

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

Slobodan Ristić^{1,2}, Milica Radojković^{1,2}, Tatjana Kostić³, Vesna Spasovski³, Sonja Pavlović³, Vesna Čemerikić-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 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) [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 ($1,323 \times 10^9$ platelets/L, reference range $150-400 \times 10^9$ platelets/L). Nine years previously he was diagnosed with B-cell chronic

Correspondence to:

Slobodan RISTIĆ
Clinic of Internal Medicine
Clinical Center Dr. Dragiša Mišović
Heroja Milana Tepića 1
11000 Belgrade
Serbia
bane.ristic@gmail.com

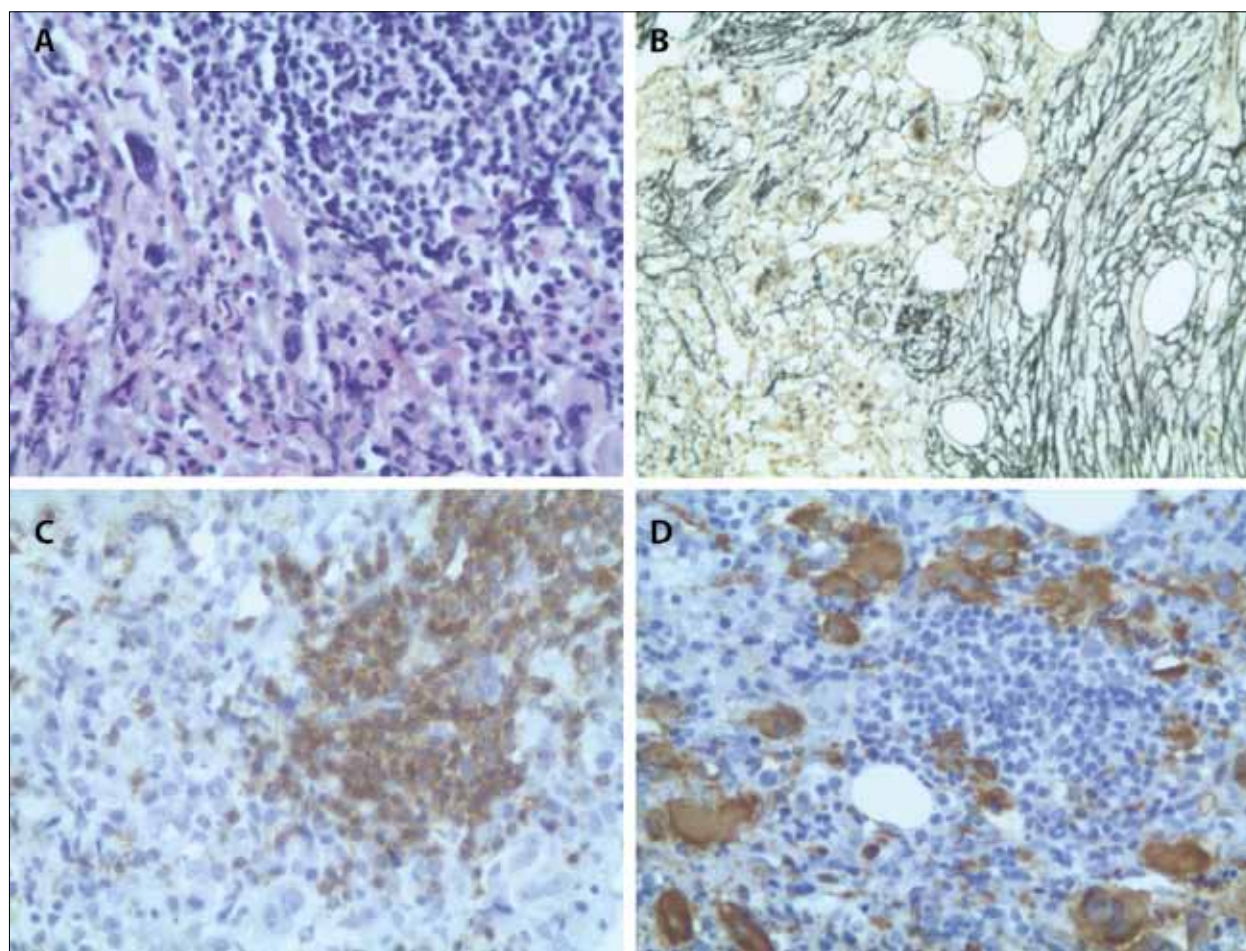


Figure 1. A) Bone marrow biopsy (hematoxylin-eosin) showing a population of small lymphoid cells, increased number of large megakaryocyte and fibrosis (magnification 400×); B) Bone marrow biopsy revealing significant reticulin fibrosis (magnification 200×); C) Bone marrow biopsy showing a population of neoplastic lymphocytes with strong CD5 immunopositivity (magnification 400×); D) Bone marrow biopsy showing increased number of CD61+ megakaryocyte (magnification 400×).

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 20×10^9 cells/L and differential count (neutrophils 8%, lymphocytes 88%, eosinophils 1%, basophils 2% and monocytes 1%, absolute lymphocyte count $17,600 \times 10^9$ cells/L). Platelet count was elevated ($1,581 \times 10^9$ platelets/L). Review of peripheral blood smear showed increased num-

ber 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-

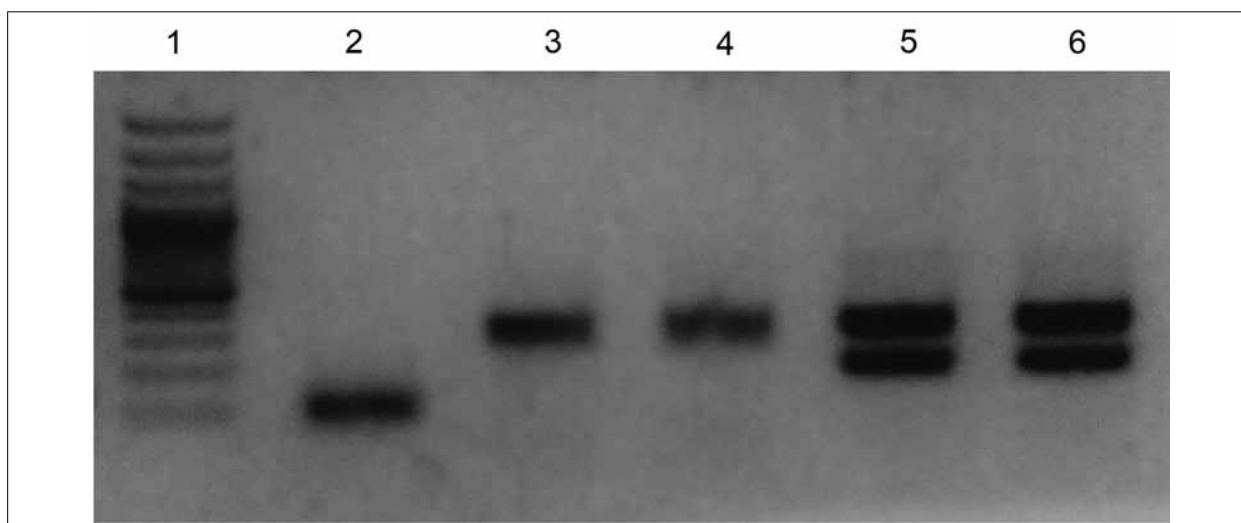


Figure 2. Detection of *JAK2V617F* mutation by allele-specific PCR (1 – 100 bp ladder; 2 – Water control; 3 – *JAK2V617F*-negative control; 4 – Patient before acquisition of myeloproliferative phenotype; 5 – Patient after acquisition of myeloproliferative phenotype; 6 – *JAK2V617F*-positive control)

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 lymphoprolifera-

tive 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 thrombocythemia 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 thrombocythemia, 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

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 polycythemia vera and chronic lymphocytic leukemia which arose independently from different hematopoietic stem cells.

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.

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

- Bartik MM, Welker D, Kay NE. Impairments in immune cell functions in B-cell chronic lymphocytic leukemia. *Semin Oncol.* 1998; 25:27-33. [PMID: 9482524]
- Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al: Cancer Genome Project. Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. *Lancet.* 2005; 365:1054-61. [DOI: 10.1016/S0140-6736(05)71142-9] [PMID: 15781101]
- Liang DC, Shih LY, Yang CP, Hung IJ, Chen SH, Jaing TH, et al. Multiplex RT-PCR assay for the detection of major fusion transcripts in Taiwanese children with B-lineage acute lymphoblastic leukemia. *Med Pediatr Oncol.* 2002; 39:12-7. [DOI: 10.1002/mpo.10092] [PMID: 12116073]
- Burgstaller S, Wimmer S, Mayrbaeurl B, Hoebing W, Thaler J. Coexistence of primary myelofibrosis and chronic lymphocytic leukaemia: treatment of two different diseases with one agent. *Blood Cancer J.* 2011; 1(5):e20. [DOI: 10.1038/bcj.2011.18] [PMID: 22829155]
- Kodali S, Chen C, Rathnasabapathy C, Wang CJ. *JAK2* mutation in a patient with CLL with coexistent myeloproliferative neoplasm (MPN). *Leuk Res.* 2009; 33:e236-9. [DOI: 10.1016/j.leukres.2009.06.027] [PMID: 19625083]
- Tabaczewski P, Nadesan S, Lim SH. Zap-70 positive chronic lymphocytic leukemia co-existing with *Jak2V617F* positive essential thrombocythemia: a common defective stem cell. *Leuk Res.* 2009; 33:854-5. [DOI: 10.1016/j.leukres.2008.09.002] [PMID: 18849073]
- Chintapatla R, Battini R, Wiernik PH. Chronic lymphocytic leukemia with essential thrombocythemia: asbestos exposure association? *Clin Adv Hematol Oncol.* 2012; 10:752-5. [PMID: 23271264]
- Hisada M, Biggar RJ, Greene MH, Fraumeni JF, Travis LB. Solid tumors after chronic lymphocytic leukemia. *Blood.* 2001; 98:1979-81. [DOI: 10.1182/blood.V98.6.1979] [PMID: 11535538]
- Laurenti L, Tarnani M, Nichele I, Ciolli S, Cortelezzi A, Forconi F, et al. The coexistence of chronic lymphocytic leukemia and myeloproliferative neoplasms: a retrospective multicentric GIMEMA experience. *Am J Hematol.* 2011; 86:1007-12. [DOI: 10.1002/ajh.22171] [PMID: 21953617]
- Tefferi A. Primary myelofibrosis: 2013 update on diagnosis, risk-stratification and management. *Am J Hematol.* 2013; 88:141-50. [DOI: 10.1002/ajh.23384] [PMID: 23349007]
- Frewin R, Dowson A. Headache in essential thrombocythaemia. *Int J Clin Pract.* 2012; 66:976-83. [DOI: 10.1111/j.1742-1241.2012.02986] [PMID: 22889110]
- Spasovski V, Tošić N, Kostić T, Pavlović S, Čolović M. *JAK2-V617F* mutation in patients with myeloproliferative neoplasms: association with *FLT3-ITD* mutation. *Srp Arh Celok Lek.* 2010; 138(9-10):614-8. [DOI: 10.2298/SARH1010614S] [PMID: 21180092]
- Leković D, Mihaljević B, Kraguljac-Kurtović N, Peruničić-Jovanović M, Bogdanović A, Čolović M, et al. Prognostic significance of new biological markers in chronic lymphocytic leukaemia. *Srp Arh Celok Lek.* 2011; 139(11-12):753-8. [DOI:10.2298/SARH1112753L] [PMID: 22338471]
- Kravić-Stevović T, Bogdanović A, Bumbaširević V. Higher percentage of in vitro apoptotic cells at time of diagnosis in patients with chronic lymphocytic leukemia indicate earlier treatment requirement: ten years follow up. *Srp Arh Celok Lek.* 2014; 142(1-2):48-53. [DOI: 10.2298/SARH1402048K] [PMID: 24684031]
- Swierczek S, Nausova J, Jelinek J, Liu E, Roda P, Kucerova J, et al. Concomitant *JAK2V617F*-positive polycythemia vera and B-cell chronic lymphocytic leukemia in three patients originating from two separate hematopoietic stem cells. *Am J Hematol.* 2013; 88:157-8. [DOI: 10.1002/ajh.23362] [PMID: 23280542]
- Yang Y-N, Qin Y-W, Wang C. *JAK2 V617F* detected in two B-cell chronic lymphocytic leukemia patients without coexisting Philadelphia chromosome negative myeloproliferative neoplasms: a report of two cases. *Oncology Lett.* 2014; 8:841-4. [DOI: 10.3892/ol.2014.2168] [PMID: 25013507]
- 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]

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

Слободан Ристић^{1,2}, Милица Радојковић^{1,2}, Татјана Костић³, Весна Спасовски³, Соња Павловић³, Весна Чемерицић-Мартинковић⁴

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 удруженост мијелопролиферативних неоплазми и ХЛЛ.

Приказ болесника Приказујемо мушкарца старог 67 година са Б ћелијском ХЛЛ код кога се након девет година развила примарна мијелофиброза (ПМФ). Болесник је лечен алкилишућим агенсима и аналозима пурина, што може бити предиспонирајући фактор за развој мијелопролиферативног 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 потичу од различитих ћелијских клонова.

Закључак Патогенетски механизми удружености мијело-пролиферативне и лимфо-пролиферативне неоплазме код болесника нису разјашњени. Потребна су даља истраживања ради утврђивања да ли ове малигне болести потичу од два различита ћелијска клона или настају од исте плурипотентне матичне ћелије хематопоезе.

Кључне речи: хронична лимфоцитна леукемија; мијелофиброза; *JAK2V617F* мутација

Примљен • Received: 14/09/2015

Прихваћен • Accepted: 25/09/2015