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Coexistance of Masked Philadelphia Chromosome and *BCR-ABL1* Rearrangement with *JAK2 (V617F)* Point Mutation-A Case Report

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM performed cytogenetic and molecular testing for this case study, wrote the draft of the manuscript and managed literature searches. Author HŠ were responsible for taking patient samples and providing clinical information. Author GŠ performed histopathologic analysis for this case study and contributed to the correction of the draft. All authors read and approved the final manuscript.

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Case Study

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ABSTRACT

In accordance with the last World Health Organization (WHO) classification, myeloproliferative neoplasms (MPNs) are classified on the basis of the presence or absence of *BCR-ABL1* gene transcript, or the presence or absence of Philadelphia chromosome (Ph) in the karyotype. Previously, from a diagnostics perspective, it was thought that the *JAK2V617F* point mutation and *BCR-ABL1* rearrangement were used to differentiate and unmask chronic myelogenous leukemia (CML) from other MPNs (and vice versa). In contradiction with this opinion, to date, several cases with the coexistence of *BCR-ABL1* fusion gene and *JAK2V617F* mutation in bone marrow and blood samples has been reported. With this case study we report a rare case of essential thrombocytemia with masked Philadelphia chromosome harboring both *BCR-ABL1* rearrangement and *JAK2V617F* point mutation, simultaneously.

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1. INTRODUCTION

In accordance with the last published World Health Organization (WHO) classification, myeloproliferative neoplasms (MPNs) are classified on the basis of the presence or absence of *BCR-ABL1* gene transcript, or the presence or absence of Philadelphia chromosome (Ph) in the karyotype. For this reason, Philadelphia chromosome negative MPNs (Ph- MPNs) include polycythemia vera (PV), essential thrombocytemia (ET), and primary myelofibrosis (PMF). Chronic Myeloid Leukemia (CML), on the other hand, is classified in the second group, comprising the Philadelphia chromosome positive MPNs (Ph+ MPNs) [1]. The diagnostic criteria of these disorders include the identification of clinical signs, symptoms, blood count changes and bone marrow histology. It is very important to determine the genetic background of these disorders and identification of specific molecular markers such as *BCR-ABL1* oncogene and *JAK2* mutation [1,2].

The Ph chromosome is an abbreviated chromosome 22, as a result of reciprocal exchange of material with chromosome 9. Large portion of a proto-oncogene, called *ABL1*, on chromosome 9 is translocated to the *BCR* gene on chromosome 22 where gene segments are fused and *BCR-ABL1* fusion gene ultimately produces the corresponding protein with deregulated tyrosine kinase activity [3].

The *JAK2* is a tyrosine kinase gene, located on the short arm of chromosome 9 and has an important role in the cell signaling pathways [4]. Detection of these genetic markers may be a potential major breakthrough for understanding the pathobiology of MPNs, and play an essential role of the diagnostic algorithm [5].

Previously, from a diagnostics perspective, it was thought that the *JAK2V617F* point mutation and *BCR-ABL1* rearrangement were used to differentiate and unmask CML from other MPNs (and vice versa). In contradiction with this opinion, to date, several cases with the coexistence of *BCR-ABL1* fusion gene and *JAK2*V617F mutation in bone marrow and blood samples at the same time have been reported [6-13].

A small percentage of patients (approximately 1.0%) with CML will have the *BCR-ABL1* gene sequence but not the Ph chromosome. These cases either have variant translocations that involve other chromosomes or have a hidden translocation involving 9 and 22 that cannot be identified by routine chromosomal analysis.

Hidden *BCR-ABL1* sequence, in the Ph negative cells, can be located by fluorescent *in situ* hybridization (FISH). The origin of this fusion gene, in the so-called “masked Ph chromosome”, is an insertion of *ABL1* sequence into the *BCR* region (or vice versa). There is yet another multiple step version where a classical translocation is followed by a translocation of both products, thus reintergrating the normal chromosome structure [14].

We report here an unusual case of essential thrombocytemia with masked Philadelphia chromosome harboring both *BCR-ABL1* rearrangement and *JAK2V617F* point mutation, simultaniously.

2. PRESENTATION OF CASE

In December 2014, the 65-year-old male patient with mild leukocytosis and thrombocytosis was hospitalized at the Department of Hematology, Oncology and Radiotherapy, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina. Initial blood cell count revealed: white blood cells (WBC) 12.0×0^{9} /L, hemoglobin (Hb) 130.0 g/L, erythrocytes 4.2×10^{12} /L and platelets 629.0×10^{9} /L. Physical examination was normal with no palpable spleen enlargement.

For the establishment of a diagnosis, histopathologic, cytogenetic and molecular analysis were performed.

Histopathologic examination of bone marrow biopsy revealed 75.0% cellularity, increased megakaryopoiesis with moderate formation of small megakaryocytes clusters and the beginning of fibrosis. Erythroid cell lineage were also numerous, with a relatively higher number immature forms, while estimates of the number and maturity of myeloid lineage are not reliable due to the damage of the sample during technical processing. The residue cells consist of T and B lymphocytes (up to 2.0%).

A sample of the bone marrow aspirate was collected for chromosome and FISH analysis.

This sample was introduced into complete RPMI 1640 medium (Euroclone, Milano, Italy) with 10.0% fetal bovine serum (FBS) (Euroclone, Milano, Italy). Direct and overnight cell culture methods were used.

After harvesting, chromosomes were prepared and GTG-banded for karyotyping according to standard laboratory protocol. A normal male karyotype was found in all 20 examined metaphases (Fig. 1).

We also performed metaphase FISH technique, using LSI dual color dual fusion *BCR-ABL1* translocation probe (Vysis, Downers Grove, IL, USA) whereby a 20 metaphase cells were analyzed. However, metaphase FISH analysis result was ins(22;9)(q11;q34), representing a small size insertion and the occurrence of masked Ph, as seen in 19 out of 20 (95.0%) metaphases (Fig. 2). One out of 20 (5.0%) metaphases was normal.

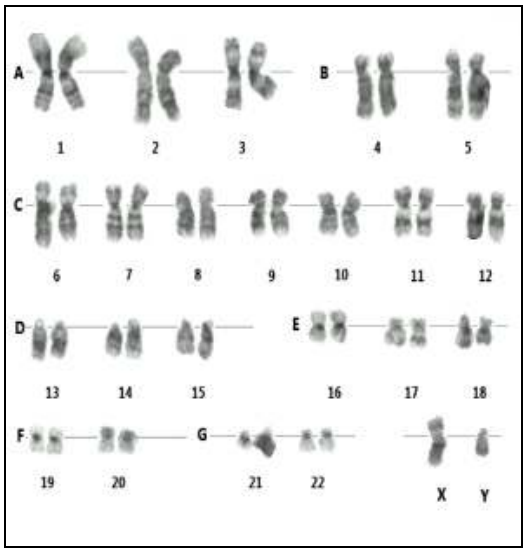

Fig. 1. The representative karyogram from a bone marrow GTG-banded metaphases of the patient showing 46,XY karyotype

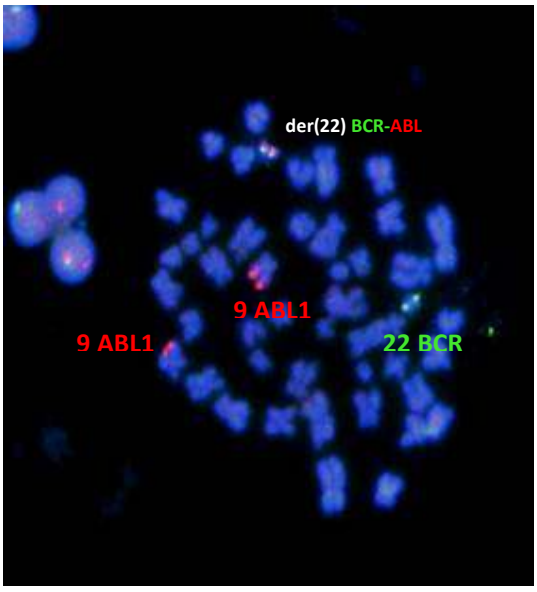

Fig. 2. A representative metaphase cell, with hybridization of LSI *BCR-ABL1* D-FISH probe (Vysis), showing the masked Ph, small size insertion ins(22;9)(q11;q34) at der(22), and the absence of green signal at chromosome 9

For the identification of *BCR-ABL1* fusion gene, we performed qualitative reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative polymerase chain reaction methods (QRT-PCR). Total RNA was extracted from $2x10^7$ peripheral blood leukocytes using Trizol-RNA isolation method [15]. RNA concentration was measured using a spectrophotometer Lambda Bio 10 (PerkinElmer, USA). Complementary DNA (cDNA) was synthesized using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, CA, USA), according to the manufacturer's manual. RT-PCR was performed with *BCR-ABL1* t(9;22) translocation Assay (In Vivo Scribe Technologies, San Diego, California) according to the manufacturer's manual. We also utilized known positive and no template (RNase/DNase-free water) control samples included with kit. The RT-PCR analyses showed that the patient was positive for the major *BCR-ABL1* fusion transcript (e14a2 molecular isoform) (Fig. 3).

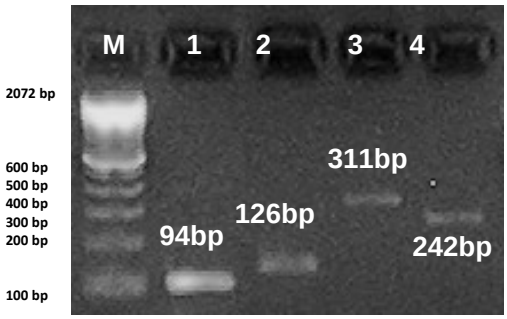

Fig. 3. Ethidium bromide-stained agarose gel with e14a2 *BCR-ABL1* transcript type (lines 2,3,4). Line 1- amplicon obtained by amplification 94 bp internal standard (Abl1 gene). M- MWM 100-2072 bp (Invitrogen Corp., San Diego, USA).

To determine the *BCR-ABL1* copy number and the fusion gene expression level, we performed QRT-PCR analysis using a LightCycler 2.0 Real Time PCR instrument (Roche diagnostics, Mannheim, Germany) with 20 µl final volume measure. We used 5 µl cDNA and 15 µl LC Roche Master mix (LC FastStart DNA MasterPlus hybridisation probes, Roche diagnostics, Mannheim, Germany), containing Ipsogen primers (Fusion*Quant* Kits for Real-Time Quantitative PCR Analysis of Fusion Gene Transcripts, Ipsogen, Marseille, France). The *BCR-ABL1/ABL1* quantitative ratio was 30.66% International Scale (IS) at diagnosis.

With regard to the referral suspected diagnosis of myeloproliferative neoplasm, we also performed Allele specific oligonucleotide PCR (ASO-PCR) for *JAK2V617F* mutation detection, according to the method of Baxter et al. [4]. Genomic DNA was extracted from the whole peripheral blood specimen of the patient using the commercially available QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The ASO-PCR test showed that the patient was positive for this point mutation (Fig. 4).

On the basis of extensive clinical evaluation and laboratory tests, especially with the the aforementioned molecular diagnostic findings, the patient was diagnosed to have essential thrombocytosis with a major *BCR-ABL1* fusion transcript, and imatinib therapy was initiated at a dose of 400 mg/day.

Three months later, the treatment with 400 mg imatinib (daily dose) returned blood cell count to normal range, while the number of *BCR-ABL1* fusion transcripts decreased to 4.57% (IS). At the time of last follow-up in October 2015 the *BCR-ABL1/ABL1* ratio was 0.0036%IS, which corresponds to a 4-log reduction from the standardized baseline [16]. The allele-specific PCR amplification still detected the JAK2 mutation. Interestingly, the patient was free of any morphological evidence of CML during the follow-up period.

3. DISCUSSION

There is a real enigma on how two genetic changes *BCR-ABL1* translocation and *JAK2V617F* mutation in the Ph+/Ph- MPNs occur simultaneously, and how these combinations can affect the pathobiology, course of disease and its prognosis.

However, there are several hypotheses and potential answers to this enigma. One answer, which has been favored in several articles, states that Ph- MPNs and CML represent two separate hematological diseases that arise from different clones of the cellular progenitors, in which case the more dominant clone will determine the disease phenotype [17]. Popescu and co-authors cited several papers supporting the second hypothesis of phenotypic diversity of patients, according to which *JAK2V617F* mutation remains positive and takes part in the suppression of *BCR-ABL1* positive clones [18]. Another hypothesis assumes the existence of a sub-clone

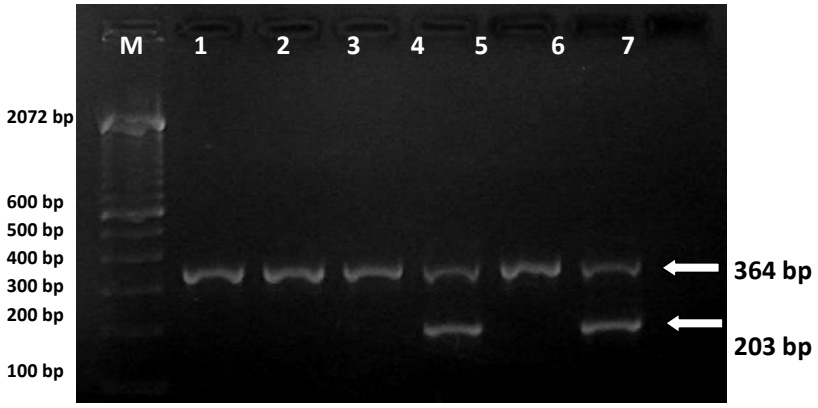

Fig. 4. Allele-specific PCR. Lines 1-3: tested patients; line 4: patient with ET- presence of the mutant allele sequence; line 5: healthy donor DNA sample ; line 6: positive control-Presence of the lower band (203bp) indicates the mutation is carried by the patient; the top band (364bp) acts as an internal PCR control; line 6: negative control (H_2O). M- MWM 100-2072bp (Life Technologies, USA)

of hematopoietic progenitor cells that acquire those two genetic anomalies within the same cell [12,19]. Furthermore, most often they occur simultaneously at diagnosis, but sometimes some of them appear as secondary changes as a result of therapeutic effect [7].

Campiotti et al. [20] give the hypothesis whereby tyrosine kinase inhibitors can cause regression of *JAK2* mutated clone as it does with Ph clone. They base their hypothesis on one patient diagnosed with CML/MPNs had recorded disappearance of *JAK2* mutations after achieving cytogenetic response to imatinib treatment. Probably, this is a sporadic case because in most other cases, imatinib does not affect the presence or absence of *JAK2* clone [9,10,12].

As previously mentioned, at the time of last follow-up, in our case, the *JAK2V617F* mutation is still detectable, but the number of *BCR-BL1* copies is in constant reducation. It seems more likely there are two independent clones initially detected at diagnosis, one sensitive to the therapy, while the other is resistant.

With regards to the presence of *BCR-ABL1* fusion transcript and Ph in myeloid malignancies other than CML, Park et al. [6] reported one interesting case, diagnosed to have ET with a major *BCR-ABL1* transcript, where a normal male karyotype with cryptic *BCR-ABL1* fusion on der (22) chromosome was found. To our knowledge, this is the first reported case diagnosed as having a rare masked Ph with *BCR-ABL1* fusion and the *JAK2V617F* point mutation at the same time.

4. CONCLUSION

In conclusion, we present a very rare case diagnosed to have ET with masked Ph, *BCR-ABL1* translocation and *JAK2V617F* mutation simultaneously. This is the first reported case from Bosnia and Herzegovina and the second reported case internationally.

Moreover, this case illustrates the importance of cytogenetic and molecular testing for *BCR-ABL1* and *JAK2V617F* mutation in patients with suspect MPNs, even in the absence of common CML clinical symptoms.

There is a crucial need for further follow-up especially in terms of monitoring these hematological chimeras, and assessing how this genetic combination can affect the course of disease and its prognosis.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this case report and accompanying images.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vardiman J, Thiele J, Arber D, Brunning R, Borowitz M, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood. 2009;114(5): 937-951.
2. Koopmans SM, van Marion AMW, Schouten HC. Myeloproliferative neoplasia: A review of clinical criteria and treatment. Neth J Med. 2012;70(4):159-165.
3. Goldman JM, Melo JV. Chronic myeloid leukemia-advances in biology and new approaches to treatment. N Engl J Med. 2003;349:1451-64.
4. Baxter EJ, Scott LM, Campbell P, East C, Forouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365(9464):1054-1061.
5. Steensma DP, Dewald GW, Lasho TL. The JAK2 V617F activating tyrosine kinase mutation is an infrequent eventh in both “atypical” myeloproliferative disorders and myelodisplastic syndromes. Blood. 2005; 106(4):1207-1209.
6. Park SH, Chi HS, Cho YU, Jang S, Park CJ, Kim DY, et al. Two cases of myeloproliferative neoplasm with a concurrent JAK2V617F mutation and BCR/ABL translocation without chronic myelogenous leukemia phenotype acquisition during hydroxyurea treatment. Ann Lab Med. 2013;33:229-232.
7. Carranza C, Tinti D, Herrera M, Rosales L, Villegas M, Silva G. Detection of Jak2 V617F mutation, secondary to the presence of Bcr-Abl1 translocation in a patient with chronic myeloid leukemia: Report of a case and review of the literature. Int J Genomic Med. 2014;116.
8. Yokus O, Kurnaz F, Balçık SO, Uz B, Albayrak M. Coexistance of JAK2V617F mutation and BCR/ABL translocation in one patient. J Clin Exp Invest. 2010;1(2): 138-140.
9. Pardini S, Fozza C, Contini S, Rimini E, Ottaviani E, Amabile M, et al. A case of coexistence between JAK2V617F and BCR/ABL. Eur J Hematol. 2008;81:75-6.
10. Cambier N, Renneville A, Cazaentre T, Soenen V, Cossement C, Giraudier S, et al. JAK2V617F-positive polycythemia vera and philadelphia chromosome-positive chronic myeloid leukemia: One patient with two distinct myeloproliferative disorders. Leukemia. 2008;22:1454–5.
11. Toogeh G, Ferdowsi S, Naadali F, Alimoghaddam K, Ghavamzadeh A, Shirkoohi R, Ghaffari SH. Concomitant presence of JAK2 V617F mutation and BCR-ABL translocation in a pregnant woman with polycythemia vera. Med Oncol. 2011;28:1555-1558.
12. Krämer A, Reiter A, Kruth J, Erben P, Hochhaus A, Müller M, et al. JAK2-V617F mutation in a patient with Philadelphia-chromosome-positive chronic myeloid leukaemia. Lancet Oncol. 2007;8:658-660.
13. Hummel JM, Kletecka MC, Sanks JK, Chiselite MD, Roulston D, Smith LB, et al. Concomitant BCR-ABL1 translocation and JAK2(V617F) mutation in three patients with myeloproliferative neoplasms. Diagn Mol Pathol. 2012;21:176–183. Go RS. Myeloproliferative disorders with coexisting BCR-ABL translocation and JAK2 V617F mutation. Leukemia. 2007;21:2051.
14. Virgili A, Brazma D, Reid GA, Howard-Reeves J, Valgañón M, Chanalaris A, et al. FISH mapping of Philadelphia negative BCR/ABL1 positive CML. Molecular Cytogenetics. 2008;1:14.
15. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate- phenol-chloroform extraction. Anal Biochem. 1987; 162(1):156-9.
16. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia. Blood. 2013;122:872–884.
17. Xu W, Chen B, Tong X. Chronic myeloid leukemia patient with co-occurrence of bcr-abl junction and jak2v617f mutation. Int J Hematol. 2014;99(1):87-90.
18. Popescu R, Dascalescu A, Danaila C, Gheorghiu D, Antohi I, Goriuc A, et al.

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19. Hussein K, Bock O, Theophile K, Seegers A, Arps H, Basten O, et al. Chronic myeloproliferative diseases with concurrent BCR-ABL junction and JAK2V617F mutation. Leukemia. 2008;22(5):1059-1062.

20. Campiotti L, Appio L, Solbiati F, Ageno W, Venco A. JAK2 V617F mutation and Philadelphia positive chronic myeloid leukemia. Leukemia Research. 2009;33: 212-3.

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