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

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**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 alkylation 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. Here, we present a patient who developed **JAK2V617F** mutation positive primary myelofibrosis (PMF) with excessive platelet count nine years after CLL.

**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].

**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].

**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 (1,323×10^9 platelets/L, reference range 150–400×10^9 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 350×10^9 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 thrombocythemia), initial genetic hit occurs early, during the pre-JAK2 phase of progenitor cell development. Stem cells...
The JAK2 mutation is rarely present in lymphoid malignancies. In most of the reported cases with MPN and CLL, JAK2 mutation was detected in myeloid, but not in lymphoid cells. Kodali et al. [5] identified JAK2V617F mutation in a patient with coexistent CLL and MPN. In 63 analyzed cases of B-cell CLL, only two were JAK2V617F-positive, but without a history of Ph-MPN [16]. JAK2V617F 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.

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JAK2V617F мутација код болесника са Б ћелијском хроничном лимфоцитном леукемијом и префибротичком примарном мијелофиброзом

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Кратак садржај
Увод Секундарни малигнитети, нарочито солидни тумори, чести су код болесника с хроничном лимфоцитном леукемијом (ХЛЛ), али ретко се среће удроженост мијелоолигореферативних неоплазми и ХЛЛ.

Приказ болесника Приказујемо мушкараца старог 67 година са Б ћелијском ХЛЛ код кога се након девет година развила примарна мијелофиброза (ПМФ). Болесник је лећен аликлишућим агенсама и аналозима пурина, што може бити предиспонирајући фактор за развој мијелоолигореферативног обољења. JAK2V617F мутација није откривена приликом постављане 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 потичу од различитих ћелијских клонова.

Закључак Патогенетски механизми удрожености мијелоолигореферативне и лимфоолигореферативне неоплазме код болесника нису разјашњени. Потребна су даља истраживања ради утврђивања да ли ове малигне болести потичу од два различитих ћелијских клонова или настaju од исте плурипotentне матичне ћелије хематопоезе.

Кључне речи: хронична лимфоцитна леукемија; мијелофиброза; JAK2V617F мутација