

Handling paroxysmal nocturnal hemoglobinuria in post-essential thrombocythemia myelofibrosis with CALR mutation: A case Report.

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ABSTRACT

The loss of erythrocyte surface proteins causes paroxysmal nocturnal hemoglobinuria (PNH), which in turn triggers complement activation and all of its consequences. In this case study, a 57-year-old man with myelofibrosis (MF) and post-essential thrombocythemia (ET) presents with symptoms of anemia and hemolysis. Despite the workup localizing hemolysis to the intramedullary region, the precise diagnosis remained unclear, necessitating a protracted course of steroid medication to control anemia. The patient was treated with complement inhibitors until the medication failed, and the hemolysis was ultimately linked to PNH, which was identified using flow cytometry. In the end, he had a successful hematopoietic cell transplant (HCT), and flow cytometry performed after the transplant revealed that his PNH had completely resolved. Although PNH has been recognized as a myelodysplastic disease progression, its uncommon development in myeloproliferative neoplasms is little understood by its mechanisms. In addition, diagnosis and treatment are difficult due to its rarity and frequently ambiguous symptoms. This is the first instance to be treated with HCT and the second case of a JAK2-negative, CALR-positive post-ET MF that has been documented. This example raises questions about the clinical importance of the relationship between MF and PNH, how it affects management, and whether HCT can be used as a curative treatment for these individuals who do not respond to complement inhibitor therapy.

Keywords: *CALR, case report, myelofibrosis, PNH, thrombocythemia*

INTRODUCTION

The rare condition known as paroxysmal nocturnal hemoglobinuria (PNH) gets its name from one of its unusual symptoms. A variety of other non-specific symptoms, such as hemolysis, cytopenias, exhaustion, and dyspnea, might be present with PNH. The PIGA gene mutates as a result, entails an inability to produce the erythrocyte surface inhibitory proteins CD55 and CD59, which results in uncontrollably activating complement [1]. There are further downstream mutations that have been found to cause PNH. Using flow cytometry, PNH is identified and treated with the monoclonal antibody eculizumab, which blocks the terminal complement. For PNH, bone marrow transplantation is still the sole treatment available.

PNH clones are rare in myeloproliferative neoplasms (MPNs), despite being a prevalent occurrence in myelodysplastic syndromes (MDS) [2]. In the context of JAK2V617F-negative CALR-1 mutant post-essential thrombocythemia myelofibrosis (post-ET MF), we report the second case of late-stage evolution of PNH. In addition, we look at the effectiveness of bone marrow transplantation in treating PNH in cases of bone marrow failure.

CASE SUMMARY

A healthy 57-year-old man with nosebleeds and an elevated platelet count in the one million range sought hematological assessment. Megakaryocytosis was observed in the initial bone marrow biopsy, along with normal cytogenetics and JAK2 negative results from molecular testing. Since the patient's diagnosis happened years before the 2013 discovery of the CALR mutation's link to MPNs, CALR mutation testing was not done at this time. Anagrelide, hydroxyurea, and aspirin were the first medications used to treat his ET, but he had unbearable side effects. Interferon therapy eventually succeeded in keeping his platelet count stable ($400-500 \times 10^3/\mu\text{L}$), but it was stopped when he and his spouse made the decision to become pregnant.

The patient chose to stop treatment and continued to have

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stable blood counts during routine checks ($6 \times 10^3/\mu\text{L}$ for white blood cells, 12–13 g/dL for hemoglobin, and $523 \times 10^3/\mu\text{L}$ for platelets). In the end, an upper respiratory infection caused a hemoglobin reduction of 11.0 g/dL with no further improvement, which prompted a bone marrow biopsy. After biopsies, the marrow was found to be hypercellular, with elevated grade 2 reticulin fibrosis, no increased blasts (<1%), abnormal placement of immature myeloid precursors, and huge, voluminous, abnormal megakaryocytes grouped together (Figure 1). MF was classified as being in the Intermediate-2 risk category with a DIPPS-plus score of 2. After starting on ruxolitinib 10 mg daily, his anemia only slightly improved, necessitating the weekly addition of 45 milligrams of pegylated interferon alpha. However, his anemia (Hgb 9.1 g/dL) persisted. He was put on darbepoetin alfa every two to three weeks, but there was no improvement, as his anemia worsened over the course of months. The patient decided to quit using pegylated interferon and ruxolitinib due to severe anemia that required red blood cell (RBC) transfusions. A repeat lab test revealed increased erythropoietin at 1880 $\mu\text{U/L}$, WBC $5.9 \times 10^3/\mu\text{L}$, Hgb 7.3 g/dL, and Plt $269 \times 10^3/\mu\text{L}$. Low hemoglobin < 10 mg/dL, high LDH 1,046 U/L, total bilirubin 1.2 mg/dL, and reticulocyte count 7.5% were all substantially higher in hemolysis labs. Other possible causes of immune-mediated extramedullary hemolysis were ruled out by the negative results of the direct coombs test and the cold hemagglutinin < 1:4. Molecular testing using interval repeats revealed a type 1 CALR mutation (52 bp del) with a 41% variant allele frequency, and showed no signs of a JAK2 mutation. Intramedullary hemolysis was the working diagnosis as the underlying cause of the patient's hemolysis was not found despite the patient's hemoglobin levels improving after prednisone was reintroduced, which is consistent with the immunological basis for the patient's hemolysis. many workups and prednisone trials. Consequently, the patient was kept under control with prednisone for about 3.5 years.

Different laboratory tests were carried out, such as flow cytometry and bone marrow biopsy, when the patient's care was transferred to a different physician.

PNH clones with 83.79% monocytes and 84.29% granulocytes were found using flow cytometry (Figure 2). With a DIPPS-plus score of 3 and no myelodysplasia, the bone marrow biopsy revealed massive, clustered hyper-lobulated hyperchromatic megakaryocytes, which are consistent with stage 2-3 MF. He began taking ravulizumab while also cutting back on the dosage of prednisone. Despite the patient not experiencing any thrombotic problems, rivaroxaban was begun for prophylactic purposes to prevent thrombosis. Before the patient became refractory after six months of treatment and switched to pegcetacoplan, which also failed after six months, hemoglobin improved to a peak of 9 g/dL while on

Ravulizumab. As needed, he was given supportive red blood cell transfusions.

DISCUSSION

The example of our patient emphasizes the difficulties and nuances of managing post-ET MF as well as the significance of taking PNH into account as a rare co-existing illness in patients exhibiting prolonged coombs negative hemolytic anemia symptoms. The illness history in this case is examined over a 23-year period, demonstrating that even seemingly insignificant changes in a patient's clinical status, like persistent anemia, can indicate new developments or disease progression and should be investigated thoroughly. As a doctor who has been treating a patient with a confirmed diagnosis for years, it is simple to ignore slow longitudinal disease progression.

Complement inhibitors are used as a treatment for hemolysis in symptomatic PNH patients. Among the first-line suggestions are eculizumab and ravulizumab, which work by binding to C5 and inhibiting activation. For individuals who do not respond to C3 inhibitors, pgcetacoplan is a second-line treatment that inhibits both intravascular and extravascular hemolysis by binding to C3. HCT is advised as a curative treatment for PNH patients with bone marrow loss brought on by MDS, however no clinical trials have compared the effectiveness of complement inhibitor therapy and allogeneic HCT. Curative outcomes were observed in PNH patients with severe MDS-related bone marrow loss, according to small studies and case reports. In light of severe MF and PNH, our patient eventually had an HCT. The first case that has been documented to determine whether HCT is successful in treating PNH patients when there is MPN and a chance of recovery. Patients with severe MF who do not respond to complement therapy or who experience bone marrow loss may benefit from hemodialysis. The fact that this is a case report concentrating on a single patient means that the results may not apply to other people in comparable circumstances, which is a limitation of the findings. Furthermore, there is no evidence to indicate any causal correlations. Additional case studies and clinical trials—which could be challenging to conduct given the rarity of this disease process—are required to demonstrate the recurring efficacy of HCT in situations like this one.

Prior case reports of patients with primary MPN caused by mutations in JAK2V617F and MPLW515L have documented the establishment of PNH clones, notwithstanding their rarity; however, there is only one other instance that reveals a CALR mutation [2]. PNH may develop in any MPN, regardless of the mutation, as a result of a gradual course of the illness that finally causes genetic alterations in hematopoietic stem cells. This lends credence to the multiple-hit theory. We propose that the CALR mutation, which has been demonstrated to induce excessive cell proliferation, may be connected to the

amplification of PNH clones into clinically relevant illnesses [3]. Another instance of late-stage PNH in CALR-positive ET lends credence to this notion.

Although the clinical relevance of PNH in MPN is unknown, one idea explains the emergence of clones in late-stage instances of MPN by proposing that PNH may have an impact on the disease's course [5]. PNH clones were discovered in a sizable percentage of cases in a study looking at the prevalence of PNH clones in different bone marrow disorders [6]. Therefore, whether or not patients with bone marrow disorders exhibit hemolysis symptoms, frequent screening for PNH may be helpful [6]. The tiny sample size of this study is one of its drawbacks.

On the other hand, another retrospective study makes the claim that lab results or symptoms indicating anemia in MPN are not indicative of PNH [7]. Despite the small sample size of this trial as well, the authors advise against routinely screening patients with MPN specifically for PNH. In spite of this, various intrinsic bone marrow disorders may still be eligible for PNH clone screening [6, 7]. A prospective study that looked at the levels of haptoglobin and LDH in MF patients who did not have liver cirrhosis or hemolytic anemia offers potential explanations for elevated hemolysis markers in MPN. They discovered a relationship between the levels of inflammatory indicators (albumin and C-reactive protein) and these aberrant markers with high JAK-2 allele load, but they neglected to examine additional mutations such CALR [8].

Conclusion

These contradictory results and imprecise Conversely, a different retrospective study asserts that test results or symptoms indicating anemia in MPN are not indicative of PNH [7]. Despite the small sample size of this trial as well, the authors advise against routinely screening patients with MPN specifically for PNH. That being said, the idea of PNH clone screening might still be relevant for other intrinsic bone marrow disorders [6, 7]. A prospective study that looked at the levels of haptoglobin and LDH in MF patients who did not have liver cirrhosis or hemolytic anemia offers potential explanations for elevated hemolysis markers in MPN. They discovered a relationship between the levels of inflammatory indicators (albumin and C-reactive protein) and these aberrant markers with high JAK-2 allele load, but they neglected to examine additional mutations such CALR [8]. These contradictory results

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