Evidence of jak2 val617phe positive essential thrombocythemia with splanchnic thrombosis during estroprogestinic treatment

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The discovery of the Janus kinase 2 Val617Phe mutation has brought new insights into the development of myeloproliferative disorders; however, the pathogenesis of essential thrombocythemia and its related thrombotic complications has not been completely understood. Although the Janus kinase 2 Val617Phe mutation confirms the initially suspected clonal character of the disease, factors influencing clonal transformation and expansion in the bone marrow have not been fully detected. Furthermore, patients affected by essential thrombocythemia who are carriers of the Janus kinase 2 Val617Phe mutation show a higher incidence of venous thromboembolism both before, and at the time of diagnosis, compared with noncarriers, and recent evidence of splanchnic and cerebral vein thrombosis in carriers of the Janus kinase 2 Val617Phe mutation has been reported. The intake of oral contraceptives is a strong and independent risk factor for venous thromboembolism. In addition, in-vitro tests showed both an altered primary haemostatic plug formation and enhanced platelet aggregation in patients taking such drugs. Little is known, though, about the influence of steroid hormones on both megakaryopoiesis and platelet function in patients with the Janus kinase 2 Val617Phe mutation. Herewith, we report the case of a 30-year-old woman who took a third generation oral contraceptive for 5 months and developed an essential thrombocythemia with spleno–portal axis and superior mesenteric vein thrombosis. She was found to carry the kinase gene Janus kinase 2 mutation. *Blood Coagul Fibrinolysis* 19:453–457 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Essential thrombocythemia (ET) belongs to the larger group of myeloproliferative disorders (MPDs), a set of haematological malignancies globally characterized by clonal haematopoiesis [1]. Clonal haematopoiesis in patients with MPDs is supported by a genetically transformed mutant clone, which retains the capacity to differentiate across multiple cell lineages. Several studies have assessed the nature of clonal haematopoiesis in this group of haematological diseases, each using different techniques including X chromosome inactivation studies [2], cytogenetic studies [3,4] and mutation screening studies. The latter studies have been of crucial importance in the last few years since the discovery of a unique acquired clonal mutation in the Janus kinase (JAK)2. JAK2 is a cytoplasmatic tyrosine kinase directly involved in survival, proliferation and maturation of erythroid progenitors by binding to erythropoietin receptors. Binding of erythropoietin to its receptor results in phosphorylation and activation of JAK2 and phosphorylation of the eryththropoietin receptor itself. The phosphorylated sites become recruitment sites for a family of cytoplasmatic proteins known as signal transducers and activators of transcription (Stats), which activate the proliferation and differentiation signal. A single valine to phenylalanine substitution at position 617 occurring in the JH2 domain of JAK2, which is involved in inhibition of the kinase activity, leads to a gain of function of the JAK2–Stat5 pathway and such effect is enhanced in the cell lines expressing the erythropoietin receptor [5]. The mutation is detectable in approximately 70–90% of patients with polycythemia vera, whereas patients affected by essential thrombocythemia and idiopathic myelofibrosis (IMF) show a positive rate of about 50% with available techniques [5]. Despite so much progress, many issues remain open in the field of MPDs. One comes from the observation that, particularly in essential thrombocythemia, the mutation detected on peripheral blood leukocytes does not exactly reflect in terms of allelic ratio the number of colonies carrying the mutation in the bone marrow [6]. According to Lippert *at al.* [7], the allelic ratio of the mutation, defined as the signal of each allele divided by the sum of signals for both alleles, increases in case of active haematopoiesis and the expression levels of the JAK2 mRNA differ both in different stages of the disease and among different MPDs. Factors

and events determining the skewing of the allelic ratio and thus the rate of clonal haematopoiesis in the bone marrow remain largely unknown and mutation carriers with similar bone marrow biopsy pattern show different clinical course, suggesting that, given the presence of a clonal disease, other events are needed for disease progression [8].

Another unsolved issue in MPDs deals with the pathogenesis of the related thrombotic and haemorrhagic manifestations, the former prevailing on the latter in this group of malignancies. Arterial thrombotic events, both cardiovascular and cerebrovascular, microcirculatory disturbances such as erythromelalgia and venous thromboembolism (VTE) are common in essential thrombocythemia. The discovery of the JAK2 Val617Phe mutation has cast new light on this burning issue. Evidence exists that enhanced platelet activation plays a key role in the pathogenesis of thrombosis in MPDs, particularly essential thrombocythemia and polycythemia vera, which is due to interactions between platelets and leukocytes [9]. The release of procoagulant and inflammatory cytokines from activated leukocytes as well as tissue factor containing microparticles would promote the formation of platelets–leukocytes aggregates, enhancing the basic coagulation threshold with consequent detectable endothelial damage [10].

It is of special clinical importance that patients with JAK2-positive essential thrombocythemia have an increased incidence of VTE compared with a similar mutation-negative population [11]. The role of increased platelet and leukocytes activation as a thrombogenic mechanism in patients with JAK2-positive essential thrombocythemia was successfully investigated in 2006 by Arellano-Rodrigo *et al.* [12], and more recently confirmed by Robertson *et al.* [13]. Nevertheless, data are insufficient to explain some peculiar features of VTE in MPDs such as the occurrence in unusual sites and before the disorder is clinically overt, suggesting that, in accordance with its multifactorial nature, other events may be necessary to produce the thrombotic event.

In the present article, we describe the case of a 30-year-old woman who came to our attention for a severe splanchnic deep vein thrombosis (DVT), which occurred after a 5-month course of therapy with a third generation steroid hormone preparation. She was diagnosed to be affected by essential thrombocythemia and to carry the JAK2 Val617Phe mutation. A possible influence of oral contraceptives intake on both essential thrombocythemia progression and the pathogenesis of thrombotic events is discussed.

Case report

A 30-year-old woman with both personal and familial negative history for VTE was admitted to our department for an almost complete thrombotic obstruction of the splenoportal axis and a superior mesenteric vein thrombosis. Five months before the hospital admission, she had started the intake of a third generation oral contraceptive (Yasmin, from Shering, Milan, Italy; 30 μg ethinylestradiol and 3 mg drospirenone). At the start of the treatment, coagulation screening including blood cell count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and antithrombin was normal. Three days before admission, she had suffered from abdominal pain not responsive to intramuscular domperidone administration. Ultrasonography and a computed tomographic scan revealed a thrombotic occlusion involving the splanchnic district deep veins. She was then referred to our centre for a better definition of the clinical picture and treatment. A duplex ultrasonography confirmed the absence of blood flow in the splenoportal axis. Blood samples revealed an increased platelet count (964×10^9/l). Therefore we performed a bone marrow biopsy that showed a striking abundance of very large megakaryocytes, often forming clusters. As such examination revealed essential thrombocythemia coexisting with an unusual site of thrombosis, we screened our patient for both the JAK2 Val617Phe mutational status and the main thrombophilic conditions in order to exactly define a prothrombotic state. Moreover, routine tests and tumour markers were carried out in order to exclude other underlying diseases. Soon after the confirmation of thrombosis, unfractionated sodium heparin was administered at the dose of 25 000 IU/day, followed by oral anticoagulation with warfarin, maintaining the prothrombin time international normalized ratio (PT-INR) between 2 and 3 (target 2.5). Hydroxyurea was also started for essential thrombocythemia treatment, modulating the daily dose in order to achieve a platelet count lower than 600×10^9/l. While on therapy, periodic abdominal ultrasonography showed a progressive resolution of the portal thrombosis until complete recanalization, and a stable platelet count was obtained within 1 month.

Materials and methods

Venous blood from the fasting patient was drawn into siliconized glass tubes containing 3.8% trisoduim citrate (1 : 9 v/v). Plasma obtained by centrifugation ($2000 \times g$ for 20 min at room temperature) was snap frozen in liquid nitrogen and stored at −80°C within 2 h after collection.

Venous blood for DNA analysis was collected in tubes containing ethylenediaminetetraacetic acid. Anticardiolipin and antiβ_2 glycoprotein 1 antibodies were measured on serum samples stored at −20°C until analysis.

Antithrombin (HemosIL antithrombin; Instrumentation Laboratory Spa, Milan, Italy), Protein C (HemosIL Protein C; Instrumentation Laboratory) and factor VIII (HemosIL factor VIII deficient plasma; Instrumentation

Laboratory) activities were determined using standard methods. Activated protein C resistance (APCR) was performed by a standard commercial assay (Chromogenix, Mölndal, Sweden). Free protein S was carried out by an immunological method (HemosIL free protein S; Instrumentation Laboratory). Lupus anticoagulant (HemosIL LAC Screen/LAC Confirm; Instrumentation Laboratory), anticardiolipin and antiβ_2 glycoprotein 1 antibodies (Diamedix Corporation, Miami, Florida, USA) were detected using commercial kits. Basal homocysteine was determined using an immunological fluorescence polarization-based assay (Abbott AxSYM System; Abbott Laboratories, Abbott Park, Illinois, USA).

Genotype analysis for the G1691A mutation of the factor V gene, the G20210A mutation of the prothrombin gene and the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene were performed using a real time polymerase chain reaction technique (Applied Biosystem, Melbourne, Australia).

The JAK2 Val617Phe mutation was detected using a DNA microchip diagnostic platform (Nanochip Molecular Biology Workstation, Nanogen Inc., San Diego, CA, USA) [14].

Tumour markers (CEA, CA 125, CA 19-9, CA 15-3, AFP) were dosed using a chemiluminescent immunologic microparticle assay (CMIA Architect System; Abbott Laboratories).

Two replicates were analysed for each test, and the mean value was calculated. Furthermore, all parameters were performed repeatedly on several occasions with similar results.

Results

Coagulation and genotypic parameters are shown in Table 1. Genetic analysis revealed homozygosity for the C677T mutation in the MTHFR gene with normal homocysteine plasma levels.

Table 1 Plasma levels at the time of hospital admission

Parameter	Result	Normal range
Platelet count(10^9/l)	964	130–400
Antithrombin (%)	90	85–125
Factor VIII (%)	117	70–120
Protein C activity (%)	72	70–120
Free protein S (%)	64	53–109
Normalized activated protein C sensitivity ratio	0.83	<0.95
Lupus anticoagulant	1.04	0.8–1.2
Anticardiolipin antibody IgM (U/ml)	4.1	<10
Anticardiolipin antibody IgG (U/ml)	3.9	<10
Antiβ_2 glicoprotein 1 IgM (U/ml)	3.1	<10
Antiβ_2 glicoprotein 1 IgG (U/ml)	3.7	<10
V617F JAK2 mutation	Heterozygosis	Absent
Factor V Leiden mutation	Absent	Absent
G20210 A prothrombin mutation	Absent	Absent
C677T MTHFR mutation	Homozygosis	Absent
Homocysteine (μmol/l)	11.4	<15

JAK, Janus kinase; Ig, immunoglobulin; MTHFR, methylenetetrahydrofolate reductase.

As mentioned, bone marrow biopsy revealed an increased number of very large clustering megakaryocytes. This finding, along with the platelet count and the presence of the JAK2 Val617Phe mutation in absence of any other bone marrow finding related to polycythemia vera or IMF, meets the 2008 WHO diagnostic criteria of essential thrombocythemia. The JAK2 Val617Phe mutation detection was positive with an allelic ratio of 11.6, which is in accordance with a lower than 50% value in the heterozygous mutational status. All other routine tests were normal.

Discussion

There was a statistically significant increased incidence of thrombosis in a subset of patients with essential thrombocythemia and who were carriers of the JAK2 Val617Phe mutation. Portal and mesenteric thrombosis, as well as cerebral DVT are the most common occurrences and the mutation can be now considered a risk factor for such events [15]. The pathogenetic mechanism of the increased thrombophilic state associated with the mutation is not clear. An intriguing observation relates to a recent retrospective study by De Stefano *et al.* [16], who detected the mutation in a significant proportion of patients with splanchnic and cerebral DVT without overt MPD, suggesting that the number of platelets is not a major determinant of thrombosis. This is in accordance with the conclusions in the study conducted by Harrison *et al.* [17], in which a comparison analysis of thrombotic and haemorrhagic events between two large groups of patients with essential thrombocythemia treated with hydroxyurea and anagrelide, respectively, was performed. Results revealed a statistically significant number of thrombotic and haemorrhagic events in the anagrelide-treated group, indicating that not only the number of platelets but also their activation status is affected by different cytoreductive strategies and that the choice of drug must take into account both the clinical history and the cardiovascular risk factors.

The intake of oral contraceptives is a strong and independent risk factor for VTE [18,19] and a large number of trials have been conducted to assess the rate of thrombotic risk connected with their use. Several studies have successfully estimated the thrombotic risk deriving from the interplay between oral contraceptives of different generations and inherited or acquired thrombophilias. In addition, the role of platelet functional status in the pathogenesis of VTE during oral contraceptives consumption is emerging. Platelet aggregation has been shown to be influenced *in vitro* by 17beta-estradiol and medroxyprogesterone acetate in postmenopausal women [20], whereas different activation rates have been reported during the ovarian cycle [21]. To clarify the influence of sex steroids on platelet function as a possible

contributing mechanism to the pathogenesis of VTE, Roell *et al.* [22] set up a platelet aggregation cross-sectional trial with five groups of healthy young women with and without intake of oral contraceptives. In the first two groups, those not taking any oral contraceptives, platelets aggregation was evaluated in the follicular phase and in the luteal phase, respectively. The remaining three groups consisted of women taking oral contraceptives of second and third generation. Women belonging to the third and the fourth groups were users of oral contraceptives with a similar progestogen component but different estradiol dose, which was lower in the fourth group. In the last group, women were taking oral contraceptives with a different progestogen, that is, with antiandrogenic effect. The authors demonstrated that platelet function is altered in two phases of the ovarian cycle, being activated in the luteal phase compared with the follicular phase. More interestingly, platelet aggregation was enhanced during oral contraceptive intake and the major determinant of the activation status was the progestogen moiety with the antiandrogen components exerting the highest effect in the last group [22]. Experimental data are in accordance with clinical reports of thrombotic episodes in women taking progestogen-only oral contraceptives whose prothrombotic potential, although they are recommended in women with a personal history of VTE, has never been investigated in large trials [23]. The molecular basis of platelet aggregation during steroid intake is a grey area that has been poorly evaluated and only one study [24] has been performed to outline a steroid hormone receptor profile on megakaryocytes and platelets in humans.

Our patient took for 5 months a drug belonging to the third generation of oral contraceptives, which were commercialized in an attempt to reduce some androgenic effects of second-generation oral contraceptives and have been shown to be more prothrombotic than those of the second generation [25]. The thrombotic risk of oral contraceptives in association with the JAK2 mutation has never been evaluated so far and one single report exists in the literature of a DVT episode with such a combination [26]. As mentioned, third generation oral contraceptives with antiandrogenic properties produce the strongest effect on platelet aggregation in normal individuals. We suppose that this effect can be enhanced in platelets of JAK2-mutation carriers in which a higher baseline activation status has been demonstrated [12]. This hypothesis is supported by the finding of no other thrombophilia in our patient. Despite detection of homozygosity for the C677T mutation of the MTHFR gene, homocysteine levels were normal in our patient and studies [27] performed on this topic did not find any association between hyperhomocysteinemia and thrombotic events in MPDs.

As our patient showed a sudden onset of clinically symptomatic essential thrombocythemia during a short course hormonal therapy, we therefore wondered if the oral contraceptive use might have contributed to the progression or establishment of the underlying haematological disorder beyond the thrombotic episode. We do not know, of course, if the JAK2 mutation was present before the hormonal treatment started, but the short course up to the clinical evidence of the disease deserves some consideration. In a recent prospective observational study, Gale *et al.* [28] showed no significant change in the mutant JAK2 expression in a large number of patients affected by essential thrombocythemia followed over a median period of 4 years, demonstrating that a minor number of mutated clones that have arisen on a polyclonal background can remain stable unless other precipitating events occur to determine disease progression. Interestingly, experimental evidence has proved that progestogens are able to constitutively induce Stat3 transcriptional activation in breast cancer cells and that this effect is mediated by JAK1 and JAK2, whose abolishment activity results in the suppression of all medroxyprogesterone acetate effects on cancer growth [29]. A possible contributing role of steroid hormones, progestins specifically, in the molecular mechanism of essential thrombocythemia might be taken into account considering that Stat3 is progesterone dependent. It is noteworthy that Stat3 and Stat5 phosphorylation is enhanced in essential thrombocythemia and polycythemia vera, and that such activation rate has been demonstrated not to be influenced by the JAK2 Val617Phe mutation [30]. Furthermore, Stat3 has been shown to be constitutively active in polycythemia vera [31]. Such observations reinforce the idea that clonal transformation in MPDs results from a sum of stepping up events.

In conclusion, given the increased thrombotic risk in carriers of the JAK2 Val617Phe mutation compared with noncarriers, the mechanism by which the mutation results in a prothrombotic state is largely unknown, even though platelet activation seems to play an important role. Its better definition will contribute to clarify the mechanism of thrombophilia and the complex relationships between genetic and environmental factors. The onset of a clinically symptomatic haematological malignancy after a short course of oral contraceptives intake suggests that great caution should be paid before administering oral contraceptives in carriers of JAK2 Val617Phe mutation. As we live in an era in which we debate the usefulness and expensiveness of thrombophilic screening, our report outlines the emerging importance of the JAK2 Val617Phe mutation testing, a matter which has been poorly evaluated so far [32].

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