JAK-2 Positive Polycythemia Vera with Paroxysmal Nocturnal Hemoglobinuria and Splanchic Thromboses: A Case Report and Review of the Literature.

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Case report

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Abstract

**Background:** Polycythemia vera (PV) is usually characterized by red cell mass expansion in the peripheral blood and can be complicated with thrombosis, bleeding, evolution to acute myeloid leukemia (AML) or a fibrotic phase. Paroxysmal nocturnal hemoglobinuria (PNH) in an acquired clonal haematopoietic stem cell disorder associated with chronic intravascular hemolysis, venous thrombosis, defective hematopoiesis, frequent episodes of infection and, rarely, leukemic transformation. Herein, we report an interesting case of a patient with co-existence of PNH clones and a \textit{JAK2V617F} positive polycythemia vera, with unusual thromboses and not overt hemolysis.

**Case presentation:** the case is a 51-year-old woman presented with increased levels of hematocrit and multiple liver, spleen, and left kidney infarctions with ascites; further investigation revealed a JAK2-positive polycythemia vera and a significant PNH population. Interestingly, our patient has experienced severe thrombotic events without reporting signs or symptoms of overt hemolysis.

**Conclusions:** This case raises questions over uncharted aspects of the PNH etiopathogenesis, its potential association with myeloproliferative neoplasms (MPN) and highlights the difficulty of dealing with patients with more than one pro-thrombotic states, especially with established and severe thromboses.

**Background**

Myeloproliferative neoplasms (MPN) usually exhibit terminal myeloid cell expansion in the peripheral blood (1). Over time, the clinical course of MPNs can be complicated with thrombosis, bleeding, evolution to acute myeloid leukemia (AML) or a fibrotic phase of the disease (2)(3).

Polycythemia vera (PV) is distinguished clinically from other MPNs by the presence of an elevated red blood cell mass over 125% of normal expressed as an increase in hematocrit (Hct) of more than 49% for men and 48% for women (4). Most patients with PV are diagnosed incidentally when an elevated hemoglobin (Hgb) or Hct is noted on a complete blood count (CBC). Others present with disease-related symptoms (eg, headache, dizziness, visual disturbances, pruritus, early satiety) or complications (eg, thrombosis, bleeding) (5). Pathologic and laboratory features generally noted include: Hgb > 18.5 g/dl in 73% of the cases, white blood cell (WBC) count > 10,5 × 10^9/L in 49%, platelet count > 450 × 10^9/L/ in 53% and platelet count > 1,000 × 10^9/L in 4%.

Paroxysmal nocturnal hemoglobinuria (PNH) in an acquired clonal haematopoietic stem cell disorder characterized by chronic intravascular haemolysis, venous thrombosis, defective hematopoiesis, frequent episodes of infection and, rarely, leukemic transformation. The mechanism of hemolysis appears to be an unregulated complement activation on the abnormal red cell surface, due to reduction or absence of regulatory membrane molecules protecting cells from the membrane attack complex of complement mediated lysis, such as CD55 and CD59 (6)(7)(8). While PNH is a non-neoplastic clonal disorder it has long been closely linked to clinical entities such as aplastic anemia (AA) and myelodysplastic syndromes.
Occasionally, PNH clones can be detected in non-cytopenic patients and patients with no apparent bone marrow failure syndromes; in about 4,1% of the normal population (6)(9).

However, by reviewing literature we identified 37 cases where PNH may be associated with an MPN(10)(11)(6), and 2 cases where thrombosis and not hemolysis was the main clinical feature like the case we present here(3). In general, PNH is usually present as a chameleon(12).

Herein, we report on an interesting case of a female patient with co-existence of PNH clones and a JAK2V617F positive polycythemia vera, with unusual thromboses and not overt hemolysis.

**Case Presentation**

A 51-year-old female - with a medical history of cholecystectomy and beta-thalassemia trait - presented to the emergency department because of discomfort and severe epigastric pain during the last three days. The patient’s history did not reveal indications of hemolytic anemia and associated symptoms, including fatigue, jaundice, or urine discoloration.

The patient had increased levels of Hct (44,8%) (considering she had β thalassemia trait), ALP, γ-GT, AST and ALT as well as a period of pruritus in extremities and her trunk seven months before.

The initial blood tests revealed an elevated Hct level of 44,8% with Hgb levels of 14,4 g/dl, WBC: 4,81 × 10⁹/L-with neutrophils 55% and PLTs: 227 × 10⁹/L. LDH was markedly elevated (410 U/L with normal range (NR) 135–214 U/L), ALP and γ-GT were also significantly elevated: 658 U/L (NR 35–104 U/L) and 459 U/L respectively, AST and ALT were 106 U/L (NR 13–35 U/L) and 123 U/L (NR < 33) respectively, and INR was 1,24. The other biochemical markers were within normal limits (Table 1).
| Laboratory parameters | Value                                      |
|------------------------|-------------------------------------------|
| Hb                     | 14,4 g/dl                                 |
| Hct                    | 44,8%                                     |
| WBC                    | $4,81 \times 10^9$/L (55% Neutrophils)   |
| PLTs                   | $227 \times 10^9$/L                      |
| INR                    | 1,73                                      |
| APTT                   | 40,8 sec                                  |
| Fibrinogen             | 456 mg/dl                                 |
| D-Dimers               | 3,83 µg/ml                                |
| Sodium                 | 140 mmol/L                                |
| Potassium              | 4,1 mmol/L                                |
| Calcium                | 8,7 mg/dl                                 |
| Glucose                | 151 mg/dl                                 |
| Urea                   | 29 mg/dl                                  |
| Creatinine             | 0,55 mg/dl                                |
| Bilirubin total        | 0,52 mg/dl                                |
| Bilirubin direct       | 0,38 mg/dl                                |
| Total protein          | 59,5 g/L                                  |
| Alboumin               | 35,9 g/L                                  |
| Amylase                | 79 U/L                                    |
| LDH                    | 399 U/L                                   |
| ALP                    | 940 U/L                                   |
| γ-GT                   | 297 U/L                                   |
| AST                    | 82 U/L                                    |
| ALT                    | 175 U/L                                   |
| CRP                    | 32,1 mg/L                                 |
Initial investigation with an abdominal ultrasound showed liver and spleen enlargement (17.5 cm and 15 cm, respectively), with ascites (grade 1) whereas a Computed Tomography (CT) scan revealed additionally multiple liver, spleen, and left kidney infarctions. At that time, splenic or portal vein thromboses were not found. A transthoracic echocardiogram revealed no additional findings. Furthermore, an upper endoscopy identified the presence of portal hypertensive gastropathy. In ascitic fluid analysis, Serum Ascites Albumin Gradient (SAAG) was 1.7 g/dl compatible with portal hypertension (Image 1).

Patient was admitted in the hospital and was administered low molecular weight heparin (LMWH) at a therapeutic dose. An autoimmune inflammatory disorder panel showed the following findings: antinuclear antibodies (ANA) positive > 1:640 AC-4, anti-ENA-La positive, anti-ENA-Ro negative, anti-ENA-RNP negative, anti-ENA-Sm negative, p-ANCA negative, c-ANCA negative, anti-dsDNA negative, antimitochondrial antibodies (AMA) negative, anti-smooth muscles (SMA) negative. Thrombophilia workup for factor V (Leiden) gene mutation, G20210A factor II gene mutation, Antiphospholipid syndrome, Antithrombin deficiency, Protein C deficiency, Protein S deficiency was negative whereas there was homozygocity for the C677T MTHFR (methylene-tetrahydrofolate-reductase) gene TT mutation with blood homocysteine level of 7 µmol/l (NR 5–15 µmol/l).

The molecular test showed that the patient was JAK-2 positive. Blood erythropoietin level were within normal ranges. A bone marrow biopsy revealed a hypercellular bone marrow (90%), with erythroid hyperplasia, mild dyserythropoiesis, a mild granulocytic hyperplasia with no dysplastic features and no increase in the blast count; consistent with the diagnosis of PV. In order to complete the diagnostic workup of thrombophilia test in unusual vascular beds, ow cytometry analysis of peripheral blood was performed, revealing a 91.8% of granulocytes, 45.5% of monocytes and 90.6% of erythrocytes CD55 and CD 59 - deficient population suggestive of PNH. At the time of diagnosis a reticulocyte count was within normal ranges, as well as haptoglobin, direct and indirect bilirubin, Direct antiglobulin (Coombs) testing (DAT) was negative and urine samples were negative for hemoglobin or hemosiderin. Lactate dehydrogenase (LDH) was markedly elevated. The patient was initially treated with hydroxyurea: 500 mg x 1 daily and LMWH at a therapeutic dose: 0.6 ml x 2. A mild drop in the Hct, AST, ALT, γ-GT, ALP was initially observed before rising up again on a new setting of abdominal pain and ascites causing marked abdominal distension. On these grounds, radiological reassessment with CT angiography of the abdomen detected a thrombosis of the hepatic veins. Then, LMWH was switched to acenocoumarol and a liver biopsy was performed which was consistent with the diagnosis of Budd – Chiari syndrome.

Dealing with a thrombotic complication of PNH, initiation of complement inhibition treatment with eculizumab was decided. Before initiation, the patient received prophylactic immunization with meningococcal and pneumococcal vaccines and vaccine against Haemophilus influenzae type b.

After eculizumab was started, the Hct and Hgb levels decreased (36% and 12 g/dl, respectively), cholestatic enzymes levels were markedly improved (ALP: 206U/l and γ-GT: 200U/l) and liver enzymes
were normalized. Ascites and abdominal pain were completely resolved (Table 2).

| Laboratory parameter | Value                        |
|-----------------------|------------------------------|
| Hb                    | 12 g/dl                      |
| Hct                   | 36%                          |
| WBC                   | $4.81 \times 10^9$/L (55% Neutrophils) |
| PLTs                  | $227 \times 10^9$/L          |
| INR                   | 1.24                         |
| LDH                   | 410 U/L                      |
| ALP                   | 206 U/L                      |
| g-GT                  | 200 U/L                      |
| AST                   | 32 U/L                       |
| ALT                   | 38 U/L                       |

On the present day, the patient is treated with hydroxyurea for PV, and acenocoumarol and eculizumab for the thrombotic complications; ten months after the initiation of full therapy.

**Discussion And Conclusion**

MPNs, particularly polycythemia vera and essential thrombocythemia, are characterized by thrombotic and hemorrhagic complications. These complications include both arterial and venous thrombosis. Patients with PV have an increased risk of thrombosis (such as cerebrovascular event, myocardial infarction, superficial thrombophlebitis, deep vein thrombosis, pulmonary embolism) or hemorrhage, as well as microcirculatory disorders, such as erythromelalgia, visual and neurologic symptoms. In a large international study, an arterial or venous thrombotic complication or major hemorrhage was noted prior to or at the time of diagnosis in 16, 7, and 4 percent of patients with PV as defined by the WHO (5).

Hyperviscosity may also contribute in the pathogenesis of thrombosis in polycythemia vera - with JAK2 mutation (13).

A high percentage of patients with idiopathic hepatic (eg, Budd-Chiari syndrome) or portal vein thrombosis, but not those with idiopathic lower extremity deep vein thrombosis DVT(13)(14)(15)(16), have the JAK2 mutation suggestive of an occult MPN (17)(18)(19)(20). Approximately 40% of patients with visceral vein thrombosis are JAK2 mutated but the same sources showed that there was another risk factor for thrombosis, either a hypercoagulable disorder or a predisposing condition(21). Although the
mechanisms involved in this hypercoagulable state are unclear, abnormalities in blood viscosity, platelets, and leukocytes have been implicated (12). Major thrombotic events can occur in patients who otherwise have few clinical and laboratory features of PV. Examples include the Budd-Chiari syndrome and portal, splenic, or mesenteric vein thrombosis (22), in whom the ensuing portal hypertension and hypersplenism may mask the increase in blood cell counts (23)(17)(24)(25)(26)(21). PV should be excluded in patients with these diagnoses, particularly women under the age of 45.

PNH patients over time present with chronic intravascular hemolysis and hemoglobinuria accompanied by leukopenia and thrombocytopenia. Nevertheless, acute and chronic venous thrombosis is the leading cause of death in PNH. PNH is associated with an approximately 40% prevalence (in the United States and Europe) of venous thrombosis in the intra-abdominal veins such as the mesenteric, hepatic, portal, splenic, and renal veins- and cerebral vessels, as opposed to deep vein thrombosis or pulmonary embolism (22). The pathogenesis of thrombosis in PNH is multifactorial and incompletely understood. However, there are several theories under investigation (28) (29). Complement inhibition seems to reduce both hemolysis and thrombotic complications (57). In addition, the risk of thrombosis correlates with the size of the PNH clone; however, it is not well investigated whether this correlation reflects the degree of hemolysis or other mechanism (28).

The reason thrombosis occurs in atypical locations rather than typical locations such as deep veins of the leg is not well understood. Relatively low flow rates in intra-abdominal vessels have been postulated as a potential mechanism.

PNH clones are present in different hematological diseases like aplastic anemia (AA) where deficiency in both CD55 and CD59 molecules were detected in 33.3% of AA patients, in 16.5% of MDS patients (50% with a hypoplastic bone marrow) and 4.1% of normal individuals (6)(8). The association between PNH and MPNs is rare and difficult to ascertain. The coexistence of PNH and PMF with JAK2 mutation has been reported (6)(27)(34)(35)(36), as well as a PNH case associated with an MPN, possibly chronic neutrophilic leukemia (37). Although "PNH-like“ defects have been described in five series of patients with any form of MPN [50% of patients with PMF (38) and 59% of 22 patients with MPN in general] only one patient with CML and one with PMF among 50 patients had a positive sucrose lysis test of 5% or greater with no clinical evidences of thrombosis or hemolysis (39)(40).

Two cases where MPN can be associated to a PNH clone without overt hemolysis at diagnosis have been reported. These PNH clones were detected in JAK2 V617F-mutated patients and were characterised by a GPI deficiency ranging between 0.05 and 99 percent (3)(41). These reports clearly showed that JAK2V617F mutation was not in the germline and it co-existed within in the PNH clone (3)(41)(42)(43)(44). This association may be attributed to the PNH clone arising either in the JAK2 mutated population or in parallel to the JAK2 mutated population.

The observation that the JAK-2 mutation is not always present in a MPN does not provide a clear explanation of this co-finding. PNH and MPN coexist regardless of the detection of a known MPN
mutation. For example, in our case we have a patient with polycythemia vera where the JAK-2 mutation is the main molecular finding. There are other cases like one of a patient with essential thrombocytosis and CALR mutation, where the co-finding of a PNH clone and MPN is also present and cases where a molecular finding of an MPN is absent but a PNH clone is detectable (42).

Both MPN and PNH are thrombophilic conditions, with a high risk of developing major thromboses in approximately 50% for both conditions (32)(45). This observation about thrombotic predisposition in PNH can be attributed to different factors such as: nitrogen oxide depletion, complement activation and to a larger number of inflammatory cytokines; without the presence of overt hemolysis in patients (3)(32).

Moreover, no increased congenital thrombotic risk has been recorded in patients with PNH and thrombosis(46) (47). Independent risk factors for thrombosis are the age (> 55 years), the number of transfusions, and the presence of thrombosis at diagnosis. Interestingly, it seems that the risk of a thrombotic event is directly related to the size of the PNH clone, and in particular to the percentage of granulocytes with a lack of GPI anchored proteins. A size above 50% is associated with a thrombosis rate of about 45%, while a size below 50% with a thrombosis frequency of about 5.8% - higher than the general population (5 thrombotic events occur per 10,000 patients per year) (49). The risk increases by 1.64 for each additional increase in size by 10%, so that patients with more than 70% deficiency have a 12 times higher risk than those with a 20% strand (50).

The PNH clone of the platelets is significantly correlated with that of the granulocytes and appears to contribute to the thrombotic risk (51). PNH cases indicate that thrombotic episodes, even in patients with large clones, may occur with or without minimal hemolysis (32).

There are studies demonstrating that CD59 - mediated signals via antibody cross-linking may induce the activation of protein-tyrosine kinases leading to a rapid increase in the tyrosine phosphorylation of several proteins like p120(48). The identification of such CD59-mediated signals may explain why patients with PNH might be susceptible to proliferative disorders and may provide a possible joining link between PNH and MPN. Moreover, other studies like that from Shen et al (52) showed that PIG-A mutation can occur alone, or be followed by one or more secondary subclonal mutations. That is possibly how a PNH clone can expand in parallel with an MPN clone. In any case the pathogenesis of PNH is highly heterogeneous.

The most effective initial treatment of patients with PNH presented with a new thrombotic event is anticoagulation therapy with unfractionated or low-molecular weight heparin. Also, eculizumab (Soliris) is the only approved treatment for PNH. Additional inhibition therapy with eculizumab may be initiated within 24 hours of the occurrence of any thrombotic event, in order to reduce the risk of an extension of a thrombotic site or relapse of it (32). In any case, the development of PNH-related thrombosis is one of the main indications for initiation of treatment with eculizumab.

The most effective initial treatment of patients with MPNs who develop a thrombotic event is the initiation of anticoagulant therapy with oral anticoagulants (acenocoumarol, DOACs). The modifying
therapeutic interventions in subjects with MPN who also have PNH include co-administration of complement inhibition therapy (eculizumab) and allogeneic bone marrow transplantation when this is required as necessary due the patient's clinical presentation.

According to all previous data, an appropriate therapeutic algorithm for patients with co-existence of PNH and MPN is the following:

If eculizumab is not available, initial prophylaxis with warfarin or acenocoumarol should be considered. The patient should be monitored and informed of the risk of bleeding and re-occurrence of thrombosis. (53) (7) (8)

If eculizumab is available, co-administration of eculizumab and warfarin-or acenocoumarol-is a possible option. (40) If there is improvement in the clinical picture following the administration of eculizumab anticoagulant therapy may be discontinued. (54)

Allogeneic bone marrow transplantation may be a therapeutic option if eculizumab is not available. Immunosuppressive therapy appears to have a protective effect, although the underlying mechanisms remain unclear (48).

In conclusion, the clinical significance of the coexistence of PNH and MPN, until nowadays, has not been thoroughly investigated. Studies have shown that small “PNH like” clones are present in approximately 2% of patients with MPN (44). Larger patient cohorts with long-term follow-up are needed. In any case, every patient with MPN and an unusual pattern of thrombosis—such as splanchnic veins or cerebral sinuses—needs to be tested for PNH according to Consensus Recommendations (55) (56).

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

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

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Citations are included in the reference list.

**Competing interests**

The authors have no conflicts of interest to disclose.

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Authors' contributions

All authors contributed to the design of the case presentation, GD and SC drafted the article. NV, PD, EG revised the manuscript. All authors read and approved the final manuscript.

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