Med & Health Jun 2023; 18(1): 233-240 https://doi.org/10.17576/MH.2023.1801.21

CASE REPORT

Concomitant *BCR-ABL* and *JAK2 V617F* in a Patient with Myeloproliferative Neoplasm: A Case Report

ALIA SUZANA A[1], AZLIN I[1], HAFIZA A[1], NOR RAFEAH T[3], SITI MARIAM Y[2], NORUNALUWAR J[2], SALWATI S[1], AZMA RZ[1]

[1]Department of Pathology, [2]Laboratory Diagnostic Services, and [3]Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak 56000 Cheras, Kuala Lumpur, Malaysia

ABSTRAK

Neoplasma myeloproliferatif (MPN) ialah barah darah yang menyebabkan aktiviti isyarat tyrosine kinase diaktifkan. MPN dikelaskan kepada BCR-ABL positif leukemia myeloid kronik (CML) dan BCR-ABL negatif MPN yang mempunyai mutasi JAK2 V617F dalam kebanyakan kes. Gabungan genetik mutasi BCR-ABL dan JAK2 V617F jarang berlaku dengan anggaran kekerapan 0.4% berdasarkan kajian terkini. Di sini, kami melaporkan kes seorang lelaki yang didiagnosis dengan CML yang dikesan oleh teknik sitogenetik molekular (FISH) menunjukkan corak gabungan BCR-ABL atipikal dalam 29% sel bernukleus dengan kehadiran mutasi JAK2 V617F. Walau bagaimanapun, transkrip BCR-ABL tidak dikesan oleh kaedah tindak balas rantai transkripase-polimerase konvensional (RT-PCR). Respon dari segi parameter hematologi tidak dicapai pada skala masa yang ditetapkan walaupun dengan rawatan ubat Imatinib. Kesimpulannya, hal ini adalah penting untuk meneliti kes-kes CML untuk mutasi JAK2 V617F terutamanya pesakit dengan penemuan atipikal dari aspek klinikal atau makmal. Oleh itu, pemantauan rapi dengan teknik klinikal dan sampingan terutamanya FISH dan kaedah molekular seperti penjujukan DNA adalah penting untuk membantu mencapai respon hematologi, sitogenetik dan molekular yang lengkap melalui terapi yang jitu.

Kata kunci: BCR ABL, hibridisasi in situ berpendaflour, Janus Kinase 2, neoplasma myeloproliferatif, tindak balas rantai polimerase

ABSTRACT

Myeloproliferative neoplasm (MPN) is a clonal proliferation of the haematopoietic

Address for correspondence and reprint requests: Dr Alia Suzana Asri. Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-91748320 Email: aliasuzana@ukm.edu.my

stem cells leading to activated tyrosine kinase signaling activity. Myeloproliferative neoplasm is classified into *BCR-ABL* positive chronic myeloid leukemia (CML) and *BCR-ABL* negative MPN which harbors *JAK2 V617F* mutation in most cases. The genetic combination of *BCR-ABL and JAK2 V617F mutation* is rare with estimated frequency of 0.4% based on recent study. Herein, we reported a case of a man diagnosed with CML detected by fluorescence in-situ hybdridisation (FISH) showing atypical *BCR-ABL f*usion pattern in 29% nucleated cells (cut-off levels ≥5% for positive signals) in the presence of *JAK2 V617F* mutation. However, the *BCR-ABL* transcript was not detected by the conventional reverse transcriptase-polymerase chain reaction (RT-PCR) method which was specific for major fragments. Interestingly, complete hematological remission was not achieved despite initiation of tyrosine kinase inhibitor (Imatinib). In conclusion, it is imperative to scrutinise CML cases for concomitant *JAK2 V617F* mutation especially patients with atypical clinical or laboratory findings. Therefore, close monitoring with clinical and ancillary technique especially FISH and molecular methods such as DNA sequencing were crucial to help achieve complete hematological, cytogenetic and deep molecular response alongside targeted therapy.

Keywords: *BCR-ABL*, fluorescence in-situ hybridisation, Janus kinase 2, myeloproliferative neoplasm, polymerase chainreaction

INTRODUCTION

Myeloproliferative neoplasm (MPN) is a clonal proliferation of one or more of myeloid lineage in the bone marrow. According to the World Health Organisation (WHO), MPN is classified into *BCR-ABL* positive chronic myeloid leukemia (CML) and *BCR-ABL* negative MPN (Ph-MPN) which are polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) (Swerdlow et al. 2017). Chronic myeloid leukemia is caused by reciprocal translocation of chromosomes 9 and 22 resulting in the characteristic Philadelphia chromosome (Ph^+) forming *BCR-ABL* fusion oncoproteins which leads to autonomous cellular proliferation via activation of tyrosine kinase.

The Ph^- MPN usually involves Janus Kinase 2 (*JAK2*) *V617F* mutation with approximately more than 90% occurrence in PV and to a lesser extent, 50% of all cases of MF and ET (Swerdlow et al. 2017). Janus kinase proteins are responsible for cellular signaling. However, due to somatic mutation of JH2 domain in *JAK2* there is *V617F* substitution rendering constitutional activation of the JAK/STAT pathway causing uninhibited cell proliferation and survival (Hubbard 2018). Previous studies suggested that *JAK2 V617F* and *BCR-ABL* mutations were mutually exclusive (Pastore et al. 2013). Recent reports described some paradoxical coincidence of these two mutations (Bader & Dreiling 2019).

This case report highlighted a rare case of MPN with coexistence of *BCR-ABL* and *JAK2 V617F* mutation.

CASE REPORT

The patient was a 70-year-old man with known hypertension, dyslipidaemia and ischemic heart disease, presented with intermittent fever, weight loss and lethargy for a year. Clinical examination showed splenomegaly with no hepatomegaly or lymphadenopathy. His abdominal computed tomography (CT) scan demonstrated marked splenomegaly (22.7 cm).

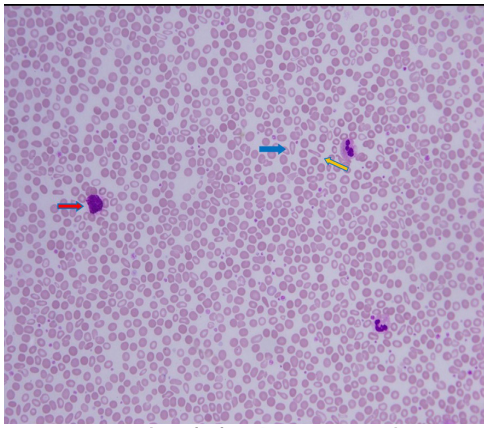

Figure 1: Peripheral blood smear (Wright's stain, 400x magnification) showed hypochromic microcytic cells with anisopoikilocytosis, tear drop (blue arrow), and pencil cells (yellow arrow). Basophils were easily seen (red arrow).

Full blood count (FBC) showed high red blood cell count (7.9 x 10^{12}/L), raised HCT (52.2%), normal hemoglobin level (15.2 g/dL) with microcytic indices (MCV 66.1 fl, MCH 19.2 pg), leukocytosis (15.3 x 10^{9}/L) and mild thrombocytosis (432 x 10^{9}/L). The peripheral blood smear revealed leukoerythroblastic picture with anisopoikilocytosis and hypochromic microcytic erythrocytes, pencil cells, tear drop cells and occasional target cells (Figure 1). White cell features included leukocytosis with neutrophilia, basophilia and occasional myelocytes with no blast cells was seen. Platelets increased with presence of large and giant forms.

Bone marrow aspiration and trephine biopsy (Figure 2) demonstrated hypercellular marrow with trilineage hyperplasia and grade 2 to 3 fibrosis (Swerdlow et al. 2017). The granulocytic series was increased with prominent eosinophilia. There was increased thickness of the immature myeloid cells of 5 to 6 cell thickness in the paratrabecular

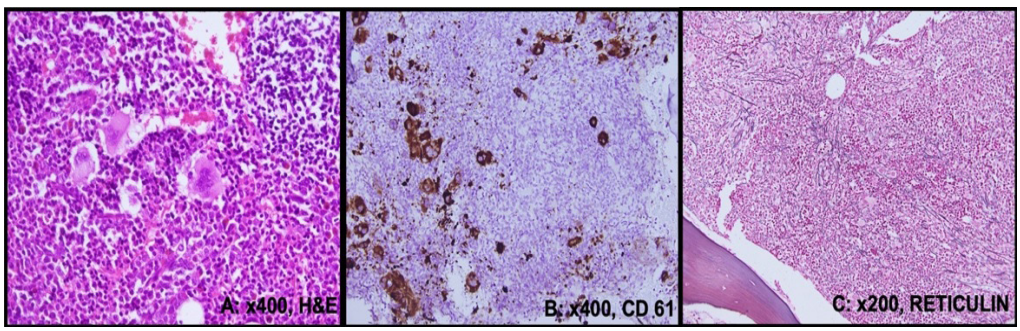

Figure 2: Trephine section. (A and B) Megakaryocytes and dwarf megakaryocytes were frequent, arranged in clusters highlighted by CD61 stain. (C) Grade 2 to 3 fibrosis was highlighted by reticulin stain

region in contrast to the normal 2 to 3 cell layers (Swerdlow et al. 2017). Megakaryocytes were frequent and arranged in clusters whilst some showed dwarf megakaryocytes and there was no excess of blasts seen. A diagnosis of MPN with fibrosis was made at this point of time.

His molecular cytogenetic study showed presence of *BCR-ABL* rearrangement in 29% of the cells after analysed by fluorescence *in situ* hybridisation (FISH) (Figure 3) on 200 nuclei which was sufficient to diagnose CML. This FISH signal pattern was unlike the common pattern, with one green, one red and two yellow signals found in CML. On the contrary, this pattern may suggest a variant type of deletion of the *BCR* and *ABL* fusion. Furthermore, conventional karyotyping showed normal male karyotype (46, XY) with only one metaphase spread available.

Interestingly, the peripheral blood for reverse transcriptase polymerase chain reaction (RT-PCR) *BCR-ABL* fusion gene was negative whilst his peripheral blood for *JAK2 V617F* mutation was positive (Figure 4). Venesection was performed followed by regular oral Imatinib 400 mg daily and Hydroxyurea. After three months of Imatinib and Hydroxyurea initiation, the completehematological remission was not attained despite compliance to therapy. Fortunately, the cytogenetic remission was finally achieved after two years of treatment which was monitored via FISH using peripheral blood. Polymerase chain reaction method for monitoring purpose was not utilised in view ofno primers available for the variant *BCR-ABL* transcript.

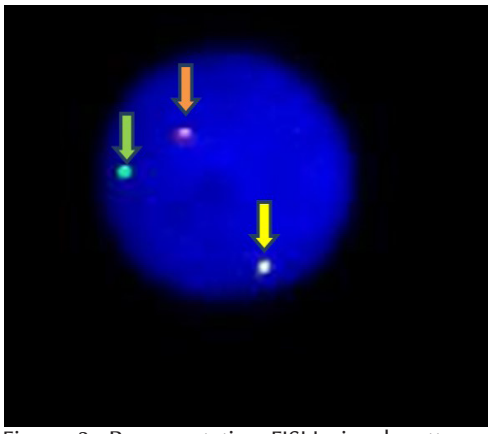

Figure 3: Representative FISH signal patterns using Vysis LSI BCR/ABL1 Dual Colour Dual Fusion Translocation Probe. One (1) green (green arrow), one (1) orange (orange arrow) and one (1) fused green (yellow arrow) signal pattern were seen in the nuclei

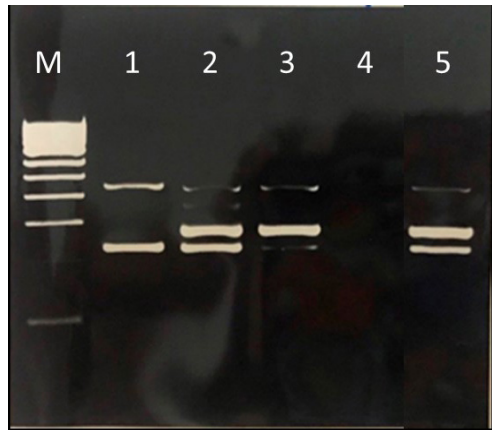

Figure 4: *JAK2 V617F* mutation study using allele-specific PCR: Lane M was the marker, lane 1 was *JAK2 V617F* (wild type) having an amplified product of 229 base pair, lane 2 was the positive control (heterozygous), lane 3 was positive control (homozygous) and lane 4 was negative control. Lane 5 was the patients's result showing heterozygous *JAK2 V617F* mutation with amplified DNA of 229 base pair and 279 base pair

DISCUSSION

This case depicted a rare clinical entity in the MPN spectrum. World Health Organisation classified MPN into 2 major groups; (i) CML which is *Ph/BCR-ABL* positive MPN, and (ii) *Ph/BCR-ABL* negative MPN mostly involving *JAK2 V617F* mutation (Swerdlow et al. 2017). The hallmark of CML is the Philadephia chromosome caused by reciprocal translocation of chromosomes 9 and 22 resulting in the formation of *BCR-ABL* fusion oncogene and later oncoproteins p190, p210, or p230 depending on the breakpoints. In CML, the most commonly found oncoprotein is p210 transcript also known as the major fragments (Mousinho et al. 2018). This fusion oncoprotein leads to ABL tyrosine kinase activation causing unopposed proliferation of the immature and mature granulocytes leading to triphasic clinical course (chronic, accelerated, blast phase) if left untreated (Swerdlow et al. 2017).

On the contrary, the leukemogenic event of the Ph-negative MPN is the somatic point mutations in *JAK2* gene, specifically JH2 domain with *V617F* mutation comprising approximately 95% of cases of PV and 50% cases of ET and MF. The *JAK2 V617* mutation activated the JAK/STAT pathway leading to signal transduction, activating transcription thus promoting the cellular proliferation and restricted apoptosis of the affected cell lineages (Hubbard 2018).

The prevalence of double mutation of *BCR-ABL* and *JAK2 V617F* is 0.4% based on a multi-institutional study from the Bone Marrow Pathology Group (Soderquist et al. 2018). There have been reports illustrating the double mutation of *BCR-ABL* and *JAK2 V617F* may arise from a single cell clone (Hubbard 2018). However, others reported that there are independent clones harboring each mutation. Nonetheless, there is no definitive clonal analysis done to prove the theory (Bader & Dreiling 2019).

On a clinical note, patient's symptoms were non-specific to MPN subtypes. The patient's initial manifestation was mainly constitutional with striking splenomegaly. Typically, CML patients are detected during chronic phase upon routine medical screening with hyperleukocytosis. To a lesser extent, some will present at accelerated or blast phase with anemic symptoms, leukostasis, infection, or bleeding. Major symptoms of PV include hyperviscosity syndrome including cardiovascular diseases such as stroke. Whilst in ET, usual presentation is asymptomatic with persistent thrombocytosis or symptoms of thrombosis or hemorrhage.

Based on previous studies, double mutation MPN typically affects elderly population as in our case. According to Hummel et al. (2012) study, two out of three patients with double mutations were detected during routine medical check-up with persistent thrombocytosis and moderate leukocytosis leading to hematological review and MPN investigation. Imaging also revealed significant splenomegaly. Similarly, a case series by Mousinho et al. (2018) on two patients with double mutations showed that they were asymptomatic during their initial

presentation and both of them were under the investigation for MPN due to sustained moderate thrombocytosis and leukocytosis. Interestingly, both patients did not have splenomegaly as evidenced by imaging modalities.

Morphologically, it was difficult to distinguish the specific types of MPN which explained why the case was concluded as MPN with fibrosis. Moreover, the cytogenetic and molecular results of the patient were not available upon morphological evaluation. There were no classical atypical bizarre megakaryocytes commonly found in ET. Nonetheless, the panmyelosis and megakaryocytic changes may suggest PV. However, the presence of fibrosis in this case cannot be attributed to either the end spectrum of MPN subtypes for instance Post PV-MF, Post ET-MF or fibrosis following CML because there was no prior documentation. One of the morphological hints for possible coexistence of *BCR-ABL* and *JAK2 V617F* mutations was mixed bone marrow cytological features for example the presence of both dwarf and cloud-like forms of megakaryocytes based on previous study (Soderquist et al. 2018).

According to a study by Hummel et al. (2012), the morphology of double mutations cases illustrated an expansion of the megakaryocytic series with atypical forms such as lagre, hyperchromatic and bulbous nuclei and they were arranged in clusters. Most of the cases also showed moderate to marked fibrosis when stained with the reticulin and trichrome stains which had similar finding in our patient. (Hummel et al. 2012).

Cytogenetically, the diagnostic FISH analysis of the bone marrow was done with specific probes for *BCR-ABL* (Vysis LSI BCR-ABL Dual Color Dual Fusion Translocation Probe) on 200 nuclei which demonstrated presence of 29% of *BCR-ABL* translocation.

Interestingly, the FISH signals were atypical, revealing only one yellow fusion signal, one red (normal chromosome 22) and one green signal (normal chromosome 9) termed 1F1R1G as opposed to the common one green, one red and two fusion signals pattern of *BCR-ABL* translocations in CML. The FISH result may depict the presence of *BCR-ABL* fusion deletion hence the atypical pattern (Švabek et al. 2018). Additionally, this case demonstrated that FISH analysis has the ability to detect subpopulation of cells with rare minor breakpoint of *BCR-ABL* translocations that may not be amplified by molecular methods. Some chromosomal changes are submicroscopic thus masking the translocation and could be revealed only by molecular analysis or by FISH (Haidary et al. 2019).

The RT-PCR was performed for the *BCR-ABL* transcripts identification but the result was negative. This incidence may be due to the unique FISH signal pattern mentioned previously which was caused by cryptic translocation (Švabek et al. 2018). According to a study involving a case with simultaneous *BCR-ABL* and *JAK2 V617F* mutation, they detected e1a2 *BCR-ABL* transcripts instead of the expected major fragments (Bader & Dreiling 2019; Xu et al. 2013). Our laboratory used commercially

available primers which were specific for major BCR-ABL fragments (b2a2, b3a2) but cannot detect variant BCR-ABL with rare breakpoints. Similarly, a study by Azma et al. (2012) revealed that the variant translocations of *BCR-ABL* cannot be detected via RT-PCR technique and they recommended advanced molecular methods such as sequencing to be performed in suspected variant cases.

With regards to the molecular genetic study, *JAK2 V617F* mutation was determined by multiplex amplification refractory mutation system (ARMS PCR) (Applied Biosystem Gene AMP 9700) using allele-specific primers. The findings were presented as qualitative results showing mutant type or wild type of *JAK2 V617F* mutation. In this case, the *JAK2 V617F* mutation was confirmed by the amplified DNA of 229 and 279 bp which was heterozygous (Figure 4). Previous study suggested to consider CML cases that are associated with other MPN subtypes is a rather poor prognosis due to higher tendency to progress to MF and blast transformation (Soderquist et al. 2018; Lurlo et al. 2014; Xu et al. 2013). Some authors suggested that the WHO should amend the diagnostic criteria for MPN to consider this "double mutation" as a possible novel clinical entity (Bader & Dreiling 2019). The *BCR-ABL* and *JAK2 V617F* genetic combination also raise some issues regarding the best treatment strategy. Tyrosine kinase inhibitors such as Imatinib alone may not be sufficient to treat these complex cases unless it is used in concert with *JAK2*-inhibitor drugs (Yi & Kim 2019). Other adjunctive treatments including cytoreduction using Hydroxyurea may be needed to tailor to patient's symptoms and cell counts. However, clinicians must be aware of profound drug-induced cytopenia which could be detrimental towards the patients.

CONCLUSION

In conclusion, it is imperative to scrutinise CML cases for concomitant *JAK2 V617F* mutation especially among patients with atypical clinical or laboratory findings. Therefore, close monitoring with clinical and ancillary technique especially FISH and molecular methods such as DNA sequencing are crucial to help achieve complete hematological, cytogenetic anddeep molecular response alongside targeted therapy.

REFERENCES

Azma, R.Z., Jaapar, N.A., Alauddin, H., Aziz, S.A., Ithnin, A., Hussin, N.H., Kit, C.W., Tumian, R., Zakaria, Z., Akmal, S.N., Shuib, S. 2012. A rare t (9; 12; 22) (q34; q23; q11) translocation in a patient with typical chronic myeloid leukemia: A case report. *J Hematol Malig* **2(**4): 17-21.

Bader, G., Dreiling, B. 2019. Concurrent JAK2-positive myeloproliferative disorder and chronic myelogenous leukemia: A novel entity? A case report with review of the literature. *J Investig Med High Impact Case Rep* **7**: 2324709619832322.

Haidary, A.M., Azma, R.Z., Ithnin, A., Alauddin, H., Tumian, N.R., Tamil, A.M., Razak, N.F.A., Abu Amis, S.H., Zin, N.M., Shuib, S. 2019. FISH versus real-time quantitative PCR for monitoring of minimal residual disease in chronic myeloid leukaemia patients on tyrosine kinase inhibitor therapy. *Malaysian J Pathol* **41**(2): 149-60.

Hubbard, S.R. 2018. Mechanistic insights into regulation of JAK2 tyrosine kinase. *Front Endocrinol* **8**: 361.

Hummel, J.M., Kletecka, M.C., Sanks, J.K., Chiselite, M.D., Roulston, D., Smith, L.B., Czuchlewski, D.R., Elenitoba-Johnson, K.S., Lim, M.S. 2012. Concomitant BCR-ABL1 translocation and

JAK2(V617F) mutation in three patients with myeloproliferative neoplasm. *Diagn Mol Pathol* **21**(3): 176-83.

Lurlo, A., Gianelli, U., Rapezzi, D., Cattaneo, D., Fermo, E., Binda, F., Cortelezzi, A. 2014. Imatinib and ruxolitinib association: First experience in two patients. *Haematologica* **99**(6): e76-7.

Mousinho, F., Azevedo, A.P., Mendes, T., Santos, P., Cerqueira, R., Matos, S., Santos, S., Ramos, S., Viana, J.F., S., Lima, F. 2018. Concomitant presence of JAK2V617F mutation and BCR-ABL translocation in two patients: A new entity or a variant of myeloproliferative neoplasms (Case report). *Mol Med Rep* **18**(1): 1001-6.

Pastore, F., Schneider, S., Christ, O., Hiddemann, W., Spiekermann, K. 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.

Soderquist, C.R., Ewalt, M.D., Czuchlewski, D.R., Geyer, J.T., Rogers, H.J., Hsi, E.D., Bagg, A. 2018. Myeloproliferative neoplasms with concurrent BCR– ABL1 translocation and JAK2 V617F mutation: A multi-institutional study from the bone marrow pathology group. *Mod Pathol* **31**(5): 690-704.

Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiele, J. 2017. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Revised 4th edition. Lyon: IARC.

Švabek, Ž.T., Josipovi , M., Horvat, I., Zadro, R., Davidovi -Mrsi , S. 2018. The incidence of atypical patterns of BCR-ABL1 rearrangement and molecular- cytogenetic response to tyrosine kinase inhibitor therapy in newly diagnosed cases with chronic myeloid leukemia (CML). *Blood Res* **53**(2): 152-9.

Xu, W., Chen, B., Tong, X. 2013. Chronic myeloid leukemia patient with co- occurrence of BCR-ABL junction and JAK2 V617F mutation. *Int J Hematol* **99**(1): 87-90.

Yi, J.H., Kim, H.R. 2019. Coexistence of BCR/ABL1-positive chronic myeloid leukemia and JAK2 V617F-mutated myelofibrosis successfully treated with dasatinib and ruxolitinib. *Blood Res* **54**(1): 77-9.

Received: 01 Dec 2021
Accepted: 09 Sept 2022