Published in final edited form as:
J Thromb Thrombolysis. 2018 January 1; 45(1): 106–113. doi:10.1007/s11239-017-1566-1.

High Prevalence of Heparin Induced Thrombocytopenia with Thrombosis Among Patients with Essential Thrombocytemia Carrying V617F Mutation

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Abstract

Background—arterial and venous complications are major causes of morbidity and mortality in myeloproliferative neoplasms (MPNs). MPNs patients, frequently receive heparin. Heparin-induced thrombocytopenia (HIT) is a rare but potentially life-threatening complication resulting in a severe acquired thrombophilic condition.

Patients and methods—we carried out a retrospective analysis to evaluate occurrence of new thrombotic events during heparin therapy in essential thrombocythemia (ET) patients. We studied 108 ET patients on heparin for treatment of previous thrombotic events or in thromboprophilaxis. Fifty-eight of them carried JAK 2 V617F mutation while fifty patients were without V617F mutation.

Results—Ten patients, among those with JAK 2 V617F mutation after a median of 10 days from heparin treatment presented a platelet drop, new thrombotic events and in 10/10 cases heparin-related antibodies were found. In the other group, 2 patients (4%) presented a platelet drop, thrombotic manifestations and heparin related antibodies.

Conclusions—Our data show that HIT is more frequent, during heparin treatment, in patients with ET carrying V617F mutation, as compared with patients without mutations (P=0.029). ET with V617F mutation seems to be associated with higher risk of thrombotic complications during heparin treatment. Monitoring platelet counts very closely during the course of heparin is essential especially in ET patients in which platelet drop may be hidden by constitutional thrombocytosis.

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Disclosure:
The authors have stated that they have no conflicts of interests

Ethical approval: This article have ethical approval of committee of University Hospital

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Keywords

myeloproliferative neoplasms (MPNs); essential thrombocythemia; Heparin Treatment; Heparin-induced thrombocytopenia (HIT); V617F mutation

Introduction

Patients with Philadelphia chromosome negative myeloproliferative neoplasms are at increased risk of arterial and venous thrombosis. The cumulative incidence of all thromboembolic events amount to 2.5% to 5% per patient/year in Policytemia Vera (PV) and to 1.9% to 3% per patient in Essential thrombocythemia (ET) [1][2][3]. Only 30% of all vascular events in BCR-ABL1 negative MPNs patients are venous thromboembolism (VTE), while arterial thromboembolism is more frequent [2[3] [4][5][6]. The JAK2V617F mutation, commonly found in MPN, correlates with several clinical and laboratory characteristics. The frequency of JAK2 V617F mutation for PV, idiopathic myelofibrosis (IMF), and ET is 92%, 58%, 50% respectively [7]. The role of JAK2 V617F allele burden as risk factor for thrombosis in MPNs remains debated to date [8][9][10][11][12][13][14]; however, recent advances showed that higher JAK2 mutation allele burden (> 75%) predispose patients to higher thrombotic and haemorrhagic risk [15] [16]. Recently other recurrent mutations have been identified in MPN including the MPL W 515 L/K mutation and mutations of Calreticulin (CALR). MPL mutations are present in 8.5% of JAK2 V617F negative patients while CALR mutations in around 20-25% [18][19]. CALR mutations are associated with younger age, male sex, higher platelet count, lower haemoglobin level, lower leukocyte count and lower incidence of thrombotic events [20].

Because frequently MPNs patients have had previous exposure to heparin, the presence of heparin-related anti-platelet antibodies typical for heparin-induced thrombocytopenia (HIT) is one underlying possible mechanism of thrombosis despite masked normal platelet count.

HIT is an adverse drug reaction caused by immunoglobulin (Ig) G platelet-activating antibodies that bind to platelet factor 4/heparin (PF4/H) complexes on the surface of platelets [21]. Although a significant proportion of patients exposed to heparin will develop anti-PF4/heparin antibodies, a much smaller fraction will develop clinical features of HIT with overt thromboembolic manifestations (HITT). Platelet factor 4 (PF4) displaced from endothelial cells, or directly from the platelets, binds to heparin molecule to form an immunogenic complex. The anti-heparin/PF4 Ig G immune-complexes activates platelets through binding with the Fc gamma RII a (CD32) receptor inducing endothelial lesions. The resulting thrombin generation causes consumptive thrombocytopenia and can lead to devastating venous and arterial thromboembolic complications. Cytokines are generated during this process and inflammation could play an additional role in the pathogenesis of thromboembolic manifestations [22] [23]

The diagnosis of HIT is generally established when a platelet drop of at least 50% occurs in the absence of obvious explanations for thrombocytopenia, and by demonstration of heparin-dependent IgG antibodies. However, HIT in the presence of normal-high platelet counts presents a diagnostic challenge and requires a high index of suspicion. It has been suggested

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that the observed risk of HIT is higher in patients with MPNs. [24] [25][26][27]. The aim of our study is to evaluate the occurrence of new thrombotic events among patients with ET with JAK2 V617F mutation treated with un-fractioned (UFH) or low molecular weight heparin (LMWH).

Patients and methods

This retrospective analysis considered a consecutive series of ET patients exposed to heparin because of previous thrombotic events in usual or in unusual sites or in thromboprophilaxis for surgery This retrospective study lasted from March 2006 to March 2017. The patients were divided into two subgroups : group 1: ET patients carrying JAK2 V617F mutations; group 2: ET patients without JAK2 V617F mutation (control group).

All patients were followed in two University Hospitals. The study was conducted according to the ethical principles stated in the Declaration of Helsinki, and the Institutional Review Board of our University Hospital approved the protocol.

The diagnosis of ET was based on the 2008 WHO criteria [28]. All of their available medical charts were reviewed, and a record was made of the data relating to their clinical characteristics, laboratory data, and bone marrow biopsy findings at the time of the diagnosis of thrombotic events and/or MPN. Informed consent to treat laboratory and clinical data was obtained from all individuals participants included in the study.

Coagulation Screening

A careful family and personal thrombotic history was collected for each patient, history of miscarriages and obstetrical history was collected in female's patients. Coagulation tests including serum fibrinogen, prothrombin time (PT) and partial thromboplastin time (PTT), anti-thrombin III, Protein C and S (total and Free) and APC resistance, Leiden factor V, prothrombin G20210A mutations and anti-phospholipids autoantibodies were performed in order to rule out additional prothrombotic risks.

Molecular Analysis

The JAK2 V617F mutation was detected by means of allele-specific PCR using the protocol of Baxter et al. [29] and confirmed by means of direct sequencing (ABI PRISM 310 Genetic Analyser, Applied Biosystems, Warrington, UK) using the Big Dye Termina-tor Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). The allele burden of the mutation was quantitatively analyzed using the protocol recently published by Klampfl et al [19].

In the JAK-2 negative group MPL/CALR mutations were detected by PCR [30]

Thromboembolic complications

The clinical suspicion of venous or arterial thromboembolism was confirmed by the following objective tests: compression ultrasound or venography in case of suspected DVT. Ventilation/perfusion lung scanning, spiral computed tomography (CT) or pulmonary angiography in case of suspected pulmonary embolism. Electrocardiography with enzymatic

and troponin T support in case of suspected myocardial infarction, and cerebral CT scan or magnetic resonance imaging in case of suspected stroke.

Diagnostic criteria for new thrombotic events consisted in: 1) onset of any group of clinical symptoms compatible with arterial or venous thrombosis in patients previously asymptomatic, subsequently confirmed by objective tests listed above 2) contralateral onset of DVT in patients with confirmed ipsilateral DVT 3) non-compressibility > or = 4 mm of a previous normalized venous segment and enlargement of thrombus thickness > or = 2 mm were considered diagnostic of proximal DVT.

Clinical Probability of HIT

The clinical probability of HIT was defined according to the 4 T's score proposed by Lo et al.[31]; a score higher than six was used to predict a high clinical likelihood of HIT.

Development of HIT

A platelet count was performed at baseline conditions and thereafter at least every 2 or 3 days. The diagnosis of HIT was suspected in all cases of platelet drop of at least 50% of pre-treatment value, if this was confirmed by a second determination. In all patients in whom a likely reason for thrombocytopenia (hemodilution from fluids/blood, sepsis, disseminated intravascular coagulation (DIC), other drug reactions, etc.) could not be found, a blood sample was obtained for the subsequent determination of heparin-dependent antibodies.

HIT diagnosis is based on the combination of a compatible clinical picture correlated with the presence of platelet-activating anti-PF4 antibodies (Ab).

Laboratory determination of heparin-dependent Ig G antibodies

Both an antigen and a functional assay were performed to detect heparin-dependent antibodies according to previously described methods at clinical suspicion of HIT.

Blood samples were collected from a brachial vein with a fine needle in sodium citrate 1:10 (antigen assay) and without anticoagulant (functional assay). Samples were then centrifuged at 10 000 *g* for three minutes and stored at – 70°C. [32].

The samples were screened by an antigen assay that detects antibody mixture of IgG, IgA, and IgM antibodies against platelet factor 4 complexed with polyvinyl sulfonate (Genetic Testing Institute, Brookfield, WI).Absorbance of the substrate of alkaline phosphatase was measured at 405 nm. This test was followed, in case of a positive result, by the search for HIT antibodies of the IgG class using a single anti-IgG alkaline phosphatase conjugate Fc specific (Sigma Chemical, St Louis, MO). The cut-off value of optical density (OD) for the antibody determination was assessed using plasma samples of 43 patients who did not develop thrombocytopenia within 48 hours of LMWH start; the value was considered positive for values >0.10 OD.

The functional test was done with a modification of the visual evaluation of heparin-induced platelet activation (HIPA) assay.[33] on patients with clinical suspicion of HIT and negative OD. HIPA is a platelet-activation test in which the patient's serum is mixed with donor

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platelets in the presence of heparin. Aggregation of the donor platelets indicates the presence of antibodies to the heparin–PF4 complex.

Statistical Analysis

The study group included ET patients carrying JAK2 (V617F) mutation, and ET patients without JAK2 (V617F) mutation. The differences in thrombotic events during heparin treatment or prophylaxis between the two groups was calculated by using the chi-square test. Student T test for unpaired values was used to assess the statistical significance of the differences between groups. Wilcox on matched-pairs signed rank test was used to assess any significant changing in platelet count before and after the occurrence of HIT. To assess changes in platelet count we computed an index of variations by subtracting the patient's platelet count after HIT from the patient's platelet count after the first episode of thrombosis. Any correlation between platelet count or its changes and occurrence of HIT was evaluated with simple logistic regression. A P-value of < 0.05 was considered statistically significant.

All the analyses were made using SPSS PC statistical package, release 20.0 (IBM SPSS Inc, Chicago; IL, USA), and statistical software R, release 3.0.0 (R Foundation for statistical computing, Vienna Austria).

Results

Laboratory findings

A search for H/PF4 Abs was performed using immune and functional assays, and the presence of specific and platelet-activating IgG confirmed the clinical suspicion of HIT in twelve patients who presented thrombosis during heparin treatment (See table 2). The patient 9 had negative OD, but the heparin-induced platelet aggregation assay (HIPA) was performed and confirmed diagnosis of HIT.

Clinical Pathological features of MPNs associated with thrombotic manifestation

We selected 108 patients with ET. Fifty-eight patients carried JAK2 V617F mutation, 27 males and 31 females who presented an unusual splanchnic vein thrombosis or thrombosis in usual or /unusual site needing heparin treatment. Median age was 41 years (range 16-65).

Fifty ET patients not carrying JAK2 V617F mutation, exposed to heparin for thrombosis served as control group. Coagulation screening was negative for 104 patients, while two patients presented Leiden factor V mutation and two patients presented prothrombin G20210A mutations. Molecular analysis showed JAK2 V617F mutation in 58 patients, JAK2 median allele burden 27.7% range (4.8-97.0). The main clinical-pathological features of the 58 study patients and 50 controls are summarized in Table 1. The two groups did not significantly differed for sex, age, duration of follow up, reasons for heparin treatment, initial platelet count was higher in JAK2 V617F mutation positive patients.

Thrombotic manifestations—In the group I all patients presented usual site or unusual site thrombotic complication requiring heparin treatment, or were on heparin prophylaxis for surgery: ten patients (17.2%) had cranial sinus vein thrombosis; four patients (6.9%) had

coronary artery disease. Four patients (6.9 %) had mesenteric and portal vein thrombosis, five patients (8.6%) had Budd Chiari syndrome, six patients (10,3 %) had ischemic stroke, seven patients (12 %) had deep vein thrombosis, twenty-two (38 %) were on heparin prophylaxis for surgery.

Forty-six patients (79.3%) were treated with low molecular weight heparin, while 12 (20,6%) received un-fractioned heparin. During heparin treatment, at a median of 7.5 days (range 5-10 days) after beginning of heparin treatment, 10 patients developed new thrombotic manifestations characterized by rapid onset of symptoms. Two patients presented new neurological symptoms while receiving heparin, one for DVT and one for splacnic vein thrombosis. One patient presented arterial limb ischemia, one patient presented sudden headache and CT scan documented sinus venous thrombosis, two patients during heparin treatment presented central line venous thrombosis, one patient presented hypotension and sudden syncope together with dyspnoea and CT scan documented bilateral pulmonary embolism. Three patients presented unusual splacnic vein thrombosis.

Of the ET patients with JAK2 V617F mutation who presented thrombosis and heparin dependent antibodies during exposure to heparin, 3 patients were on un-fractioned heparin and 7 patients received low molecular weight heparin. In the control group, the two patients with HITT were on LMWH.

All patients had a platelet drop, and in all cases, no alternative causes of thrombocytopenia were found. Platelet drop and thrombotic manifestations were concomitant. Seven of ten patients who had thrombotic manifestations had previous exposure to heparin. All patients treated with UFH were in therapeutic range at the time of onset of thrombotic manifestation. Heparin-related antibodies were found in ten patients with heparin-related thrombotic complications in the group I and in two patients in the control group. Table 2 reports clinical characteristics of ten patients with MPNs and new thrombotic events during heparin treatment. In the control group (consisting of 50 patients with ET without JAK2 V617F mutation undergoing UFH or LMWH), 2 of them presented thrombotic manifestation consisting in cerebral thrombosis and in acute coronary syndrome, platelet drop and heparin-dependent Ig G antibodies were documented. Statistical analysis showed a significant increase of thrombotic manifestation in ET patients carrying JAK2 V617F mutation compared with ET patients without the mutation. (P= 0.029).

Discussion

The study presented here is aimed to determine if the thrombosis or recurrence of thrombosis, that arise in the course of heparin treatment in patients with ET are due, at least in part, to the heparin–induced thrombocytopenia. Patients with MPN are prone to develop thrombosis being this the major cause of morbidity and mortality among MPN patients. UFH and LMWH therapy are associated with a high rate of drug-related problems and side effects due to either their inherent pharmacological properties or human errors. Thrombocytopenia, bleeding events, and osteopenia are the 3 most common drug-related problems associated with unfractionated heparin and LMWH therapy [34]. Beside hemorrhagic complications also thrombotic events are described while patients assume

unfractionated heparin or LMWH. HIT occurs in 3 to 5 percent of patients who receive intravenous unfractionated heparin compared to the 0.5 percent incidence rate with subcutaneous LMWH, catheter flushes, and even the minuscule amounts of heparin that leaks from coated catheters [35]. Absolute risk for HIT was only 0.2% with LMWH and was 2.6% with unfractionated heparin.[36] HIT can precipitate an extreme prothrombotic diathesis known as HITT, resulting in venous or arterial thromboembolism in 50 percent of patients. The pathophysiology of HIT may be different in UFH from in LMWH. The smaller molecule size of LMWH at least in part determines its affinity for PF4, as has been shown experimentally. Presumably, this makes LMWH less likely to induce HIT than the larger unfractionated heparins. Nevertheless HITT, although rare is also seen in patients treated with LMWH. The interesting finding, observed in our case series that 9/12 patients developed HITT during therapy or prophylaxis with LMWH may be explained with the fact that actually UFH is reserved only to patients with severe renal failure, or to patients at high risk for bleeding complications or to candidates to invasive procedures. LMWH is largely preferred in all other patients both for prophylaxis and for therapy of thrombotic complications.

Anecdotal data on relationship between HIT and MPNs are reported [26], but few studies have systematically approached the problem. [27][25]. Thus to differentiate if a thrombotic event, occurring during heparin treatment, may be related to HIT or due to thrombotic complication of MPNs as natural course of disease may be very challenging. The masked normal platelet count, in a subset of patients with thrombocytosis, can further accentuate the problem hiding a heparin-induced thrombocytopenia. For these reasons, the real incidence of heparin-induced thrombocytopenia in the pathogenesis of new thrombotic events in the setting of patients of MPNs treated with heparin remains unclear and might be under-estimated. Spectre et al reported three patients with MPNs who developed HIT; all presented with a relative fall of platelet counts (although without an absolute thrombocytopenia), thrombosis or skin necrosis and a positive test for HIT antibodies (particle gel immunoassay)[37]. Bovet et al. [25] highlighted that the observed risk of HIT in MPNs was 10 times higher than in general population. Since HIT occurs in approximately 1 in 5000 hospitalized patients [38] the incidence of HIT is relatively high in our study population, confirming the suspect that HIT may be more frequent in patients with MPNs. The same authors hypothesize that platelet activation markers have been reported in ET patients, which could explain the predisposition for HIT in these patients. High PF4 levels have been observed in ET patients, thus leading to the formation of PF4/heparin complexes, which predispose MPNs patients to HIT [39]. In the same way, HIT has already been reported in PV, thus reinforcing a potential link between MPNs and HIT. In our series, we have evaluated 58 patients affected by MPNs with JAK 2 V617F mutation, which, for prophylaxis or for treatment of thrombotic events, were exposed to any heparin treatment (UFH or LMWH) and 50 patients with ET exposed to any type of heparin without JAK 2 V617F mutation. Ten of these patients (17.2%) presented a new thrombotic event after exposure to heparin in the group one. Only 2 patients in the control group presented thrombotic events during heparin treatment. Considering the clinical manifestations (sudden thrombotic events in- patient’s previously asymptomatic), the high clinical probability, the presence of Ig G heparin dependent autoantibodies and the platelet drop, the clinical picture is highly

consistent with diagnosis of heparin-induced thrombocytopenia. The time from heparin exposure and platelet drop corroborates the diagnosis of HIT. It should be considered that platelet drop might be, in patients with thrombotic events, or thrombotic recurrence, a mere platelet consumption, but all patients in our series had heparin related platelet antibodies suggesting an immune-mediated platelet drop like HITT. Statistical analysis showed that the prevalence of thrombotic events, in ET patients exposed to heparin is significantly related with the presence of *JAK2* V617F mutation suggesting that the presence of such mutation could be a risk factor both for thrombosis and for HITT. The *JAK2* V617F mutation unequivocally divides the disease into two subtypes, patients with V617F mutations have a pattern of disease characterized by significantly increased haemoglobin, bone marrow erythropoiesis and granulopoiesis, more venous thrombosis, and a higher rate of polycythaemic transformation than those without the mutation [40]. Rumi et al. [14] have recently suggested that JAK2 V617F mutation represent a strong additional risk factor for thromboembolic events in patients with MPNs as compared with patients with CALR mutation who rarely develop anemia, thrombocytopenia, marked leukocytosis and thrombosis.

Limitations

Our study has not the statistical power to evaluate in absolute the prevalence of HIT in patients with MPNs exposed to heparin treatment due to the rarity of phenomenon, it is a retrospective study, but strongly suggest that HIT may play a role, in the pathogenesis of thrombosis or recurrence of thrombosis that arise in the course of heparin treatment in patients with JAK 2 mutated myeloproliferative neoplasms. Essential thrombocythemia carrying JAK2 V617F mutation seems to be associated with higher risk of thrombotic complications during heparin treatment and this finding should be confirmed in wider studies due to the rarity of the disease.

Conclusions

Our data show that HIT is more frequent, during heparin treatment, in patients with ET carrying JAK2 V617F mutation. ET and JAK2V617F mutation seems to be associated with higher risk of thrombotic complications during heparin treatment.

This paper suggests the relationship between heparin-induced thrombocytopenia with thrombosis (HITT) and thrombotic events during heparin treatment in the setting of patients with ET carrying JAK 2 V617F mutation. This observation illustrates the need to monitor platelet counts very closely during the course of heparin—even subcutaneous heparin—therapy especially in patients in which platelet drop may be hidden by constitutional thrombocytosis such as in ET patients. HIT can lead to HITT, which can cause severe harm. The early identification of HIT and subsequent termination of heparin therapy can prevent complications especially in a setting of patients prone to thrombotic complications.

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Clinical Characteristic of patients with Essential Thrombocythemia (ET) and heparin induced thrombocytopenia according to JAK2 (V617F) Mutation.

	ET JAK2 mutation positive	ET JAK2 mutation negative	P value
N	**58 (100%)**	**50 (100%)**	
Male/Female	27/31	27/23	**ns**
Median age (Years/range)	41(16-65)	43 (18-67)	**ns**
Median Follow up (Years)	4 (1-5)	4(1-5)	**ns**
Low molecular weight heparin	46 (79%)	37 (74%)	ns
MPL mutation	Not detected	2 (4%)	na
CALR mutation	Not detected	10 (20%)	na
Triple negative patient		7 (14%)	na
Prior Heparin treatment	11 (19%)	9 (18%)	ns
Cytoreduction with Hydroxyurea	16 (27.5%)	10 (20%)	ns
Cytoreduction with Interferon	1 (1.7%)	1 (2%)	ns
Reason for heparin treatment			
Cranial sinus Thrombosis	10 (17,2 %)	8 (16%)	ns
Coronary artery Disease	4 (6.9%)	2 (4%)	ns
Mesenteric and portal vein thrombosis	4 (6.9%)	2 (4%9	ns
Surgery Prophylaxis	22 (38%)	25 (50%)	ns
Budd Chiari Syndrome	5 (8.6 %)	3 (6%)	ns
Deep vein thrombosis	7(12%)	4 (8%)	ns
Initial platelet count (x 10^{9}/L) mean	1076	980	p< 0.001
Total	10 (17.2%)	2 (4%)	P=0.029
New neurological signs	2 of 10 (20%)	1 of 2	
Arterial limb ischemia	1 of 10 (10%)		
Sinus vein thrombosis	1 of 10 (10%)		
Pulmonary embolism	1 of 10 (10%)		
Central vein thrombosis	2 of 10 (20%)		
Unusualsplanchnic vein thrombosis	3 of 10 (30%)	1 of 2	
Low Molecular weight heparin while thrombosis	7	2	

Ns: not significant, na: not applicable

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tient n/gender	Type of Myeloproliferative disease	First thrombosis	Second thrombosis	Additional prothrombotic risk factor/ JAK 2 status	Type of Heparin	Anticoagulant after heparin withdrawal	Days elapsing between 1st and 2nd thrombosis	Platelets at 1st thrombosis, 109/l	Platelet nadir, !109/l	Total 4 T's score	H/P F4 Ab IgG OD
	ET	Coronary artery disease	ictus	None/ V617F	LMWH	Lepirudin/oral anticoagulants	14	456	90	7	> 2
	ET	Mesenteric thrombosis	ictus	F V Leiden/ V617F	UFH	Lepirudin/oral anticoagulants	12	729	300	8	> 2
	ET	Coronary artery disease	Arterial limb ischemia	None/ V617F	LMWH	Lepirudin/oral anticoagulants	10	859	268	8	> 2
M	ET	Portal vein thrombosis	Sinus vein thrombosis	none /V617F	LMWH	Lepirudin/oral anticoagulants	12	426	87	8	> 2
M	ET	Portal vein thrombosis	Pulmonary embolism	none /V617F	LMWH	Lepirudin/oral anticoagulants	12	830	422	7	> 2
	ET	Coronary artery disease	Central vein thrombosis	None/ V617F	UFH	Lepirudin/oral anticoagulants	15	236	64	7	> 2
M	ET	Coronary artery disease	Central vein thrombosis	Prothrombin G20210A mutations/ V617F	LMWH	Lepirudin/oral anticoagulants	14	657	275	7	> 2
	ET	DVT	Splacnic vein thrombosis	None/ V617F	UFH	Fondaparinus	15	67	12	8	> 2
	ET	Surgery prophylaxis	Splacnic vein thrombosis	None/ V617F	LMWH	oral anticoagulants	8	230	87	7	o.10
M	ET	DVT	Splacnic vein thrombosis	None/ V617F	LMWH	Fondaparinus	12	342	67	7	> 2
M	ET	DVT	Ictus	None/negative	LMWH	Lepirudin/oral anticoagulants	15	360	78	7	> 2
F	ET	Surgery prophylaxis	Arterial limb ischemia	None/negative	LMWH	Lepirudin/oral anticoagulants	16	398	58	7	> 2

ogical and Clinical Features of 12 patients affected by Essential Trombocytopenia (ET) who presented thrombotic complications during heparin treatment DVT. Deep vein thrombosis, LMWH. Low cular weight heparin. UFH.: Unfractioned heparin.