

MYELOPROLIFERATIVE NEOPLASMS WITH CONCURRENT BCR-ABL FUSION GENE AND JAK2V617F MUTATION

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Abstract: This study investigates the occurrence of BCR-ABL fusion gene and JAK2V617F mutation in myeloproliferative neoplasms (MPN) patients at diagnosis, in order to evaluate the clinical features, and compare them to the literature data. The study was conducted between January 2012 and February 2014 and included 190 cases of MPN from Regional Institute of Oncology Iasi. Molecular evaluation of BCR-ABL transcript and JAK2V617F mutation by RT-PCR, were assessed for diagnosis and monitorization. Only 2 patients showed simultaneous occurrence of both the JAK2V617F mutation and the BCR/ABL translocation. Patient 1 presented a complex clinical picture with clinical signs of CML and essential thrombocythemia, while the second patient had a clinical picture suggestive of polycythemia vera. The screening for the JAK2V617F mutation and BCR-ABL should be considered at MPN diagnosis. Following these patients up might provide new data regarding the long term evolution of such cases.

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of diseases characterized by the clonal proliferation of hematopoietic stem cells with the expansion of one or more myeloid lines. These diseases display typically increased number of medullar cells, maturation of cellular lines and organomegaly and are classified according to phenotypic and clinical characteristics and genetic abnormalities.

According to the data provided by the World Health Organization (WHO) in 2008, a classification of the major entities includes chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) (Vardiman et al., 2009).

CML is specifically associated with Philadelphia (Ph) chromosome resulting from the reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11) which lead to the formation of the fusion gene *BCR-ABL*.

JAK2 is a tyrosine kinase that has a major role in the signaling pathways of many hematopoietic growth factors receptors. In patients with BCR-ABL-negative MPN, it was identified a point mutation in the *JAK2* gene causing a substitution of a valine to phenylalanine at codon 617 which leads to a constitutive activation of JAK2 kinase and induces cellular proliferation and resistance to apoptosis. The discovery of the JAK2V617F mutation in 90% of PV and 50-60% of ET and PMF was the molecular evidence for the common pathogenesis of PV, ET and MF (Levine et al., 2005).

It was initially thought that *BCR-ABL* fusion gene and JAK2V617F mutation are mutually exclusive (Jelinek et al., 2005). Yet, in the last few years, some rare cases of patients with the coexistence of these two anomalies have been reported in the literature (Hussein et al., 2007, Inami et al., 2007), raising questions about their phenotypic and prognosis relevance.

This study aims to investigate the occurrence of this double mutated phenotype in MPN patients at diagnosis, to evaluate the clinical features, and compare them to data provided by literature.

MATERIALS AND METHODS

This study was conducted between January 2012 and February 2014 and included 190 MPN cases from Regional Institute of Oncology Iasi. Hematological parameters including complete blood picture and differential count were taken from patients' records. Bone marrow morphology, cytogenetic, molecular evaluation of BCR-ABL transcript and JAK2V617F mutation were assessed at diagnosis. Informed consent was signed by the patients under the protocol approved by the Ethics Committee of the University of Medicine and Pharmacy, Iasi.

Methods for quantitative assessment of BCR-ABL transcripts

The peripheral blood lymphocytes were isolated using red blood lysis solution (Promega Inc, Madison, WI, USA), then washed with phosphate buffer saline solution, and resuspended at a concentration of 2×10^7 cells in 1 mL of Guanidin Thiocyanate reagent (RNAzol® RT, Sigma-Aldrich, US). RNA was afterwards isolated according to the

manufacturer's instructions by alcohol precipitation, washing and solubilization. RNA was quantified using a NanoDrop 2000 (Thermo Fischer Scientific) spectrophotometer.

Reverse transcription

2 µg of RNA (4 µl of total RNA 500ng/µl) was used in a reverse transcription reaction using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The annealing, extension, and enzyme inactivation parameters were 25°C/ 5 min, 42°C/ one hour, and 70°C/ 5 min, respectively. cDNA was then diluted 1/5 (with 80 µl nuclease free water) in order to be used in PCR amplification.

Quantitative assessment of BCR-ABL expression

The assessment of BCR-ABL p210 transcript (b2a2, b3a2) was performed by quantitative Taqman Real Time PCR using Translocation Kit t(9;22)/M-BCR-RQ (Experteam, Italy) according to the manufacturer's instructions.

ABL gene transcripts were used as internal control and for the calculation of BCR-ABL/ABL ratio. Expression of BCR-ABL transcript was calculated as a ratio of BCR-ABL transcripts over ABL transcripts. Samples showing a Ct > 28 for ABL were excluded from the analysis.

The assessment of JAK2V617F mutation

DNA was extracted from peripheral blood using a Wizard Genomic DNA Purification kit (Promega, USA). The assessment of JAK2V617F mutation consisted of Taqman Real-Time PCR genotyping, using Clonit qualitative detection kit (Milano, Italy).

The 5µL DNA was amplified in a 25 µL reaction volume (12.5 µl Amplification mix and 7.5 µl JAK2 Probe mix - fluorescence FAM for wild type and VIC for mutant) using the Stratagene Mx3005P thermocycler (Agilent Technologies, Inc., USA). The allelic discrimination was performing making a scatter plot of mutated allele's fluorescence (y axis) versus wild type alleles' fluorescence (x axis) and discriminating this way the tree possible genotypes: homozygote wild-type (100% WT DNA), homozygote mutated (100% JAK2V617F DNA) and heterozygote mutated (reference control, limit of detection 2% JAK2V617F DNA).

RESULTS AND DISCUSSIONS

Out of the 190 patients in which both the *BCR-ABL* fusion gene and the *JAK2* mutation were evaluated, 94 patients (49.4%) displayed *JAK2V617F* mutation (79 heterozygote, 15 mutant) without the presence of *BCR-ABL* gene, 68 patients (35.8%) displayed wild type *JAK2* genotype and were negative for *BCR-ABL* and 26 patients (13.7%) tested negative for the *JAK2* mutation (wild type genotype) and positive for *BCR-ABL*. Only 2 patients showed simultaneous occurrence of both the *JAK2V617F* mutation and the *BCR/ABL* translocation, accounting for 1.05% of the total number.

Case 1 was a 62 year old female who was investigated in April 2013 for thrombocytosis after a thrombotic stroke. The first diagnosis assumption was essential thrombocythemia. The patient's laboratory findings at admission were as follows: hemoglobin - 14.2 g/dL; platelets- $579 \times 10^9/L$; white blood cells- $11.4 \times 10^9/L$ with a differential blood count revealing 79% segmented neutrophils, 4% eosinophils, 1% basophils, 12% lymphocytes, and 4% monocytes. The peripheral blood smear revealed erythrocyte anisocytosis, rare elliptocytes, platelet anisocytosis and enlarged (macro) platelets. Splenomegaly (160 mm) was detected by ultrasound at diagnosis.

The patient's GTG karyotype revealed the presence of Ph chromosome in all of twenty analyzed metaphase cells. Real Time PCR for the detection of *BCR/ABL* transcript proved positive for b2a2 type transcript.. The *BCR-ABL/ABL* ratio was 99.54% at diagnosis. The patient was found positive also for *JAK2V617F* heterozygous mutation. On the basis of these findings, the patient was diagnosed with *JAK2V617F*- and Ph-positive CML.

Starting with August 2013 treatment was initiated, consisting of Imatinib mesylat (IM) 400mg/day. After one month a complete hematologic response (CHR) was obtained, while a complete molecular response (CMR) was recorded after three months.

In February 2014, CHR was maintained, while the repeated cytogenetic examination revealed the occurrence of Ph chromosome in three of twenty analyzed metaphase cells

(46,XX[17]/46,XX,t(9;22)(q34;q11)[3]) and molecular analysis showed an increased level of BCR-ABL transcript (BCR-ABL/ABL ratio: 8.4%). The JAK2V617F heterozygous mutation remained present. The patient continued the treatment with IM 400mg/day.

In September 2014 (one year after the diagnosis) a complete blood count revealed hemoglobin 13.5g/L, normal white blood cells ($7.74 \times 10^9/L$) and slight thrombocytosis ($450 \times 10^9/L$). The differential blood count showed 73% segmented neutrophils, 3% eosinophils, 15% lymphocytes, 6% monocytes and 1% basophils. The first patient's evolution is indicated in figure 1.

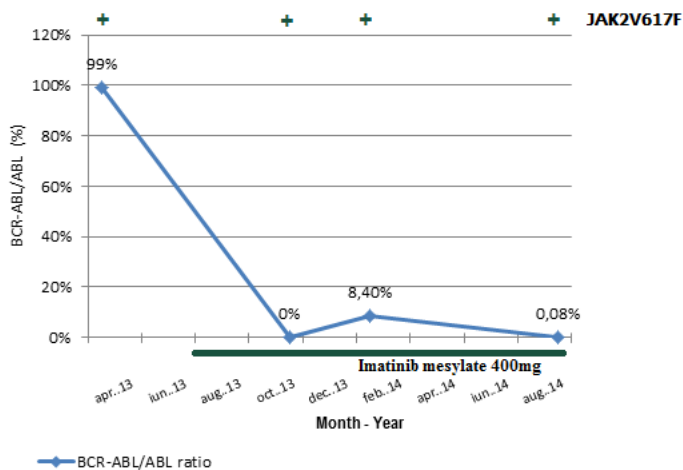


Figure 1. Evolution of BCR-ABL/ABL ratio and of the JAK2V617F mutation in Case 1;

Repeated molecular analyses indicated a major molecular response - MMR (BCR-ABL/ABL ratio 0.08%). The evaluation of the JAK2 mutation indicated that the mutation is still present as a heterozygous status (Fig. 2).

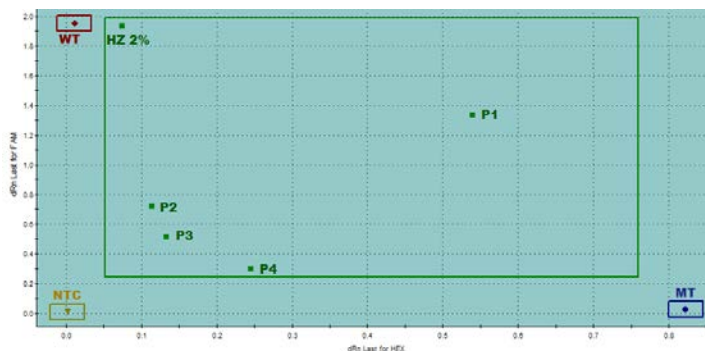


Figure 2. Real Time PCR aspect, with a scatter plot graphic obtained during the interpretation of the JAK2V617F mutation status for case 1; WT - homozygote wild-type, MT - homozygote mutated, HZ 2% - heterozygote mutated 2%, P1-P4 – Case 1 evaluations

Case 2 was a 45 year old male patient who presented dyspnea on moderate efforts and palpitations. A complete blood count revealed polycythemia (hemoglobin 19.8 g/L), normal white blood cells ($8.8 \times 10^9/L$) and normal platelet counts ($314 \times 10^9/L$). The differential blood count showed 87% segmented neutrophils, 3% eosinophils, 8% lymphocytes and 2% monocytes. The patient was found positive for the JAK2V617F heterozygous mutation and the BCR-ABL/ABL ratio was 2.6% at diagnosis. The patient was diagnosed with Polycythemia vera with a major BCR/ABL fusion transcript. No further data were available.

In this study, the occurrence of cases displaying BCR-ABL fusion gene and the JAK2V617F mutation was 1.05% (2/190). A similar result was reported by Cappetta et al., who analyzed 1320 cases of MPN suspicion and identified 5 patients (0.37%) with atypical MPN, who displayed both anomalies (Cappetta et al., 2013). A low frequency of the occurrence of JAK2V617F mutation was reported in Ph+ CML patients by Pieri et al. (2.25%, X/314) (Pieri et al., 2011) and Campiotti et al. (7.7%, 1/13) (Campiotti et al., 2009). A higher frequency of cases with both mutations was reported by two studies which included CML patients: 26.7% (12/45) (Pahore et al.) and 44% (11/25) (Tabassum et al., 2014, Pahore et al., 2011). The higher frequency reported in the two studies could be explained by means of the evaluated population and the different inclusion criteria. In order to determine the real frequency and the role the two anomalies play in determining the phenotype, further studies are required.

Patients can display variable, unspecific phenotype (leukocytosis, splenomegaly, as well as increased Ht, thrombocytosis), the evaluation of bone marrow biopsy being a valuable test in MPN differentiation. Initially they can display a suggestive phenotype for Ph- MPN, most frequently PV (Mirza et al., 2007, Bocchia et al., 2007, Pingali et al., 2009, Ursuleac et al., 2013) or PMF (Bornhauser et al., 2007, Jallades et al., 2008) and more seldom ET (Curtin et al 2005), associated with BCR-ABL positive CML throughout the evolution of the disease. Another category is represented by those who display specific CML phenotype and later develop Ph-MPN, PV (Tefferi et al., 2010, Inami et al., 2007, Cambier et al., 2008), PMF (Hussein et al., 2007, Kramer et al., 2007) or TE (Pastore et al., 2013, Veronese et al., 2010, Lee et al., 2013) (Supplemental material 1). There is no connection between the treatment administered prior to diagnosis and the occurrence of CML or Ph- MPN clinical signs in these patients.

From the perspective of the two anomalies, the JAK2V617F mutation might precede the acquisition of the BCR-ABL fusion gene (Bocchia et al., 2007, Jallades et al., 2008, Bornhauser et al., 2007, Mirza et al., 2007), it can follow it (Tefferi et al., 2010, Nadali et al., 2009) or, even more frequently, the two anomalies might appear at the same time (Inami et al., 2007, Cambier et al., 2008, Hussein et al., 2008, Kramer et al., 2007, Hussein et al., 2007, Pahore et al., 2011). The retrospective evaluation of the JAK2V617F mutation, at the CML diagnosis, has proved its presence, concomitantly with the BCR-ABL fusion gene, in 8 cases reported in the literature (Supplemental material 1).

Generally, the occurrence of the BCR-ABL fusion gene changes the phenotype in CML, which sustains the hypothesis of an advantage of this clone in determining the phenotype. During the evolution there seems to be a phenomenon of competition between the proliferations of the two abnormal clones, so that the mutant JAK2V617F clone decreases or disappears when the BCR-ABL transcript increases and reappears possibly in a dominant position, with clinical manifestations, when the BCR-ABL+ clone is inhibited by the TKI treatment (Inami et al., 2007, Cambier et al., 2008, Hussein et al., 2008, Kramer et al., 2007).

The two patients presented in detail in this study displayed both anomalies concomitantly at diagnosis, yet had different phenotypes.

Patient 1 displayed a phenotype which was untypical for CML (unspecific blood smear, discrete leukocytosis without left deviation in the leukocyte formula, without myeloblasts, without basophilia, despite displaying a high level of expression of the BCR-ABL at diagnosis) associating specific TE elements. Cases associating CML and TE phenotype are rare. Payande et al. reported one case of co-existence of the JAK2V617F mutation and the BCR-ABL fusion gene at diagnosis, the patient displaying intricate phenotype of CML and TE (leukocytosis, thrombocytosis, anemia, high LDH level) (Payande et al., 2011). Lee et al. reported two cases diagnosed with CML, where the phenotype evolved towards TE after 119 and respectively 30 months after the occurrence of the JAK2V617F mutation. Other two cases are reported with CML diagnosis, which displayed low levels of the JAK2 mutation at diagnosis (0.12 and 0.2%, respectively), the TE phenotype appearing at 67 and respectively 6 months, after the levels of the JAK2V617F mutation increased up to 10% and 9% respectively (Supplemental material 1) (Veronese et al., 2010, Pastore et al., 2013).

As far as response to treatment is concerned, in most reported patients the suppression of the positive BCR-ABL clone was obtained under TKI treatment (complete cytogenetic response - CCyR) or MMR/CMR), yet the hematologic response was partial or medullar fibrosis progression was reported. In most cases, IM did not affect the co-existence or the acquisition of the JAK2V617F clone (Inami et al., 2007, Hussein et al., 2007, Kramer et al., 2007, Veronese et al., 2010, Cambier et al., 2008, Pardini et al., 2008). In the case of the first patient presented in this study, the response to the treatment with IM 400 mg/day was very good, CHR and CMR being achieved within three months. A slight increase of the transcript was recorded afterwards, MMR (BCR-ABL/ABL 0.08%) being maintained after 9 months. Hematologic parameters kept within normal limits except a slight thrombocytosis.

Case 2 was diagnosed with PV without phenotypic alterations specific to CML, the BCR-ABL/ABL ratio being as reduced as 2.6%, which provides an explanation for the lack of CML clinical manifestations. Only two reported cases display the BCR-ABL fusion gene (with reduced levels of expression) without clinical signs of CML throughout the Ph- MPN evolution (Park et al., 2013, Bornhauser et al., 2007). Unfortunately, there is no data available about the further evolution of the patient. Another case of BCR-ABL and JAK2V617F co-existence was described in our country in a patient diagnosed with PV, who developed a CML phenotype after 7 years of disease progression. The JAK2V617F mutation was identified after 24 months of IM treatment, when the Ph+ clone was no longer detectable (Ursuleac et al., 2013).

Two hypotheses were proposed for explaining the presence of JAK2V617F and the BCR-ABL fusion in the same patient.

The first hypothesis states that Ph- MPN and CML are two separate diseases, developed from different clones of the cellular progenitors, the phenotype being determined by the dominant clone. This hypothesis is supported by both the phenotypic heterogeneity of the patients and the fact that the JAK2V617F mutation stays positive, constant or it increases at the suppression of the BCR-ABL+ clone (Hussein et al., 2008, Xu et al., 2014, Veronese et al., 2010, Bee et al., 2010, Pastore et al., 2013). We do not exclude the possibility that the two anomalies superpose independently over an unknown founding mutation capable of generating genomic instability at the hematopoietic stem cell level (Tefferi et al., 2010, Hussein et al., 2007, Inokuchi et al., 2012, Nadali et al., 2009).

The second hypothesis supposes the existence of a single sub-clone of hematopoietic progenitor cells that acquire the two anomalies within the same cell, at different moments. The concomitant presence of the two anomalies at the moment of CML diagnosis and the

maintenance of a constant level of the JAK2V617F mutation arguments in favor of the occurrence of the JAK2V617F mutation as an initial step, a sub-clone acquiring the BCR-ABL translocation afterwards, the latter providing a proliferative advantage to the double mutant clone. The suppression of the BCR-ABL bearing clone after the TKI treatment allows the proliferation of positive JAK2V617F cells, allowing for the clinical manifestation of the Ph-MPN. (Bornhauser et al., 2007, Bocchia et al., 2007, Jallades et al., 2008, Kramer et al., 2007, Hussein et al., 2008, Inami et al., 2007). This hypothesis is supported by a few studies on cellular cultures from patients presenting the two anomalies. The JAK2V617F mutation is identified in myeloid progenitor cells, mostly BFU-E (erythroid burst-forming units) and CFU-GM (colony-forming unit granulocyte macrophage) alone or together with the BCR-ABL transcript, yet none of the colonies display the BCR-ABL gene in isolation (Bocchia et al., 2007, Wang et al., 2013). After the IM treatment, when MMR is achieved, most colonies display JAK2V617F and none of them display BCR-ABL (Bocchia et al., 2007). Bornhauser et al. notes both mutations in a limited number of granulocyte colonies, while the JAK2 mutation is identified in both types of colonies (erythroid and granulocyte). This aspect does not exclude the existence of the initial mutation that leads to the acquisition of several genetic anomalies (Kramer et al., 2007, Bocchia et al., 2007).

In both cases reported in this study, the JAK2V617F mutation was detectable in heterozygous status, at the diagnosis, together with the *BCR-ABL* fusion gene and remained positive during the whole evolution of the disease in patient 1. Unfortunately there is no data regarding the evolution of the second patient. Clinically, the first patient displayed intricate CML and TE signs, while the second patient's phenotype was PV (decreased expression of BCR-ABL). This fact supports the second hypothesis, namely of the co-existence of the two mutations in the same clone, with the occurrence of BCR-ABL in a sub-clone of positive JAK2V617F cells, the latter gaining proliferative grounds. However, we cannot exclude the possibility of the existence of two different mutant clones.

The evolution of CML does not seem to be influenced by the presence of the JAK2V617F mutation, but it can be a rare cause of the lack of hematologic response to ITK treatment (Xu et al., 2014). The differentiation between relapse, disease progression or IM resistance and the superposition of a MPN Ph- can be done by means of evaluating both anomalies in these patients. Similarly, the occurrence of clinical signs of CML in patients displaying mutant JAK2V617F indicates the evaluation of the presence of BCR-ABL fusion gene, in order to initiate the TKI treatment.

CONCLUSIONS

Besides other cases described in literature, the cases presented by us support the co-existence of the *BCR-ABL* gene and the JAK2V617F mutation in the same patient. The screening for the JAK2V617F mutation and BCR-ABL should be considered at the very first suspicion of a MPN diagnosis, as well as in the case of patients with a CML diagnosis who keep developing myeloproliferations despite obtaining MMR.

Following up patients with JAK2V617F mutation and BCR-ABL in clinical trials might provide new data regarding the long term evolution of such cases.

REFERENCES

- Bee, P. C., Gan, G. G., Nadarajan, V. S., Latiff, N. A., et al., (2010): *A man with concomitant polycythemia vera and chronic myeloid leukemia: The dynamics of the two disorders*. Int J Hematol, 91(1), 136-139.
- Bocchia, M., Vannucchi, A. M., Gozzetti, A., Guglielmelli, P., et al., (2007): *Insights into jak2-v617f mutation in cml*. Lancet Oncol, 8(10), 864-866.
- Bornhauser, M., Mohr, B., Oelschlaegel, U., Bornhauser, P., et al., (2007): *Concurrent jak2(v617f) mutation and bcr-abl translocation within committed myeloid progenitors in myelofibrosis*. Leukemia, 21(8), 1824-1826.
- Cambier, N., Renneville, A., Cazaent, T., Soenen, V., et al., (2008): *Jak2v617f-positive polycythemia vera and philadelphia chromosome-positive chronic myeloid leukemia: One patient with two distinct myeloproliferative disorders*. Leukemia, 22(7), 1454-1455.
- Campiotti, L., Appio, L., Solbiati, F., Ageno, W., et al., (2009): *Jak2-v617f mutation and philadelphia positive chronic myeloid leukemia*. Leuk Res, 33(11), e212-213.
- Cappetta, M., Perez, V., Zubillaga, M. N., Elizondo, V., et al., (2013): *Concomitant detection of bcr-abl translocation and jak2 v617f mutation in five patients with myeloproliferative neoplasm at diagnosis*. Int J Lab Hematol, 35(1), e4-5.
- Hussein, K., Bock, O., Seegers, A., Flasshove, M., et al., (2007): *Myelofibrosis evolving during imatinib treatment of a chronic myeloproliferative disease with coexisting bcr-abl translocation and jak2v617f mutation*. Blood, 109(9), 4106-4107.
- Hussein, K., Bock, O., Theophile, K., Seegers, A., et al., (2008): *Chronic myeloproliferative diseases with concurrent bcr-abl junction and jak2v617f mutation*. Leukemia, 22(5), 1059-1062.
- Inami, M., Inokuchi, K., Okabe, M., Kosaka, F., et al., (2007): *Polycythemia associated with the jak2v617f mutation emerged during treatment of chronic myelogenous leukemia*. Leukemia, 21(5), 1103-1104.
- Inokuchi, K., Yamaguchi, H., Tamai, H., Dan, K., (2012): *Disappearance of both the bcr/abl fusion gene and the jak2v617f mutation with dasatinib therapy in a patient with imatinib-resistant chronic myelogenous leukemia*. J Clin Exp Hematop, 52(2), 145-147.
- Jallades, L., Hayette, S., Tigaud, I., Johnston, A., et al., (2008): *Emergence of therapy-unrelated cml on a background of bcr-abl-negative jak2v617f-positive chronic idiopathic myelofibrosis*. Leuk Res, 32(10), 1608-1610.
- Jelinek, J., Oki, Y., Gharibyan, V., Bueso-Ramos, C., et al., (2005): *Jak2 mutation 1849g>t is rare in acute leukemias but can be found in cmml, philadelphia chromosome-negative cml, and megakaryocytic leukemia*. Blood, 106(10), 3370-3373.
- Kramer, A., Reiter, A., Kruth, J., Erben, P., et al., (2007): *Jak2-v617f mutation in a patient with philadelphia-chromosome-positive chronic myeloid leukaemia*. Lancet Oncol, 8(7), 658-660.
- Lee, Y. J., Moon, J. H., Shin, H. C., Seo, J. W., et al., (2013): *Two cml patients who subsequently developed features of essential thrombocythemia with jak2-v617f mutation while in complete cytogenetic remission after treatment with imatinib mesylate*. Int J Hematol, 97(6), 804-807.
- Levine, R. L., Wadleigh, M., Cools, J., Ebert, B. L., et al., (2005): *Activating mutation in the tyrosine kinase jak2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis*. Cancer Cell, 7(4), 387-397.
- Mirza, I., Frantz, C., Clarke, G., Voth, A. J., et al., (2007): *Transformation of polycythemia vera to chronic myelogenous leukemia*. Arch Pathol Lab Med, 131(11), 1719-1724.
- Nadali, F., Ferdowsi, S., Karimzadeh, P., Chahardouli, B., et al., (2009): *Jak2-v617f mutation and philadelphia positive chronic myeloid leukemia*. IJHOSCR, 3(3), 43-45.
- Pahore, Z. A., Shamsi, T. S., Taj, M., Farzana, T., et al., (2011): *Jak2v617f mutation in chronic myeloid leukemia predicts early disease progression*. J Coll Physicians Surg Pak, 21(8), 472-475.
- Pardini, S., Foza, C., Contini, S., Rimini, E., et al., (2008): *A case of coexistence between jak2v617f and bcr /abl*. Eur J Haematol, 81(1), 75-76.
- Park, S. H., Chi, H. S., Cho, Y. U., Jang, S., et al., (2013): *Two cases of myeloproliferative neoplasm with a concurrent jak2 (v617f) mutation and bcr/abl translocation without chronic myelogenous leukemia phenotype acquisition during hydroxyurea treatment*. Ann Lab Med, 33(3), 229-232.
- Pastore, F., Schneider, S., Christ, O., Hiddemann, W., et al., (2013): *Impressive thrombocytosis evolving in a patient with a bcr-abl positive cml in major molecular response during dasatinib treatment unmasks an additional jak2v617f*. Exp Hematol Oncol, 2(1), 24.
- Payande, M., Zare, M. E., Shure, S. G. H., Zadeh, F. S., (2011): *Jak2-v617f mutation combined with philadelphia chromosome-positive chronic myeloid leukaemia: A case report*. International Journal of Hematology-Oncology and Stem Cell Research, 5(2), 34-37.
- Pieri, L., Spolverini, A., Scappini, B., Occhini, U., et al., (2011): *Concomitant occurrence of bcr-abl and jak2v617f mutation*. Blood, 118(12), 3445-3446.

- Pingali, S. R., Mathiason, M. A., Lovrich, S. D., Go, R. S.,** (2009): *Emergence of chronic myelogenous leukemia from a background of myeloproliferative disorder: Jak2v617f as a potential risk factor for bcr-abl translocation.* Clin Lymphoma Myeloma, 9(5), E25-29.
- Tabassum, N., Saboor, M., Ghani, R., Moinuddin, M.,** (2014): *Frequency of jak2 v617f mutation in patients with philadelphia positive chronic myeloid leukemia in pakistan.* Pak J Med Sci, 30(1), 185-188.
- Tefferi, A., Levitt, R., Lasho, T. L., Knudson, R. A., et al.,** (2010): *Postimatinib therapy emergence of a new jak2v617f clone and subsequent development of overt polycythemia vera in a patient with chronic myelogenous leukaemia.* Eur J Haematol, 85(1), 86-87.
- Ursuleac, I., Colita, A., Adam, T., Jordan, C., et al.,** (2013): *The concomitant occurrence of jak2v617f mutation and bcr/abl transcript with phenotypic expression - an overlapping myeloproliferative disorder or two distinct diseases? - case report.* J Med Life, 6(1), 34-37.
- Vardiman, J. W., Thiele, J., Arber, D. A., Brunning, R. D., et al.,** (2009): *The 2008 revision of the world health organization (who) classification of myeloid neoplasms and acute leukemia: Rationale and important changes.* Blood, 114(5), 937-951.
- Veronese, L., Tchirkov, A., Richard-Pebrel, C., Ledoux-Pilon, A., et al.,** (2010): *A thrombocytosis occurring in philadelphia positive cml in molecular response to imatinib can reveal an underlying jak2(v617f) myeloproliferative neoplasm.* Leuk Res, 34(4), e94-96.
- Wang, X., Tripodi, J., Kremyanskaya, M., Blouin, A., et al.,** (2013): *Bcr-abl1 is a secondary event after jak2v617f in patients with polycythemia vera who develop chronic myeloid leukemia.* Blood, 121(7), 1238-1239.
- Xu, W., Chen, B., Tong, X.,** (2014): *Chronic myeloid leukemia patient with co-occurrence of bcr-abl junction and jak2 v617f mutation.* Int J Hematol, 99(1), 87-90.

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Supplemental material 1: Characteristics of reported cases with JAK2V617F MPN discovered in initially diagnosed Ph+ CML.

Authors	Age (years) /sex	MPN Ph- Second diagnosis (time to Ph- MPN-mo)	JAK2V617F mutated allele		Chronology of molecular findings	Treatment	Evolution of BCR-ABL and JAK2 V617F mutation
			At CML diagnosis	At MPN diagnosis			
Veronese et al. 2010	82/F	PMF (12)	0.5%	92%	concomitant (retrospective)	HUR, IM, hydroxycarbamide, salicylic acid	MMR/NA
	62/M	ET (6)	0.2%	9%	concomitant	IM, salicylic acid	MMR/NA
Conchon et al. 2008	52/F	PMF (0)	High levels	High levels	concomitant	HUR, IM	Good response/positive
Cambier et al. 2008	64/M	PV (0)	High levels	High levels	concomitant	IM, phlebotomy	MMR/positive
Inami et al. 2007	43/M	PV(>6a)	20%	20%	concomitant (retrospective)	INF α , HUR, IM	CCyR/positive
Inokuchi et al. 2012						flebotomy, dasatinib	CMR/negative
Hussein et al. 2008	32/M	Ph- MPN (44)	-	21	BCR then JAK2	anagrelide, HUR,IM	CMR/positive
	58F/	PMF (12)	ND	23	JAK2 ND at diagnosis	HUR, INF α	NA/positive
	45/M	No Ph- MPN (0)	43	43	concomitant	NA	CMR/positive
Hussein et al.2007	55/M	PMF	5%	23%	concomitant (retrospective)	IM	CMR/positive
Krämer et al. 2007	50/M	PMF (>3a)	35%	≈ 50%	concomitant (retrospective)	Hydroxycarbamide, IM	CMR/positive
Caocci et al 2010	70/M	Ph- MPN (84)	ND	+	JAK2 ND at diagnosis	INF α , IM	CCyR/positive
Lee et al. 2013	53/M	TE (119)	-	+	BCR then JAK2	HUR, INF α ,IM; anagrelide and HUR,	MMR/positive
	60/F	TE (30)	-	+	BCR then JAK2	IM, nilotinib, anagrelide	MMR/positive
Payande et al.2011	82/M	TE (0)	+	+	concomitant	HUR, IM, INF α	NA
Nadali et al. 2009	39/M	No MPN Ph-(48)	ND	+	BCR then JAK2	HUR,IM	NA
Campiotti et al. 2009	na	No MPN Ph- (0)	12%	-	concomitant	IM	PCyR/negative
Pastore et al. 2013	42/F	TE(67)	0.122	>10	concomitant (retrospective)	HUR, IM, dasatinib	MMR/positive
Pardini et al. 2008	67/M	PMF	HZ	+	concomitant (retrospective)	IM,HUR	MMR/positive
Xu et al. 2014	21/F	No MPN Ph- (0)	+	+	concomitant	IM	CMR/positive
Kim et al. 2008	49/M	PMF (6)	77.3%	>50%	concomitant (retrospective)	IM, dasatinib	CMR/positive
	64/M	PMF (0)	61%	61%	concomitant (retrospective)	IM, nilotinib	CCyR /positive

MPN - myeloproliferative neoplasms; PV - polycythemia vera, ET - essential thrombocythemia, PMF - primary myelofibrosis, NA - not available, ND – not determined; IM- imatinib mesylate, HUR- hydroxiurea, INF α - Interferon alfa., CMR- complete molecular response, MMR- major molecular response, CCyR- complete cytogenetic response

