Diagnostic value of fluorescence *in situ* hybridization for the detection of genomic aberrations in older patients with acute myeloid leukemia

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Background and Objectives. Karyotype is one of the most important prognostic factors in acute myeloid leukemia (AML).

Design and Methods. To assess the diagnostic value of molecular cytogenetics in AML patients older than 60 years, we compared the results of chromosome banding with those of fluorescence *in situ* hybridization (FISH) applying a comprehensive DNA-probe set for the detection of the most relevant AML-associated chromosome aberrations in a prospective series of 283 patients registered for the multicenter treatment trial AML HD98-B.

Results. Four cases of inv(16)/t(16;16) and 2 cases of t(11q23) were only detected by FISH. Molecular cytogenetic analysis was also more sensitive for the detection of genomic imbalances, in particular 7q–, +11q, 17p–, and 20q–, but virtually all cases of aneuploidy or deletions that were missed on banding analysis were identified in patients without assessable metaphases, in patients with normal karyotypes but poor chromosome morphology, in patients with a leukemia-specific balanced rearrangement, or in patients with complex karyotypes.

Interpretation and Conclusions. Our results support the use of FISH as a complementary method for the detection of inv(16)/t(16;16) and t(11q23) in all older AML patients eligible for intensive therapy. Molecular cytogenetics should also be considered in cases with insufficient yields of metaphase cells, poor chromosome morphology, or both. Routine screening for chromosomal imbalances by FISH does not improve cytogenetic risk assessment in patients with adequate pretreatment karyotype information.

Key words: AML, genomic aberrations, chromosome banding, FISH.

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Pretreatment karyotype is one of the key determinants of outcome in acute myeloid leukemia (AML),[1-5] and differential treatment of certain cytogenetic subsets has resulted in markedly improved prognosis.[6-11] As a consequence, several cytogenetic abnormalities, t(8;21), inv(16)/t(16;16), t(15;17), and abn(11q23), are considered in the recent World Health Organization classification of AML.[12,13]

In younger adults, chromosome banding reliably yields the leukemia karyotype, provided that sufficient metaphase cells are assessable. Molecular techniques, such as fluorescence *in situ* hybridization (FISH) and reverse-transcriptase polymerase chain reaction, have proven useful for the identification of selected chromosome aberrations, for instance, inv(16)/t(16;16) and t(11q23), which are sometimes missed on standard cytogenetic analysis, especially in metaphase preparations of suboptimal quality.[14-18] Compared with AML in younger patients, AML in the elderly is characterized by profound biological differences, including the distribution (but not the spectrum) of karyotypic abnormalities.[3,19,20] However, a previous study[3] indicated that the cytogenetic risk groups defined in younger adults[1,2,5] are also predictive of outcome in older individuals. The value of molecular diagnostics to improve cytogenetic risk assessment in older AML patients has not been systematically evaluated.

In the present study, we compared the results of chromosome banding with those of interphase FISH, applying a comprehensive DNA-probe set for the detection of the most relevant AML-associated chromosome aberrations, in a prospective series of 283 older AML patients.

Design and Methods

Patients

Two hundred and eighty-three consecutive patients older than 60 years with AML, *de novo* or secondary (after treatment for a primary malignancy or following myelodysplasia), or refractory anemia with excess blasts in transformation, as defined by the French-American-British classification,[21] were studied centrally by chromosome banding and FISH in the Laboratory for Cytogenetic and Molecular Diagnostics of the AML Study Group, Ulm. All patients were registered for the AML HD98-B treatment trial.[22] The study was approved by the institutional review boards of the participating centers. Informed consent was obtained from all patients according to the Declaration of Helsinki.

Chromosome banding

G-banding was performed using standard techniques. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature.[23]

FISH

The DNA clones selected for the detection of AML-associated chromosome aberrations by FISH are listed in Table 1. The criteria identifying gene fusions and the method used to define cut-off levels for the diagnosis of chromosomal imbalances were reported previously.[14] Preparation of DNA clones and labeling of probes by nick translation followed standard protocols. Dual-color FISH and visualization of hybridization signals by fluorescence microscopy were performed as described previously.[14,15]

Table 1. DNA clones selected for the detection of AML-associated genomic aberrations by FISH.

Aberration	*DNA clone*	*Localization*	*Gene/ locus*	*Source/ reference*
inv(3)/t(3;3)	858_c_9/14EE12	3q26	EVI1, D3S1212	CEPH/ICI
t(8;21)	464_h_8/72_h_9	21q22	RUNX1	CEPH
	+P1 164	8q22	CBFA2T1	
t(9;22)	361_d_9	22q11	BCR	CEPH
t(11q23)	785_c_6/856_b_9	11q23	MLL1, THY1	CEPH
t(6;11)	+C-109F0645	6q27	MLLT4	24
t(9;11)	+48/55	9p22	MLLT3	25
t(15;17)	356_c_12	15q22	PML	CEPH
	+RARAcos121/124	17q21	RARA	
inv(16)/t(16;16)	854_e_2	16p13	MYH11	CEPH
	+LA2-2/LA4-1	16q22	CBFB	
+3q	858_c_9/14EE12	3q26	EVI1, D3S1212	CEPH/ICI
+4q	F06184	4q22		RZPD
5q–	773_d_3	5q31	IL9, D5S89	CEPH
	yPR411	5q33	CSF1R	
7q–	HSC7E506	7q22	RELN, D7S240	HSC
	B2021/B17259	7q22	D7S1799	RZPD
	HSC7E124	7q35	D7S688	HSC
+8q	K19268	8q24	MYC	RZPD
+11q	785_c_6/856_b_9	11q23	MLL1, THY1	CEPH
abn(12p)	964_c_10	12p13	TEL	CEPH
13q–/+13q	A12173	13q14	RB1, D13S25	RZPD
17p–	K0189	17p13	TP53	RZPD
20q–	808_c_5	20q12	D20S99	CEPH
+21q	464_h_8/72_h_9	21q22	RUNX1	CEPH
+22q	361_d_9	22q11	BCR	CEPH
Xq–	IE018	Xq28	DXS304	RZPD

CEPH, YAC library of the Fondation Jean Dausset – Centre d'Etude du Polymorphisme Humain (http://www.cephb.fr). RZPD, PAC library RPCIP704 of the Resource Center and Primary Database established within the German Human Genome Project (http://www.rzpd.de). ICI, YAC library of the United Kingdom Human Genome Mapping Project Resource Centre (http://www.hgmp.mrc.ac.uk). HSC, chromosome 7-specific YAC library of the Hospital for Sick Children, Department of Genetics, Toronto, Ontario, Canada (http://www.genet.sickkids.on.ca/chromosome7).

Results

Overall comparison of chromosome banding and FISH

Assessable metaphases for conventional cytogenetic analysis were obtained in 257 (90.8%) of the 283 patients. Of these 257 patients, 136 (52.9%) exhibited clonal chromosome aberrations. Evaluable interphase preparations for FISH were obtained in all 283 patients. Chromosomal abnormalities were detected in 130 (45.9%) of the 283 patients. The combined results of chromosome banding and FISH are given in Table 2; the distribution of individual aberrations is shown in Table 3.

Detection of translocations and inversions

An inv(16)/t(16;16) was identified in 4 additional patients by FISH. These included 1 patient without assessable metaphases, 2 with normal karyotypes, and 1 with a t(X;17)(q28;q21). Retrospective analysis of the 2 cases with normal karyotypes showed that the inv(16) was likely present but had been missed due to poor chromosome morphology. Rearrangements involving 11q23 were identified in 2 additional cases by FISH: 1 patient with an add(6p) on banding analysis had a t(6;11)(q27;q23); 1 patient with a –Y and a +8 on banding analysis demonstrated a t(9;11). Retrospective analysis of the latter case showed that the t(9;11) was likely present but that it,

Table 2. Incidence of AML-associated genomic aberrations in 283 AML patients older than 60 years as assessed in a central reference laboratory.

	Chromosome banding	*FISH*	*Chromosome banding or FISH*
	Number of cases (%)	*Number of cases (%)*	*Number of cases (%)*
Not assessable	26 (9)	0 (0)	0 (0)
No clonal aberrations	121 (43)	153 (54)	138 (49)
Clonal aberrations	136 (48)	130 (46)	145 (51)

Table 3. Distribution of AML-associated genomic aberrations in 283 AML patients older than 60 years.

	Number of aberrations (%)		
Aberration	*Chromosome banding*	*FISH*	*Chromosome banding or FISH*
inv(3)/t(3;3)	1 (0.4)	1 (0.4)	1 (0.4)
t(8;21)/t(8;21)var	7 (2.5)	7 (2.5)	7 (2.5)
t(9;22)	1 (0.4)	1 (0.4)	1 (0.4)
t(11q23)	5 (1.8)	7 (2.5)	7 (2.5)
t(15;17)	6 (2.1)	6 (2.1)	7 (2.5)
inv(16)/t(16;16)	7 (2.5)	11 (3.9)	11 (3.9)
+4/+4q	3 (1.1)	5 (1.8)	5 (1.8)
–5/5q–	42 (14.8)	40 (14.1)	44 (15.5)
–7/7q–	30 (10.6)	34 (12)	34 (12)
+8/+8q	21 (7.4)	25 (8.8)	28 (9.9)
+11/+11q	7 (2.5)	15 (5.3)	15 (5.3)
–12/12p–	11 (3.9)	14 (4.9)	16 (5.7)
–13/13q–/+13/+13q	23 (8.1)	21 (7.4)	26 (9.2)
–17/17p–	12 (4.2)	16 (5.7)	16 (5.7)
–20/20q–	9 (3.2)	16 (5.7)	16 (5.7)
+21/+21q	10 (3.5)	11 (3.9)	13 (4.6)
+22/+22q	6 (2.1)	6 (2.1)	8 (2.8)
–X/Xq–	3 (1.1)	4 (1.4)	4 (1.4)

Percentages may not add up to 100 because of rounding.

too, had been missed due to inadequate chromosome morphology. One patient without assessable metaphases demonstrated a t(15;17) by FISH; on the other hand, 1 case of a t(15;17) was not detected by FISH (number of metaphases carrying a t(15;17), 13 of 13). All cases of t(8;21)/t(8;21)var, inv(3), and t(9;22) were detected by both methods.

Detection of genomic aberrations in patients without assessable metaphases

Chromosome aberrations were detected by FISH in 6 (23%) of the 26 patients without assessable metaphase preparations: +4q; 5q–, 13q–, 20q–, +22q; 20q–; t(15;17) (*see above*); inv(16) (*see above*); and +8q.

Detection of genomic aberrations in patients with normal karyotypes

Chromosome aberrations were detected by FISH in 4 (3.3%) of the 121 cases with normal karyotypes. Two patients had an inv(16)/t(16;16) (*see above*). Two patients had single chromosomal imbalances: in 1 patient with 22 normal metaphases, a +8q was present in 41% of the interphase nuclei; in 1 patient with 20 normal metaphases and poor chromosome morphology, 31% of the interphase nuclei carried a 12p–.

Detection of genomic aberrations in patients with complex karyotypes

Forty-six (16.3%) of the 283 patients had at least 3 unrelated chromosome abnormalities in the absence of t(8;21), inv(16)/t(16;16), and t(15;17). Without the additional use of FISH, 1 (2.2%) of these 46 patients would have been classified as having less than 3 aberrations: in this patient with an inv(3) and a –7, an additional 5q– was detected by FISH (proportion of interphase nuclei carrying a 5q–, 53% and 59%, respectively). Thirty-four (12%) of the 283 patients exhibited a complex karyotype, defined according to the criteria proposed by the British Medical Research Council (MRC), that is, 5 or more unrelated chromosome abnormalities in the absence of t(8;21), inv(16)/t(16;16), and t(15;17).[2,3] Without the additional use of FISH, 1 (2.9%) of these 34 patients would have been classified as having a non-complex karyotype: in this patient, 5 of 7 analyzable mitoses demonstrated a tetraploid karyotype with a –5, a +22, and a marker chromosome, whereas 4 additional aberrations (7q–, 8q–, 17p–, 20q–) were detected by FISH.

Detection of chromosomal imbalances

Molecular cytogenetic analysis was more sensitive for the detection of the following genomic imbalances: +4q, 7q–, +8q, +11q, 12p–, 17p–, 20q–, +21q, and Xq–. However, virtually all cases of aneuploidy or deletions that were missed on conventional cytogenetic analysis were identified in patients without assessable metaphases (*see above*), in patients with normal karyotypes but poor chromosome morphology (*see above*), in patients with complex karyotypes, as defined by the presence of at least 3 unrelated

chromosome abnormalities in the absence of t(8;21), inv(16)/t(16;16), and t(15;17), or in patients with a leukemia-specific balanced rearrangement (a 12p– in 31% of interphase nuclei and a 20q– in 24% of interphase nuclei in a patient with an inv(16) and a +13q in 58% of interphase nuclei in a patient with a translocation involving 11q23). The 2 remaining chromosomal imbalances, a +11q that was present in 21% of interphase nuclei and a +11q that was present in 58% of interphase nuclei, occurred in combination with chromosome changes (5q– and –7, respectively) that are associated with primary resistance to intensive chemotherapy and poor long-term outcome in younger[1,2,5] and older[3] AML patients. Some genomic imbalances were only detected by conventional cytogenetics, mainly in a small proportion of metaphases (*data not shown*). Since chromosome banding is restricted to the analysis of dividing cells, this may indicate that these genomic changes were only present in leukemic subclones with a high mitotic activity.

Discussion

Ours is the first study to evaluate systematically the diagnostic value of a molecular technique for the detection of genomic aberrations in a large prospective series of older AML patients entered into a multi-institutional treatment trial. Given the paramount importance of karyotype for prognostication in AML, our findings have important clinical implications.

In accordance with our findings in younger adults,[14,15] FISH was more sensitive for the detection of inv(16)/t(16;16) and t(11q23). An inv(16)/t(16;16) can be detected in up to 10% of younger AML patients and predicts a favorable clinical outcome.[1-5,9,26] In contrast, inv(16)/t(16;16) is present in less than 5% of older AML patients;[3,19,20] nonetheless, its presence is also associated with a relatively good prognosis in intensively treated elderly patients.[3] Therefore, we favor a diagnostic approach with a high sensitivity for the detection of inv(16)/t(16;16), such as a combination of chromosome banding and FISH, to identify those elderly patients who are most likely to benefit from intensive therapy.

We also found that FISH provides a valuable tool for cases without assessable metaphases. Consequently, we suggest that elderly AML patients without evaluable mitoses who are candidates for induction chemotherapy be screened for genomic aberrations by FISH to allow accurate cytogenetic risk assessment.

Molecular cytogenetic analysis identified clonal aberrations in 4 (3.3%) of 121 cases with normal karyotypes: inv(16), inv(16), 12p–, and +8q. In the first 3 cases, chromosome morphology was poor. Accordingly, the +8q was the single aberration detected by FISH, indicating that molecular cytogenetics does not add relevant information to that gained from chromosome banding in patients with normal cytogenetics and metaphase preparations of sufficient quality.

Different cytogenetic classification schemes have defined complex karyotypes by the presence of at least 3[1,5] or at least 5[2,3] clonal aberrations in the absence of t(8;21), inv(16)/t(16;16), and t(15;17). Two of the aforementioned studies included older patients.[1,3] Byrd *et al*. assessed the prognostic impact of cytogenetic abnormalities in patients with *de novo* AML who were enrolled in 5 consecutive treatment trials of the Cancer and Leukemia Group B.[1] The median age of the study population was 52 years, and the proportion of patients above the age of 60 was 36%. Although patients with 3 or 4 chromosome aberrations had a significantly better cumulative incidence of relapse and overall survival than did patients with 5 or more abnormalities, their outcome was significantly worse than that of the cytogenetically normal group. Grimwade *et al*. analyzed the predictive value of pretreatment cytogenetics in patients older than 55 years of age (median age, 66 years) who were entered into a single MRC trial.[3] As in the study by Byrd *et al*., the subgroup of patients with 5 or more aberrations had the worst prognosis, but the outcome of patients with *non-complex adverse* abnormalities, that is, –5, 5q–, –7, and abn(3q), alone and in combination with up to 3 other aberrations, was also extremely poor (relapse risk at five years, 81%; overall survival at five years, 3.9%). In our study, the combination of chromosome banding and FISH using DNA probes for the detection of genomic imbalances allowed reclassification of less than 1% of the 283 patients to the complex-karyotype category, irrespective of the definition of a complex karyotype.

Although routine screening for chromosomal gains or losses by FISH did not improve cytogenetic risk assessment in patients with adequate pretreatment karyotype information, FISH provided more precise information on the prevalence of specific genomic imbalances. The use of modern techniques, such as matrix-based comparative genomic hybridization, will aid further refinement of the interpretation of complex karyotypes and the identification of novel genomic regions recurrently involved.[27-32]

This study defines the diagnostic value of a comprehensive cytogenetic analysis using chromosome banding and FISH in older AML patients entered into a multicenter treatment trial. Based on our results, we propose that FISH be used as a complementary method for the identification of inv(16)/t(16;16) and

t(11q23) in all elderly patients considered eligible for intensive therapy. In addition, patients with inadequate numbers of evaluable metaphase cells or poor chromosome morphology should be screened for 5q–, 7q–, +8q, 12p–, 17p–, and 20q– by FISH, given the dismal prognosis associated with these aberrations.

Appendix

The following AML Study Group Ulm institutions and investigators participated in this study.

Universitätsklinikum Bonn, Germany, A. Glasmacher; Universitätsklinikum Düsseldorf, Germany, U. Germing; Universitätsklinikum Giessen, Germany, H. Pralle; Universitätsklinikum Göttingen, Germany, D. Haase; Allgemeines Krankenhaus Altona, Hamburg, Germany, H. Salwender; Universitätskliniken des Saarlandes, Homburg, Germany, F. Hartmann; Universitätsklinikum Innsbruck, Austria, G. Gastl; Städtisches Klinikum Karlsruhe, Germany, J.T. Fischer; Universitätsklinikum Kiel, Germany, M. Kneba; Klinikum rechts der Isar der Technischen Universität München, Germany, K. Götze; Städtisches Krankenhaus München-Schwabing, Germany, C. Waterhouse; Städtische Kliniken Oldenburg, Germany, F. del Valle; Caritasklinik St. Theresia Saarbrücken, Germany, J. Preiß; Bürgerhospital, Stuttgart, Germany, W. Grimminger; Katharinenhospital Stuttgart, Germany, H.G. Mergenthaler; Krankenhaus der Barmherzigen Brüder, Trier, Germany, W. Weber; and Hanusch-Krankenhaus, Wien, Austria, E. Koller.

SF, RFS, HD, KD contributed to conception/design of the study, collection/analysis/interpretation of the data, drafting/revising the article. SK, CM, SM, CW, SS contributed to collection and analysis/interpretation of the data. All authors approved the final version of the article to be published. The authors declare that they have no potential conflicts of interest.

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