Molecular Genetic Aspects of the Diagnosis of Myeloproliferative Diseases: the Example of Polycythemia Vera

Miyeloproliferatif Hastalıkların Tanısının Moleküler Genetik Yönü: Polisitemi Vera Örneği

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Abstract

Molecular genetic testing is a reliable method for the definitive diagnosis of polycythemia vera (PV). The use of interferon preparations in addition to hydroxyurea and antiplatelet agents could be associated with better therapeutic outcomes. In this report, we present the clinical case of a young patient with asymptomatic PV to demonstrate the effectiveness of molecular genetic analysis in PV diagnosis.

Keywords: janus kinase 2; myeloproliferative; polycythemia vera

Öz

Moleküler genetik testler polisitemi veranın (PV) kesin teşhisi için güvenilir bir metottur. Hidroksiüre ve antiplatelet ajanlara ilaveten interferon preparatlarının kullanımı ile daha iyi terapötik sonuçlar gözlenebilir. Bu raporda, moleküler genetik testlerin PV teşhisindeki etkililiğini göstermek üzere asemptomatik PV'den muzdarip genç bir hastaya dair klinik vaka sunulmuştur.

Anahtar Sözcükler: janus kinaz 2; miyeloproliferatif; polisitemi vera

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INTRODUCTION

Myeloproliferative disorders (MDs) are a group of clonal disorders of the hematopoietic stem cells and are characterized by the neoplastic proliferation of one or more lineages of myelopoiesis, with a change in peripheral blood indices (1,2). They can be classified according to the presence of the Philadelphia chromosome. Philadelphia-positive MDs include chronic myeloid leukemia (CML), which is driven by the Philadelphia chromosome, while polycythemia vera (PV), myelofibrosis, and essential thrombocythemia, which are not associated with the Philadelphia chromosome, are called Philadelphia-negative MDs (2,3). MDs also include a nonclassifiable group, which is referred to as "unclassified myeloproliferative disorders".

According to the 2008 World Health Organization (WHO) recommendations, the PV diagnosis should be made based on the results of clinical, laboratory, and histological tests (bone marrow biopsy), ruling out any other nosological form of Philadelphia-negative MDs. However, there can be discrepancies between clinical, laboratory, and histological data in the early stages of the disease, leading to difficulty in identifying the MD form. Such discrepancies could also be present if an inflammatory, metabolic or neoplastic pathology is masking the main MD signs and symptoms in any form of the disease.

PV is a clonal MD characterized by the neoplastic proliferation of mature myeloid cell lineages, predominantly erythroid cell lineages. An increase in the red blood cell (RBC) count, hemoglobin (Hb) concentration, platelets (PLT), and leukocytes (WBC) in the peripheral blood referred to as pancytosis is also seen. The erythropoiesis tends to be independent of the physiological regulatory mechanisms. Almost all patients with PV are carriers of a janus kinase 2 (JAK2) V617F mutation or another functionally similar mutation. PV is a rare (orphan) disease with annual prevalence of 0.4 to 2.8 cases per 100,000 population and typically presents in late adulthood, although it has also been reported in younger patients, with a female predominance (1,2).

CASE

In April 2019, a 38-year-old female patient was undergoing treatment for acute bronchitis in the outpatient setting. The laboratory data were as follows: RBC, 5.64×10^{12}/L; Hb, 174 g/L; red cell distribution width (RCW), 16.8%; hematocrit (HCT), 51.1%; PLT, 1102×10^{9}/L; WBC, 22.9×10^{9}/L; stab neutrophil%, 14%; lymphocyte%, 14%; basophil%, 1%; eosinophil%, 1%; monocyte%, 3%; and erythrocyte sedimentation rate, 1 mm/hr. The patient was then referred to the Hematology Center of Smolensk for consultation. In May 2019, she denied any complaint during the hematology consultation. Although the bronchitis symptoms improved, laboratory data showed erythrocytosis, leukocytosis, and thrombocytosis. Reviewing the patient's medical history, it was found that she had been exposed to excessive sun exposure for 10–14 days, 2–3 times a year during the last five years, and experienced extreme stress a year before the detection of the blood changes.

Thereafter, a comprehensive laboratory examination was carried out and the complete blood count (CBC) revealed trilineage hyperplasia. The myelogram showed hyperplasia of the granulocytic and megakaryocytic germ cell lineages. An increase in the number of segmented neutrophils and normoblastic hematopoiesis were also noted. Due to increased granulocytopoiesis, a slight disturbance in the leukocyte/erythrocyte ratio was noted: 5/1. The number of megakaryocytes per high-power field was 50–250 and they were in different maturation stages. Enhanced platelet accumulation was observed. No change was noted in the coagulation profile. Biochemical analysis showed a decrease in erythropoietin (2.3 mU/ml; normal: 4–24 mU/ml). Abdominal ultrasound revealed hepatomegaly (length on midclavicular line: 165 mm; normal: 84 mm), elongated gall bladder with folds, polyp in the gallbladder fundus, splenomegaly (165×57 mm, area 92 cm^2), and mild ascites. A decrease in the amplitude of the T wave was noted on the electrocardiogram.

Based on the follow-up examination results, the initial diagnosis "Myeloproliferative disorder (polycythemia vera not ruled out for initial diagnosis)" was made. Hydroxycarbamide (one 500-mg capsule every other day) and allopurinol (100 mg 3 times a day) were prescribed as a symptomatic treatment. The patient was then referred to the Almazov National Medical Research Center in Moscow, Russia, for better clarification about the nature of the pathology.

Table 1. Patient laboratory values

Date	HCT %	RBC× 10^{12}/L	Hb g/L	CI	WBC 10^9/L	EOS %	BAS %	NEUT %	LYM %	MON %	PLT 10^9/L	ESR mm/hr
04.19	51.1	5.64	174	0.90	22.91	1	1	81	14	3	1102	1
06.19	46.7	5.07	154	0.92	28.90	0.2	0.5	94.2	2.6	2.5	762	8
07.19	50.2	5.23	163	0.96	17.59	2	1	81	11	5	548	
08.19	52.1	5.31	168	0.98	13.47						521	
09.19	50.3	4.91	161	0.92	13.53						513	
12.19	46.2	4.50	155	0.93	11.53						498	6

BAS: basophils; CI: color index; EOS: eosinophils; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; LYM: lymphocytes; MON: monocytes; NEUT: neutrophils; PLT: platelets; RBC: red blood cells; WBC: white blood cells/leukocytes

Table 2. Diagnostic criteria for polycythemia vera (PV) *

Major criteria	Minor criterion
Hemoglobin >165 g/L (men), hemoglobin >160 g/L (women); or hematocrit >49% (men), hematocrit >48% (women); or increased red cell mass (RCM)	Serum erythropoietin level below the normal reference range.
Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size). This criterion may not be required in patients who have sustained absolute erythrocytosis (men, hemoglobin/hematocrit >185 g/L/>55.5%; women, >165 g/L/>49.5%) if major criterion 3 and the minor criterion are present.	
JAK2 V617F mutation in exon 14 or exon 12.	

* The diagnosis of PV requires meeting either all three major criteria or the first two major criteria and the minor criterion (World Health Organization, 2017).

A polymerase chain reaction (PCR) in real-time aimed at detecting genetic markers of MDs was performed at the Almazov National Medical Research Center. Relative expression of the BCR-ABL gene (p210 protein) was 0.000% (IS), which ruled out a diagnosis of chronic myelogenous leukemia. Mutations in the exon 9 of the calreticulin (CALR) gene were studied by PCR to detect markers of chronic myelofibrosis. The analysis of the PCR products was done using electrophoresis under denaturing conditions, followed by direct Sanger sequencing of the PCR products. No mutation was found in the CALR and MPL-1 genes. Mutations of the JAK2 V617F gene, a marker of chronic MDs, indicating the presence of PV, were identified.

After a cytogenetic examination, the diagnosis of stage II-B PV was made. The treatment was continued with hydroxycarbamide (Hydrea, 500 mg every other day, per os) and allopurinol (100 mg per day, per os), starting from June 6, 2019. A month later, an improvement was seen in the CBC (PLT count decreased from 1196×10^9/L to 762×10^9/L). Alpharone (interferon [IFN] alpha 2b) 3 million IU, 3 times a week, IM, and Cardiomagnyl (aspirin) 0.75 mg per day, per os, were added to the treatment. The dose of hydroxyurea was adjusted. The patient tolerated the treatment well and did not have any complaint. A decrease in the number of WBCs and PLTs was noted, although the other indicators remained elevated (Table 1). Abdominal ultrasound was performed after four months, which showed a further increase in the size of the liver and spleen. Due to the insufficient cytological improvement and worsening hepatosplenomegaly, a decision was made in October 2019 to replace Alpharone with 90 μg PEGylated (PEG) IFN alpha 2a once a week, including the patient in a phase II multi-center clinical trial. PEG IFN alpha 2a is associated with a better hematological and molecular response in PV compared with IFN alpha 2b. It is less toxic and, in some cases, can also eliminate the JAK2 mutant clone (1,3).

After the combined prescription of PEG IFN alpha 2a, hydroxyurea, and aspirin, the patient presented with a positive outcome. The laboratory values are shown in Table 1.

Report ethics

Written informed consent was obtained from the patient for the publication of this report.

DISCUSSION

MDs may initially be asymptomatic and can be an incidental finding. Also, similar changes are often found in standard laboratory tests. In the present study, we aimed to demonstrate that PV's clinical diagnosis and differential diagnosis from the other MDs could be complicated. Our findings can contribute to the understanding and management of the disease, its early identification before the onset of clinical manifestations, and its differential diagnosis. The clinical diagnosis of PV in a 38-year-old woman is a rare case. Despite the high CBC values (Hb, >170 g/L; PLT, $>1000\times10^9$/L; WBC, 20×10^9/L), the patient had no characteristic symptom and it was an incidental finding. Moreover, trilineage hyperplasia is also a rare finding in the CBC of a patient with PV.

MD development is multi-stage and MD predisposition is influenced by external factors that damage the genome of a normal cell and lead to its malignant transformation. This is the main hypothesis in the MD etiology and pathogenesis (4,5). Although significant progress has recently been made in the understanding of the molecular genetic mechanisms of Philadelphia-negative MDs, including PV, essential thrombocythemia, and primary myelofibrosis, the initial mutation leading to hematopoietic cell malignancy remains unknown (4). In 2005, it was established that the cause of clonal proliferation in PV was a mutation in the JAK2 V617F, located on Chromosome 9 (5–7). This mutation, an essential criterion for the differential diagnosis of symptomatic PV, is found in almost all patients with PV: in 96% of them it is located in the exon 14, and in 2%, in the exon 12 (5,6). In addition to the mutations of the JAK2 gene, mutations in other genes are also detected. MPL gene mutations are rare. The most frequent mutations of the MPL W515L/K are located in exon 10 (6,7). These mutations are not strictly specific for MDs and are of secondary genesis in the chain of genetic events.

In 2013, data were reported on the diagnostic significance of somatic mutations in the exon 9 of the CALR gene encoding calreticulin protein (3). Mutations in this gene are detected in 67% of cases of PV and 88% of cases of primary myelofibrosis in the absence of other mutations, including JAK2 and MPL gene mutations. Other authors also confirm the extremely high mutation frequency of the CALR gene in patients with MDs (in 70 to 84% of cases with no JAK2 gene mutation). Also, CALR mutations were detected in 8% of cases with myelodysplastic syndrome and other myeloid neoplasms but were absent in PV. Molecular genetic abnormalities in Philadelphia-negative MDs lead to the activation of the JAK-STAT signaling pathway (3–5). This activation increases the number of RBCs, WBCs, and PLTs in the peripheral blood of patients with PV. Thus, mutations in the JAK2 and CALR genes have a significant diagnostic value. Their presence indicates the clonal nature of the disease and helps in the differential diagnosis of PV, essential thrombocytopenia, and primary myelofibrosis from several other myeloid neoplasms, as well as from secondary erythrocytosis and thrombocytosis.

The clonal proliferation of myeloid cells in Philadelphia-negative MDs can be accompanied by secondary inflammation with changes in bone marrow stroma and pathological production of cytokines. The pathological production of cytokines, chemokines, and metalloproteinases may participate in the pathological intercellular interaction of neutrophils, monocytes, and megakaryocytes, leading to the release of CD34+ myeloid progenitors and endothelial cells into the peripheral blood (4).

Symptoms of PV depend on the developmental stage of the disease, the number of morphological blood elements of each type, an increase in circulating blood volume, and the thromboembolic–hemorrhagic complications. The disease can be asymptomatic for a long time (5,6). PV is an incidental finding which is suspected due to abnormal CBC in most patients.

The 2017 WHO criteria for the diagnosis of PV is established based on a comprehensive assessment of the clinical picture and the laboratory parameters (level of evidence A) (Table 2). Thus, the results of molecular genetic analysis play a fundamental role in the diagnosis (1,3).

CONCLUSION

PV can have an asymptomatic course and can be an incidental finding, as described in the present study. Standard laboratory blood tests limit the ability to dif-

ferentiate PV from other MDs. It is necessary to identify the presence of BCR-ABL, CALR and MPL-1 gene and JAK2 C617F mutations. Therefore, molecular genetic analysis could be a reliable method for the diagnosis of various forms of MDs, although larger studies are required.

Conflict-of-Interest and Financial Disclosure

The authors declare that they have no conflict of interest to disclose. The authors also declare that they did not receive any financial support for the study.

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