**JAK2V617F Mutation in a Patient with B-cell Chronic Lymphocytic Leukemia and Prefibrotic Primary Myelofibrosis**

Slobodan Ristić1,2, Milica Radojković1,2, Tatjana Kostić3, Vesna Spasovski3, Sonja Pavlović3, Vesna Ćemerikić-Martinović4

1Clinic of Internal Medicine, Clinical Hospital Center Dr. Dragiša Mišović, Belgrade, Serbia;  
2University of Belgrade, School of Medicine, Belgrade, Serbia;  
3Institute of Molecular Genetic and Genetic Engineering, University of Belgrade, Belgrade, Serbia;  
4Beolab, Belgrade, Serbia

**SUMMARY**

**Introduction** Secondary malignancies, particularly solid tumors, are common in patients with chronic lymphocytic leukemia (CLL), but association of myeloproliferative neoplasms and chronic lymphocytic leukemia in the same patient is very rare.

**Case Outline** We report of a 67-year-old man with B-cell chronic lymphoid leukemia (B-CLL) who developed primary myelofibrosis (PMF) nine years after initial diagnosis. Patient received alkylating agents and purine analogue, which can be a predisposing factor for the development of myeloproliferative neoplasms. JAK2V617F mutation was not present initially at the time of CLL diagnosis, but was found after nine years when PMF occurred, which indicates that B-CLL and PMF represent two separate clonal origin neoplasms.

**Conclusion** Pathogenic mechanisms for the development of myeloproliferative and lymphoproliferative neoplasms in the same patient are unknown. Further research is needed to determine whether these malignancies originate from two different cell clones or arise from the same pluripotent hematopoietic stem cell.

**Keywords:** chronic lymphocytic leukemia; myelofibrosis; JAK2V617F mutation

**INTRODUCTION**

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Europe. Patients with CLL are predisposed to develop a secondary malignancy due to impaired immune system or chemotherapy [1]. Secondary neoplasms, mainly solid tumors, are common in CLL, but coexistence of myeloproliferative neoplasms (MPN) and CLL is very rare. Janus kinase 2 (JAK2) is a cytoplasmic protein tyrosine kinase which plays an important role in cellular proliferation and survival. JAK2V617F mutation has been detected in patients with Philadelphia chromosome negative myeloproliferative neoplasms (Ph-MPN) and CLL is very rare. Janus kinase 2 (JAK2) is a cytoplasmic protein tyrosine kinase which plays an important role in cellular proliferation and survival. JAK2V617F mutation has been detected in patients with Philadelphia chromosome negative myeloproliferative neoplasms (Ph-MPN) [2]. Here, we present a patient who developed JAK2V617F mutation positive primary myelofibrosis (PMF) with excessive platelet count nine years after CLL.

**Materials and methods**

**Detection of JAK2V617F mutation**

Peripheral blood granulocytes were isolated on Ficoll gradient (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s instructions. Genomic DNA was extracted from granulocytes using the QIAampDNA BloodMini Kit (Qiagen, Hilden, Germany). The JAK2V617F mutation was detected using allele-specific polymerase chain reaction (PCR) described elsewhere [2].

**Detection of BCR-ABL fusion transcript**

Peripheral blood mononuclear cells were isolated on a Ficoll gradient according to the manufacturer’s instructions. RNA extraction was performed using TRI Reagent solution (Ambion, Waltham, MA, USA) according to the manufacturer protocol. Complementary DNA (cDNA) was prepared from 1 μg of RNA using RevertAid Reverse Transcriptase (Thermo Scientific, Waltham, MA, USA) and random hexamer primers. RT PCR for BCR-ABL fusion transcript was performed using protocol described elsewhere [3].

**CASE REPORT**

A 67-year-old male patient was admitted to the Department of Hematology (Clinical Hospital Center Dr. Dragiša Mišović, Belgrade) in April 2014 with severe headache and elevated platelet count (1,323×10⁹ platelets/L, reference range 150–400×10⁹ platelets/L). Nine years previously he was diagnosed with B-cell chronic
lymphocytic leukemia in 0/I Rai stage. The patient was monitored without therapy for four years. Subsequently, due to the elevation of white blood cell (WBC) count, he was occasionally treated with chlorambucil. In April 2012 CLL progressed to IV Rai stage. The bone marrow biopsy showed 60% nodular/interstitial infiltration with small mature lymphocyte, with expression of CD5, CD20, CD23, CD79a, and zeta chain associated protein kinase 70 (ZAP 70). The patient was treated with COP (cyclophosphamide, vincristine, prednisone) chemotherapy, and from May 2013 received FC (fludarabine, cyclophosphamide), VI cycles with partial response. The patient was in good condition until March 2014, when he felt fatigue and permanent headache. Physical examination showed cervical and axillar lymphadenopathy and splenomegaly, 2 cm below the costal margin. Splenomegaly with a diameter of 16 cm was present on ultrasound examination. Neurological examination, electroencephalogram and endocranial scan were normal. The hemoglobin (Hb) was 80 g/L, WBC count was 20×10⁹ cells/L and differential count (neutrophils 8%, lymphocytes 88%, eosinophils 1%, basophils 2% and monocytes 1%, absolute lymphocyte count 17,600×10⁹ cells/L). Platelet count was elevated (1,581×10⁹ platelets/L). Review of peripheral blood smear showed increased number of small lymphocytes, numerous platelets, anisocytosis and poikilocytosis. Erythrocyte sedimentation rate, fibrinogen level and C-reactive protein level were within normal range. The serum lactate dehydrogenase activity was elevated (877 U/L, normal range 160–410 U/L). Direct and indirect Coombs tests were negative. Coagulation status and D-dimer level were normal. Markers of neoplasm (CEA, CA19-9, PSA) were negative. Serum iron level and iron binding capacity were normal. Quantitative immunoglobulin test showed decreased serum immunoglobulin level (IgG 2.5 g/L, IgM 0.37 g/L, IgA 0.10 g/L). Causes for secondary thrombocytosis were excluded.

The bone marrow biopsy was performed, and showed hypercellularity with 30% nodular and interstitial infiltration by small lymphocytes, the megakaryocyte compartment was increased, with dysplastic megakaryocytes and reticulin proliferation grade II (Figure 1). The finding was consistent with diagnosis of CLL and prefibrotic phase of myelofibrosis.

Cytogenetics analysis detected normal male karyotype (46XY). Molecular assay revealed JAK2V617F mutation (Figure 2) and the absence of BCR-ABL fusion gene. When detection of JAK2V617F mutation was performed on a DNA sample which was obtained and preserved when di-
agnosis of CLL was established, JAK2V617F mutation was not detected. Cytoreductive treatment with hydroxyurea (2 g/day) was started with a low dose of aspirin, as well as management of anemia with red blood cell transfusions. Platelet count decreased to 350×10⁹ platelets/L after one month, hydroxyurea dose was reduced to 1 g/day and discontinued after three months. Normalization of platelet count was associated with the disappearance of headaches. Platelet count stayed within normal range, but due to low hemoglobin concentration the patient received blood cell transfusions and prednisone therapy. The patient died in February 2015 because of progression of leukemia and associated pneumonia.

DISCUSSION

The development of chronic myeloproliferative disorder in a patient with lymphoproliferative neoplasm is very rare. Sequential or simultaneous occurrence of CLL and PMF in the same patient has been reported in literature in only 17 cases, with particular male predominance [4]. Simultaneous diagnosis of both diseases at presentation was noticed in nine patients [5], and in the case of subsequent diagnoses of diseases, myelofibrosis preceded CLL in the majority of patients [6, 7]. Our patient suffered from CLL and after nine years developed prefibrotic PMF. Impaired immune surveillance in chronic lymphocytic leukemia might be a triggering factor for the development of secondary malignancy [1]. In this case myelofibrosis occurred subsequent to previously treated CLL, and might be induced by the chemotherapy. The increased risk of therapy-related myeloid malignancies is reported in patients who received purine analogue [8]. However, in most patients with co-occurrence of myelo- and lymphoproliferative diseases, CLL patients were in Rai stage 0/I, without administered chemotherapy. Our patient had a progressive CLL and severe anemia, in contrast to literature data according to which patients having a combination of lymphoproliferative and myeloproliferative disease often show indolent clinical course [9].

Myelofibrosis is a very heterogeneous disease. A characteristic of prefibrotic myelofibrosis is elevated serum lactate dehydrogenase level, increased peripheral blood CD34+ cell count and a leucoerythroblastic peripheral blood smear [10]. Early prefibrotic myelofibrosis can mimic essential thrombocytopenia and careful morphologic examination is necessary for distinguishing between the two diseases. Elevated platelet count is found in about one third of patients with PMF. In essential thrombocythemia megakaryocytes are giant with cluster formations, while those in prefibrotic PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei. Our patient had very high platelet count, intense headaches, resistant to analgesics. Thrombohemorrhagic complications were ruled out, and the normalization of platelet count led to disappearance of headaches. Causes of headache associated with elevated platelet count and platelet dysfunction include increased plasma levels of serotonin, hypersensitivity of serotonin receptors, increased levels of platelet adenosine diphosphate and microcirculatory disturbance [11].

JAK2V617F mutation has been described in patients with Philadelphia chromosome negative myeloproliferative neoplasms (Ph-MPN), in majority of patients with polycythemia vera, in 50% of patients with primary myelofibrosis and essential thrombocytopenia, in a small number of other myeloid malignancies, and rarely in lymphoid malignancies [2, 12]. The role of JAK2V617F mutation in B cell CLL is controversial. ZAP-70 expression, which is present in 30% of CLL cases, correlates with non-mutated immunoglobulin genes and predicts poor prognosis [13]. In most reported MPN cases which coexist with CLL, ZAP-70 was positivity present, as in our patient. Tabaczewski et al. [6] proposed hypothesis that in cases of co-existence of CLL with MPN (JAK2V617F-positive essential thrombocytopenia), initial genetic hit occurs early, during the pre-JAK2 phase of progenitor cell development. Stem cells...
then differentiate to lymphoid and myeloid cells, but due to genomic instability, acquire additional molecular mutations, as JAK2 mutation within the myeloid lineage. JAK2 mutation was not detected in B-cell lineage, which means that the two diseases arise from the same pluripotent stem cell but different cellular lineages. Our case favors this hypothesis because JAK2 mutation was not present on CLL at presentation, and mutation is acquired during development of myeloproliferative disease, which suggests that B-CLL and PMF are two distinct clonal hematologic malignancies. Additionally, latency period between CLL and PMF appearance was very long, which favors hypothesis that impaired T-cell immunity might predispose the development of a second malignant clone [14]. Thus, neoplastic effect of received chemotherapy may be of importance. Different mutagenic events would independently induce the lymphoid and myeloid malignant proliferation, and the development of separate clonal origin malignant diseases. In contrast to this finding, Swierczek S. et al. [15] reported of three patients with concomitant development of polyclonal cells. Kodali et al. [5] identified JAK2-V617F mutation in a patient with coexistent CLL and MPN. In 63 analyzed cases of B-cell CLL, only two were JAK2-V617F-positive, but without a history of Ph-MPN [16]. JAK2-V617F mutation was detected at low level in the peripheral blood of healthy donors, which indicates that mutation alone is not sufficient to induce Ph-MPN [17].

Pathogenesis of associated sporadic occurrence of myelo- and lymphoproliferative neoplasms is unclear and further studies are needed to find out whether these malignancies represent two distinct clonal hematological disorders or both derive from the same pluripotent stem cell.

ACKNOWLEDGMENTS

This study was supported by grant No. III 41004 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES


17. Sidon P, El Housini H, Dessars B, Heimann P. The JAK2V617F mutation is detectable at very low level in peripheral blood of healthy donors. Leukemia. 2006; 20:1622. [DOI: 10.1038/sj.leu.2404292] [PMID: 16775613]

doi: 10.2298/SARH1512739R
**JAK2V617F мутација код болесника са Б ћелијском хроничном лимфоцитном леукемијом и префибротичком примарном мијелофibrозом**

Слободан Ристић1,2, Милица Радојковић1,2, Татјана Костић3, Весна Спасовски1, Соња Павловић3, Весна Чемерикић-Мартиновић4

1Клиника за интерну медицину. Клиничко-болнички центар „Др Драгиша Мишовић“, Београд, Србија;  
2Универзитет у Београду, Медицински факултет, Београд, Србија;  
3Институт за молекуларну генетику и генетско инжењерство, Универзитет у Београду, Београд, Србија;  
4„Беолаб“, Београд, Србија

**КРАТАК САДРЖАЈ**

Увод Секундарни малигнитети, нарочито солидни тумори, чести су код болесника с хроничном лимфоцитном леукемијом (ХЛЛ), али ретко се среће удроженост мијелои профилератних неоплазама и ХЛЛ. 

Приказ болесника Приказујемо мушкарца старог 67 година са Б ћелијском ХЛЛ код кога се након девет година развила примарна мијелофibrоза (ПМФ). Болесник је лечен аликлишућим агенсима и аналозима пурина, што може бити предиспозицијући фактор за развој мијелои профилеративног обољења. JAK2V617F мутација није откривена приликом постављања дијагнозе ХЛЛ, али је утврђена после девет година, када се развила ПМФ, што указује на то да су Б ћелијска ХЛЛ и ПМФ неоплазме које почињу од различитих ћелијских клонова. 

Закључак Патогенетски механизми удрожености мијелои профилеративне и лимфои профилеративне неоплазама код болесника нису разјашњени. Потребна су даља истраживања ради утврђивања да ли ове малигне болести почињу од два различитих ћелијских клонова или настaju од исте плурипотентне матичне ћелије хематопоеze. 

Кључне речи: хронична лимфоцитна леукемија; мијелофиброза; JAK2V617F мутација