Original Article

Passenger Lymphocyte Syndrome (PLS): A Single-center Retrospective Analysis of Minor ABO-incompatible Liver Transplants

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Abstract

Background and Aims: Due to the shortage of donor livers, minor ABO-incompatible liver transplantations are commonly performed. Together with the allograft, immunocompetent B-lymphocytes, called passenger lymphocytes, are transplanted. In case of minor ABO-incompatibility, these passenger lymphocytes produce antibodies directed towards the recipient's red blood cells, which causes immune-mediated hemolysis, also known as the passenger lymphocyte syndrome (PLS). Although this is a self-limiting disorder, serious complications can occur, including graft failure. Retrospectively, we evaluated the role of PLS in minor ABO-incompatible liver transplantations performed at our center. ***Methods:*** A retrospective analysis was conducted for all minor ABO-incompatible liver transplantations performed at the Antwerp University Hospital between 2003 and 2015. All patient files were inspected for clinical and laboratory findings. In cases of PLS diagnosis, the applied treatment was also studied. ***Results:*** In total, 10 patients underwent a minor ABO-incompatible liver transplantation and 4 showed signs of PLS. All 4 PLS patients were treated with different therapeutic strategy, corresponding to the severity of hemolysis. In all 4 cases, PLS resolved following treatment. ***Conclusion:*** When performing minor ABO-incompatible liver transplantations, knowledge of PLS is elemental. Next to a high index of clinical suspicion, we suggest routine screening for markers of hemolysis, with emphasis on haptoglobin level and direct antiglobulin test, weekly in the first 4 weeks post-transplantation as well as in case of a sudden hemoglobin drop within the first 3 months after transplantation. Peri- and postoperative transfusion support using donor-compatible blood has been suggested to prevent the occurrence or limit the extent of hemolysis.

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Introduction

It is well known that full ABO-identical grafts have a statistically significant survival advantage.[1] Nevertheless, due to the shortage of deceased donor liver grafts, minor ABO-incompatible liver transplantations are commonly performed. Minor ABO-incompatibility can be described by the presence of anti-A and/or -B isohemagglutinins in the donor's plasma directed against the recipient's red blood cell antigens.[2] In other words, the donor plasma type is incompatible with the recipient's ABO type;[3] for example, when a patient with blood group A receives an organ from a donor with blood group O, immunocompetent B lymphocytes are transplanted together with the allograft. These so-called passenger lymphocytes are able to produce antibodies against the recipient's red blood cells, causing varying degrees of immune-mediated hemolysis, which is clinically identified as the passenger lymphocyte syndrome (PLS). This syndrome is reported to occur in around 40% of minor ABO/Rhesus (Rh)-incompatible liver transplants[3,4] and is seldom the result of non-ABO/Rh antibodies such as Jka, Kpb, Fya and M.[5–10] The antibodies typically appear 1 to 3 weeks after transplantation. PLS is characterized by the abrupt onset of immune-mediated hemolysis and the presence of allo-antibodies. Usually, the hemolysis is mild and the antibodies spontaneously disappear approximately three months after transplantation. Even though PLS is a self-limiting disorder and outcomes are generally good, it may be accompanied by graft failure, disseminated intravascular coagulation or even death.[3,4]

In this retrospective study, we analyzed all minor ABO-incompatible liver transplantations performed at the Antwerp University Hospital in order to identify the incidence of PLS,

Keywords: Passenger lymphocyte syndrome; ABO-incompatible; Liver transplantation; Direct antiglobulin test.

Abbreviations: CT, computed tomography; DAT, direct antiglobulin test; dL, deciliter; ELAS, Eurotransplant Liver Allocation System; g, gram(s); Hb, hemoglobin; HCC, hepatocellular carcinoma; HIT, heparin-induced thrombocytopenia; Ig, immunoglobulin; LDH, lactate dehydrogenase level; l, liter(s); IVIG, intravenous immunoglobulin; MELD, model for end-stage liver disease; mg, milligram; MMF, mycophenolate mofetil; PLS, passenger lymphocyte syndrome; PLT, platelet transfusion; PRBCs, packed red blood cells; RAI, rejection activity index; Rh, Rhesus; SD, standard deviation; TIPSS, transjugular intrahepatic portosystemic shunt; U, unit(s).

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the characteristics of the patients who suffered from this complication, and the applied treatments.

Methods

A retrospective analysis of the medical records of all patients who underwent a minor ABO-incompatible liver transplantation between 2003 and 2015 at the Antwerp University Hospital was performed. The collected clinical variables included age, sex, underlying liver disease, model for end-stage liver disease (MELD) score at time of transplantation, and transplantation date. All patient files were inspected for the donor and recipient's ABO blood type and Rh status, and for the laboratory data of immune-mediated hemolysis (by interpreting plasma hemoglobin (Hb) level, reticulocyte count, serum lactate dehydrogenase level (LDH), indirect bilirubin, haptoglobin level and direct antiglobulin test (DAT)). For cases with PLS, we recorded all clinical and laboratory findings as well as the applied treatment. The Bio-Rad IH-1000 Automated Analyzer System (Switzerland) was used to perform the DAT, based on principles of column agglutination technology and gel filtration; the reaction was graded from 1+ to 4+, which was semi-quantitatively analyzed by a card reader.[11]

Results

Between 2003 and 2015, 10 minor ABO-incompatible liver transplantations were performed. The characteristics of these 10 patients are summarized in Table 1.

Non-group O patients (3 in group A, 4 in group B and 1 in group AB) received a group O liver in 8 of the 10 cases (80%). Two other patients (20%) with group AB respectively received a group A liver and a group B liver. Three Rh-negative patients (30%) received a liver from a Rh-positive donor, and one Rh-positive acceptor (10%) received a liver from a Rh-negative donor. The mean age of our patient population was 58 years-old (SD ± 7.48) with a male to female ratio of 2.3. Four patients (numbers 2, 4, 8 and 10) received a liver transplantation because of alcoholic liver cirrhosis, one patient (number 9) because of primary biliary cholangitis, one patient (number 3) because of homozygous alpha 1-antitrypsin deficiency, one patient (number 5) because of hereditary hemochromatosis, one patient (number 7) because of hepatitis B infection and two patients (numbers 1 and 6) because of hepatitis C infection. End-stage liver disease was complicated by a hepatocellular carcinoma (HCC) in 3 of 10 patients and diagnosed according to international criteria. The MELD score varied from 7 to 22. Of the two patients with a MELD score of 22, one received a liver transplant because of high urgency and the other because of standard exception. Five patients (50%) developed a sudden reduction in Hb within 2 weeks after the liver transplantation. In four patients (numbers 3, 4, 9 and 10) PLS was the cause of this Hb reduction, with an average time of 12 (SD ± 1.71) days after transplantation. The mean nadir Hb value of these four patients was 5.93 g/dL (SD ± 0.68). The clinical and laboratory findings of these four cases are described below, as well as the applied treatment. In patient 6, bleeding from a branch of the hepatic artery was found to be the source of the Hb reduction.

Patient 3: In April 2006, an O Rh-positive orthotopic liver transplantation was performed on an AB Rh-negative 49 year-old male diagnosed with homozygous alpha 1-antitrypsin deficiency that had resulted in liver cirrhosis Child-Pugh score C. In 2006, the MELD score was not yet used as a criterion within the Eurotransplant Liver Allocation System (ELAS) for assigning a donor. The patient presented with acute-on-chronic liver failure complicated with acute renal failure due to a hepatorenal syndrome, coagulopathy, respiratory failure and coma caused by hepatic encephalopathy. In January 2006, a transjugular intrahepatic portosystemic shunt (TIPSS) was placed to decrease the hepatic venous pressure gradient. Persisting thrombocytopenia, with an average count of 50×10^9/L platelets, required platelet transfusion prior to the transplantation procedure. After surgery, the immunosuppressive therapy consisted of corticosteroids, mycophenolate and sirolimus. Two episodes of acute rejection with a rejection activity index (RAI) score of 5 occurred, starting 10 days post-transplantation, which was treated with high pulse doses of corticosteroids. Platelet transfusion was performed on day 10, prophylactically, prior to a planned liver biopsy. On day 12, a reduction in Hb was noticed, from 8.1 g/dL on day 11 to 6.9 g/dL. The highest level of indirect bilirubin was 1.5 mg/dL on day 15, while the LDH level increased to 943 U/L on that same day. Haptoglobin was below the limit of detection and the DAT tested positive for IgG but not for C3d. Finally, the eluate turned out to be positive for both type IgG anti-A and anti-B antibodies. No specific treatment was given. The patient had already received high-dose corticosteroids to treat the acute rejection, which may have contributed to the resolution of PLS. Hb levels raised slowly, and 30 days after transplantation a value of 8.8 g/dL was measured. Although there was a Rh status discordance, this did not contribute to the hemolysis. Since, in this case, the donor was Rh-positive, the passenger lymphocytes will not produce anti-Rh antibodies (Fig. 1A).

Patient 4: A 57 year-old female with known alcoholic liver cirrhosis, Child-Pugh score C and blood type A Rh-positive underwent a successful orthotopic liver transplantation from an O Rh-positive donor in October 2007. The liver cirrhosis was complicated with portal hypertension, and earlier that year she had developed hepatic encephalopathy, variceal bleeding, ascites and spontaneous bacterial peritonitis with *Escherichia coli*. A MELD score of 13 was determined at the time of transplantation. Platelet transfusion was required prior to the transplantation because of a low platelet count (60×10^9/L), which was due to portal hypertension and hypersplenism. The immunosuppressive medication scheme included corticosteroids and tacrolimus. Seven days after transplantation, the patient developed a fever that was caused by an acute cholangitis associated with *Enterococcus faecalis* bacteremia. Treatment with broad spectrum antibiotics (meropenem and vancomycin) was initiated with good results. Persistent pleural effusion complicated the postoperative course and multiple pleural punctures were performed. The Hb level dropped from 7.4 g/dL on day 10 to 5 g/dL on day 11. An abdominal CT scan showed a mild and stable postoperative hematoma at the hepatic hilum. An explorative laparotomy did not reveal signs of active hemorrhage. The highest level of indirect bilirubin was 0.9 mg/dL, detected on day 12. On day 11, the LDH level increased to 1514 U/L, while this was only 854 U/L on day 10. No schistocytes were found and the haptoglobin level was normal, but there was an increased level of reticulocytes (59×10^9/L). Heparin-induced thrombocytopenia (HIT) was excluded. A polyvalent DAT was positive on day 11, with anti-A IgG antibodies present in the eluate. Multiple blood transfusions, non-compatible with the donor blood type, were given on days 2, 17 and 24. On days 2 and 17, platelet transfusions were

Table 1. Patients with ABO-incompatible liver transplants

Patient No. (year)	Age/Sex	Donor ABO/Rh group	Recipient ABO/Rh group	Reason for Tx	MELD score	DAT (IgG)/ Antibody eluate	Immunosuppressive protocol	Change in hemoglobin, g/dL (day[a])
1 (2003)	54/M	O +	B +	hepatitis C and HCC	/	–	corticosteroïds, tacrolimus	None
2 (2005)	53/M	A +	AB +	alcoholic liver cirrhosis	/	–	corticosteroïds, tacrolimus	None
3 (2006)	49/M	O +	AB –	homozygous alpha 1-antitrypsin deficiency	/	+ / anti-A, anti-B	corticosteroids, MMF, sirolimus	From 8.1 (d11)–6.9 (d12)
4 (2007)	57/F	O +	A +	alcoholic liver cirrhosis	13	+ / anti-A	corticosteroids, tacrolimus	From 7.4 (d10)–5.0 (d11)
5 (2008)	70/M	O –	A +	hereditary hemochromatosis and HCC	7	–	corticosteroïds, tacrolimus	None
6 (2009)	68/F	B +	AB -	hepatitis C	18	–	corticosteroïds, tacrolimus	From 10.9 (d8)–4.5 (d9)
7 (2010)	49/M	O +	B +	Hepatitis B	21	–	corticosteroïds, tacrolimus	None
8 (2013)	60/M	O +	B –	alcoholic liver cirrhosis	22 (high urgency)	–	corticosteroïds, tacrolimus	None
9 (2015)	64/F	O +	B +	primary biliary cholangitis and HCC	22 (standard exception)	+ / anti-B	basiliximab, corticosteroids, MMF, delayed tacrolimus[b]	From 8.8 (d11)–6.2 (d14)
10 (2015)	54/M	O +	A +	alcoholic liver cirrhosis	17	+ / anti-A	basiliximab, corticosteroids, MMF, delayed tacrolimus[b]	From 7.2(d9)–6.4 (d10)

Abbreviations: Tx, transplantation; HCC, hepatocellular carcinoma; DAT, direct antiglobulin test; MMF, mycophenolate mofetil.
[a]Day after transplantation.
[b]As of 2015, the immunosuppressive protocol was changed to basiliximab, with tacrolimus introduction on post-transplant day 5 instead of day 1.

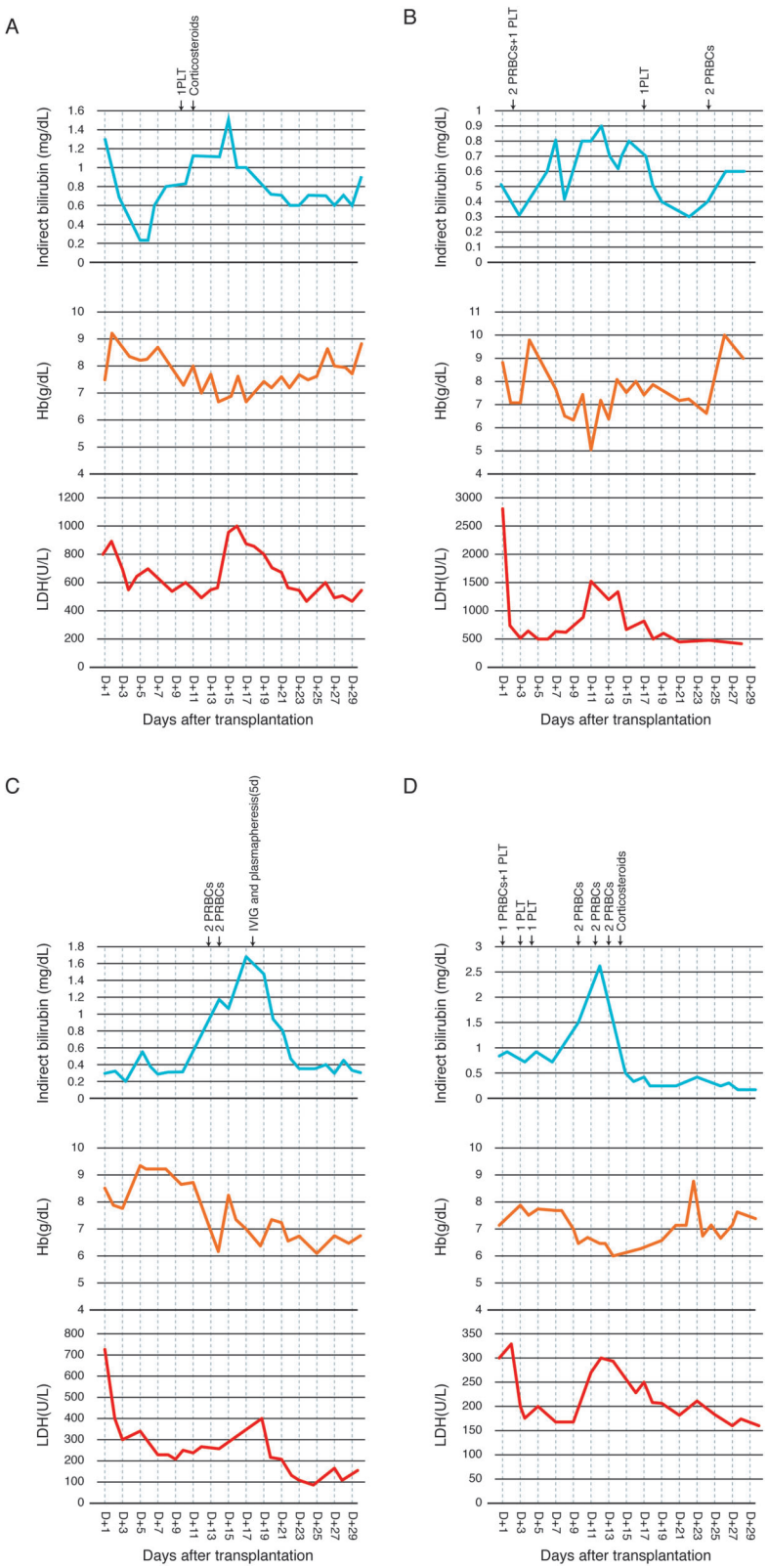

Fig. 1. Evolution of hemoglobin (Hb), indirect bilirubin and lactate dehydrogenase (LDH) in the four patients with PLS. (A) Patient 3; (B) Patient 4; (C) Patient 9; (D) Patient 10.
Abbreviations: PRBCs, packed red blood cells; PLT, platelet transfusion; IVIG, intravenous immunoglobulin; 5d, during 5 days.

also given, prophylactically, prior to pleural punctures. No additive treatment was given, and after day 24 no further blood transfusions were needed. The Hb level reached 9 g/dL at 28 days after transplantation (Fig. 1B).

Patient 9: In August 2015, a B Rh-positive 64 year-old woman with known liver cirrhosis and Child-Pugh score B due to primary biliary cirrhosis and complicated by portal hypertension and HCC in segment 6, underwent an O Rh-positive orthotopic liver transplantation. The single lesion had a diameter of 23 mm, for which—according to the Eurotransplant rules for HCC lesions within Milan criteria—a standard exception MELD score was obtained, reaching 22 at the time of transplantation. While the patient was on the waiting list, local control of tumor growth was performed by laparoscopic radiofrequency ablation. After transplantation, immunosuppression consisted of basiliximab, corticosteroids and mycophenolate, with delayed introduction of tacrolimus on day 5. Besides pneumonia, which was diagnosed on day 8, the immediate postoperative course was uncomplicated. However, 14 days after the transplantation, the patient developed a sudden reduction in Hb from 8.8 g/dL on day 11 to 6.2 g/dL, without any evidence of active hemorrhage on abdominal CT scan and by gastroscopy. On day 12, blood analysis showed a high reticulocyte count (344×10^9/L), a slightly elevated total bilirubin (1.2 mg/dL), LDH of 266 U/L and an haptoglobin level below the limit of the detection. LDH reached 402 U/L on day 19. DAT tested positive, with an eluate reactive for anti-B IgG antibodies, confirming the diagnosis of PLS. Because no improvement was seen after blood transfusion with 2 U of donor compatible blood cells on days 13 and 14, treatment with intravenous immunoglobulin (IVIG) and plasmapheresis was started on day 18 and continued for 5 consecutive days, after which the hemolytic parameters as well as the Hb level gradually improved. There was no need for platelet transfusion (Fig. 1C).

Patient 10: In September 2015, a 54 year-old man, blood type A Rh-positive, underwent an orthotropic liver transplantation from an O Rh-positive donor, because of alcoholic liver cirrhosis, Child-Pugh score C and a MELD score of 17. The year before, transplantation was complicated by ascites, spontaneous bacterial peritonitis, thrombocytopenia and portal hypertensive gastropathy. Because of pre-existing thrombocytopenia, platelet transfusion was given prior to the transplantation. Immunosuppressive therapy consisted of basiliximab, corticosteroids and mycophenolate, with delayed introduction of tacrolimus on day 5. Postoperatively, the patient suffered from a urinary tract infection, which was treated with temocillin. Besides 1 U of packed red blood cells (PRBCs), postoperative platelet transfusions were needed on days 1, 3 and 4. Ten days after his transplantation, the Hb level decreased from 7.2 g/dL to 6.4 g/dL, without any clinical signs of bleeding. Further work-up revealed an indirect bilirubin level of 2.2 mg/dL, LDH of 274 U/L, a haptoglobin level below the limit of detection and a reticulocyte count of 166×10^9/L. The DAT performed on postoperative day 12 turned out positive with presence of type IgG anti-A antibodies in the eluate, confirming the diagnosis of allo-immune hemolytic anemia based on PLS. Despite supportive therapy with donor compatible PRBCs transfusions on days 10, 11 and 13, the Hb level decreased further to 5.9 g/dL on day 14. On that day, treatment with an immunosuppressive dose (1 mg/kg) of corticosteroids was initiated, leading to improvement of hemolytic parameters. The dose of steroids was tapered from day 19 onwards, without any signs of relapse (Fig. 1D).

Discussion

In this case series, we describe 4 patients out of 10 who received minor ABO-incompatible liver transplants and developed PLS within 2 weeks post-transplantation. Although transplantation with minor ABO-incompatible donors is associated with a slight decrease in survival, it is now frequently performed due to the growing demand for donor organs.[3,12,13] PLS occurs in 30–40% of minor ABO-incompatible liver transplantations, with 29% of such resulting in clinically relevant hemolysis. Ramsey *et al*.[7] reported on the first series of unexpected anti-A or anti-B isoagglutinins from minor ABO-incompatible donors in kidney allografts in 1980.

PLS can be regarded as a type of graft-versus-host reaction and most of the time the serum antibodies produced are of type IgG, but they may also be IgM. Because both IgG and IgM can fix complement, PLS is thought to be complement-mediated.[4,14] IgG antibodies were detected in all of our four affected patients. Rarely, non-ABO/Rh antibodies, such as Jka, Kpb, Fya and M, result in PLS.[5,6,8–10] Fung described a case report of severe anti-Rh-mediated hemolysis in a liver transplantation with a Rh-positive recipient and a Rh-allo-immunized donor.[15] A Rh-status discordance was noticed in patient 3. Since it concerned a Rh-positive donor and a Rh-negative acceptor, the passenger lymphocytes will not produce anti-Rh antibodies. The antibodies typically occur 1 to 3 weeks after transplantation and usually disappear after approximately 3 months, except for the anti-D antibodies which can remain present for up to 1 year.[10] The origin of the antibodies can be confirmed by immunoglobulin allo-typing, flow cytometry and nested polymerase chain reaction.[3,4]

Severity of the hemolysis depends in general upon three factors: the amount of lymphoid tissue transplanted, the titer of red cell isohemagglutinins in the donor's serum, which can be influenced by previous sensitization of the donor, and the kinetics of antibody production after transplantation.[14] However, the exact contribution of the antibody titer on subsequent recipient hemolysis remains to be proven, particularly in liver and heart/lung transplants.[16] Because of the correlation between PLS and the transplanted volume of lymphoid tissue, PLS is seen more frequently in heart-lung transplants (antibody in 70%, hemolysis in 70%), compared to liver (antibody in 40%, hemolysis in 29%) and kidney transplants (antibody in 17%, hemolysis in 9%). The risk of hemolysis is not influenced by the sex, age, ethnicity and type of the allograft (cadaveric vs. living-related).[3,4,10,15–18]

PLS is very heterogeneous in its clinical presentation and does not occur in all minor ABO-incompatible organ transplantations. It is proposed that viable donor B-lymphocytes require a trigger after their transfer, such as an infection or a transfusion with stored blood, in order to induce an immune response. This theory is supported by the fact that donor antibodies are not detected in a recipient immediately after transplantation.[8,14,18] Three of our four patients who were diagnosed with clinically relevant PLS developed an infection before the onset. The fourth patient was diagnosed with an acute rejection. The type of immunosuppressive regimen can also influence the incidence and severity of PLS. It is hypothesized that immunosuppressive agents that cause deep recipient T-lymphocyte suppression, such as basiliximab and calcineurin inhibitors, relatively spare humoral immunity. This could lead to donor B cell activation and thus

enhancement of antibody production.[19] Only two of our patients received basiliximab and both developed PLS. Agents noted to reduce B cell antibody production, such as azathioprine and mycophenolate mofetil, may be more beneficial.[12,18,19]

Although PLS is a self-limiting often subclinical disorder, complications like graft failure, disseminated intravascular coagulation or even death can occur.[3,4] Additionally, a sudden reduction in Hb may lead to unnecessary investigations and even surgical procedures to rule out underlying hemorrhage. Therefore, knowledge of PLS and its risk factors is extremely important for transplant physicians. Unfortunately, it is not possible to predict which recipients of minor ABO-incompatible transplantations will develop PLS.[17,19] Cserti-Gazdewich *et al*.[20] described a variability in PLS severity, even in recipients of paired allografts derived from the same previous sensitized donor. Therefore, it is not recommended to search for irregular antibodies in recipient and donor tissue before transplantation.[14] We propose weekly determinations of isohemagglutinins by DAT and other hemolysis markers in the first 4 weeks post-transplantation, as well as in case of a sudden Hb reduction in the first 3 months after transplantation. This will help to identify immune hemolysis by PLS in a timely manner and to prevent unnecessary diagnostic modalities or severe complications.[15] Different authors are working towards prevention of PLS by transfusion of donor compatible PRBCs postoperatively and even during surgery in minor ABO-mismatched liver transplants.[8,15,21] Booth *et al*.[22] recommend to keep the Hb level at least 9 g/dL with donor compatible PRBCs in case of minor ABO-incompatible allogeneic stem cell transplantation. Based on their findings, we suggest that implementation of peri- and postoperative transfusion support with donor compatible blood might be able to prevent the occurrence or limit the extent of hemolysis in patients who received minor ABO-incompatible grafts.

There is no specific treatment for PLS. It has been successfully managed with supportive care and blood transfusions of the donor organ ABO blood type to replace susceptible RBCs with cells that will not be hemolyzed. Other treatment options consist of immune modulation by corticosteroids, IVIG and plasmapheresis to decrease antibody titer or monoclonal antibodies that cause B cell immunosuppression (e.g. rituximab) in severe cases. It is assumed that destroying the passenger B cells with this CD20 monoclonal antibody would prevent further production of antibodies. Unfortunately, most current therapeutic options are derived from anecdotal case reports and small case series, but randomized studies are lacking.[4,21,23–25] Usually a combination of the above options is applied, as in patient number 9, who received high doses of corticosteroids, IVIG and plasmapheresis. However, in view of the known possible adverse effects of these therapies we suggest withholding of these treatments until regular blood transfusions with the donor's blood type fails.

Conclusions

Full ABO-identical liver grafts have a statistically significant advantage for survival. However, it is likely that the number of minor ABO-incompatible organ donations will increase due to the growing demand for donor organs. Therefore, knowledge of PLS is of great importance. Unfortunately, there are no reliable factors to predict which recipients will develop PLS. Besides a high clinical suspicion, we suggest performing routine screening for hemolysis markers, with emphasis on haptoglobin level and DAT, weekly in the first 4 weeks post-transplantation as well as in case of a sudden Hb reduction within the first 3 months after transplantation. Finally, support with donor compatible blood transfusions peri-operatively as well as in the first 3 months post-transplantation is suggested to prevent the occurrence or limit the extent of hemolysis.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the study (SB, EP, TV, AV), acquisition of data (SB, EP), analysis and/or interpretation of data (SB, EP, TV, AV), drafting the manuscript (SB, EP, TV, AV), revising the manuscript critically for important intellectual content (MMC, BB, TV, AV), approval of the version of the manuscript to be published (SB, EP, MMC, BB, DY, PM, SF, TV, AV).

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