Heparin-induced thrombocytopenia leading to a diagnosis of essential thrombocythemia

Dear Editors,

Essential thrombocythemia (ET) belongs to the “BCR-ABL-negative” subcategory of myeloproliferative neoplasms (MPN) along with polycythemia vera (PV) and primary myelofibrosis (PM). Various recurrent molecular alterations have been described in classical MPN, such as JAK2 V617F, MPL W515L/K mutations, and deletion or insertions in the calreticulin (CALR) gene. MPN are known for high incidence of thrombotic complications, with a predominance of arterial rather than venous events (16.2% vs 6.2%). Indeed, the prevalence of overall thrombosis has been described in 28.6%, 20.7%, and 9.5% of patients with PV, ET, and PM, respectively.

In addition to the traditional risk factors of thrombosis, blood cells count, mutational profile, chronic inflammation, and abnormal cell adhesion appear to be specific risk factors of thrombosis in MPN-patients. The classical initial treatment of these thrombotic complications includes unfractionated heparin (UFH).

Heparin-induced thrombocytopenia (HIT) is a rare but potentially life-threatening complication of heparin therapy. Its incidence has been lowered by the use of low-molecular-weight heparins (LMWH). Indeed, it occurs in 0.1%-0.8% of patients treated by LMWH and in 1%-2.6% of patients under UFH. HIT diagnosis comprises the use of the clinical 4Ts scoring system and the detection of antibodies against the heparin/platelet factor 4 (PF4) complex. Diagnosis confirmation is based on platelet activation in the presence of heparin, classically measured by radiolabeled serotonin release assay (SRA).

Treatment of HIT relies on the withdrawal of heparin and administration of an alternative non-heparin anticoagulant agent.

In the current work, we report the case of a patient developing multiple thrombosis in a context of HIT, who was diagnosed with ET CALR-mutated.

A 45-year-old man referred to our hospital for worsening of exertional dyspnea was diagnosed with a massive bilateral pulmonary embolism. He had no specific medical history. His initial hemoglobin was 13.1 g/dL, platelet count 306 G/L, and creatinine 118 µmol/L. The patient improved on UFH therapy, but 1 week after treatment initiation a fall in platelet count to 64 G/L was noticed (Figure 1). Since no obvious cause of thrombocytopenia was found (sepsis, disseminated intravascular coagulation, immune thrombocytopenia, drugs, and viral infection), HIT was suspected. On day 8, the “4Ts” score was high (6 points) and the anti-PF4 IgG antibodies were positive by immunoassay (optical density = 1.09). The functional assay SRA was then found positive. UFH was switched to argatroban. Four days later, platelet count reached its nadir: 35 G/L. The day after, the patient presented superficial (basilica vein) as well as deep (ulnar and brachial veins) venous thrombosis of the left arm, and deep venous thrombosis of the right lower limb (femoropopliteal and sural veins) while on therapeutic dose of argatroban (anti-IIa activity between 0.88 and 1.08 µg/mL). The argatroban dose was increased with a target anti-IIa of 1.5 µg/mL. Despite this treatment, the patient worsened and developed a complete deep venous thrombosis.

FIGURE 1 Synthesis graph of the main thromboembolic and biological events during the patient’s hospitalization. The chronology of treatments is represented under the graph. SRA, Serotonin Release Assay; UFH, Unfractionated Heparin.
| Case number | Age/sex | Disease treated with heparin | Initial platelet count ×10^9/UL | Platelet Nadir ×10^9/UL | New thrombotic event | Type of heparin | Mutation | Biological test | Author (year) |
|-------------|---------|------------------------------|--------------------------------|-------------------------|-----------------------|-----------------|----------|----------------|--------------|
| 1           | 52/F    | Cranial sinus thrombosis    | 1008                           | 109                     | None                  | UFH             | NA       | NA             | Walther et al (1996) |
| 2           | 57/M    | Transient ischemic attack   | 1235                           | 633                     | None                  | UFH             | NA       | ELISA          | Risch et al (2000) |
| 3           | 78/F    | Subclavian and axillary thrombosis | 965                            | 194                     | None                  | UFH             | NA       | SRA            | Houston (2000) |
| 4           | 71/F    | Spleen infarction           | 640                            | 383                     | Heparin-induced skin necrosis | UFH + LMWH | NA       | ELISA          | Spectre et al (2008) |
| 5           | 38/F    | Budd-Chiari syndrome        | 175                            | 78                      | Heparin-induced skin necrosis | UFH           | V617F    | HIPA           | Randi et al (2010) |
| 6           | 66/F    | DVT and pulmonary embolism  | 700                            | 6                       | Not described         | UFH             | Not V617F | ELISA          | Lapecorella et al (2010) |
| 7           | 61/F    | Cerebral venous thrombosis  | 517                            | 42                      | Extension of cerebral venous thrombosis | UFH           | V617F    | ELISA          | Richard et al (2011) |
| 8           | 67/F    | Cerebral infarction         | 1270                           | 7                       | Cerebral infarct, femoral artery occlusion, and thrombophlebitis of extremities | UFH           | NA       | ELISA          | Murawaki et al (2012) |
| 9           | 48, F   | Ischemic stroke             | 665                            | 135                     | None                  | UFH             | V617F    | ELISA + PAT    | Bovet et al (2015) |
| 10          | 47, F   | Cerebrovascular thrombosis  | 444                            | 85                      | Thrombosis in central venous line and in radial artery | UFH           | V617F    | ELISA + PAT    | Bovet et al (2015) |
| 11          | 67/F    | NSTEMI                       | 750                            | 216                     | Cerebral infarct, popliteal vein thrombosis | UFH           | NA       | SRA            | Noel et al (2015) |
| 12;>23      | F: n = 7 M: n = 5 Age: NA   | Arterial thrombosis: n = 5     | Arterial: n = 3           | V617F: n = 10           | HIPA: n = 1       | CALR           | ELISA + SRA | Present case   | Castelli et al (2018) |
|             |         | Venous thrombosis: n = 5     | Venous: n = 7                 | LMWH: n = 9             |  |                  |                |                |                |
|             |         | Surgery prophylaxis: n = 2   |                               |                         |  |                  |                |                |                |

**Abbreviations:** CALR, Calreticulin; DVT, Deep venous thrombosis; ELISA, Enzyme-Linked Immunosorbent Assay; F, Female; HIPA, Heparin-Induced Platelet Aggregation; LMWH, Low-molecular-weight heparin; M, Male; NA, Not available; NSTEMI, Non-ST Elevation Myocardial Infarction; PAT, Platelet Aggregation Test; SRA, Serotonin Release Assay; UFH, Unfractionated heparin.
of his lower limb. His platelet count remained low around 70 G/L for 7 days. Warfarin, a vitamin K antagonist (VKA), was started at day 21 as platelet count improved and reached 163 G/L. On day 25, the INR was of 6.47, leading to a decrease in argatroban dose. The next day, skin necrosis appeared on the patient’s hip so VKA was stopped and vitamin K was administered per os while argatroban was increased again to the previous anti-IIa target (1.5 µg/mL). Thrombophilia screening was negative (Protein C, protein S, antithrombin, activated protein C resistance, FV Leiden mutation, FII 20210 mutation, lupus anticoagulant, and antiphospholipid antibodies). Screening for MPN was also performed and a CALR type I mutation (deletion of 52-bp) was found. Bone marrow biopsy showed a megakaryocytic hyperplasia with reticuliculn myelofibrosis (state 2) confirming the diagnosis of ET. Antithrombotic treatment with dabigatran (150 mg twice daily) and aspirin (160 mg daily) was initiated, in association with hydrocarbamide, after collegial discussion with hematologists. The patient was discharged from hospital on day 33 with a platelet count of 220 G/L. To date, the patient is still on antithrombotic treatment (VKA and aspirin) and he has not experienced any new thrombotic event.

Mutations of CALR, a gene encoding the calcium-binding chaperone calreticulin, are the predominant mutations found in patients with non-JAK2 V617F-mutated ET, accounting for 20%-25% of the overall somatic mutations in ET. Compared with JAK2 V617F-mutated ET, CALR-mutated ET has a distinct clinical presentation: it is associated with male sex, younger patients, higher platelet count (>1000 G/L), lower hemoglobin levels, lower leukocyte count, and a significantly lower risk of thrombosis. In our case, the clinical presentation was marked by venous thromboembolic events, which is uncommon for a CALR-mutated ET. This atypical thrombotic presentation may be in relation with the HIT developed by the patient.

HIT is characterized by an immune reaction caused by IgG antibodies recognizing macromolecular complexes of PF4 and heparin. The pathogenicity of these IgG antibodies is linked to their activation of platelet Fcɤ receptors, particularly FcɤRIIA. In “classical” HIT, activated platelets release PF4 from their α granules, leading to the formation of more PF4/heparin complexes. This leads to powerful platelet activation, release of procoagulant microparticles and intense thrombin generation. It has been reported that ET is associated with a 173% increase in concentration of circulating PF4. Whether this potential high level of plasmatic PF4 participates to the genesis and/or severity of HIT in patients with ET has to be established.

Patients with MPN seem to have a higher incidence of HIT. The first case of HIT after heparin therapy in patient with ET was described in 1996 by Walther et al. Twenty-three cases have been then reported (Table 1), all in patients previously diagnosed with HIT.

Recently, Castelli et al suggested that HIT could be more frequent in JAK2-mutated ET rather than non-JAK2-mutated ET, in a retrospective study concerning twelve patients. In their study, the diagnosis of HIT was based on a high 4Ts score (≥7) and the detection of IgG anti-heparin/PFA antibodies by optical density, with no confirmation by functional assays. Thus, we cannot eliminate a non-clinically relevant positivity of the anti-PF4 antibodies. In our case, the detection of antibodies against the heparin/PF4 complex by ELISA was confirmed by radiolabeled SRA.

HIT can be responsible for severe and extensive thrombotic events, but the originality of our case is that these complications, of particular severity, led to the screening for underlying MPN and the diagnosis of CALR-mutated ET. This association is quite uncommon, as no HIT in CALR-mutated ET has been described so far. HIT in ET patients can be underdiagnosed or delayed with a risk of a worse prognosis, due to a high baseline platelet count and a nadir platelet count over 100 G/L. Whether this holds true for CALR-mutated ET, where baseline platelets counts are higher than in others ET, can be suspected but has not been proved so far. Interestingly, in our case, the platelet level at admission was particularly low considering the presence of CALR-mutated ET. This is probably in relation with the acute massive thrombosis (bilateral pulmonary embolism) presented by the patient, likely responsible of an important platelet consumption. Unfortunately, no platelet count history was available before hospitalization. Moreover, the nadir in platelet count was reached 4 days after stopping UFH. Overt platelet consumption in the context of multiple thrombosis may have contributed to this delayed nadir.

The association of superficial and deep vein thrombosis is uncommon in HIT but has already been described. We can thus wonder whether this superficial thrombosis is related to the patient’s ET or if the HIT contributed to its onset. Moreover, HIT is known to be associated with an hypercoagulability state. In our case, the patient had to face a real "thrombotic storm" with multiple venous thrombotic manifestations at first and then skin necrosis. The combination of HIT and ET likely played an important role in the severity of these extensive thrombotic complications.

Concerning the occurrence of skin necrosis, although VKA was introduced when the platelet count was over 150 G/L, the overlap may have been too short, regarding the international recommendations, before the argatroban dose was decreased (4 days). However, skin necrosis may also be related to the use of argatroban at high therapeutic dose. The specific strong anti-IIa activity of argatroban at this dose may have drastically lowered the protein C activation by thrombin-thrombomodulin complex, thus worsening the VKA-related activated protein C depletion and contributing to the skin necrosis. Argatroban alone was administered for 7 days and was then switched to the unusual combined treatment of dabigatran and aspirin, at usual posology.

In conclusion, this is the first report of a severe case of HIT that led to the diagnosis of CALR-mutated ET. In case of HIT with extended thrombosis while on therapeutic anticoagulant dose, a screening for all MPN, including CALR-mutated ET, should therefore be considered even in the absence of high baseline platelet count.

Several cases of HIT diagnosed in patients with ET have been described. Thus, it would be interesting to further explore the potential association between ET and HIT, with particular focus on levels of circulating PF4 in ET patients.
CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
FNG and IGT supervised the study. FNG, IGT, and PG designed the study. AP, JV, and MK collected data and wrote the manuscript. MK, MB, and PM provided clinical information. All authors critically read and approved the manuscript.

DATA AVAILABILITY STATEMENT
Data available on request from the authors.

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