

# Philadelphia-positive case negative for JAK2 V617F mutation with hyperdiploidic karyotype: A case report

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**Abstract.** Chronic myeloid leukemia (CML) is one of the most common hematological malignancies and accounts for 15-20% of all leukemia cases. The cytogenetic marker of CML is the presence of Philadelphia chromosome (Ph) in >95% of patients. The current case reports a 83-year old woman who was directed to the genetic laboratory for a cytogenetic and molecular-genetic analysis suspected to be Ph positive [(+)]. Karyotype analysis of a bone marrow sample revealed a hyperdiploid karyotype in a part of Ph (+) cells with additional chromosomes 8, 10 and 12. Restriction analysis for V617F *JAK2* mutation was negative, while the quantitative RT-qPCR assay indicated BCR-ABL/ABL transcript at the level of 120% International Scale (IS). Generally cytogenetic complexities are important in the prognostic evaluation of CML. Besides the Ph chromosome, a variety of chromosomal aberrations may be associated with CML. A total of 5-10% of these cases show complex translocations involving another chromosome. The current case is Ph(+) demonstrating an additional hyperdiploid karyotype clone with three additional autosomes (8, 10 and 12). This case highlights the significance of cytogenetic abnormalities on the prognosis of CML.

## Introduction

Chronic myeloid leukemia (CML) is one of the most common hematologic malignancies and accounts for 15% of all cases of leukemia in adults (1). The incidence of CML is approximately 1.6/100,000. The cytogenetic marker of the disease is the presence of a distinctive Philadelphia chromosome (Ph) in more than 95% of the patients (2). It is a reciprocal translo-

cation between the long arms of chromosomes 9 and 22. The translocation involves the transfer of the Abelson or *ABL1* gene on chromosome 9 to the breakpoint cluster region, *BCR*, of chromosome 22, resulting in a fusion *BCR/ABL* gene. The fusion gene produces BCR/ABL, a tyrosine kinase with deregulated activity that plays a key role in the development of CML.

Our case is an 83-year old woman who is directed to the genetic laboratory for a cytogenetic and molecular-genetic analysis suspected to be Ph positive [(+)]. Her initial diagnosis is primary aplastic anemia with additional diagnosis of primary (essential) hypertension. The anamnesis is taken both from medical documentation and the statements of patient's relatives. She is accepted for the first time in the clinic having leukocytosis with neutrophilia, moderate-to-severe anemic syndrome-microcytic, hypochromic anemia; the thrombocytes are in the reference ranges. She has a consuming syndrome accompanied with a preserved and/or increased appetite and sore throat. Fever and feverish night sweats are lacking, there is no bleeding.

High levels of leucocytes have been diagnosed February, 2018. The leucocyte numbers increase in the following several months up to 85G/l. The levels from March, 2005 are documented as follows: St 12%; Sg 17%; Mo 2%; Ly 10%; Eo 5%; Bl + ProM 25%; M 21%; Meta M 9%.

Upon her acceptance in the hematological ward she is adequate and orientated. Her skin and mucosal membranes are pale pink with isolated suffusions and hematomas; no icterus is present. Peripheral lymph nodes are not enlarged on palpation; breathing is clear vesicular, double-sided, without wheezing. Cardiovascular system-there is arrhythmic cardiac activity, clear tones, systolic noise at Ao and cardiac apex. Stomach-soft, painless; liver-1-2 cm below the ribs' arch; spleen-non-enlarged. There are no swellings of the limbs.

## Materials and methods

At the Clinic of Hematology the patient is subjected to routine diagnostic procedures: Whole blood count test and biochemical analysis (Table I), morphological analysis of the blood cells, restriction analysis for V617F in *JAK2* gene (Fig. 1), quantitative molecular-biological analysis (Real-time PCR) at the time

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Table I. WBC and biochemical analysis result of the patient at the time of her acceptance in the ward.

Biochemical characteristic	Result	Units	Reference values	Parameter	Result	Units	Referent values
WBC	69.77	$\times 10^9/l$	3.50-10.50	Gluc	5.58	mmol/l	3.50-6.10
RBC	4.34	$\times 10^{12}/l$	3.70-5.30	Creatinine	161.00	$\mu\text{mol/l}$	up to 96.00
HGB	87	g/l	120-160	TBil	8.50	$\mu\text{mol/l}$	up to 21.00
HTC	0.295	l/l	0.360-0.480	ASAT	27.60	U/l	up to 32.00
MCV	67.8	fl	80.0-96.0	ALAT	7.00	U/l	up to 33.00
MCH	19.90	pg	27.0-33.0	LDH	2783.00	U/l	up to 460
MCHC	294	g/l	300-360	Na	143.00	mmol/l	135-151
Platelets	182	$\times 10^9/l$	130-360	K	3.20	mmol/l	3.5-5.6
% lymphocytes	3.60	%	20.0-48.0	Cl	103.00	mmol/l	93-112
% monocytes	6.30	%	1.0-11.0	Fe	15.80	$\mu\text{mol/l}$	6.60-28.00
% eosinophils	0.80	%	Up to 6.5	TIBC	50.30	$\mu\text{mol/l}$	42.00-78.00
% basophils	7.40	%	Up to 2.0	UIBC	34.50	$\mu\text{mol/l}$	27.8-63.6
% neutrophils	88.00	%	40.0-70.0	CRP	0.20	mg/l	<6
No lymphocytes	2.54	$\times 10^9/l$	1.00-4.00				
No monocytes	4.36	$\times 10^9/l$	Up to 0.80				
No eosinophils	0.59	$\times 10^9/l$	Up to 0.50				
No basophils	5.14	$\times 10^9/l$	Up to 0.14				
No neutrophils	61.39	$\times 10^9/l$	2.00-7.00				
MPV	9.40	fl	6.3-12.5				

WBC, whole blood count; RBC, red blood cells; HGB, hemoglobin; HTC, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; Gluc, glucose; TBil, total bilirubin; ASAT, aspartate transaminase; ALAT, alanine transaminase; LDH, lactic acid dehydrogenase; Na, sodium; K, potassium; Fe, iron; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; CRP, C-reactive protein.

of her first visit and three months after treatment (Fig. 2), and karyotyping (Fig. 3). For all procedures and tests the patient has provided a written informed consent.

**PCR amplification and restriction analysis for JAK2 V716F mutation detection.** DNA is extracted from 200  $\mu\text{l}$  venous blood taken in K<sub>2</sub>EDTA tube using QIAamp DNA Mini kit (Qiagen). JAK2 mutation has been checked as a part of the routine diagnostic procedure of the Clinic of Haematology when chronic myeloproliferative process is suspected. DNA is amplified using JAK2 codon 617 mutation specific primers (V617F) (*JAK2F* 5'-GGGTTTCCTCAGAACGTT-3' and *JAK2R* 5'-TCATTGCTTTCCTTTTC-3') for 32 cycles using Taq polymerase (Qiagen), annealing temperature of 60°C, and standard amplification conditions as described previously by Baxter *et al* (3). The amplified 460-bp fragment is enzymatically digested using *BsaXI* restriction enzyme (BioLabs™). Fragments of three different sizes (241, 189 and 30 bp) are received after digestion of the wild type allele, while the mutant allele remains undigested (Fig. 1). Digested fragments are separated in 2% agarose gel. Visualization of the restriction fragments is achieved by ethidium bromide (Fig. 1).

**RT-qPCR analysis.** It is carried out using the Cepheid GeneXpert® platform. RNA has been automatically extracted from 4 ml venous blood and converted to cDNA. Xpert BCR-ABL kit is used for quantitative detection of BCR-ABL chromosomal translocation mRNA transcripts and the ABL

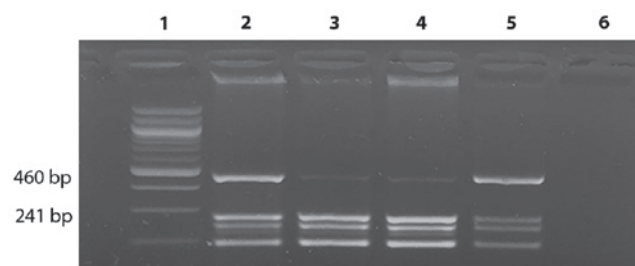


Figure 1. Restriction analysis following reverse transcription-quantitative PCR of JAK2 exon 12 gene. *BsaXI* endonuclease is used to specifically cut the PCR product on 2% agarose gel. Lane 1 indicates 100 bp ladder, lane 2 and 5 indicate individuals, heterozygote for JAK2 V617F mutation, lane 3 and 4 indicates individuals homozygous by the normal allele (lane 3 corresponds to the patient from the presented case) and lane 6 indicates the negative control.

endogenous control mRNA transcripts in peripheral blood specimen from patients with CML. The Xpert BCR-ABL Ultra quantifies the BCR-ABL mRNA level on the *IS* and is calibrated to the first World Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL mRNA. The GeneXpert software calculates the %BCR-ABL/ABL (*IS*) using the following equation where the Delta Ct ( $\Delta\text{Ct}$ ) value is obtained from ABL Ct minus BCR-ABL Ct:  $\%BCR-ABL/ABL\ IS = E_{\Delta\text{Ct}}^{(\Delta\text{Ct})} \times 100 \times \text{Scaling Factor (SF)}$  (4). The efficiency value is embedded in the barcode of the Xpert BCR-ABL Ultra cartridge; the SF is lot-specific (Xpert® BCR-ABL Ultra Handbook) (Fig. 2).

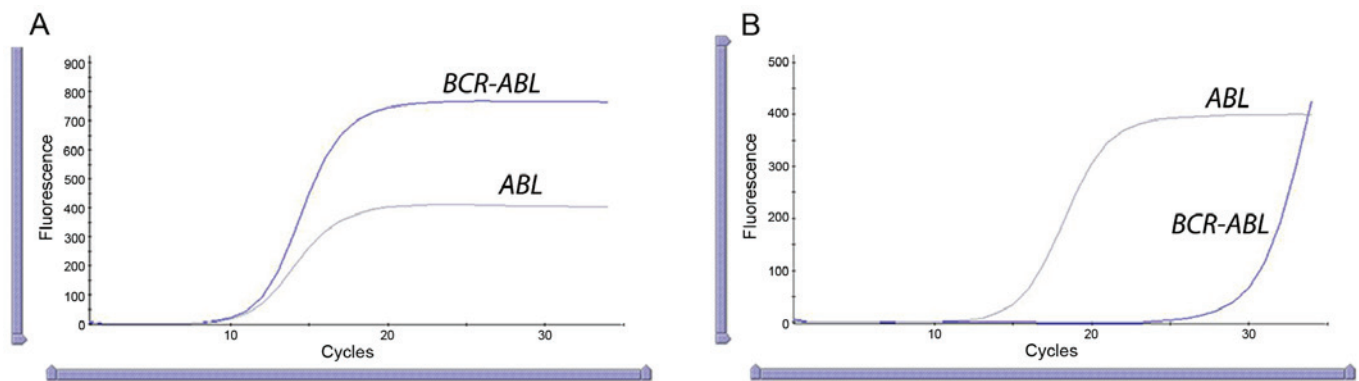


Figure 2. Cepheid® RT-q PCR analysis of the patient showing a positive amplification curve (A) Result from the RT-q PCR analysis at the time of diagnosis. (B) Result from the RT-q PCR after three months of Tasigna treatment. Primary curve of the patient showing amplification of ABL (internal control) and the BCR-ABL gene is represented in both cases. RT-q, reverse transcription-quantitative; BCR, breakpoint cluster region; ABL, Abelson.

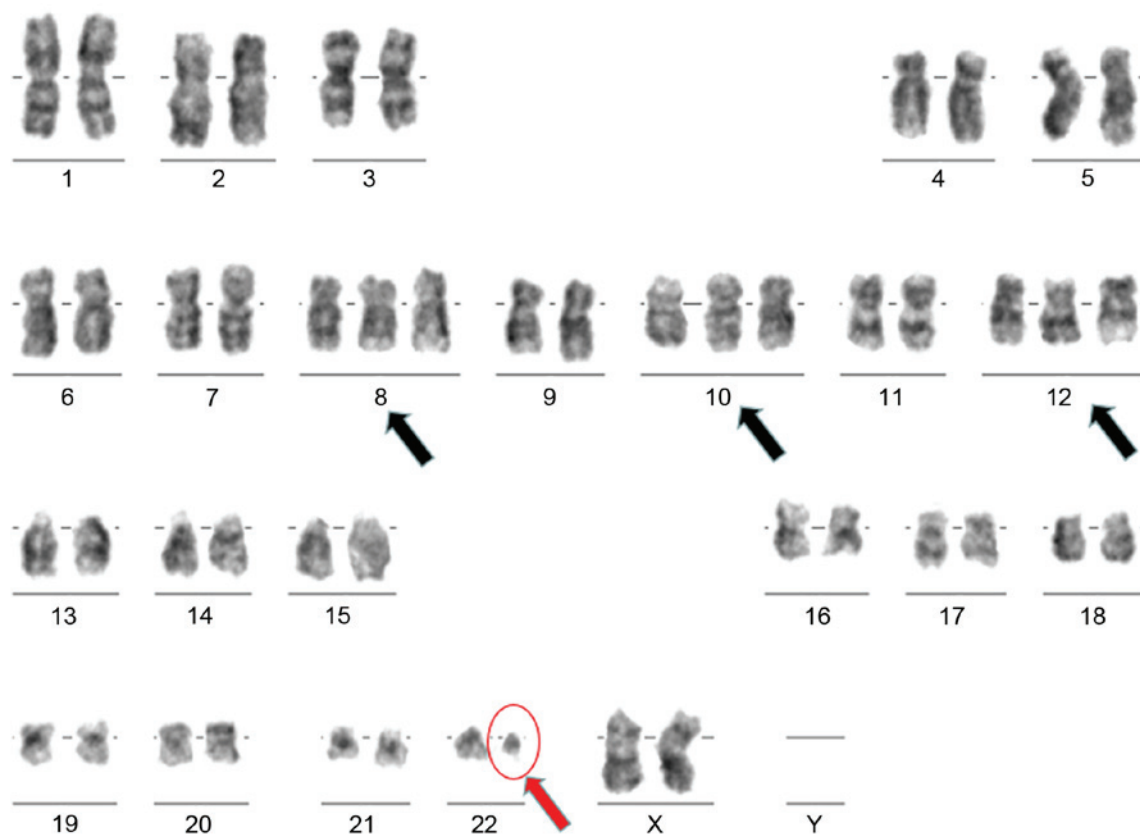


Figure 3. Chromosome banding analysis indicating a complex hyperdiploid karyotype with numerical aberration and a total number of 49 chromosomes (49, XX, +8, +10, +12). Additional autosomes are indicated by black arrows while Philadelphia chromosome is encircled and indicated by a red arrow.

**Cytogenetic analysis.** The bone marrow sample of the patient is taken by trepanobiopsy and sent to the cytogenetic laboratory of the Military Medical Academy (Sofia, Bulgaria). The cells are cultured in RPMI culture medium supplied with antibiotics and growth factors at 37°C for 24 h. 70 µl of colcemid is added to the sample and incubated in 37°C for 20 min. After centrifugation at 1,500 rpm, 0.075 M KCl is added and the sample is incubated at 37°C for 10 min. After two times fixation and centrifugation, several microscopic slides are prepared. They undergo standard Giemsa staining and are analyzed under microscope (Fig. 3).

## Results

The morphological analysis of cells' populations shows hypercellular bone marrow with an altered ratio between the neutrophil, granulocyte and erythroblast clones up to 12.1:1 (granulocytes 0.90; erythroblasts 0.07); hyperplasia of the neutrophil population with sustained maturation, increase of myelocytes and promyelocytes. Index of maturation 1.3 (reference values 0.5-0.8). Cytological features of gigantism in a part of the metamyelocytes. Substantially reduced erythroblast population with single representatives of all

maturation forms. Well-presented megakaryocyte apparatus with a dominating population of polyploidic granulated megakaryocytes. The conclusion of the morphological analysis is 'Chronic myeloproliferative process'. The ratio of different cell populations in the bone marrow is as follows: 1.5% lymphocytes, 87.9% granulocytes, 0.1% myeloblasts, 6.2% mature monocytes, 4.2% promonocytes. The whole blood count test of the patient and the biochemical analysis result at the time of her acceptance in the haematological ward of 'St. Ivan Rilski' Hospital is presented in Table I.

Based on the myelography the patient is directed for molecular-biological analysis for *JAK2* mutation and Ph chromosome. The result of the restriction analysis is presented in Fig. 1 showing the genotype status of the patient (homozygous carrier of two wild type alleles for V617F *JAK2* gene mutation).

Quantitative Real-time PCR analysis of the patient is performed (May, 2018) to confirm the presence of the molecular marker of CML-fusion gene *BCR/ABL*. The graph clearly shows the positive result of the amplification for both *ABL* (endogenous control) and *BCR-ABL* genes. The software estimates automatically the level of the fusion gene of 120% (*IS*) on the International Standardized Scale (Fig. 2A). Real-time analysis is performed second time (September, 2018) after four months' treatment with Tasigna (Nilotinib) to monitor the molecular improvement of the patient. The result demonstrates 0.0019% (*IS*) (Fig. 2B) level which is much less compared to the patient's earlier data.

Karyotyping is performed after 24-h cultivation time of stimulated bone marrow specimen. Chromosomes are obtained, stained following Giemsa standard protocol and subsequently analyzed under light microscope (5). The cytogenetic analysis shows two different clones of cells: Hyperdiploid with additional chromosomes 8, 10 and 12 and Ph chromosome; and second clone which is Ph(+) with no hyperdiploidy (Fig. 3).

## Discussion

Hyperdiploidy is a phenomenon of having additional chromosomes rather than the diploid chromosome number in the karyotype. According to Onodera *et al*, hyperdiploid karyotype arises by a simultaneous gain of multiple chromosomes from a diploid karyotype during a single abnormal cell division (6). Generally hyperdiploidy is not a common event in CML patients (7). Other chromosome changes, such as chromosomal translocations, are more common, especially at the time of blast transformation (8). Hyperdiploidy could be a bad prognostic factor in CML (7,9). Further investigation on the topic shows that the existing statements about hyperdiploidy are ambiguous.

A case report (10) presents a Ph(+) CML case with hyperdiploid karyotype and an additional T315I kinase domain (KD) mutation in the *BCR-ABL* gene. The patient responded well after therapy with Nilotinib. Though T315I mutation remained after the treatment, targeted drug eliminated the hyperdiploid clone (10).

In a study on acute lymphoblastic leukemia (ALL) in children high hyperdiploidy is characterized by a favourable prognosis (11). A paper studying chromosomal aberrations in pediatric and adult ALL defines high hyperdiploidy as present in 25-30% of children with favorable prognosis and 7-8% in

adults with favorable/intermediate prognostic value (12). In multiple myeloma patients hypodiploidy but not hyperdiploidy, is a poor indicator for the disease's development (13). According to some authors (14-16) hypodiploid DNA content is related to a poor response to chemotherapy and a very short survival time; according to others (17) hyperdiploid karyotype is associated with a better prognosis. The chromosomes which usually appear in addition in ALL karyotypes are 21, X, 14, 6, 18, 4, 17 and 10 which tend to be gained in blasts (18). The acquisition of chromosome 21 is seen in 95% of hyperdiploid cases.

There are only few reports about hyperdiploid karyotypes in CML cases. Rojas *et al* analyzes 63 cases with CML and not even one additional chromosome is found (19). There is only one case with i(17q); and one case with 21q deletion (19). Roland and Blahey reported a case of a patient with breast cancer who developed hyperdiploidy at the accelerated phase of the disease, near blast crisis, presenting with seven additional chromosomes besides Ph chromosome (+6, +8, +11, +18, +19, +20, +21) (8). Gains of chromosomes 6 and 19 are common in CML hyperdiploid karyotypes (8). Other commonly reported chromosomes are 8 and 19 in Ph(+) cases of CML (20). In a study of 256 patients with CML only one is with 51 chromosomes and trisomy +6, +10, +13 and +19 (21). Chromosomes +7, +8, +9, +10, +12, +15, +19 are reported in a case with near triploid karyotype in pre-imatinib mesylate CML patient with T315I mutation in *BCR-ABL* kinase domain (10).

The reported case in this study has been diagnosed as CML as for of an absence of a blast infiltration in the bone marrow. The myelogram and the immunophenotyping characteristics of the cells reveal the percentage of myeloblasts in the bone marrow estimated at 0.1%. Besides being Ph(+), the patient shows a hyperdiploid karyotype with additional chromosomes 8, 10 and 12 (see 'Results'). To our knowledge, this is the only report of chromosome 12 in a hyperdiploid CML patient.

The underlying mechanism of hyperdiploidy and how this phenomenon can potentially influence the expression profile of the genes is still unknown (22). The influence of hyperdiploidy in the course of CML remains a disputable question in literature. From clinical point of view, the chromosomal gain is associated with increased risk for blast crisis (23,24). To our knowledge, there is only one more report which presents higher percentage level of *BCR-ABL* transcript detected quantitatively and expressed in % (*IS*) (25). The detected level of the *BCR-ABL* transcript was measured to be 187% (*IS*) in bone marrow and 152% (*IS*) in peripheral blood of a 28-year-old patient.

As a whole the cytogenetic complexities play major role in the prognostic evaluation of CML. Along with the Ph chromosome, various chromosomal aberrations can be associated with CML. 5-10% of these cases showing complex translocation involving another chromosome in addition to the Ph chromosome. Our case is Ph(+) with an additional hyperdiploid clone with trisomy 8, 10, 12. Cases like this could sometimes present with higher results of *BCR-ABL/ABL* transcripts detected quantitatively.

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## Availability of data and materials

All data generated or analyzed during the present case study are included in this article.

## Authors' contributions

DN performed the restriction analysis, Real-time PCR test, performed the literature review and wrote the manuscript, VD assisted in the laboratory data interpretation, VH and MM interpreted the clinical data of the patient and monitored the treatment, LM and AA performed the cytogenetic analysis, AR and DT acquired relevant data and revised the manuscript critically. All authors have read and approved the manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Written informed consent was obtained from the patient for the publication of the obtained data.

## Competing interests

The authors declare that they have no competing interests.

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