total no. of changes made[a]	446	404	42
Type of revision			
Recognition of a missed abnormality or change in abnormality description (%)	195 (44)	176 (43)	19 (45)
Change in breakpoint(s) assignment (%)	100 (22)	83 (21)	17 (40)
Correction of misidentified or upside-down chromosome(s) (%)	98 (22)	95 (24)	3 (7)
Correction in ISCN (1995) nomenclature (%)	53 (12)	50 (12)	3 (7)

[a]Some karyotypes required two or more corrections.

(P=0.007). These improvements in quality can be attributed to many factors, including progress in cell culturing, harvesting and banding techniques, as well as increase in proficiency in analyzing specimens from patients with acute leukemias. The latter has been achieved in part through feedback provided to cytogeneticists from the submitting laboratories after central karyotype review sessions and through educational workshops organized and conducted by the members of the Karyotype Review Committee during CALGB Group Meetings.

Among karyotypes deemed to have adequate banding at 8 recent Central Karyotype Review sessions, changes in karyotype interpretation were made in 26% of both AML and ALL cases (Table II). The revisions included identification of an unrecognized chromosome abnormality or reinterpretation of an abnormality incorrectly interpreted by the submitting laboratory (44% of all revisions), reassignment of breakpoints in structural aberrations (22%), correction of misidentified or upside-down chromosomes (22%), and correction of errors in the ISCN (1995) nomenclature (12%).

In several instances, major changes in karyotype interpretation made on central karyotype review resulted in moving patients from one diagnostic category in the current

WHO classification (1) to another. For example, AML patients whose karyotypes submitted as normal were revised to abnormal ones that harbored t(9;11)(p22;q23), t(11;19)(q23;p13.1) or inv(16)(p13q22), as well as patients whose submitted karyotype was changed from 46,X,-Y,+8 to 46,X,-Y,+8,t(9;11)(p22;q23), from 46,XX,t(10;11)(p14;q13) to 46,XX,ins(10;11)(p12;q23q13), from 46,XY,del(5)(q13q33),del(11)(q23) to 46,XY,del(5)(q13q33),t(6;11)(q27;q23), and from 46,XY,del(3)(p13),add(11)(q23),-18,-21,+mar to 46,XY,t(3;11)(p13;q23),add(18)(q21),-21 would be reclassified from having 'AML not otherwise categorized' to 'AML with recurrent cytogenetic abnormalities'. Conversely, the patient whose submitted complex karyotype included an inv(16)(p13q22) that was revised on central karyotype review to der(16)add(16)(p13)del(16)(q22) would be reclassified from having 'AML with recurrent cytogenetic abnormalities' to 'AML not otherwise categorized'. There would be multiple additional changes in diagnostic categories once the new 2008 edition of the WHO classification (2) is published, including, for example, a reclassification from 'AML with myelodysplasia related changes' to 'AML with recurrent genetic abnormalities' for the patient whose karyotype submitted as normal was found on central karyotype review to contain an inv(3)(q21q26).

Many of both the aforementioned and other karyotype revisions made on central karyotype review resulted in changes in prognostic group assignment of the patients. For instance, revisions from a normal karyotype to a karyotype containing inv(3)(q21q26) or t(11;19)(q23;p13.1), or from del(11)(q23) to t(6;11)(q27;q23) meant that these patients were no longer classified as having an intermediate risk but were included in the adverse risk category instead (5). Poor prognosis associated with an isolated trisomy of chromosome 13 (49) was no longer predicted in a patient whose karyotype was revised from 47,XY,+13 to 47,XY,+15 when the reviewers discovered that an extra chromosome submitted as chromosome 13 was in fact an extra chromosome 15 placed upside-down in the karyotype of borderline quality. To date, prognostic significance of isolated trisomy 15 is unclear and it has been suggested to represent a benign, age-related abnormality in older male patients (50,51). Moreover, identification of an initially unrecognized inv(16) alters the patient's risk assignment from intermediate to favorable, whereas revision of the submitted inv(16) to der(16)add(16)(p13)del(16)(q22) as part of a complex karyotype means that the patient's risk-group assignment is no longer favorable but becomes adverse. Importantly, documentation of the presence or absence of inv(16) is of major clinical importance (52) because the postremission therapy of AML patients with inv(16) on CALGB protocols is administered in a risk-adapted fashion and includes three courses of high-dose cytarabine, whereas patients without inv(16) [or t(8;21)] receive autologous peripheral stem-cell transplantation (15).

Finally, we evaluated both the data on karyotype revisions made in the accepted cases and data on rejection rates during the recent 4-year period. Overall, 32% of AML and 38% of ALL samples submitted were either rejected or revised on central karyotype review.

While several studies have addressed the issue of proficiency testing and quality control in clinical cytogenetics (53-55), to our knowledge only one other study (from the Children's Oncology Group), published in abstract form, has reported the cooperative group experience concerning central review of karyotypes from acute leukemia patients (Heerema, *et al*, Cytogenet Genome Res 106: 136, 2004). Results of this study are remarkably similar to ours, showing that the karyotype was either changed or rejected in 31% of AML and 49% of ALL pediatric cases submitted. Therefore, we conclude that central karyotype review plays a vital role in ensuring the validity of the clinical trials and correlative studies conducted by both adult and children's cooperative groups.

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Appendix

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