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Case report

Molecular characterization of complex chromosomal changes in *de novo* acute myeloid leukemia: a case report

Caracterizarea moleculară a modificărilor cromozomiale complexe în leucemia mieloidă acută *de novo*: prezentare de caz

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Abstract

De novo acute myeloid leukemias (AML) represent a heterogeneous group of clonal hematopoietic disorders in which chromosomal abnormalities are detected in a majority of patients. At present, cytogenetic changes are recognized as important diagnostic markers and prognosis determinants. Complex karyotype changes are associated with resistance to treatment and unfavorable evolution. We report on an AML case with complex karyotype changes characterized by molecular genetic techniques (fluorescence in situ hybridization - FISH and array-based comparative genomic hybridization – array-CGH) and an extremely poor outcome. A 72 year-old female patient was admitted for genetic investigations with a clinical diagnosis of AML. Classical and molecular cytogenetic tests as well as array-CGH were performed. Complex chromosomal abnormalities were identified at diagnosis, consisting of genomic imbalances involving chromosomes 6, 7, 9, and 17. AML with complex karyotype changes is a heterogeneous disease, as a variety of genomic abnormalities are detected, involving virtually all chromosomes. The pathogenesis of AML with complex karyotype is poorly understood. The complexity of karyotypic changes in our case highlights the importance of using complementary genetic investigation in order to obtain a comprehensive view of AML genome.

Keywords : *acute myeloid leukemia, array-based comparative genomic hybridization, complex karyotype*

Rezumat

Leucemiile mieloide acute de novo (LAM) reprezintă un grup heterogen de afecţiuni hematopoietice clonale, majoritatea pacienţilor prezentând anomalii cromozomiale. Modificările citogenetice sunt considerate în momentul actual factori importanţi de diagnostic şi prognostic. Modificările complexe ale cariotipului sunt asociate cu rezistenţa la tratament şi evoluţie nefavorabilă. Raportăm cazul unei paciente cu AML şi modificări complexe de cariotip identificate prin tehnici citogenetice şi moleculare (hibridizare in situ fluorescentă – FISH

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şi hibridizare comparativă genomică bazată pe microarray – array-CGH) la care evoluţia a fost extrem de nefavorabilă. Pacientei, în vârstă de 72 de ani, i-au fost recomandate investigaţii citogenetice în contextul unui diagnostic clinic şi hematologic de LAM. Au fost efectuate teste citogenetice şi moleculare, incluzând array-CGH. Au fost identificate modificări cromozomiale complexe reprezentate de dezechilibre genomice implicând cromozomii 6, 7, 9 şi 17. Pacienţii cu LAM cu modificări complexe de cariotip reprezintă un grup heterogen, la care sunt detectate numeroase anomalii genomice ce pot afecta practic orice cromozom. Patogeneza LAM cu cariotip complex este incomplet înţeleasă. Complexitatea modificărilor genetice la pacienta noastră subliniază importanţa utilizării de investigaţii genetice complementare pentru a obţine o imagine cât mai comprehensivă a anomaliilor genomice la pacienţii cu LAM.

Cuvinte cheie: *leucemie mieloidă acută, hibridizare comparativă genomică bazată pe microarray, modificări cromozomiale complexe.*

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Introduction

Acute myeloid leukemia (AML) is a clonal disorder originating in the hematopoietic progenitor cells, characterized by an accumulation of leukemic blasts and maturation arrest. AML is a heterogeneous group of disorders in terms of acquired genetic changes and clinical features (e.g. response to chemotherapy and outcome). Genetic and molecular biology research have had major contribution in deciphering the events underlying the pathogenesis of AML. A better understanding of disease biology has led to the identification of prognostic determinants in AML (1, 2). The pretreatment karyotype remains the strongest established factor with predictive value for the response to induction treatment and survival for AML patients (3, 4). The technological progresses in the field, mainly the advances of genome-wide high-throughput methods, led to the identification of new clinically significant genetic markers, such as gene mutations or gene expression anomalies in AML (5, 6, 7). Thus, while the complete picture of AML genetic architecture is still to be unraveled, genetic testing became a gold standard for AML diagnostic investigation and genetic data have been included in the World Health Organization classification of AML (8).

Fifty to seventy percent of acute myeloid leukemia (AML) patients have cytogenetic abnormalities and 10-15% of adult AML show complex karyotypes (1, 3, 9), defined as at least three abnormalities or presence of a clone with more than three chromosomal anomalies (1, 10). Complex chromosomal changes consist mainly in genomic losses or gains, and are often difficult to dissect by conventional cytogenetics only. The most frequently detected genome imbalances are: monosomies of chromosomes 5, 7, and 17p, partial deletions of 5q, 7q, and 17p, trisomies of chromosomes 8, 21 and partial duplications of 8q, 21q, 11q, and 22q (11).

Besides karyotype, another important predictive factor with respect to survival in AML proved to be the age at presentation (12). There is a higher association of unfavorable chromosomal changes, especially complex karyotype changes, with increased age (10, 12). Whereas significant progress has been made in the treatment of younger adults, the prospects for elderly patients have remained dismal, with median survival time of only few months. This difference is related to comorbidities associated with ageing and to disease biology (13).

We report an AML case with complex karyotype changes identified by classical and molecular genetic techniques; array-based comparative genomic hybridization (array-CGH) played an important role in the accurate description of chromosomal anomalies in our patient.

Materials and methods

The patient, a 72 year-old female, was hospitalized with signs of respiratory infection. Biochemical, hematologic (complete blood

count test - CBC, bone marrow morphological examination) and immunophenotypic tests were performed at diagnosis.

Karyotype examination was done on GTG-banded bone marrow metaphases obtained from direct preparations and short term cultures (24 and 48 hours). FISH with painting probes (chromosome 7 and 9 – Kreatech Diagnostics, The Netherlands; Chromoprobe 1,2,4 & 3,5,6 – Cytocell, UK), locus specific probes (TP53 and BCR/ABL dual color, dual fusion translocation probes – Vysis, Abbott Molecular Diagnostics, USA; subtelomeric 17pter Kreatech Diagnostics) and BAC FISH probes (RP11-525O11 - 17p11.2, RP11-129E3 - 17q24) were applied for molecular characterization. Cytogenetic and FISH analyses were performed on a motorized Axio Imager Z1 Zeiss Microscope (Carl Zeiss, Germany) equipped with CCD camera, appropriate filters and dedicated software (Ikaros and Isis, MetaSystems, Germany). Bone marrow fixed cytogenetic cell suspension (methanol/glacial acetic acid), stored at -20 °C, was used for genomic DNA isolation (Wizard Genomic DNA purification kit, Promega, USA). The DNA was used for array-based comparative genomic hybridization – array-CGH on an Agilent Human genome CGH 4x44K platform (Agilent Technologies, USA, hg 18), following the manufacturer's recommendations. Data were extracted using Agilent Feature Extraction software v.10.1.1.1 and analyzed using Genomic Workbench v 6.5.0.18 software (Agilent Technologies).

Informed consent for research and data publication was obtained from the patient according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

Results

A 72 year-old female patient without previous history of hematologic disorders, environmental and drug exposure was admitted to hospital for right basal pneumonia. On physical examination she presented pallor, purpura, a performance status of 3; no hepatosplenomegaly was noted at presentation.

CBC showed severe anemia (6.2 g/dL), leukocytosis (WBC = 22.7 x 10^9/L) with 38% blast cells, thrombocytopenia (PLT = 10 x 10^9/L). The bone marrow examination revealed hypercellularity, 30% blast cells, and no signs of dysplasia. Flow cytometric analysis showed a population of blasts with strong reactivity with MPO, HLA DR, CD13, CD33 , CD117, moderate expression of CD45, and reduced expression of SS; cytoplasmic CD79A, CD3 and CD22, as well as CD14, CD11b, CD20, CD16, CD64 were all negative.

The clinical, morphological and immunological features were consistent with AML M_1 FAB type.

Radiological features confirmed the diagnosis of right basal pneumonia. Biochemical tests showed an elevated lactate dehydrogenase serum level.

Bone marrow chromosomal analysis showed a hypodiploid karyotype (45 chromosomes) with monosomy 6 and der(17), due to an unbalanced translocation t(6;17)(p12;p11.2) in all the analyzed cells (*Figure 1A*). In addition, a structural anomaly of chromosome 9 was observed (partial deletions of both short and long arms). Conventional karyotyping, as sole test, could not accurately describe the complex chromosomal changes in our case, thus molecular techniques were applied. FISH studies allowed a better description of the visible cytogenetic changes, as well as the discovery of a new cryptic anomaly. Thus, both homologous chromosomes 17 proved to be involved in different abnormalities: an unbalanced t(6;17)(p12;p11.2) affecting one chromosome, generating der(17)t(6;17)(p12;p11.2), and an interstitial deletion, not encompassing TP53, affecting the homologous chromosome 17, del(17)(p11.2p13.1) (*Figure 1B and C*). The derivative chromosome 9 was generated through partial deletions of p and q arms, der(9)del(9)(p2?)del(9)(q22q32). While painting and locus specific FISH confirmed the terminal 9p deletion and interstitial 9q deletion in 18% of

the cells, cryptic BCR/ABL1 fusion was not observed in any of the analyzed cells.

Oligonucleotide array-CGH was used for further refinement of the genomic imbalances. The DNA extracted from the fixed cytogenetic preparation was of good quality; quality control metrics for the raw microarray data showed a derivative log ratio spread of 0.17, within the category "excellent" as defined by the software Agilent Genomic Workbench, warranting further analysis of the data. The use of fixed cytogenetic preparations for array CGH applications was previously reported (14). An ADM1 (aberration detection module-1) algorithm was used for the detection of copy number changes. Array-CGH accurately described the partial monosomy of chromosome 6q, and 17p due to t(6;17) combined with the interstitial deletion of the homologous chromosome 17; in addition it revealed two abnormalities, not previously observed through karyotype examination. The first one was a partial deletion of chromosome 7q21.11 - q34, encompassing a known commonly deleted region in myeloid leukemias, 7q22-q31.1. The second one was a small deletion of approximately 1.7 Mb (17q11.2) containing the NF1 gene (*Figure 1D*). However, the anomalies of chromosome 9 were not detected by array-CGH, due to the low percentage of the affected cells, as assessed by FISH (18%). One malignant clone (bearing the above described abnormalities of chromosomes 6, and 17) and two subclones (first subclone with one additional anomaly, 7q partial deletion, and the second subclone with two additional anomalies, 7q partial deletion and derivative chromosome 9) were detected (*Figure 1E and F*).

In conclusion, the karyotype of our patient was:

45,XX,-6,der(17)t(6;17)(p12;p11.2)(2)/45,idem,del(7)(q21)(14)/45,idem,del(7)(q21),der(9)del(9)(p2?)del(9)(q22q3?2)(4).ish der(17)t(6;17)(wcp6+,D17S643-,TP53-,RP11-525O11+),del(17)(p11.2p13.1)(D17S643+,TP53+,RP11-525O11-)(7)/idem,del(7)(wcp7+)(67)/idem,del(7)(wcp7+),der(9)del(9)(p2?)del(9)(q22q32)(wcp9+,RP11-675G5-, ABL1+)(17).arr 6q12q27(68,742,686-170,734,368)x1, 7q21.11q34(78,474,218-140,692,336)x1,17p13.3q11.1(84,087-22,335,303)x1, 17q11.2(25,441,504-27,155,772)x1.

Considering the patient´s age, performance status and the coexisting infectious process, a treatment with daily low dose cytosine arabinoside (Cytosar) associated with antibiotics and supportive measures (transfusions) was started. No apparent improvement was observed; the patient died of infectious complication after 10 days.

Discussion

Genetic investigation in AML is instrumental for an accurate diagnosis and best patient care. In our case, the combined use of karyotyping and molecular techniques revealed the complexity of the chromosomal anomalies and allowed the refinement of the genomic regions involved as well as the discrimination of malignant clones/subclones.

Genomic losses of chromosomes 6q, 7q, 9p, 9q, 17p and 17q were detected in our case. Deletions of 17p, affecting the TP53 locus, have been associated with genetic instability and a proneness to complex changes (15). Chromosome 7q deletion is significantly more frequent among complex karyotype AML with alterations of TP53 (16) One other recurrent genomic loss, frequently detected in complex karypotype AML, is 17q deletion encompassing the NF1 gene. Taking into account the biological roles of NF1, the loss of this gene might be considered a cooperating event in the leukemogenesis of complex karyotype AML (11). Our case harbors disruptions of TP53 and NF1 loci, besides other genomic regions losses. We consider the complexity of the genomic changes a reflection of the profound genetic instability of leukemic blast cells.

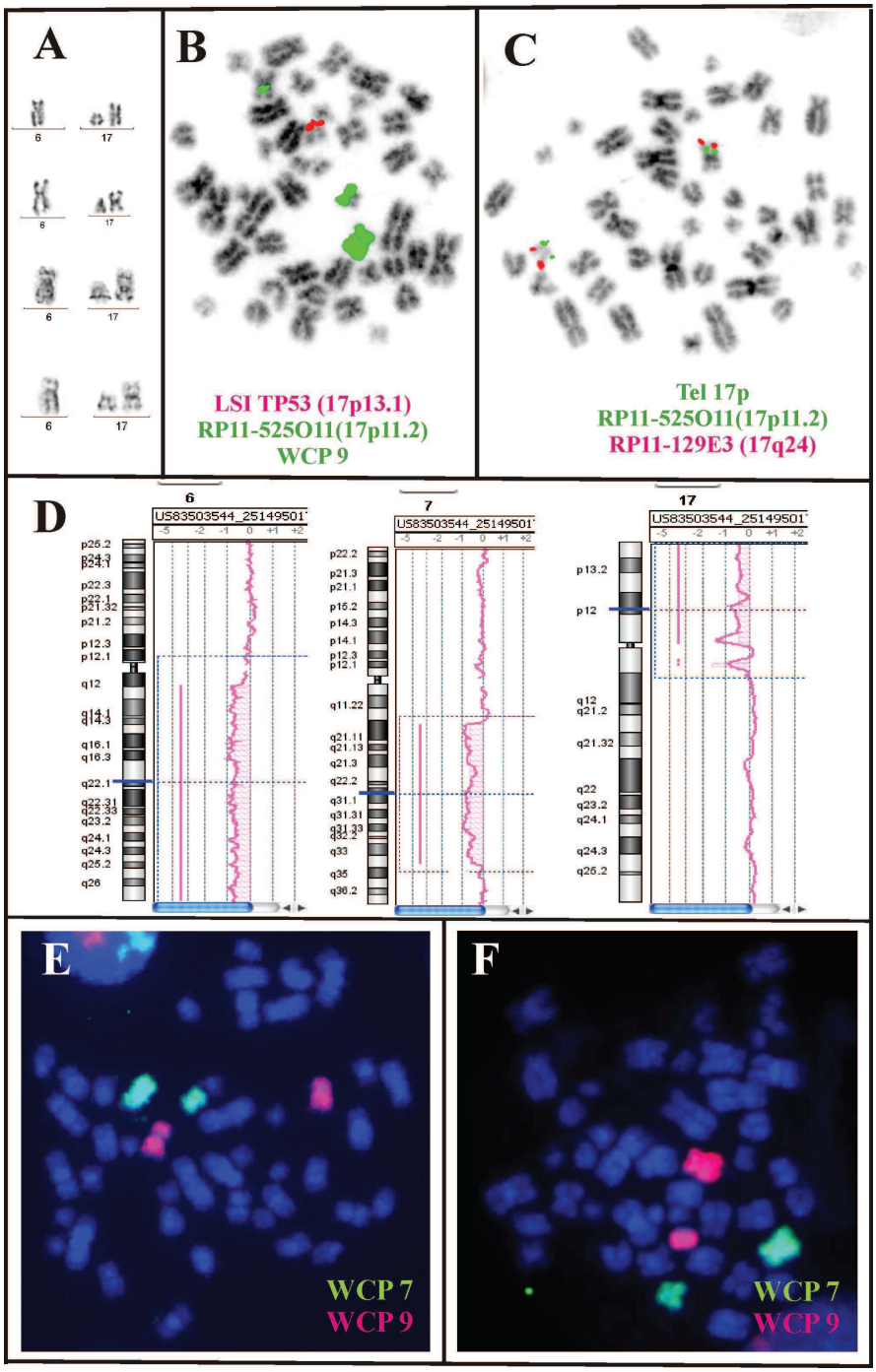

Figure 1. Chromosomal abnormalities in our case: A. Partial karyotype showing monosomy 6 and der(17)t(6;17)(p12;p11.2); B. BAC-FISH test showing the deletion of TP53; C. BAC-FISH test showing the interstitial deletion of 17p11.2; D. Array-CGH profiles of chromosomes 6, 7 and 17; E and F. Painting FISH for chromosomes 7 and 9 describing two different subclones: partial deletion 7q in one subclone, and partial deletion 7q associated with partial deletions of chromosome 9p and 9q in the other subclone.

Array-CGH allows the screening of the entire genome, at a high resolution, being thus a useful tool in complex karyotype AML analysis. However, the limitations of array-CGH (lack of detection of balanced anomalies or small subclones) make this technology particularly useful in conjunction with established genetic methods (conventional karyotyping and FISH). Array-CGH analysis proved to be essential in deciphering the genetic constitution of our case, by unfolding submicroscopic anomalies (17q-) and chromosomal changes, not detected by standard analysis, due to the type, size and multitude of anomalies.

The prognostic impact of age in AML is well recognized, older patients having worse outcomes when compared to younger patients (17). It is also recognized that there are differences in the incidence of distinct cytogenetic anomalies as age increases (1, 10). It seems however, that both age and cytogenetics have an independent prognostic impact in *de novo* AML, making thus the karyotype investigation mandatory in all age groups (12).

As with most forms of cancer, performance status plays a major role in prognosis; elevated lactate dehydrogenase serum levels were also associated with poorer outcomes (18). Our patient had both poor performance status and elevated lactate dehydrogenase level, adding to the dismal prognostic impact of complex cytogenetic abnormalities and increased age.

The pathogenesis of AML with complex karyotype is poorly understood when compared with AML associated with specific genetic defects. Thus, unravelling new cytogenetic abnormalities in AML with complex karyotype might bring some important insights in understanding this heterogeneous disease. To our knowledge, this is the first reported case with an unbalanced translocation t(6;17)(p12;p11.2) as part of a complex karyotype in *de novo* AML. The complexity of karyotypic changes in our case highlights the importance of using complementary genetic investigation in order to obtain a comprehensive view of genomic abnormalities in AML.

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Declaration of interest

There is no conflict of interests among the authors.

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