Cytogenetic analysis in acute myeloid leukemia in Tîrgu Mureş

Analiza citogenetică în leucemia acută mieloidă în Tîrgu Mureş

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Abstract

Acquired clonal chromosome aberrations can be observed after metaphase banding analyses in 50–60% of patients with acute myeloid leukemia (AML). The cytogenetic results at diagnosis provide the most important parameter for determining prognosis so far. Numerous recurrent karyotype abnormalities have been described in AML. Our aim is to evaluate the frequency of chromosomal abnormalities in patients with acute myeloid leukemia admitted in the Hematology Clinic in Tîrgu Mureş and to compare our results with those reported elsewhere.

We received for chromosomal analysis 28 samples of AML patients from the Hematology Clinic in Tîrgu Mureş between January 2006 and July 2008. We carried out bone marrow and/ or peripheral blood cell culture according to standard methods.

The leukemic karyotypes of 24 patients were successfully analyzed. Abnormal clones were detected in 66% of cases AML. In our study, the most frequent abnormality was hyperdiploidy. These findings are similar to the results obtained in other studies using a similar approach.

Key words: *acute myeloid leukemia, cytogenetics, chromosomal abnormalities*

Rezumat

Anomaliile cariotipice clonale sunt detectate prin analize citogenetice în 50-60% dintre pacienţii cu leucemie acută mieloidă (LAM). Analizele citogenetice în momentul diagnosticului reprezintă parametrul cel mai important pentru stabilirea prognosticului. În LAM au fost descrise numeroase anomalii cromozomiale recurente. Scopul studiului nostru este să stabilim frecvenţa anomaliilor cromozomiale la pacienţii cu LAM din Clinica de Hematologie din Tîrgu Mureş şi de a compara datele obţinute cu cele descrise în literatura de specialitate.

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Au fost primite 28 de probe biologice de la pacienții cu LAM internați în Clinica de Hematologie din Tîrgu Mureş în perioada ianuarie 2006 - iulie 2008. S-au efectuat culturi celulare din măduva osoasă hematogenă şi/ sau sânge periferic, conform metodelor standard.

Analiza citogenetică (cariotipuI) a putut fi efectuată în 24 dintre cazuri. În 66% dintre acestea au fost evidențiate anomalii cariotipice clonale. Aceste date sunt similare cu rezultatele obținute în alte studii. Hiperdiploidia a fost cea mai frecventă anomalie cromozomială întâlnită în studiul nostru.

Cuvinte cheie: *leucemie acută mieloidă, citogenetică, anomalii cromozomiale*

Introduction

Acute myeloid leukemia (AML) is a very heterogeneous disease with regard to clinical features and acquired genetic alterations, microscopically detectable both as structural and numerical chromosome aberrations [15]. At present, cytogenetic aberrations detected at the time of AML diagnosis constitute the most common basis for prediction of clinical outcome [5, 12]. Several parameters provided by cytomorphology, immunophenotyping and especially cytogenetics are needed to classify AML into biological entities in order to establish the diagnosis and to understand the pathogenesis, as well as to develop specific treatment approaches.

Pattern of Cytogenetic Abnormalities in AML

Clonal chromosome abnormalities, defined as an identical structural aberration or gain of the same structurally intact chromosome detected in at least two metaphase cells, or the same chromosome missing from a minimum of three cells, are consistently found in the majority of AML patients at diagnosis. However, in contrast to patients diagnosed with chronic myeloid leukemia, who are invariably positive for t(9;22) or its variants, the cytogenetic picture of AML is much more complex [11]. To date, approximately 200 different structural and numerical aberrations such as reciprocal translocations, inversions, insertions, deletions, unbalanced translocations, isochromosomes, isodicentric chromosomes, isolated trisomies and monosomies have been found to be recurring chromosome changes in AML. Many of these aberrations are very rare, being so far detected in few patients worldwide, whereas others occur more frequently. These more common abnormalities, together with their frequencies among adults and children with AML, are presented in *Table 1*.

The incidence of abnormal karyotypes in AML has been reported to be 55% to 78% in adults and 77% to 85% in children [1, 6]. However, a substantial proportion of patients show no chromosome abnormalities.

Prognostic significance of cytogenetic abnormalities in AML

The karyotype of the leukemic blasts is the most important independent prognostic parameter in AML [7]. A favorable outcome under currently used treatment regimens was observed in several studies in patients with t(8;21)(q22;q22), inv(16)(p13q22) or t(15;17)(q22;q11-12). Chromosome aberrations with an unfavorable clinical course are inv(3)/t(3;3), -5/del(5q), -7/del(7q) and complex aberrant karyotype. All others, i.e. patients with abnormal karyotype and rare chromosome aberrations, are assigned to an intermediate prognostic group [3,15].

The incidence of distinct chromosome abnormalities varies with age, but the prognosis of defined cytogenetic aberrations is age-independent [1].

The higher incidence of the chromosomal abnormalities demonstrates the importance of cytogenetic evaluation in patients with acute myeloid leukemia. Our findings suggest that cytogenetic analysis is a useful tool in the investigation of these patients for confirmation of clin-

Table 1. Frequencies of the most common cytogenetic abnormalities in patients with acute myeloid leukemia in different study groups [3, 11, 17].

Cytogenetic Abnormality	MRC study n=2337	CALGB study n=1311	SWOG/ ECOG study n=609
-5	79	26	-
-7	136	47	-
-Y	-	58	20
del(5q)	104	42	-
del(7q)	73	19	-
del(9q)	37	33	17
del(11q)	-	12	-
+8	211	123	53
+21	51	28	-
t(15;17)(q22;q21)	210	88	27
t(8;21)(q22;q22)	104	81	50
inv(16)(p13q22); t(16;16)(p13;q22)	53	96	53
t/inv(11q23)	45	54	42
t(9;22)(q34;q11)	16	10	8
Complex karyotype	222	234	124

MRC- United Kingdom Medical Research Council; CALGB- Cancer and Leukemia Group B; SWOG/ECOG -Southwest Oncology Group/Eastern Cooperative Oncology Group;

ical diagnosis. The karyotype of the leukemic blast has been shown to be the most important independent prognostic parameter in AML. These techniques should be performed in each patient with AML at diagnosis as well as in the case of relapse.

Our aim was to evaluate the frequency of chromosomal abnormalities in patients with acute myeloid leukemia in the Hematology Clinic in Tîrgu Mureş and to compare our results with those reported elsewhere. We also included our patients in different cytogenetic risk groups. There are few reports about cytogenetic analysis in malignant hemopathies in Romania. Most of them were performed in children with acute leukemia. It is the first study about the frequency of chromosomal abnormalities in AML in this region of the country.

Materials and Methods

Patients

Between January 2006 and July 2008, 28 samples of bone marrow and/or peripheral blood from patients with acute myeloblastic leukemia were sent to the Genetic Laboratory of the University of Medicine and Pharmacy in-Tîrgu Mureş, Romania, for cytogenetic evaluation. The study included children and adults with a diagnosis of acute myeloblastic leukemia. Their ages ranged from 2 to 62 years (median 35 years). All the cases were classified according to the French-American-British (FAB) classification [2]. There were 13 males and 15 females from the Hematology Clinic in Tîrgu Mureş. The patients were from the central part of Romania.

Cytogenetic analysis

Heparinized bone marrow and/or peripheral blood samples were collected in syringes or test tubes and sent to the laboratory at room temperature. Three different cultures (for direct harvest, 24-hour culture, and 72-hour culture with hematopoietic growth factors**)** were prepared as described elsewhere [8], starting from these samples. Culture media contained RPMI 1640 medium, 20% FCS (fetal calf serum), L-glutamine and penicillin/streptomycin (50 IU/ml and 50 μg/ml, respectively). Any samples without anticoagulant or in which EDTA was used as an anticoagulant, were classified as unsuitable and excluded from the analysis. Metaphases were harvested by adding colcemid (10μg/ml) solution followed by hypotonic KCl (0.075 M) treatment and fixation using standard 3:1 methanol: glacial acetic acid fixator. We used the conventional Giemsa banding (GTG banding) technique. Five to ten slides were screened in each case and 10 - 20 metaphases were analyzed for each sample. At least three cells were karyotyped according to the International System for Human Cytogenetic Nomen-

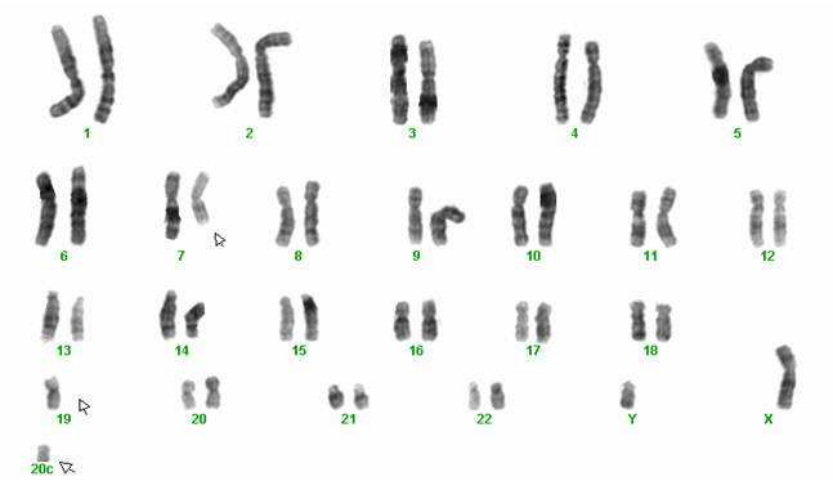

Figure 1. Karyotype 46,XY,del(7q),-19,+mar

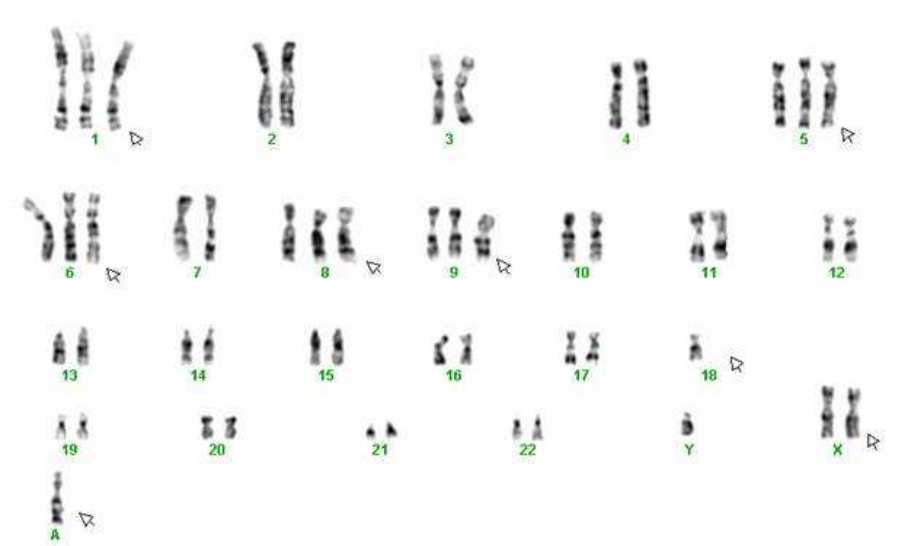

Figure 2. Hyperdiploidy. Karyotype: 52,XXY, +1,+5,+6,+8,+9,-18,+mar

clature (ISCN 1995) [11]. Analysis was carried out using a BX51 Olympus microscope and images captured with an automated image analysis system (Cytovision, Applied Imaging). Cell culture failure was defined as cases with poor-quality metaphases or with less than 10 analyzable metaphases.

Results

The 28 analyzed samples from patients with acute leukemia included: 26 cases at the moment of diagnosis and 2 after relapse. Among the 28 samples received, 2 were unsuitable for cytogenetic analysis due to clotting or the use of an unsuitable anticoagulant; these samples were consequently excluded from the study. A karyotype was not definite in 7.6 % of the cases because of a very low mitotic index or poor-quality metaphases obtained from the cell culture.

We successfully analyzed the leukemic karyotype of 24 patients, and identified 16 cases with chromosomal abnormalities. The results

Table 2. Distribution of FAB groups by ploidy in AML at diagnosis in our patients

	No. of cases	FAB groups							
		M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
Hyperdiploidy (>50)	4	1	-	-	-	-	2	-	1
Hyperdiploidy (47-50)	4	1	1	2	-	-	-	-	-
Diploidy (normal)	8	1	1	2	2	1	-	1	-
Pseudodiploidy	4	-	1	1	1	1	-	-	-
Hypodiploidy	4	1	-	1	1	-	-	-	1
Total	**24**	**4**	**3**	**6**	**4**	**2**	**2**	**1**	**2**

Table 3. Cytogenetic Risk Groups

Risk Group	**Cytogenetic abnormality**	**No. of cases**
Favorable	t(15;17)	1
Intermediate	del(7q)	1
	+21	2
	+19	1
	t(14;21)	1
	Normal karyotype	8
	All other structural/numerical abnormalities (hyperdiploidy, hypodiploidy)	8
Unfavorable (adverse)	Complex karyotype	2

were obtained from overnight cultures and from cultures with additional growth factors, as described in the methods.

Cytogenetic Findings

Clonal abnormalities were detected at diagnosis in 16 patients (66%), of wich 2 cases (12.5%) had complex karyotype and 14 patients (87.5%) had one or two clonal chromosome changes. An abnormal clone carrying del(7q) was detected in 1 patient with AML (*Figure 1*). In this patient, del(7q) was part of a complex karyotype and was associated with monosomy 19 and an extra chromosome marker (mar). A complex karyotype is defined by the presence of at least 5 clonal aberrations (12,16) or at least 3 abnormalities in the absence of t(8;21), inv(16)/t(16;16) and t(15;17). [3, 17, 18]

Isolated trisomy 19 was present in 1 case. Isolated trisomy 21 was documented in 2 cases. Hyperpdiploidy with more than 50 chromosomes (*Figure 2*) was detected in 4 cases.

Table 2 shows the distribution of structural abnormalities and ploidy according to FAB subgroups in AML at diagnosis. The structural rearrangements were the Robertsonian translocations t(14;21) in the M4 (1 case), reciprocal translocation t(15;17) in the M3 (1 case) and deletion del(7q) in the M1 (1case) FAB sub - groups.

On the whole, our results showed that the most frequent clonal karyotype alteration in AML was hyperdiploidy, detected in 50% of cases, while metaphases with structural anomalies were found in 4 cases (25%); another 4 (25%) presented a hypodiploid karyotype.

Cytogenetic Risk Groups

We included our patients in different cytogenetic risk groups, according to the hierar-

chical system of karyotype classification of Medical Research Council (MRC) AML 10 Trial (*Table 3*).

Discussion

Cytogenetic analysis of acute leukemia yields important information which has been demonstrated to be correlated with patient survival. Cytogenetic analysis is consequently standard practice in the diagnostic approach to leukemia, but very accurate culturing methods and considerable technical expertise are needed for proper karyotype analysis of acute leukemia.

In our study, acquired clonal chromosome abnormalities, structural aberrations or trisomy observed in at least 2 and monosomy found in at least 3 metaphase cells, were detected in 66% of patients with *de novo* AML. In 12.5% of patients, the abnormal karyotype was complex, i.e. contained at least 3 chromosome aberrations, whereas in almost 40% of patients no cytogenetic abnormality could be found using standard banding methods. The chromosome abnormalities frequency (66%) found in our study are within the limits reported by other authors [4, 5, 15].

We successfully analyzed the leukemic karyotype of 24 patients (85%) with AML. These results are similar to the findings of a BFM (Berlin-Frankfurt-Munster Group) multicenter study conducted on patients with acute leukemia. Lampert reports a cytogenetic success in 70% of AML, with a proportion of abnormal karyotypes of 68% in AML [9]. Sainati et al. have observed abnormal clones in 66% of AML cases [16].

Our results indicate that in AML, good metaphases are obtained from the three short-term cultures, and the addition of growth factors is useful. Sainati et al. also demonstrated the relationship between cytogenetic success in AML and the method with three different cultures [16].

In our study, the most frequent clonal karyotype alteration in AML was hyperdiploidy, detected in 50% of cases, while metaphases with structural abnormalities were found in 4 cases (25%); another 4 (25%) presented a hypodiploid karyotype. In other studies like the Medical Research Council (MRC) AML 10 Trial, AIEOP protocols (Associazione Italiana Emato-Oncologia Pediatrica), GALGB 8461 (Cancer and Leukemia Group B) and SWOG/ECOG (Southwest Oncology Group/ Eastern Cooperative Oncology Group), the most frequent karyotype alterations were represented by chromosomal structural abnormalities [3, 5]. We think that the different results concerning the most frequent clonal karyotype in AML obtained in our study are due to the low number of patients.

In our study the most frequent trisomy isolated or associated with other chromosome abnormalities was trisomy 21. According to Mrozek et al. [2], the most common trisomies in *de novo* AML are, in decreasing order of frequency, +8, +22, +13, +21 and +11. Trisomy 22 is a non-random secondary aberration accompanying inv(16)/t(16;16) and is rarely seen as an isolate chromosome abnormality. Although each of the remaining trisomies can be found as a secondary aberration, +8, +13, +11 and +21 are also detected recurrently as isolate karyotypic changes at diagnosis, with a frequency among adults with *de novo* AML of 4% for trisomy 8; 1% each for trisomy 13 and trisomy 11, and 0.4% for trisomy 21. [4, 12]

It is also very important to continue looking for other abnormalities that might be relevant to the diagnosis, treatment and follow-up of leukemia confirming the relevance of cytogenetic analysis in acute leukemia.

Cytogenetic analyses are used not only in diagnosis. According to Slovak et al., karyotypic analysis predicts outcome of pre-remission and post-remission therapy in adult acute myeloid leukemia [18].

We included our patients in three dif-

ferent cytogenetic risk groups (favorable, intermediate and unfavorable) according to the hierarchical system of karyotype classification of Medical Research Council (MRC) AML 10 Trial. According to this classification, most of our patients had an intermediate risk.

According to published criteria adopted by SWOG (Southwest Oncology Group), four cytogenetic categories were defined [17]. The favorable risk category included patients with abnormalities inv(16)/ t(16;16)/ del(16q), or t(15;17) with any additional abnormalities, or t(8;21) either without del(9q) or being part of a complex karyotype. The intermediate risk category included patients characterized by +8, -Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of inv(3q)/ t(3;3), -5/del(5q), -7/del(7q), del(9q), t(6;9), t(9;22) and complex aberrant karyotype defined as 3 or more abnormalities. The unknown risk category included all other abnormalities. All SWOG karyotypes of unknown prognostic significance are designated as intermediate risk by MRC. Other major differences between these 2 systems are: the classification of 11q abnormality as intermediate risk by MRC but unfavorable risk group by SWOG and the classification by MRC of all t(8;21) studies as favorable, despite the presence of del(9q) or complex karyotypes. According to SWOG risk category definitions, most of our patients had an unknown risk.

During the last 30 years, cytogenetic analyses of patients with AML have discovered a great number of recurrent chromosome abnormalities. Several of the more common abnormalities have been associated with specific laboratory and clinical characteristics, and are used as diagnostic and prognostic markers. However, the prognostic importance of less frequent recurrent aberrations, both primary and secondary, is still unknown.

Because cytogenetic findings are among the most important prognostic factors, cytogenetic analysis of bone marrow is now mandatory in the diagnostic workup of newly diagnosed patients with AML.

The genetic alterations in AML include chromosome abnormalities detectable by cytogenetic analyses i.e. translocations and numerical abnormalities, as well as subtle gene alterations that are identified by molecular techniques such as small duplications/insertions and point mutations. Recent molecular analyses of leukemic blasts from patients with AML and a normal karyotype, have revealed a striking heterogeneity with regard to the presence of acquired gene mutations and changes in gene expression. Multiple submicroscopic genetic alterations have been discovered, including internal tandem duplication of the FLT3 (Fms-related tyrosine kinase3) gene, mutations in the NPM1 (Nucleophosmin - nucleolar phosphoprotein B23, numatrin) gene, partial tandem duplication of the MLL (Myeloid/lymphoid or mixed-lineage leukemia) gene, high expression of the BAALC gene, and mutations in the CEBPA (CCAAT/ enhancer binding protein, alpha) gene [14].

According to the latest studies, FLT3 and NPM1 aberrations show apparently opposite prognostic significance, FLT3 mutations are correlated with poor outcome and *NPM1* mutations are associated with a more favorable response to therapy.

Other chromosomal aberrations, which have not yet been well defined at the gene level (e.g. numerical abnormalities such as –7, –5, +8, and others) are detectable by cytogenetic or fluorescence in situ hybridization (FISH) analysis only, and are equally important in the clinic because of their association with specific entities (e.g. therapy-related AML) and with unfavorable outcomes.

Based on the above considerations, modern genetic characterization of AML combines conventional karyotyping and molecular methods – FISH, reverse transcriptase polymerase chain reaction (RT-PCR), sequencing – with the aim of analyzing all major types of

clinically relevant alterations [10].

Application of gene-expression profiling has also identified a gene-expression signature that appears to separate cytogenetically normal AML patients into prognostic subgroups, although gene expression signature based classifiers predicting outcome for individual patients with greater accuracy are needed. These and similar future findings are likely to have a major impact on the clinical management of cytogenetically normal AML not only in terms of prognosis but also in the selection of appropriate treatment, since many of the identified genetic alterations already constitute or will probably become targets for specific therapeutic intervention [14]. Because of the different results concerning the most frequent clonal karyotype in AML (hyperdiploidy) obtained in our study we will continue this study and we will look for other abnormalities.

Conclusion

The cytogenetic investigation in AML is important because it enabled us to include our patients in different cytogenetic risk groups.

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