Passenger Lymphocyte Syndrome as a rare cause of hemolysis in a patient after small intestine transplantation, A case report and review of the literature

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Abstract:
Passenger lymphocyte syndrome (PLS) is a well-described phenomenon causing immune hemolytic anemia, mostly in non-ABO identical transplantations. The syndrome occurs when donor lymphocytes produce antibodies against the recipient’s red blood cells. Although the syndrome is usually self-limited, further management with blood transfusions, immunosuppression, or plasmapheresis might be needed. A 23-year-old female with AB⁺ blood group underwent small intestine transplantation from a deceased donor with O⁻ blood group. She received rituximab, thymoglobin, and methylprednisolone as immunosuppressive induction. In the 9th postoperation day, she developed hemolysis which was primarily managed with blood transfusions and finally ceased by plasmapheresis and intravenous immunoglobulin. Few cases of PLS have been previously described in intestinal transplantation recipients. Correct diagnosis and management prevents severe hemolysis outcomes. Previous cases have been successfully treated with a combination of immune suppression, plasma exchange, and transfusions.

Keywords:
Immune hemolysis, intestinal transplantation, passenger lymphocyte syndrome

Introduction
Passenger lymphocyte syndrome (PLS) is a well-defined but rare complication of the ABO blood group—mismatched stem cell or solid organ transplantations. PLS occurs as a result of the anti-recipient’s red blood cell (RBC) antibody production by B-lymphocytes of the donor, transferred within the transplanted organ and resulting in antibody-mediated RBC destruction. PLS usually occurs in nonidentical ABO/Rh grafts; however, in rare cases, it occurs against minor RBC antigens namely, Kidd and Lewis. The syndrome usually presents between the 1st and 2nd post organ transplantation weeks when the antibodies are sufficiently produced to cause a clinically apparent hemolytic anemia. PLS is diagnosed with abrupt onset anemia, laboratory signs of hemolysis, positive direct antiglobulin test (DAT) with or without the presence of serum antibodies against recipient blood group antigens, and exclusion of other more likely diagnoses. The severity of the hemolysis depends on the lymphoid content of the transplanted organ. Among the solid organs, PLS is more frequently reported in heart and lung transplantations. Only a few cases have been reported describing PLS in intestinal transplantation. In severe hemolysis cases, blood transfusion may be required and if
not properly managed, hemolysis-related renal failure occurs.\textsuperscript{3} As the donor lymphocytes do not engraft, PLS is self-limited and usually vanishes within the weeks.\textsuperscript{4} Despite the self-limiting course of the syndrome, hemolysis increases the hospital stay. In addition, to compensate the anemia, repeated blood transfusions may be needed. Patients might experience the potential side effects of blood transfusions including viral and bacterial infections and transfusion-related hemolysis, lung injury, and allergic reaction.\textsuperscript{5} In the current report, we present the diagnosis and treatment of PLS as a cause of hemolysis in an isolated small intestine recipient who received rituximab as an induction immunosuppressive agent. A review of the previous cases in the context of the current literature for PLS in small intestine transplantation is included in this study.

**Case Report**

**Presentation**

A 23-year-old female with AB\textsuperscript{+} blood group presented in the emergency room 3 days following a C-section delivery. The patient’s past medical history included pseudotumor cerebri being treated by acetazolamide. She was immediately transferred to the operation room for an emergent laparotomy due to diffuse abdominal rebound tenderness and guarding. During the operation, the surgeons observed small bowel gangrene from 5 cm distal to the ligament of Treitz to the middle of transverse colon. The gangrened bowel segments were resected and a stoma was created for the patient. During the following days, she developed septic shock and gangrene of the stoma. She was transferred to our center where we resected the remnant of the small intestine from the Treitz ligament and inserted a gastroduodenostomy tube for decompression.

**Postoperation days**

After the operation, she was transferred to the intensive care unit. She was then admitted to the intestinal rehabilitation unit (IRU) for 227 days and received parenteral nutrition. She was listed on the waiting list for small intestinal transplantation from deceased donor. Her blood group was AB\textsuperscript{+} and she had negative flow panel reactive assay and anti-human leukocyte antigen antibodies. During the admission days in the IRU, the patient had two time positive bacterial culture from central venous catheters and one episode of pulmonary thromboembolism.

**Small intestine transplantation**

After 8 months of admission in the IRU, a donor was found, a 16-year-old brain-dead patient with no underlying disease or history of blood products transfusion. The donor’s blood group was O\textsuperscript{+}; therefore the donor organ was compatible with the patient but not identical. The operation lasted for 210 min; the patient bled 200 cc and received no RBC transfusion. The cold phase was estimated to be 30 min. She was successfully weaned from the ventilator 6 h after the operation. The patient received methylprednisolone 1 gr for 3 days, 60 mg daily dose of thymoglobulin for 5 days, and 500 mg of rituximab, which was repeated in the 5\textsuperscript{th} postoperation day (POD) as the induction immunosuppression. The recorded laboratory data of the patient in one day before the operation are presented in Table 1.

**Posttransplantation course**

An immunosuppressive maintenance regimen with the combination of tacrolimus, mycophenolate mofetil, and prednisolone was started for the patient. On the 5\textsuperscript{th} POD, small bowel biopsy was done which showed no rejection and oral nutrition was started for her. The patient’s kidney and liver function tests were normal and her hemoglobin was 9 g/dL. On the 10\textsuperscript{th} POD, her hemoglobin level dropped to 7.2 g/dL with hypotension and tachycardia and she received two units of AB + RBC transfusion as we supposed the drop was due to bleeding. The next day, despite the previous correction, the hemoglobin level had reached 6.2 g/dL and she received another AB\textsuperscript{+} RBC transfusion and the hemoglobin was corrected to 8 g/dL. The next day, the hemoglobin had another drop to 6 g/dL and she developed acute jaundice with a total bilirubin level of 22 mg/dL [Figure 1]. She was transferred to the operation room for an exploratory laparotomy. During the operation, a small intestinal hernia was observed which was reduced, the bowel seemed intact, and no internal bleeding was noted. A hematologist visited the patient and demanded the lab data with the following results; Lactate dehydrogenase (LDH) = 935 U/L (normal range: 140–280 U/L), reticulocyte count = 10%, and haptoglobin level of

![Figure 1: The hemoglobin and total bilirubin trend during the first 30 postoperation days](image-url)
0.149 g/L (normal range: 0.334–1.532 g/L). Meanwhile, the total bilirubin was 34.9 mg/dL with indirect bilirubin level of 18 mg/dL and 2+ bilirubin was detected in the patient’s urine. In the peripheral blood, smear fragmented RBCs were reported and the mean corpuscular volume of the RBCs were reported to be 97 fl. The laboratory data were in favor of hemolysis. The patient’s medications, past medical history, and hospital documents were thoroughly reviewed and no medication, preexisting hemolytic disease, or infection was found to cause the hemolysis. In previous workups, she had a normal range of G6PD enzyme and normal hemoglobin electrophoresis. Meanwhile, kidney function tests and platelet count were within the normal range, opposing the diagnoses of thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome. As PLS was suspected, a direct coombs test was demanded on the 12th POD, which was negative. During the following days, the patient suffered ongoing hemolysis and received RBC transfusion identical to the donor’s blood group (O). However, due to the high suspicion of PLS a second direct coombs test was done on the 14th POD which turned positive. In the following days, the total bilirubin level had a decreasing trend and reached 11 mg/dL on the 18th POD. As the patient was still dependent on blood transfusions, intravenous immunoglobulin (IVIG) transfusion, and 7 cycles of plasmapheresis were started for her. Consequently, the severity of hemolysis decreased by adopting the new treatment strategy. On the 43rd POD, after 320 days of hospitalization, she was discharged in a fairly good condition. During postoperation hospitalization, she had an episode of cytomegalovirus infection and acute rejection which were successfully treated. At the time of discharging, her hemoglobin level was 8.7 g/dL. The patient was admitted a month later with a neck soft tissue abscess near to central venous catheter site and died of consequent sepsis. The death was unrelated to the prior hemolysis.

**Discussion and Conclusions**

Due to the high demand for organ transplantation and health compromise of prolonged organ failures, compatible nonidentical organ transplantation is frequently being performed. In this type of transplantations, the lymphocytes within the donor organ might become sensitized to the recipient’s antigens and produce antibodies against the recipient’s RBCs, causing a form of graft versus host disease (GVHD) called PLS. Among the various causes of postorgan transplantation anemia, PLS should be considered a relatively rare but important etiology, especially in non-ABO identical cases. The risk of PLS is higher in previously sensitized donors which would have occurred by pregnancy or blood product transfusions. The severity of PLS varies in individuals and in some cases two recipients of allograft organ from the same donor have showed different degrees of symptoms. In some cases, donor-derived anti-recipient RBC antibodies are detected without clinical manifestations. The diagnosis of PLS is made on evidences of hemolysis, including indirect bilirubinemia, increased reticulocyte count and serum LDH, as well as decreased haptoglobin with the presence of positive Direct antiglobulin test (DAT) or anti-recipient’s RBC antibodies. Other causes of hemolysis such as septicemia, delayed transfusion-related hemolytic reaction, and drug-induced hemolysis should be excluded from the study. Our patient’s first DAT was negative, which turned positive in re-check. The principle of DAT is to detect IgG and complement components bound on the RBC membrane. Initially, RBCs are washed to remove the unbound antibodies, then and anti-human agglutinate is added to agglutinate the RBCs in case of IgG or complement bonding. False-negative DAT occurs in cases of technical errors, severe hemolysis with rapid destruction of the affected RBCs, and low level of antibodies. Technical errors include under washing or under-centrifuging of RBCs and delayed adding of agglutinates. In the presence of high suspicion of immune hemolysis and negative DAT, a second test referred to an immunohematology laboratory is recommended. We suppose the first negative DAT could have been caused by severe hemolysis of the affected RBCs or laboratory errors. The condition is usually treated with supportive care such as blood transfusion and hydration to prevent the sequelae of hemolysis. Blood transfusions should be identical to the donor’s blood group to avoid further hemolysis. Despite the large content of bowel lymphoid tissue, PLS has been less frequently reported in small intestine transplantation comparing to other organs. Up to this date, seven studies have reported PLS in intestinal transplantation recipients [Table 2]. As previous studies have reported 9% occurrence of PLS in kidney transplantation recipients, we excluded a report of PLS in a patient with simultaneous kidney and small intestine transplantation. According to the current data, PLS is extremely rare in isolated small intestine transplantation recipients and our case is the 5th one to be reported. In previous cases, the average POD of the presentation was 6.6 with minimum of 4 and maximum of 14.

In our report, the first episode of anemia was mistakenly supposed to have been caused by bleeding and was
treated with blood transfusion similar to the recipients. In the following days, the hemolysis exacerbated. In drastic hemolysis forms with renal damage or prolongation of the hemolysis beyond 2 weeks, further management is suggested. Up-to-date, no confirmed guideline for the treatment of PLS has been presented. Previous studies have successfully treated the patients with IVIG (to decrease the antibody production by B-lymphocytes with various mechanisms), plasmapheresis (to remove the circulating antibodies), rituximab (an anti-CD20 drug targeting B-lymphocytes), and increasing the dose of immune suppression with corticosteroids. In one other report, IVIG combined with Alemtuzumab (anti-CD52 targeting B-lymphocytes). In the latter report, Alemtuzumab was used to treat liver Graft versus host disease (GVHD), which could have also ameliorated the PLS. The hemolysis in all patients was successfully treated; however, two patient died of encephalitis and brain infarction, unrelated to hemolysis. In our case, we primarily planned to alleviate the hemolysis symptoms by transfusions; however, the severe ongoing hemolysis additional managements with IVIG administration and plasma exchange were successfully applied.

Table 2: Previous reports of PLS in intestinal transplantations

| Reference/Year | Donor/Recipient Blood Group | Graft Type/isolated vs multivisceral | First presentation | Treatment | Outcome |
|----------------|-----------------------------|-------------------------------------|-------------------|-----------|---------|
| Cohen et al., 1986 | O/A | Deceased/iso | 4th POD | Transfusion | Died of encephalopathy |
| Sindhai et al., 1996 | O/A | Deceased/multi | 14th POD | Methylprednisolone | Successful |
| Holtermann et al., 2003 | O+/A+ | Living related (Mother)/iso | 7th POD | Supportive | Successful |
| Panaro et al., 2004 | O+/A+ | Living related/iso | 6th POD | Plasmapheresis | Successful hemolysis treatment/died of brain infarct |
| Davis et al., 2010 | O+/A+ | Deceased/multi | 4th POD | Transfusion | Successful |
| Foell et al., 2017 | O+/A+ | Deceased/iso | 9th POD | Plasmapheresis | Successful |
| Our Case | O+/AB+ | Deceased/iso | 9th POD | Transfusion | Successful hemolysis treatment/died of sepsis |

The efficacy of rituximab in preventing PLS is controversial. Lee et al., reported shorter and milder duration and the severity of PLS in a stem cell transplantation patient who had received rituximab. In a study by Tsujimura et al., no occurrence of PLS in 85 ABO-mismatched renal transplantation patients who had received rituximab as part of their induction immunosuppressive regimen was reported. In the study, they used 200 mg of rituximab in the operation room as the induction immunosuppressive agent. In a report of blood type A to B kidney transplantation, the patient received a single-dose of 200 mg/m² rituximab combined with sessions of plasmapheresis 2 weeks before the transplantation but eventually developed PLS; however, in this report, rituximab was administered 2 weeks earlier to the operation and sessions of plasmapheresis might have lowered the serum level of the drug resulting in minimal effect on donor passenger lymphocytes. In the current report, our patient developed PLS despite receiving a total dose of 1000 mg rituximab (divided into two equal doses, infused in the operation room and on the 5th POD. With these inconsistent reports, further studies on the efficacy of rituximab to prevent and treat PLS are needed.

Although rare, PLS should be kept in mind when facing hemolysis in small intestine transplantation recipients. Prompt diagnosis and management prevents hemolysis-related sequelae and shortens hospital stay and costs. In our case, rituximab did not prevent PLS. More studies should be conducted on the efficacy of rituximab to treat and prevent PLS.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.
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