

## Clinical case of acute myeloblastic leukemia with t(8;21)(q22;q22) in a patient with Klinefelter's syndrome

Vanya Slavcheva,<sup>1</sup> Tzvetan Lukanov,<sup>2</sup> Gueorgui Balatsenko,<sup>3</sup>

Svetlana Angelova,<sup>3</sup> Antonio Antonov,<sup>1</sup> Lachezar Bogdanov,<sup>1</sup> Nikolay Tsvetkov<sup>1</sup>

<sup>1</sup>Hematology clinic, University Hospital, Pleven, Bulgaria;

<sup>2</sup>Flow cytometric laboratory, University Hospital, Pleven, Bulgaria;

<sup>3</sup>Laboratory of cytogenetic and molecular biology, National Hospital of Hematological Disease, Sofia, Bulgaria

### Abstract

Klinefelter's syndrome is characterized by abnormal karyotype 47, XXY and a phenotype associated with hypogonadism and gynecomastia. Often the disease can be diagnosed accidentally, when carrying out cytogenetic analysis in cases of a malignant blood disease. We present the clinical case of a patient diagnosed with acute myelomonoblastic leukemia-M4 Eo (AML-M4), where by means of classic cytogenetics a karyotype was found corresponding to Klinefelter's syndrome. Three induction courses of polychemotherapy were made, which led to remission of the disease, documented both flowcytometrically and cytogenetically.

### Introduction

Klinefelter's syndrome was first described in 1942 as an endocrine disorder characterized by gynecomastia, hypogonadism and higher levels of follicle-stimulating hormone. It is usually associated with 47 chromosomes, including two X and one Y (47,XXY).<sup>1,2</sup> Infertility and gynecomastia are the two most common symptoms pointing to the diagnosis.<sup>3</sup> Mamunes describes a patient with Klinefelter's syndrome and acute leukemia for the first time in 1961.<sup>2</sup> Consequently some medical manuals and clinical reports suggest that there is an increased risk of leukemia and lymphoma in patients with Klinefelter's syndrome,<sup>4,5</sup> but such a risk has never been proved. In cytogenetic tests of bone marrow of 5366 patients with malignant hematological diseases, only six of the patients had a karyotype compatible with Klinefelter. Out of these six patients three had myelodysplastic syndrome, two had non-Hodgkin's lym-

phoma and one was with acute myeloblastic leukemia. The fact that a cytogenetic analysis is routinely carried out in patients with leukemia and lymphoma by means of which constitutional abnormalities of the karyotype may be detected and should not be related with a connection between Klinefelter's syndrome and malignant hematologic diseases.<sup>6</sup> According to one retrospective study in Denmark in 696 patients with Klinefelter's syndrome not a single case of leukemia or lymphoma was established.<sup>7</sup>

### Clinical Presentation

KEK- 34-year-old man diagnosed with acute myelomonoblastic leukemia (AML-M4), determined for the first time during his stay in the clinic in July 2006, when he entered with complaints of weakness, fatigue at normal physical exercise and fever to 38°C. The physical examination did not show any pathological abnormalities. The conducted laboratory tests – complete blood count and differential count showed: Hb 76.0 g/dL, RBC  $2.2 \times 10^{12}/L$ , PLT  $46 \times 10^9/L$ , WBC  $3.5 \times 10^9/L$ , band 2%, segmented neutrophils 3%, eosinophils 1%, lymphocytes 76%, monoblasts 18%. The flow cytometric

Correspondence: Vanya Slavcheva, Hematology clinic, University hospital 8 A G. Kochev, blv Pleven 5800, Bulgaria. E-mail: slavcheva@abv.bg

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analysis of bone marrow during diagnostic-tion showed: myeloblasts expressing: CD13, CD33, CD34, CD117 and monoblasts with immuno-phenotype CD11b, CD11c, CD13, CD14, CD33, CD64, HLA-DR. Conclusion: the described flow cytometric finding is characteristic of AML-M4 (Figure 1). Cytogenetic analysis was performed of 18 metaphase plates of bone marrow which showed: 46, XX,(7)-

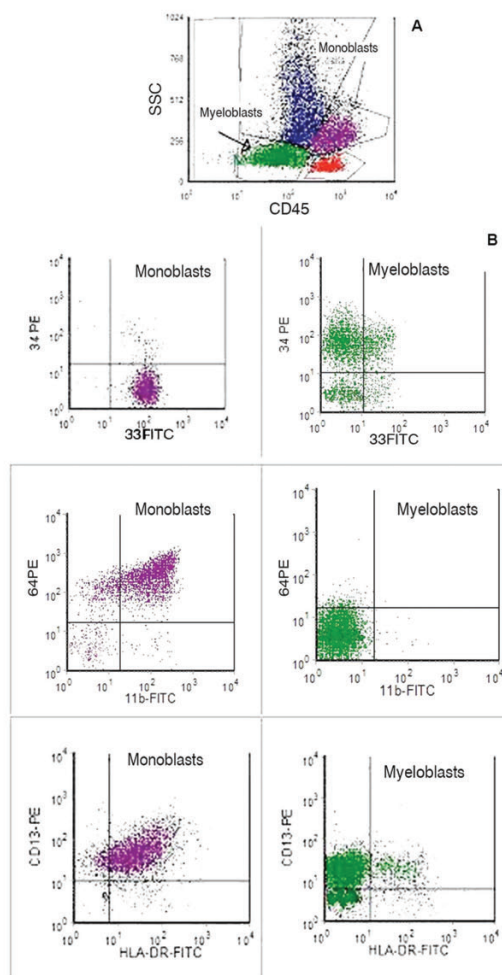
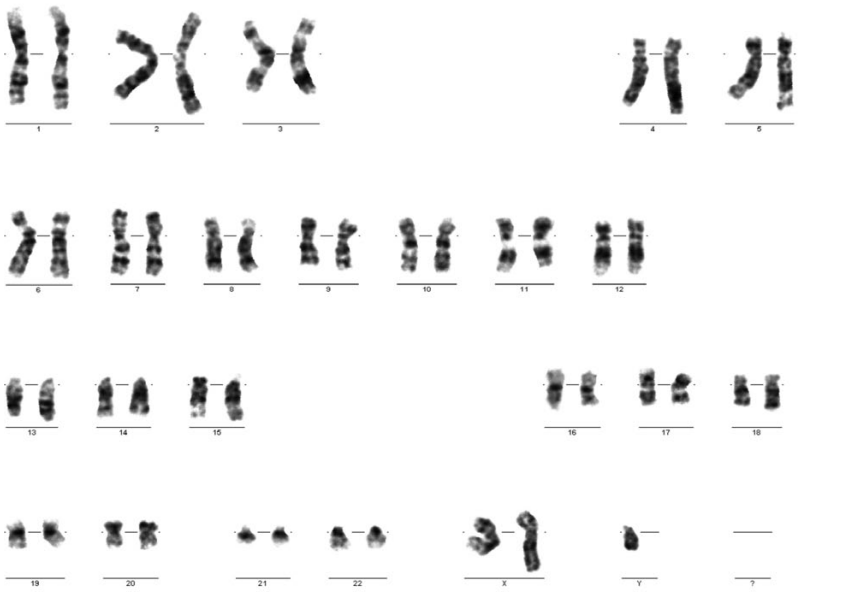


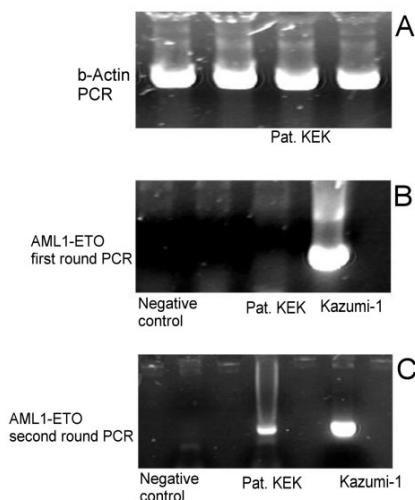
Figure 1. Flow cytometric analysis of bone marrow. Flow cytometric diagnosis of AML M4 was established. (A) CD45/Side light scatter (SSC) dot plot picture with two gates set around myeloblast cells and monoblast cells. (B) Fluorescence (FI) 1/FI2 dot plot presentation of myeloblast cells (right dot plots - CD34<sup>+</sup>/CD33<sup>-</sup>/CD13<sup>+</sup>/CD11b<sup>+</sup>/CD64<sup>+</sup>/HLADR<sup>+</sup>) and monoblast cells (left dot plots - CD34<sup>-</sup>/CD33<sup>+</sup>/CD13<sup>+</sup>/CD11b<sup>+</sup>/CD64<sup>+</sup>/HLADR<sup>+</sup>).

(q32;q36),t(8;21)(q22;q22),del(7)(q32;q36). A complex aberrant karyotype was found and not conforming to the sex, which led to a cytogenetic analysis of peripheral blood lymphocyte culture stimulated with phytohemagglutinin, due to which the karyotype 47, XXY was found, corresponding to Klinefelter's syndrome. Two induction courses were carried out including: epirubicin and cytarabine at conventional doses and mitoxantron with cytarabine, which resulted in reduction of the blast population in the bone marrow. Hematological remission with complete cytogenetic response (CCyR) of the disease was achieved after the third induction course containing mitoxantron, ete

and cytarabine, documented by means of flow cytometric and cytogenetic analysis in October 2006 (Figure 2). Consolidation was carried out with mitoxantrone and cytarabine, followed by maintenance therapy with mercaptopurine and methotrexate, and monthly reinduction courses (epirubicin and cytarabine, mitoxantrone and cytarabine – in turns) throughout the first year, every two months throughout the second year and every three months throughout the third year. At the end of the second year flow cytometric, cytogenetic and molecular analysis (Figure 3) was carried out once again of bone marrow, which confirmed the achieved remission.



**Figure 2.** The karyotype corresponds to Klinefelter's syndrome. No structural chromosomes were detected: complete cytogenetic response regarding t(8;21)(q22;q22). Routine cytogenetic analysis was performed on metaphase chromosomes from bone marrow samples using a direct method and after short-term 24 hours or 48 hours culturing. Metaphases were analyzed using GTG differentially stained chromosomes at a discriminatory level of 300–400 bands per haploid count. Karyotypic findings were interpreted and described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2009).<sup>8</sup>



**Figure 3.** Detection of residual AML1-ETO transcripts by Nested Primers (NP) Reverse Transcription Polymerase Chain Reaction (RT-PCR). (A) Control amplification of  $\beta$ -Actin cDNA as a marker for RNA integrity and efficiency of copy DNA synthesis. (B) First round PCR for AML1-ETO transcripts – absence of product of amplification. (C) Second round PCR for AML1-ETO transcripts – weak positive reaction corresponding to relatively low number of AML1-ETO transcripts. PCR assay for AML1-ETO fusion transcripts.

## PCR assay for AML1-ETO fusion transcripts

The presence of AML1-ETO rearrangement was determined by nested primers Reverse Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from bone marrow cells using TRizol Reagent (Invitrogen BRL) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis and amplification was done according to the protocol, recommended by the European BIOMED 1 Concerted Action for standardization of MRD studies in acute leukaemia.<sup>8</sup> In addition, an amplification of *beta-Actin* mRNA with primers 5'-GGCATCGT-GATGGACTCCG-3' and 5'-GCTGGAAGGTG-GACAGCGA-3' was performed as a marker for RNA integrity and efficiency of copy DNA synthesis. Amplification products were run in a 2% agarose gel (Invitrogen BRL) after staining with ethidium bromide, visualized after UV irradiation and photographed. Kasumi-1 cell line was used as a positive control and RNA from healthy donors as a negative control. In addition, RNA from each sample using the same RT-PCR conditions and reagents omitting the reverse transcriptase, and a mixture, containing all the reagents except the nucleic acids have also been used as negative controls

## Discussion

The described clinical case is of interest due to the fact that to the moment of manifestation of the acute leukemia, Klinefelter's syndrome was not diagnosed. For the period from 2003 to 2007, 72 men, (average age 59,78), entered the hematological clinic in Pleven with newly diagnosed acute leukemia, Klinefelter's syndrome was found in only one of them, who also had a second chromosomal abnormality t(8;21)(q22;q22). According to some authors two mutational events are required for the development of cancer: the first step may be a constitutional event and the second an acquired genetic mutation.<sup>9</sup> It is an open question whether the presence of three sex chromosomes is an event that leads to chromosomal instability. Some of the national and international cooperative research groups (Medical Research Council group; CALGB and GIMEMA/AML10) consider that complementary aberration, even the complex karyotype, do not influence significantly the prognosis in cases of favorable aberrations. Other research groups ( SWOG/ ECOG, HOVON/ SAKK) have found reliable differences in the prognosis of patients with "good" features in the karyotype with or without complex karyotype.<sup>10</sup>

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