

Case Report

Use of intravenous immunoglobulin in the treatment of paroxysmal nocturnal hemoglobinuria: a case report and literature review

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Abstract: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of hematopoietic stem cell (HSC) that makes blood cells more sensitive to active complement system. Eculizumab is considered as primary treatment for PNH nowadays. Because of the commercial unavailability of eculizumab in China, the treatment is still at a conventional level, such as using steroid and blood transfusion. However, PNH treated with intravenous immunoglobulin (IVIg) has never been reported. Here, we reported a patient treated with IVIg and got better therapeutic effects, presenting with obviously improved hemoglobin and dropped lactate dehydrogenase (LDH) close to normal level after IVIg. We think IVIg for PNH is worth being concerned and verified as an alternative therapy when eculizumab is not in hand.

Keywords: Intravenous immunoglobulin, paroxysmal nocturnal hemoglobinuria, complement, eculizumab

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by intravascular hemolysis [1], having the clinical manifestations of intermittent hemoglobinuria associated with sleepiness, bone marrow failure and thrombosis [2]. C5 monoclonal antibody, named eculizumab, is the primary treatment of PNH now. But it is not available commercially in China at present. In this report, we report a patient treated with intravenous immunoglobulin (IVIg) and discuss the possible mechanisms.

Case presentation

A 58-year-old male was admitted to our hospital due to fatigue and dark-colored urine in 2015. Anemia countenance and slight yellow sclera were documented by physical examination. Laboratory tests revealed white blood cell (WBC) $3.98 \times 10^9/L$, hemoglobin (Hb) 8.4 g/dL, platelet (PLT) $80 \times 10^9/L$, reticulocyte $238.2 \times 10^9/L$, lactate dehydrogenase (LDH) 2565 IU/L and free Hb 0.1 g/dL. Hypercellular with relative erythroid hyperplasia were documented on

bone marrow aspiration. Coombs test was negative. Flow cytometry analysis revealed CD55 negative cells were 29% in erythrocyte and 31% in granulocytes, and CD59 negative cells were 38% in erythrocyte and 42% in granulocytes. On these bases and according to Chinese experts consensus on diagnosis and treatment of PNH, the patient was diagnosed as PNH. Prednisone was initiated, but the effect was not good. After informed consent, he got IVIg treatment (0.4 g/kg/day, day 1-5). Five days later, the blood examination revealed that WBC, Hb, PLT started moving up. Two weeks later, the laboratory tests showed that WBC, Hb, PLT increased to $5.59 \times 10^9/L$, 9.2 g/dL, $105 \times 10^9/L$, respectively. Then, he was discharged.

Three months later after his discharged, he was readmitted to our hospital because of severe hemoglobinuria and perianal abscess. Laboratory tests showed serious anemia (Hb 3.8 g/dL), thrombocytopenia ($56 \times 10^9/L$) with normal WBC, high LDH (3465 IU/L) and reticulocyte ($144.40 \times 10^9/L$) level. IVIg (0.4 g/kg/day, day

Use of IVIG in PNH treatment

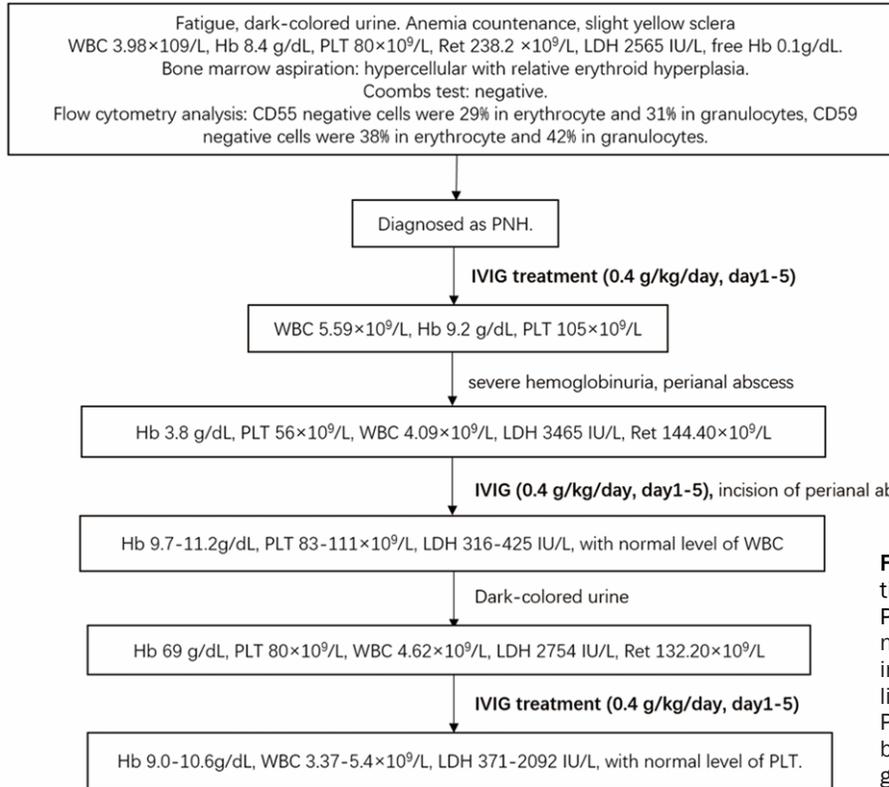


Figure 1. Sketch of the patient's condition changes. PNH, paroxysmal nocturnal hemoglobinuria; IVIG, intravenous immunoglobulin; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; LDH, lactate dehydrogenase; Ret, reticulocyte.

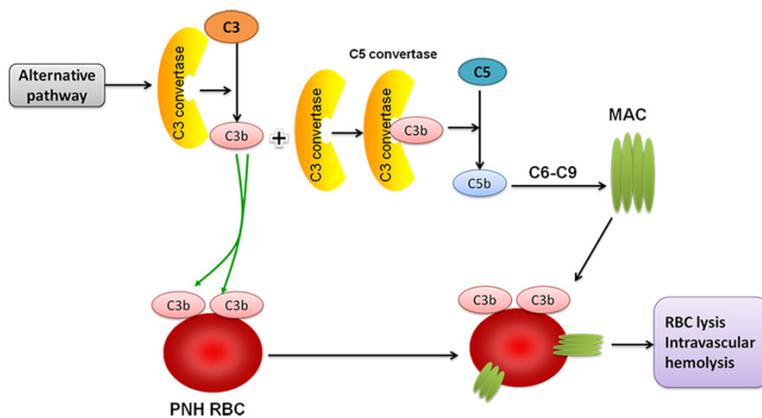


Figure 2. Mechanism of intravascular hemolysis in PNH patients. CD55 and CD59 deficiency makes the red blood cells (RBCs) sensitive to complement attack, resulting in hemolysis. MAC, membrane attack complex.

1-5) therapy was initiated again as well as incision of perianal abscess and anti-infection treatment. Two weeks later, the blood examination showed that Hb increased to 9.4 g/dL. Regular examination in the next month showed Hb level as 9.7-11.2 g/dL, PLT $83-111 \times 10^9/L$, LDH 316-425 IU/L, with normal level of WBC.

Two months later after the second discharged, he got dark-colored urine again and admitted to

our hospital. IVIG treatment was adopted and proved effective again. After two weeks, he took flow cytometry analysis, which showed CD55 negative cells were 74% in erythrocyte and 75% in granulocytes, and CD59 negative cells were 72% in erythrocyte and 77% in granulocytes. The blood examination showed that Hb 10.4 g/dL, PLT $107 \times 10^9/L$, WBC $3.93 \times 10^9/L$ and LDH 573 IU/L. In the next seven months till Jan. 12, 2017, he took danazol (100 mg/day) and vitamin E & B9 (300 mg/day & 0.8 mg/day, respectively), and the regularly re-examination showed Hb level as 9.0-10.6 g/dL, WBC $3.37-5.4 \times 10^9/L$, LDH 371-2092 IU/L, with normal level of PLT (**Figure 1**).

Discussion

PNH has an acquired somatic mutation in phosphatidylinositol glycan class A (PIG-A) which is involved in the synthesis of the glycosyl phos-

Use of IVIG in PNH treatment

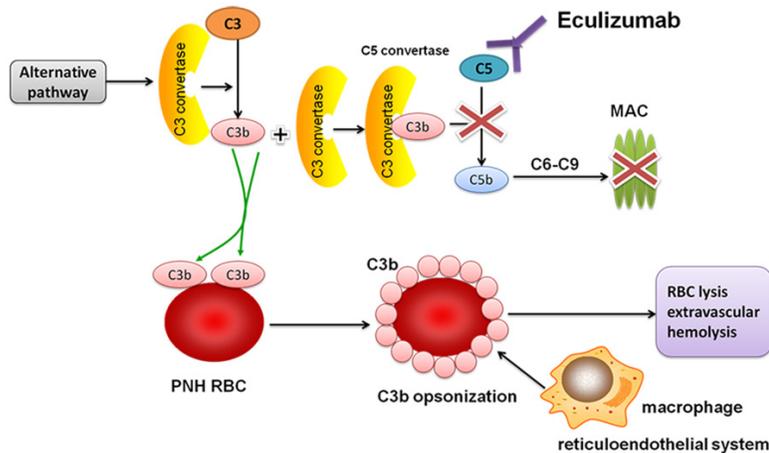


Figure 3. Mechanism of extravascular hemolysis during eculizumab treatment. With eculizumab, the MAC is not formed and PNH RBCs are not destroyed in this way. However, C3b opsonizes the cells, which become prey for macrophages from reticuloendothelial system (RET).

phatidylinositol (GPI) anchor who is required for attaching some protein to the cell membrane [3].

Failure to synthesize GPI anchors leads to an absence of all proteins that utilize GPI to attach to the plasma membrane, including two important complement regulative proteins: decay-accelerating factor (DAF), also named CD55 and membrane inhibitor of reactive lysis (MIRL), also named CD59 [4]. CD55 inhibits the formation of C3 convertase and CD59 interrupts the combination of C9 and membrane attack complex (MAC), leading to the inactivation of the complement system [5]. Therefore, the absence of CD55 and CD59 on the surface of PNH cells leads to complement-mediated intravascular hemolysis (**Figure 2**) [1]. As a consequence, a high concentration of free hemoglobin is released into plasma, responsible for the nitric oxide (NO) scavenging. NO depletion gives rise to smooth muscle dystonia and vascular endothelium injury, responsible for abdominal pain, dysphagia and venous thrombosis [6, 7]. In addition, the absence of CD55 and CD59 on PNH platelets leads to prothrombotic microparticles which contribute to thrombosis [8]. As for bone marrow failure, the leading hypothesis is T cell-mediated autoimmune attack that targets the bone marrow, leading to pancytopenia [3].

The treatment of PNH in China is just based on symptoms, including corticosteroids, membrane stabilizing agents and alkaline drugs [9].

Besides, supportive treatments include blood transfusion if necessary and suitable anti-infection when combining infection [10]. Chemotherapy is used for refractory and relapsed PNH patients who have good bone marrow proliferation [11]. But severe complications would show in bone marrow depression phase after chemotherapy.

Allo-HSCT is the only curative option for PNH patients and recommended for PNH patients with disabling hemolysis or thrombosis that is not controlled by eculizumab [12, 13]. Recent research

showed that 15 among 26 patients could survive for 10 years with disease free survival (DFS) condition after allo-HSCT [14]. Unfortunately, allo-HSCT could not be recommended as initial therapy for the most PNH patients on account of high rates of transplant related morbidity and mortality, as well as the presence of eculizumab [3]. In addition, allo-HSCT is not a suitable treatment option for life-threatening thrombosis in PNH patients [15].

Eculizumab, a humanized monoclonal antibody against complement C5, blocks terminal activation of the complement system at C5, avoiding intravascular hemolysis [3], reduces transfusion dependency and thromboembolic complications, improves the quality of life and prolongs the survival of patients [16]. Administration of eculizumab to PNH patients with renal dysfunction or damage is well tolerated and is usually associated with clinical improvement [17]. In addition, eculizumab could prevent thrombosis and avoid the risk of anti-coagulation or thrombolytic drugs when thrombosis is the key cause of death in PNH patients [18]. For the patients who are resistant to steroid, eculizumab could improve the situation impressively, and eradicate complication significantly [19].

Although eculizumab can control intravascular hemolysis of PNH patients, it cannot cure PNH, and sometimes it even aggravates hemolysis [8]. This should be due to an increased extravascular hemolysis during eculizumab therapy.

Use of IVIG in PNH treatment

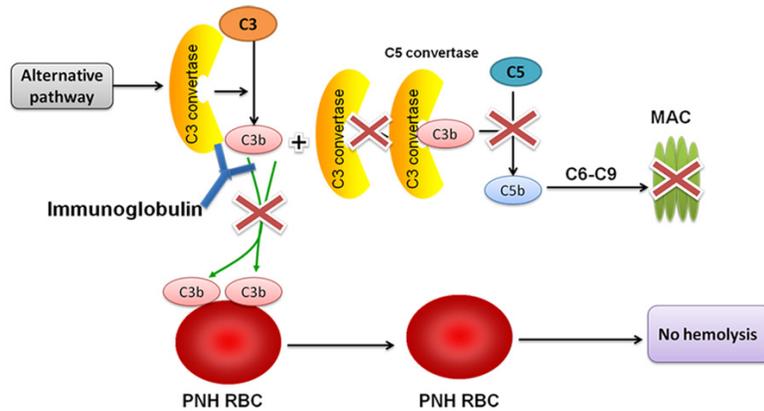


Figure 4. Possible mechanism of immunoglobulin treatment in PNH patients. With immunoglobulin, the MAC is not formed and PNH RBCs are not destroyed in this way. Furthermore, immunoglobulin decrease the deposition of C3b and PNH RBCs are not destroyed by reticuloendothelial system (RET).

Since intravascular hemolysis is blocked by eculizumab, the number of PNH red blood cells which carry the early components of the complement system (C3 opsonins) increased. Indeed, the formation of C3 convertase is not blocked by eculizumab because the deficiency of CD55. These opsonized red blood cells are recognized by reticuloendothelial cells, leading to serious extravascular hemolysis localized mostly in the spleen (**Figure 3**) [20, 21]. Moreover, it is important to keep in mind that PNH patients receiving eculizumab have a higher risk of neisserial sepsis because of the deficiency or decrease of C5 [12, 22]. The infection rate can be decreased by giving current vaccination or antibiotic prophylaxis strategies, but it cannot be completely avoided. Yet no neisserial sepsis occurred in our case of PNH receiving IVIG treatment.

Immunoglobulin, consisting of mainly IgG [23], has many functions, such as binding to Fc receptor and autoantibody [24, 25], regulating the synthesis and release of inflammatory factors [26], inhibiting the activation of complements and regulating the proliferation and apoptosis of lymphocytes [27, 28]. At present, IVIG is administered as replacement therapy for certain immunodeficiency [29] and immunomodulating therapy for some autoimmune diseases [30, 31].

Immunoglobulin blocks the complement activation in earlier phase than eculizumab by combining C3b and inhibiting the formation of C5 convertase, avoiding intravascular hemolysis

[27, 32, 33]. At the same time we speculate that immunoglobulin may decrease the deposition of C3b in PNH RBCs so that extravascular hemolysis is prevented either (**Figure 4**) [32, 33]. For these reasons, we chose IVIG to be an alternative therapy for PNH patient. As shown in the part of case presentation, severe infection is an important inducement of hemolysis, while immunoglobulin could strengthen patients' immunity to reduce the risk of getting infected again [32]. However, some research showed high-

dose (exceeding 0.5 g/kg/day) IVIG could induce hemolysis in several patients [34]. Therefore, we tried to apply middle dose IVIG (0.4 g/kg/day, day 1-5) as the treatment for PNH patient. Surprisingly, the treatment led to a significant elevation of Hb level and a decrease of LDH (monitoring intravascular hemolysis) approaching to normal levels. In addition, no IVIG-related hemolysis happened. After intermittent IVIG treatment for three times, his flow cytometry analysis showed that the percentage of CD55 and CD59 negative cells was increased. The reason could be resulted from the accumulation of CD55 and CD59 negative cells because that IVIG blocked the activation of the complement system and prevented the occurrence of hemolysis.

Eculizumab must be taken for the whole life, and the dosing intervals cannot be longer than 17 days [35]. And its price is extremely high. In America, one dose of 300 mg eculizumab costs 4,547 dollars and the price of one-year treatment per patient is about 380,000 dollars. Accordingly, one dose of 25 g IVIG costs only 428 dollars, and the price of one-year treatment per patient is just 22,256 dollars if the IVIG treatment has to be administered every four weeks (based on the half-time of IVIG, which is 23 days). Obviously, IVIG has price advantage.

Conclusion

Our case of applying IVIG to treat PNH demonstrated that it had better outcomes and no side

effect was found yet. Immunoglobulin could control intravascular and extravascular hemolysis by integrating complement C3b and decrease the deposition of C3b in the PNH cell surface. So we think that IVIG for PNH is worth to being concerned and verified by case-control study in future, especially when eculizumab is not in hand.

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Disclosure of conflict of interest

None.

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Use of IVIG in PNH treatment

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