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Classic and molecular cytogenetic findings in leukemia patients from the Western part of Romania

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in childhood and rare in adults, while acute myeloid leukemia (AML) is less common in children and more common in older adults. The aim of the study was to present our experience for the diagnostic of leukemia by using the classic and molecular cytogenetic methods. The study was conducted between 2009 and 2019 within the Classic and Molecular Genetic Laboratory of the Oncohematology Department from the Louis Ţurcanu Emergency Hospital for Children, Timişoara, Romania. The study group included 337 children and adults, evaluated between 2009 and 2019. By using the conventional and molecular cytogenetic technique, the cytogenetic anomalies found were 35 numerical chromosomal abnormalities, 10 (9;22)(q34;q11) [four ALL, one AML, five chronic myeloid leukemia (CML)] translocations, nine (15;17)(q24;q21) translocations, three (14;14)(q11;q32) translocations, two (4;11)(q21;q23) translocations, one (1;14)(p32;q11) translocation, one (7;14)(qter;q11) translocation, one (8;21)(q22;q22) translocation, one (9;14)(p12;q32) translocation, seven rearrangements of the *MLL* gene and two rearrangements of the core-binding factor subunit beta/myosin heavy chain 11 (*CBFB/MYH11*) gene. The use of conventional and molecular cytogenetic analysis is one of the most important prognostic indicators in acute leukemia patients, allowing the identification of biologically distinct subtypes of disease and selection of appropriate treatment approaches.

Keywords: leukemia, conventional cytogenetics, molecular cytogenetics.

Introduction

Leukemia is a malignant disease, the site of its occurrence and further development is the hematopoietic cell from the bone marrow (BM). Nowadays, they are widely regarded as malignant diseases of the hematopoietic tissue, their source being an abnormal stem cell. Leukemias are defined as a group of malignant disorders of the blood and BM that may present at all ages, with different forms having different age distributions [1]. Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in childhood and rare in adults, while acute myeloid leukemia (AML) is less common in children and more common in older adults. Chronic myeloid leukemia (CML) is very rare in young children, and chronic lymphocytic leukemia (CLL) is the most common form of leukemia in adults having >40 years, with a median age at diagnosis of over 70 years [1].

Acute leukemia is a clonal disturbance due to malignant transformation of a myeloid or lymphoid progenitor cell, which allows the classification of leukemia in ALL and AML. ALL and AML are characterized by a variety of numerical and structural chromosome aberrations.

Within leukemia, the reciprocal translocation, which involves an exchange of genetic material between the non-homologous chromosomes, is the most frequent translocation encountered. In AML, usually is present at least one of a variety of recurrent chromosomal abnormalities [2]. Chromosomal abnormalities in neoplastic BM cells often correlate closely with specific clinical and biological characteristics of the disease and serve as a tool to predict the clinical outcome and develop effective therapeutic approaches [3, 4].

For both types of acute leukemia, ALL and AML, there were described genetic variants associated with favorable, intermediate and poor prognostic [5]. For AML, the cytogenetic markers of a good prognostic are (15;17)(q24;q21), *inv*(16) translocation, (16;16) and (8;21) translocations, while for ALL patients, (12;21) translocation and hyperdiploidy are associated with better prognosis. The poor prognostic markers in AML are monosomy 5, 7, 17, deletion of *q* arm of chromosomes 5 and 7, deletion of *p* arm of chromosome 17, (3;3), (6;9), (v;11)(v;q23) translocation; other monosomies, and complex rearrangements, while in ALL poor prognostic markers are (4;11)(q21;q23) and (9;22)(q34;q11) translocations [5].

Aim

The aim of the study was to present our experience in diagnostic of leukemia by using the classic and molecular cytogenetic methods.

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Materials and Methods

Between 2009 and 2019, in the Classic and Molecular Genetic Laboratory of the Oncohematology Department from the Louis Ţurcanu Emergency Hospital for Children, Timişoara, Romania, cytogenetic and molecular analyses were carried out for 202 patients with suspicion of ALL, 130 patients with clinical suspicion of AML and five patients with suspicion of CML.

For specimen collection, 1–2 mL of BM were aspirated aseptically into a syringe coated with preservative-free sodium heparin and transferred to a sterile 15 mL centrifuge tube containing 5 mL culture medium (RPMI-1640, 100 units sodium heparin). The metaphase spreads were obtained from the BM using indirect method, implying cellular cultures with the length of 24, 48, 72 hours. For blood specimens, 5 mL are drawn aseptically by venipuncture into a syringe coated with preservative-free sodium heparin.

To prepare metaphase spreads, the sample were exposed sequentially to mitotic inhibitors to accumulate cells in mitosis, hypotonic potassium chloride (0.075M KCl) to swell the cells, and fixative (absolute methanol–glacial acetic acid; 3:1, *v/v*). G banding was used for chromosome banding, in brief cells were immersed in trypsin solution (0.1–0.25%), followed by staining in phosphate-buffered Giemsa stain.

Fluorescence *in situ* hybridization (FISH) was used for diagnostic confirmation and was performed by using the MetaSystems Probes kit. Depending on the type of leukemia and the results of the conventional cytogenetic technique results, we used one of the following locus-specific probes: XL *BCR/ABL* dual fusion, XL *AML1/ETO* dual fusion, XL promyelocytic leukemia/retinoic acid receptor alpha (*PML/RARA*) dual fusion, XL core-binding factor subunit beta/myosin heavy chain 11 (*CBFB/MYH11*) dual fusion, XL *MLL* break apart, XL immunoglobulin heavy locus (*IGH*) break apart (Metasystem).

Slides were immersed in 2X SSC (saline–sodium citrate) buffer solution for 30 minutes at 37ºC and then dehydrated in 70% and 100% ethanol, two minutes each. Slides were denatured at 73ºC (in a pre-warmed water bath) for two minutes and rinsed in ice-cold series of 70%–80%–100% alcohol, two minutes each. Slides were put on a slide warmer (40ºC) and 10 µL probe was applied. After overnight hybridization at 37ºC, slides were washed in 0.4X SSC solution at 73ºC for two minutes and rinsed in 2X SSC solution at room temperature. For each case, a minimum of 300 cells were evaluated. The slides were analyzed through a fluorescent microscope. The best images were captured using the camera mounted on the microscope attached to a computer that was equipped with karyotyping and FISH software.

Results

By using the conventional and molecular cytogenetic technique, the following cytogenetic anomalies found were 35 numerical chromosomal abnormalities, 10 (9;22)(q34;q11) translocations (four ALL, one AML, five CML), nine (15;17)(q24;q21) translocations, three (14;14)(q11;q32) translocations, two (4;11)(q21;q23) translocations, one (1;14)(p32;q11) translocation, one (7;14)(qter;q11) translocation, one (8;21)(q22;q22) translocation, one (9;14)(p12;q32) translocation, seven rearrangements of the *MLL* gene and two rearrangements of the *CBFB/MYH11* gene. The ages of the patients for which a cytogenetic anomaly was found varied between two weeks and 70 years old. The highest frequency was for the patients aged one to 4.99 years old (18.46%) and 15 to 19.99 years old (18.46%), respectively, 70.77% of the patients being under 20 years old. The age distribution for the patients where cytogenetic anomalies were found is presented in Figure 1.

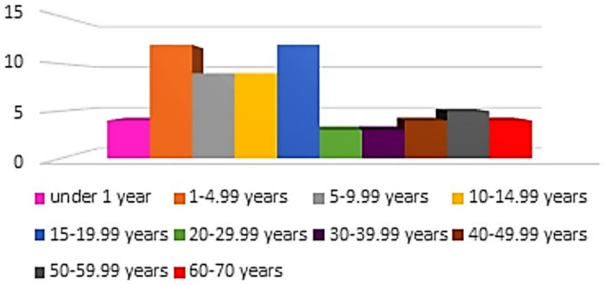

Figure 1 – ***Age distribution for patients exhibiting chromosomal anomalies.***

FISH interphase and metaphase analysis gave us a better estimation of the percent of the cells carrying hybrid genes resulting from translocation with further role in leukemogenesis.

The Philadelphia (Ph) chromosome (Figure 2, A and B), resulting from the reciprocal t(9;22)(q34;q11) translocation is usually found in CML. In the study group, the Ph chromosome was found in five cases of CML, four cases of ALL and one case of AML. Regarding the age of debut, the majority of the patients (six cases) were under 20 years old (aged between nine to 17 years old), two patients were 32 years old and the last two cases were 64 and 65 years old, respectively.

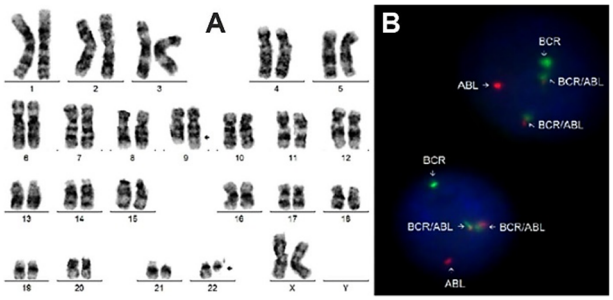

Figure 2 – ***(A) (9;22)(q34;q11) translocation; (B) FISH analysis: BCR/ABL fusion gene. FISH: Fluorescence in situ hybridization.***

Concerning the t(15;17)(q24;q21) translocation (Figure 3, A and B), it is the specific anomaly in acute promyelocytic leukemia (M3 subtype AML). In the present study, *PML/RARA* translocation was found in nine cases of M3 subtype AML, in eight cases the anomaly was present in all the analyzed cells, while in one case was found in 80% of the analyzed cells. Eight patients were under 20 years old (aged between eight to 19 years old), and one patient was 42 years old at the moment of diagnosis. In one case, a patient 18 years of age, the evolution of the diseases was lethal in less than one year from the moment of diagnosis.

Another translocation emphasized was t(4;11)(q21;q23) (Figure 4, A and B), which is specific in ALL with B precursor. The aforementioned translocation was encountered in one patient by using interphase FISH technique, while in the other patient, the translocation was diagnosed by

using both classic cytogenetic and FISH technique, the anomaly being present in all cells analyzed. The youngest patient was three months old, while the second one was 15 years old. Additionally, rearrangements involving *MLL*, located on chromosome 11q23, were found in seven cases in mosaic state, in five ALL patients and two AML (M5 subtype) patients. The patients diagnosed with ALL were aged between two and 43 years old (two cases were two years old, one case five years old, one case 12 years old, one case 43 years old). The patients diagnosed with AML were one case 50 years old and one case 68 years old. Other structural anomaly involving chromosome 11 was 11q23 deletion without rearrangement of the *MLL* gene.

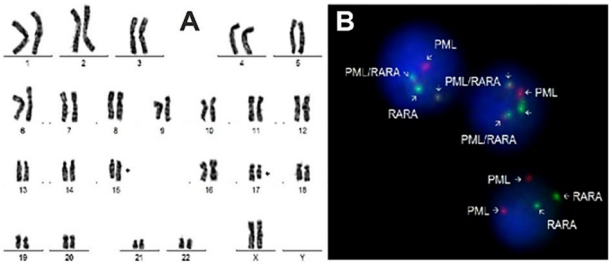

Figure 3 – ***(A) (15;17)(q24;q21) translocation; (B) FISH analysis: PML/RARA fusion gene. FISH: Fluorescence in situ hybridization; PML: Promyelocytic leukemia; RARA: Retinoic acid receptor alpha.***

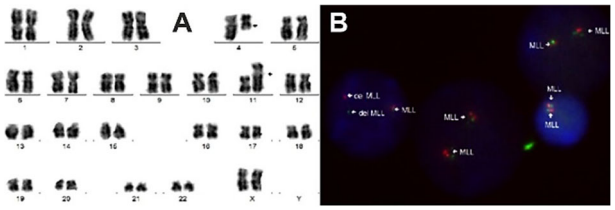

Figure 4 – ***(A) (4;11)(q21;q23) translocation; (B) FISH analysis: rearrangements involving MLL gene. FISH: Fluorescence in situ hybridization.***

The t(8;21)(q22;q22) translocation (*AML1/ETO*) (Figure 5, A and B) is the characteristic anomaly in M2 subtype AML, where myeloblasts with maturation features are present. The *AML1/ETO* was found in the case of a 3-year-old child diagnosed with M2 subtype AML.

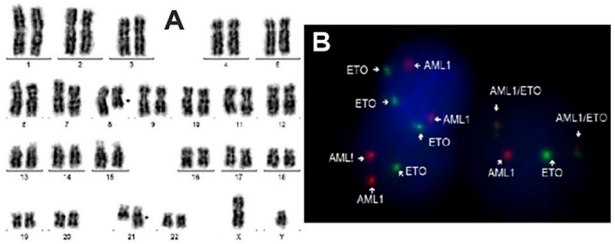

Figure 5 – ***(A) (8;21)(q22;q22) translocation; (B) FISH analysis: AML1/ETO fusion gene. AML1: Acute myeloid leukemia 1; FISH: Fluorescence in situ hybridization.***

Within our study, t(14;14)(q11;q32) translocation (Figure 6, A and B) was found in three cases of ALL. In the first case, a child seven years of age diagnosed with T-ALL, the t(14;14)(q11;q32) translocation was present as a single anomaly in mosaic state. In the second case, a 4-year-old child diagnosed with T-ALL, t(14;14)(q11;q32) translocation was found in a mosaic karyotype with a cell line exhibiting an extra r(14). The third case, a 2-year-old child diagnosed with B-ALL, presented the above mentioned translocation as part of a hyperdiploid karyotype.

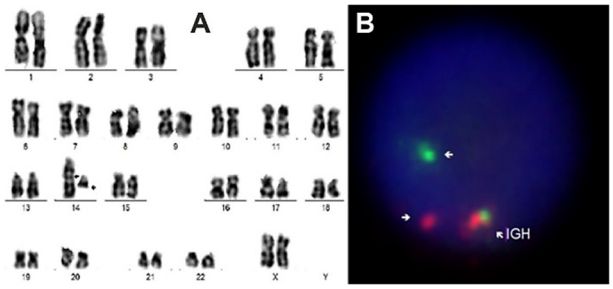

Figure 6 – ***(A) (14;14)(q11;q32) translocation; (B) FISH analysis: IGH gene rearrangement. IGH: Immunoglobulin heavy locus; FISH: Fluorescence in situ hybridization.***

Additional structural anomalies found were: one case with isochromosome (17q) present in all analyzed cells, one case of 16(p13;q21) inversion (Figure 7, A and B), one case of t(1;14)(p32;q11) translocation, one case of t(9;14)(p12;q32) translocation and one case of t(7;14)(qter;q11) translocation, all those anomalies being in mosaic state.

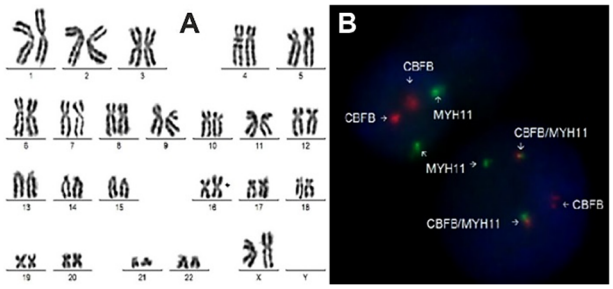

Figure 7 – ***(A) 16(p13;q21) inversion; (B) FISH analysis: CBFB/MYH11 rearrangement. CBFB: Core-binding factor subunit beta; FISH: Fluorescence in situ hybridization; MYH11: Myosin heavy chain 11.***

Numerical chromosomal abnormalities, as single anomaly or associated with structural rearrangements were found in 35 cases: 20 cases of AML, 14 cases of ALL, and one case of CML.

In AML patients, 15 cases presented hypodiploidy, only five patients having hyperdiploidy (Figure 8, A and B). Two cases of AML associated complete trisomy 21, one case presented a mosaic karyotype with two cell lines with trisomy 22 and tetrasomy 22, the fourth case exhibit mosaic trisomy 8 associated with a cell line presenting t(15;17) and the last case exhibit mosaic trisomy 9. In AML, the hypodiploidy found involved chromosomes 3, 5, 7, 8, 93, 10, 11, 12, 16, 17, 18, 19, 20, 22 and X. The most frequent monosomies were those of chromosomes 5 and 7, being present at 30.3% of the cases with ALL for which a cytogenetic aberration was found. One case presented monosomy 5 in mosaic state as single chromosomal aberration, while another case presented monosomy 7 in mosaic state as single chromosomal aberration. Regarding numerical abnormalities, all cases of ALL presented hyperdiploidy (involving chromosomes 4, 6, 8, 9, 11, 13, 14, 17, 19, 20, 21 and 22), in complete or mosaic state, three cases were also associating structural anomalies. In four cases of ALL, hyperdiploidy with over 50 chromosomes was found, three cases with homogenous karyotype [52,XX, +8,+9,+13,+19,+20,+22; 51,XY,+6,+19,+21,+21,+i(17q); 57,XY,+4,+6,+8,+9,+11,+13,+14,+17,+19,+21,+21] and one case in mosaic state (51,XX,+4,+17,+19,+21,+11/46, XX).

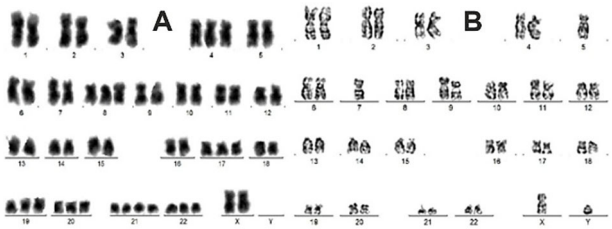

Figure 8 – ***(A) Karyotype exhibiting hyperdiploidy; (B) Karyotype exhibiting monosomy 5 and 7.***

Discussions

We pointed out the genetic anomalies found after using the following methods, classic cytogenetic and interphase and metaphase FISH technique with locus genetic probes.

Ph chromosome is the one usually found in CML but it could be also encountered in the ALL and AML [6–8]. The fusion gene with a key role in leukemogenesis is *BCR/ABL*. The election diagnostic method for identifying the presence and the percentage of Ph+ cells is FISH technique with locus genetic probes. In the present study, *BCR/ABL* fusion gene was found in 10 patients: five cases of CML, four cases of ALL and one case of AML.

Concerning the t(15;17)(q24;q21) translocation (Figure 3, A and B), it is the specific anomaly in M3 subtype AML, reported in 95% of cases diagnosed with this AML subtype [9]. In this AML subtype, it was found that the leukemogenesis is due to the *PML/RARA* fusion gene and that the prognostic of the patient is better owing to the treatment with retinoic acid [10]. In the present study, *PML/RARA* translocation was found in nine cases of M3 subtype AML, in eight cases the anomaly was present in all the analyzed cells, while in one case was found in 80% of the analyzed cells.

The t(4;11)(q21;q23) is one of the recurrent translocations being prevalent in lymphoblastic leukemia in adults and infants/children [11]. By the fusion of the two loci at 11q23 and 4q21 on the derivative chromosome 11, the translocation will lead to formation of the MLL–AF4 chimeric protein [11, 12]. Other structural anomaly involving chromosome 11 was 11q23 deletion without rearrangement of the *MLL* gene, associated with a favorable evolution [13].

The t(8;21)(q22;q22) translocation (Figure 5, A and B) is the characteristic anomaly in M2 subtype AML, where myeloblasts with maturation features are present. About 15% of adults with AML carry the t(8;21)(q22;q22) chromosomal translocation which lead to formation of *AML1/ETO* hybrid gene with role in leukemogenesis [14]. In acute leukemia, the description of the genetic lesions can vary between countries, for example it is reported a high incidence of t(8;21) in the Japanese population (13%) and African–Americans (17%) [15].

The t(14;14)(q11;q32) translocation is a rare rearrangement and it is mostly present in ALL with B or T precursors involving of the *IGH* gene at 14q32 [16]. Within our study, t(14;14)(q11;q32) translocation was found as a single anomaly (T-ALL) in one case, in another case of T-ALL was found in a mosaic state with a cell line exhibiting an extra r(14) and in the third case as part of a hyperdiploid karyotype (B-ALL).

Regarding the numerical anomalies found in AML patients, monosomy 5 and monosomy 7 are considered most prognostically relevant, being associated with a poor prognostic [17].

For the study group, in patients diagnosed with AML, monosomy 5 and monosomy 7 had a similar frequency. Monosomy 7 is frequent in pediatric myelodysplasic syndromes [17].

The poor outcome of monosomy 7 is considered to be consecutive to a higher risk of resistance to therapy (71–83% chemoresistance) [18].

Monosomy 5 and monosomy 7 are frequently associated with other chromosomal anomalies, as we also found in our study group. Only two cases presented the above-mentioned anomalies as single aberration both in mosaic state.

Structural and numerical anomalies of chromosome 21 are common in different types of hematological malignancies [19]. For the study group, trisomy 21 was found in two cases, the youngest patients that were diagnosed with AML (two weeks old and one month old, respectively). For AML cases, trisomy 8 is one of the most frequent chromosomal, either as the sole anomaly or associated with another aberration [20]. In our study, trisomy 8 was found in one case in mosaic state with a cell line exhibiting t(15;17) [karyotype 46,XX, t(15;17)(q24;q21)/47,t(15;17)(q24;q21),+8].

Complex karyotypes and as well as monosomal karyotypes are well known as an indicator of a poor outcome in adult AML but there is no consensus yet in pediatric cases of AML [17].

Hyperdiploidy is frequently encountered in ALL, the one characterized by more than 50 chromosomes leading to a favorable evolution, while hyperdiploidy with less than 50 chromosomes denotes a poor prognosis.

Complete trisomy frequently encountered in ALL are 4, 6, 8,10, 14, 17, 18, 20, 21. Trisomy 4, 6, 10 and 21 is a marker for a good prognostic along with a good survivor rate [16]. Trisomy 6 and 21 indicate a favorable prognosis. Even if trisomy 8 is a characteristic of the myeloid disorders, in our study it was also encountered in ALL.

The use of conventional and molecular cytogenetic analysis is one of the most important prognostic indicators in acute leukemia patients, allowing the identification of biologically distinct subtypes of disease and selection of appropriate treatment approaches [17].

In this study, we report the cytogenetic results from 337 Romanian patients referred with suspicion of leukemia. In our study, for AML patients, the most common structural anomaly was the t(15;17)(q24;q21) translocation (30.3% of the anomalies detected). A high incidence of the same translocation was reported for Spanish population [21]. In ALL patients, even if not a very characteristic aberration, we found that t(9;22)(q34;q11) translocation was the most common structural anomaly (14.81% of the anomalies detected), while the complex karyotype associating hyperdiploidy were more frequent (66% of the anomalies detected).

Conventional and molecular cytogenetic evaluation play an important role in the diagnosis and prediction of prognosis hematological malignancies. Lack of cytogenetic information, in case of karyotype failure or the lack of cytogenetic testing could impair the outcome of the adult patients as they could not receive a risk-adapted therapy [22].

More useful information for the patients could be

obtained by using new technologies such as new generation sequencing (NGS). It is expected that NGS might replace the cytogenetic testing in the future, as suggested by a recent cost/effect study [23].

A limitation of the present study is the numbers of cases, only for few cases with cytogenetic analysis were available data regarding the survival, as many patients were lost to follow-up during this study.

Conclusions

Nowadays, classic cytogenetics turns out to be still important providing useful information as regards the diagnostic, prognostic, and treatment of hematopoietic malignancies. Nevertheless, this technique is used usually in conjunction with FISH technique, which is performant, expensive, and laborious, therefore bringing efficiency in diagnostic and oncological treatment. The current knowledge regarding the use of cytogenetics in the management of leukemia justifies the integration of those analysis into a prognostic index applicable in risk-directed therapy decision-making for leukemia patients.

Conflict of interests

The authors declare that they have no conflict of interests.

Compliance with ethical standards

The authors declare that the procedures followed in this research conform to the ethical standards in accordance with the World Medical Association (WMA) and the Declaration of Helsinki. This research is exempt from approval by the Ethics Committee of our institution because it is a retrospective study.

Funding

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