JAK2 (V617F) Positive Latent Myeloproliferative Neoplasm Presenting with Splanchnic Vein Thrombosis

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Abstract Myeloproliferative neoplasms (MPNs) are chronic clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the granulocytic, red cell or platelet lineages in the bone marrow, with fairly normal maturation, resulting in increase in the leukocyte, erythrocytes and platelets in the blood. They also represent a common cause of splanchnic vein thrombosis (SVT). Herein, we describe a case of SVT as a presenting symptom of latent MPN. The patient has had normal complete blood counts since presentation. 3 ½ years later, she was found to have JAK2 (V617F) mutation and bone marrow biopsy was consistent with MPN. Five years later, her platelet count started to rise. In patients with a first episode of SVT, thrombophilia workup including JAK2 (V617F) mutation is warranted. Anticoagulation with heparin and warfarin is the treatment of choice for SVT.

Introduction

Myeloproliferative neoplasms (MPNs) are chronic clonal hematopoietic stem cell disorders characterized by

proliferation of one or more of the myeloid (i.e. granulocytic, erythroid and megakaryocytic) lineages in the bone marrow. The proliferation is associated with relatively normal maturation, leading to increase in leukocytes, red cells or platelets in the peripheral blood. They have the potential to undergo clonal evolution and progression which terminates in myelofibrosis, or transformation to acute leukemia. MPNs also represent a common cause of splanchnic vein thrombosis (SVT). Herein, we describe a case of SVT as a presenting symptom of latent MPN. In patients with a first episode of SVT, thrombophilia workup including JAK2 (V617F) mutation is warranted. Anticoagulation with heparin and warfarin is the treatment of choice for SVT. Low-dose aspirin may be useful in reducing the risk of thrombosis.

Case

In 2004, a 50-year-old Caucasian woman was transferred to our hospital for further management of epigastric pain. Her past history includes hyperlipidemia, gastroesophageal reflux and osteoporosis. Family history was remarkable for breast cancer in her mother and bladder cancer in her father. She had quit smoking 25 years ago. Examination was unremarkable except epigastric tenderness. Complete blood count (CBC) revealed WBC 10,300/cmm (range 4,500–10,500/cmm), hemoglobin 12.7 g/dL (range 12.0–14.0 g/dL), platelet 407,000/cmm (150,000–450,000/cmm), neutrophils 77 %, lymphocytes 14.8 %, monocytes 6.1 %, eosinophils 0.5 % and basophils 1.5 %. Chemistry profile was unremarkable except serum AST of 86 U/L (range 9–52 U/L). Abdominal ultrasound from outside hospital reported gallbladder sludge. At our hospital, endoscopic retrograde cholangiopancreatography (ERCP) revealed normal biliary anatomy. Abdominal color Doppler ultrasound at our hospital was remarkable for prominent portal

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and splenic vein and abnormal pattern of blood flow in portal vein and distal splenic vein. A contrasted computed tomogram confirmed occlusion and dilatation of the portal, splenic and superior mesenteric veins. Gadolinium-enhanced magnetic resonance imaging showed thrombosis of portal, superior mesenteric and splenic veins (Fig. 1). She was anticoagulated with intravenous heparin and later with warfarin for six months.

Thrombophilia workup (factor V Leiden and prothrombin gene mutations, protein C, protein S, antithrombin III, antiphospholipid antibodies, homocystein) was unremarkable. Antinuclear antibody and rheumatoid factor were negative. Serum protein electrophoresis showed no monoclonal protein. Acquired risk factors for thrombosis such as recent immobilization, trauma, and intra-abdominal inflammatory processes were ruled out. Of note, JAK2 (V617F) mutation was not discovered yet at that time. She continued to follow up regularly to the gastroenterology

clinic and she has had no recurrent SVT or venous thromboembolism (VTE). 3½ years later, she was referred back to hematology clinic. She has no plethora, palpable hepatosplenomegaly or lymphadenopathy. CBC remained normal. At this point, she reported occasional itching after hot showers. Since JAK2 (V617F) mutation analysis was commercially available by this time, it was requested. JAK2 (V617F) mutation was positive. Flow cytometry of the blood ruled out paroxysmal nocturnal hemoglobinuria. This prompted a bone marrow biopsy which showed hypercellular marrow with granulocytic hyperplasia (myeloid: erythroid ratio = 6:1), megakaryocytic hyperplasia and clustering and progressively maturing hematopoiesis (Figs. 2, 3, 4), consistent with early MPN. BCR-ABL gene rearrangement was not detected. She has been started on aspirin 81 mg daily to reduce the risk of thrombosis recurrence. Subsequently in 2009–2010, her platelet count

Fig. 1 MRI scan showing portal vein thrombosis

Fig. 3 Clustering of megakaryocytes in marrow

Fig. 2 Granulocytic and megakaryocytic hyperplasia in marrow

Fig. 4 Increased reticulin in marrow (reticulin stain)

rose to around $500,000 \text{ cmm}^{-1}$, but her hemoglobin and leukocyte count remained normal.

Discussion

Our patient initially presented with idiopathic SVT, treated with anticoagulation. Her CBC has been normal. However, she turned out to have latent MPN by positive JAK2 (V617F) mutation and by bone marrow biopsy findings. Subsequently, she has remained on low-dose aspirin. According to the 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, she would fit into the category of myeloproliferative neoplasms, unclassifiable [1].

JAK2 Tyrosine Kinase and JAK2 (V617F) Mutation

Janus Kinase 2 (JAK2) is a cytoplasmic tyrosine kinase that induces signal transduction especially those triggered by growth factors such as erythropoietin in normal and neoplastic cells. The JAK proteins function as intermediaries between membrane receptors and signaling molecules. Receptor activation by cytokine binding activates JAK kinases located on cytoplasmic domains of the receptors; this in turn creates docking sites for downstream signaling molecules—notably those from the STAT (Signal Transduction and Activators of Transcription) family [2]. Activated STAT molecules enter the nucleus where they act as transcription factors. JAK2(V617F) represents a G to T somatic mutation of JAK 2 at nucleotide 1849 in exon 14 resulting in substitution of valine to phenylalanine at codon 617 [3]. This in turn results in constitutively active JAK2 enzyme leading to cytokine independent proliferation of cell lines. Moreover, transfection of hematopoietic stem cells with mutant JAK2 gene causes a polycythemia-like phenotype in the murine model. In wild type cells, JAK2 activation involves tightly regulated cytokine induced phosphorylation of tyrosine residues in the kinase and negative regulatory JAK homology domains JH1 and JH2 respectively. (JH1 is the active kinase domain which is negatively regulated by an inactive pseudokinase domain-JH2). The V617F is a somatic mutation in hematopoietic cells, often only one allele carries the JAK2 mutation but the loss of heterozygosity through mitotic recombination converts the affected cell from heterozygous to homozygous carrier of mutation. This occurs most frequently in polycythemia vera (PV) as homozygosity provides the cell with a proliferation advantage by increasing the allele burden, thus allowing for a complete expression of the PV phenotype.

The association between JAK-2 and PV and other myeloproliferative neoplasms was first reported by five independent groups in 2005 [4–8]. The reported incidence of JAK-2 tyrosine kinase gene has been reported to be over

90 % in PV, 50 % in myelofibrosis (MF) and essential thrombocythemia (ET), 20 % in subcategories of atypical myeloproliferative neoplasms and 3 % in myelodysplastic syndromes [9]. Therefore, the presence of JAK2 (V617F) mutation is a strong pointer towards an underlying MPN. Hence, evaluation of JAK2 (V617F) mutation is a reasonable strategy in the evaluation of polycythemia, primary thrombocytosis, unexplained leukocytosis, marrow fibrosis or splanchnic vein thrombosis (SVT).

JAK2 (V617F) Mutation, Myeloproliferative Neoplasms and Splanchnic Vein Thrombosis

Venous thrombosis disorders are the third most common affliction of the cardiovascular system after coronary artery disease and stroke in MPN patients [10]. SVT is an uncommon event and usually occurs in the setting of an inherited thrombophilic disorders (21 %) or infectious, inflammatory, malignant disorders or cirrhosis of liver. SVT encompasses the diagnosis of portal vein thrombosis, hepatic vein thrombosis causing Budd-Chiari syndrome, splenic vein and mesenteric vein thromboses. Other well documented risk factors for development of splanchnic venous thrombosis include Philadelphia-chromosome-negative MPNs like PV and ET. Latent or overt MPN is a risk factor in the development of SVT in patients without identifiable inherited or acquired risk factors. Diagnosis of MPN in patients with SVT is challenging as most patients have portal hypertension as a consequence of venous thrombosis resulting in hypersplenism, occult gastrointestinal blood losses, iron deficiency and hemodilution, all of which can decrease the accuracy of peripheral blood counts [11]. Several cases of SVT may go on to develop overt MPN several years later. Historically, latent MPN was diagnosed by formation of spontaneous endogenous erythroid colonies (EEC) by peripheral cells or bone marrow biopsy [12]. EEC studies are specialized tests with limited availability, hence not applicable to general clinical practice. The availability of a peripheral blood test to diagnose MPN with a high degree of accuracy is thus a major advance in timely diagnosis and management of subclinical MPN. It has been reported that JAK2 (V617F) mutation is present in 50–79.3 % all cases of diagnosed MPN with SVT. On the other hand, in patients with latent MPN with SVT, the incidence of JAK2 (V617F) mutation varies from 11 to 74 % [13–23]. Only one retrospective study reported a low prevalence rate of 9.3 % [24]. Hence, patients with SVT and positive JAK2 (V617F) mutation should undergo careful surveillance for subsequent development of overt MPN. Recent studies have identified features of MPN in patients with Budd-Chiari syndrome and SVT; these include younger age at presentation, female preponderance and normal blood counts [11]. The reason for predilection of splanchnic venous circulation as a site for thrombus formation of is unknown in patients with positive

JAK2 (V617F) mutation. Hypothesis includes increased neutrophil and platelet activation, low flow rates and stasis in portal circulation and possible endothelial dysfunction from circulating JAK2 (V617F) derived stem cells. JAK2 (V617) mutation per se has also been thought to be implicated in the pathogenesis of thrombosis. Multiple theories such as modification of red cell adhesion molecules, impaired expression of cMPL signal transduction for thrombopoietin-induced platelet priming leading to chronic platelet hyper-responsiveness, elevation of soluble *p*-selectin levels leading to induction of procoagulant microparticles and platelet-leukocyte aggregates, higher levels of tissue factor-positive platelets, higher soluble thrombomodulin levels, and higher hemoglobin in JAK2 (V617F)-positive ET cases, have been proposed [25–29]. Many retrospective clinical studies have also addressed the association between JAK2 (V617F) mutation and SVT. They also highlighted that JAK2 (V617F) mutation was also observed in a portion of patients not suffering from overt MPN at the time of thrombotic events. Many authors have speculated patients with JAK2 (V617) mutation, but without overt MPN, may have latent or early MPN and be at high risk of overt disease later on. However, long follow-up studies are limited but suggest JAK2 (V617F) mutation may portend the subsequent development of MPN [11, 13–15, 20, 21, 30–32].

Recently, Dentali et al. performed a systematic review to assess the frequency of JAK2 (V617F) mutation in VTE patients and the role of JAK2 (V617F) mutation in the diagnosis of MPNs. Twenty-four studies involving 3,123 patients were included. They discovered that the mean prevalence of JAK2(V617F) mutation was 32.7 % in SVT patients and that this mutation was associated with increased risk of SVT (odd ratio, 53.98; 95 % CI, 13.10–222.45) and mean prevalence of this mutation is low in other VTE patients. Authors concluded that presence of JAK2 (V617F) mutation in SVT patients was associated with subsequent diagnosis of MPN in many patients and that routine screening of JAK2 (V617F) mutation appears to be indicated in SVT patients [33].

Conclusion

In conclusion, recent studies have indicated that the development of SVT is a marker for latent Philadelphia-chromosome-negative MPNs. JAK2 (V617F) mutation is strongly observed in overt as well as latent MPNs. The presence of this mutation also correlates with the development of SVT and in patients without evidence of MPN, may portend the future development of latent or overt MPN. Hence, patients with SVT, the thrombophilia workup should include JAK2 (V617F) mutation and patients should be thoroughly investigated for evidence of

MPNs. The subgroup of JAK2 (V617F)-positive patients without evidence of MPN require close monitoring and long-term follow-up for future development of MPNs.

Conflict of interest None.

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