RARE HEMATOLOGIC NEOPLASMS – AN ACUTE MEGAKARYOCYTIC LEUKEMIA CASE REPORT

SORINA MIHAELA CHIRIEAC[1*], AURORA ARGHIR[1], HORIA BUMBEA[2,3], ANA MARIA VLADAREANU[2,3], SANZIANA RADESI[3], ANDREEA CRISTINA TUTULAN-CUNITA[1], AGRIPINA LUNGEANU[1]

Keywords: Acute megakaryoblastic leukemia, complex cytogenetic abnormalities,
Abstract: Acute megakaryoblastic leukemia (AMKL) is a rare disorder in adults, particularly challenging due to difficulties in diagnosis, its complex genetic abnormalities and severe prognostic. A high percentage of AMKL cases bear cytogenetic abnormalities and, among them, 70-80% show complex karyotypes. We report on a 46 year-old male patient diagnosed with AMKL, after an initial evolution as acute lymphoblastic leukemia Burkitt-like. Highly complex chromosomal abnormalities were revealed by cytogenetic and molecular techniques (FISH), reflecting the underlying genetic instability. Our report emphasize the importance of both cellular and molecular approaches in onco-hematology, in order to accurately identify and refine genetic aberrations. As a consequence, better diagnosis and prognosis assessment is achieved, as well as understanding of pathogenic mechanisms.

INTRODUCTION

Acute megakaryoblastic leukemia (AMKL) is a rare form of acute myelogenous leukemia (AML), defined by the presence of ≥20% blasts, with ≥50% of them being of megakaryocyte lineage. It was first described by Van Boros and Korenyi in 1931 and, presently, is classified as M7 according to the FAB system, while the AMKL nomenclature is favored by the World Health Organization (WHO) classification.

It comprises approximately 7-10% of pediatric (Athale *et al*. 2001) and 5% of adult (Tallman *et al*. 2000) AML cases. Although the incidence of this type of AML is increased in children with Down's syndrome (Hama *et al*. 2008), it is a rare disorder in adults, instead, where it can arise *de novo* or, more rarely, as a secondary event post-chemotherapy or as a progress from myelodysplastic syndrome or chronic myelogenous leukemia (Pullarkat *et al.* 2008; Hino *et al*. 1992).

There is no specific association of any chromosomal aberration with adult AMKL; still, in 70-80% of cases, the karyotype presents 3 or more clonal anomalies. The most frequent aberrations reported in complex karyotypes are monosomy 5/deletion of 5q, monosomy 7/deletion 7q, trisomy 19, trisomy 21 and anomalies of 3q21/3q26.

The prognosis for patients with adult AMKL is severe. Although complete morphological remission rates are achieved in 30-50% in adult patients, the relapse rate is high.

Here, we describe cytogenetic and FISH characterization of complex karyotype abnormalities in a case of adult AMKL, after an initial evolution as Burkitt-like acute lymphoblastic leukemia (ALL).

MATERIALS AND METHODS

We present the case of a 46 year-old man referred for cytogenetic investigations following an AMKL diagnosis after an initial evolution of Burkitt-like ALL.

Peripheral blood and bone marrow aspirate smears were examined. Bone marrow biopsy was performed to assess the bone marrow cellularity and hematopoietic precursors. Immunophenotyping was performed on bone marrow aspirate collected in EDTA vacutainers (BD), using a four-colors BD FACS Calibur Flowcytometer. All hematopoietic lineages were analyzed, using several monoclonal antibodies: CD19, CD20, CD10, CD22, CD34, CD33, CD13, CD65, CD64, CD117, CD14, CD61, CD41, CD9, CD36, Glycophorin A, CD7, CD2, CD5, CD3, MPO, CD79a, TdT.

Karyotype analysis was performed on slides obtained from bone marrow cells cultured by standard protocols. GTG-banded metaphases were captured and analyzed using a motorized Imager Z1 Zeiss Microscope equipped with Zeiss CCD camera and Ikaros MetaSystem software. The results were interpreted according to the International System for Human Cytogenetic Nomenclature 2009 guidelines (Shaffer *et al.* 2009)

Fluorescence *in situ* hybridization (FISH) techniques were applied to elucidate and confirm complex anomalies observed by classical cytogenetics. Subtelomeric probes for chromosomes 7q and 17q (*Kreatech Diagnostics*), centromeric probe for chromosomes 7 (*Kreatech Diagnostics*) and painting probe for chromosome 7 (*Qbiogene*) were used for FISH studies. Sequential FISH on the same metaphases was used to elucidate the detected anomalies. Analysis was performed on the same microscope equipped with appropriate single band fluorescence filters and Isis MetaSystem software.

RESULTS AND DISCUSSIONS

Clinical findings in our patient were: relative good clinical status, asthenia, pale skin, no hemorrhagic syndrome, no adenopathies, no hepatosplenomegalia, two mild infectious episodes (diarrhea and pharyngitis). Hematological features consisted of mild anemia (Hb = 8.9 g/dl) and mild thrombocytopenia (89 x 10^9/L), peripheral blood blast count 1%, and other myeloid immature elements around 16%. Bone marrow aspiration was very difficult (2 assays) with smears showing normocellular bone marrow, with only 9% myeloblasts. Immunophenotyping revealed precursors with low expression of CD45 and SSC, and with coexpression of CD61 and CD41. The patient had an unfavorable evolution with short survival after AMKL diagnosis.

GTG-banding analysis of 30 metaphases revealed a complex hyperdiploid karyotype (47 chromosomes) with structural and numerical aberrations involving chromosomes 1, 5, 7, 17, 18, 19, and the presence of a marker chromosome (Figure 1A):

47,XY,t(1;18)(q32;p11.2),del(5)(q22q35),der(7)t(7;17)(q11.2;q11.2),-17,+19,+mar[27].

ish der(7)t(7;17)(wcp7+,D7Z1+,ST7q-,ST17q+)/46,XY[3]

Among the observed abnormalities, trisomy 19 was the single numerical anomaly of an entire chromosome. Alvarez S *et al.* (2001) reported total and partial trisomy 19 (19q14) as the most common abnormality detected by CGH in megakaryoblastic leukemias, with a frequency of 33.3% in patient samples and 81% in megakaryoblastic cell lines. Thus, it is likely that trisomy 19 plays a pathogenetic role in AMKL. Still, the pathogenetic impact of +19 remains elusive, possible mechanisms including the gene dosage effect generated by the trisomy, or duplication of rearranged or mutated genes present in the extra chromosome 19.

Partial deletion of chromosome 5, del(5)(q22q35) was observed by conventional banding, this anomaly being frequently reported in adult AMKL **(**Dastugue *et al.* 2002)**.** Hemizygous deletions of tumor-suppressor genes at 5q, generating haploinsufficiency of these genes, is thought to be the underlying mechanism in del(5q) associated myeloid neoplasms.

Two novel translocations, reciprocal t(1;18)(q32;p11.2) and unbalanced t(7;17)(q11.2;q11.2) were identified by karyotype and FISH studies. Although a balanced translocation t(1;18) with the aforementioned breakpoints it was not described in literature, the 1q32 and 18p11 breakpoints were reported to be involved in unbalanced and balanced abnormalities in Down syndrome–ALL (Forestier *et al*. 2008) and AML. (Bakshi *et al*. 2004). Painting, centomeric and subtelomeric FISH demonstrated that the translocation t(7;17)(q11.2;q11.2) is unbalaced, with only one derivative chromosome present, der(7)t(7;17)(q11.2;q11.2). Painting and centromeric FISH for chromosome 7 (Figure 1B, C) and subtelomeric FISH for 17q region exhibited fluorescence signals on der(7)t(7;17) confirming the cytogenetic findings. Partial or total deletions of chromosome 7 are frequently observed in myeloid neoplasm, especially in AML. Similarly to 5q deletions, haploinsuficiency of genes located in several distinct regions on 7q is supposed to be responsible for the myeloid hematopoiesis alterations.

Additionally, a marker chromosome similar in size with group C was observed by GTG-banding, and could be not elucidated by FISH studies.

CONCLUSIONS

AMKL is a rare leukemia with a very poor prognosis, thus our results add new genetic data to a relatively scarce body of evidence concerning this disease. The results of genetic analyses contribute to accurate diagnosis and are extremely important in view of prognostic implications.

Our results demonstrate a common feature of AMKL, its genetic instability. Thus, both previously reported cytogenetic anomalies, involving chromosomes 5, 7, 19, as well as new structural aberrations, t(7;17) and t(1;18), were identified in our patient. Overall, the prognostic was poor. The patient evolution was unfavorable, with short survival after AMKL diagnosis.

REFERENCES

Alvarez S., MacGrogan D., Calasanz M.J., Nimer S.D., Jhanwar S.C., (2001): *Frequent gain of chromosome 19 in megakaryoblastic leukemias detected by comparative genomic hybridization.* Genes, chromosomes & cancer, 32 (3), 285-293.

Athale U.H., Razzouk B.I., Raimondi S.C., Tong X. *et al.,* (2001): *Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience*. Blood, 97(12), 3727–3732.

Bakshi S., Roy S., Trivedi P., Brahmbhatt M. *et al,.* (2004): *AML-M2 with der(18)t(1;18)(q2?;p11.3) in addition to t(8;21) and del(9q).* Indian Journal of Human Genetics, 10 (2), 78-80.

Dastugue N., Lafage-Pochitaloff M., Pagès M.P., **Radford I. *et al,.*** (2002): *Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): a study of the Groupe Français de Cytogénétique Hématologique (GFCH).* Blood, 100(2), 618-626.

Forestier E., Izraeli S., Beverloo B., Haas O. et al., (2008): *Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study.* Blood, 111(3), 1575-1583.

Hama A., Yagasaki H., Takahashi Y., Nishio N. *et al.,* (2008): *Acute megakaryoblastic leukemia in children: a comparison of AMKL with and without Down's syndrome.* British Journal of Haematology, 140(5), 552–561.

Hino K., Sato S., Sakashita A., Tomoyasu S. *et al.,* (1992): *Megakaryoblastic transformation associated with disseminated intravascular coagulation in the course of polycythemia vera: a case report.* Rinsho Ketsueki, 33(4), 500–506.

Pullarkat S.T., Vardiman J.W., Slovak M.L., Rao D.S. *et al.,* (2008): *Megakaryocytic blast crisis as a presenting manifestation of chronic myeloid leukemia.* Leukemia Research, 32(11), 1770–1775.

Shaffer L., Slovak M., Campbell L, (2009): An International System for Human Cytogenetic Nomenclature 2009, Karger.

Tallman M.S., Neuberg D., Bennett J. M., Francois CJ *et al*., (2000): *Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience*. Blood, 96(7), 2405–2411.

Institutional affiliation
1. “Victor Babes” National Institute of Pathology, Laboratory of Medical Genetics, Bucharest, Romania
2. “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
3. Universitary Emergency Hospital, Bucharest, Romania

Corresponding address
“Victor Babes” National Institute of Pathology, Laboratory of Medical Genetics, 99-101 Splaiul Independentei, Bucharest, Romania, Tel.: +4021.319.2732/218, Email: sorina.chirieac@ivb.ro

Date of manuscript submission
26/08/2010