ILTS Travel Scholar 2010

Final Report

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From 17 September 2009 until today 78 liver transplantations were performed at Kyoto University Hospital, 4 were diseased donor liver transplantation (DDLT) and 74 were living donor liver transplantations (LDLT).Forty-seven recipients were female and 31 were male. Twenty- three transplants were pediatric transplants and55 were adult patients. Mean age at transplantation in adult patients was 49.37years and in pediatric patients 4.53 years.

Table: 1 indications for Liver transplantation:

Indication	Frequency	Percent
Biliary artesia	23	29.5%
Liver cirrhosis (LC)	6	7.7%
HBV-LC	1	1.3%
HCV-LC	11	14.1%
HBV-LV, HCC	4	5.1%
HCV-LC, HCC	9	11.5%
Acute liver failure	8	10.3%
Primary biliary cirrhosis	6	7.7%
Primary sclerosing cholangitis	2	2.6%
Re-transplantation	4	5.1%
Hepato-pulmonary syndrome	1	1.3%
Hepatoblastoma	1	1.3%
OTCD	1	1.3%
Wilson's disease	1	1.3%
Total	78	100%

Features of living donor liver transplantation at Kyoto University Hospital-

ABO incompatible liver transplantation:

Portal pressure modulation to prevent Small-for-Size Syndrome:

Kyoto Criteria for Liver Transplantation for HCC:

ABO Incompatible Liver Transplantation:

In the living-donor liver transplantation (LDLT), choice of donor is restricted to a close family member, and ABO- incompatible (ABO-I) liver transplantation turns out to be inevitable to overcome "organ demand versus supply."

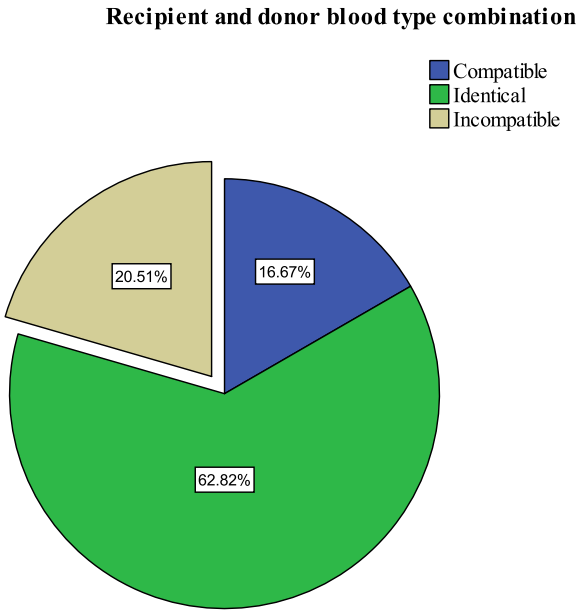

Figure 1 Blood type combination of recipient and donor.

Although ABO- Incompatible liver transplantations pioneered by Professor Starzl, current protocols for ABO incompatible liver transplantation are not

standardized, I studied history of ABO-Incompatible liver transplantation and current protocols for ABO incompatible liver transplantation. This article (Annexure: 1) is accepted for publication in Surgery Today.

Protocol for ABO Incompatible Liver Transplantation at Kyoto University Hospital:

Immunosuppression protocol for ABO-I LDLT at Kyoto University Hospital includes rituximab and plasma exchange.

Rituximab: is monoclonal antibody against CD-20 antigen. CD-20 antigen is expressed by B- lymphocytes during their proliferation and development except mature B-lymphocyte. A total of 300 mg Rituximab administered intravenously 3 week prior to transplantation. Depletion of B-cells from circulation is confirmedby flow cytometry analysis of CD-20 and CD-19 marker preoperatively.

Plasma exchange is performed with blood group AB fresh frozen plasma, one to three times depending upon anti-ABO antibody titers before transplantation; at dose of 1 unit /kg body weight to reduce anti-ABO antibody titer less than 1:16.

Mycophenolate mofetil (MMF) is a functionally cyto-toxic drug to plasma cells, which escape form Rituximab action. MMF stared 7 day before LDLT at dose of 500mg BD unless there is no risk for the administrationof MMF preoperatively.

Routine immunosuppression for ABO-incompatible LDLT at Kyoto University Hospital includes tacrolimus, steroid, and MMF. Tacrolimus trough maintained between 10-15 ng/ml in first 2 weeks, 7-10 ng/ml between 2-8 weeks, 5-7ng/ml until 6 months and below 5ng/ml thereafter. Methylprednisolone was administered via portal vein catheter

for one week, which followed by oral prednisolone from POD 8 at dose of 3mg/kg then tapered to 0.1mg/kg from 4th week and stopped after 3 moths.

Portal vein infusion: portal vein assessed through branch of superior mesenteric vein,canulated using 18 Fr central venous catheter intra-operatively to measure portal venous pressure. In ABO-Incompatible liver transplant, this catheter is kept post-operatively for intra-portal steroid therapy for acute humoral rejection.

Splenectomy is controversial in protocol for ABO- Incompatible liver transplantation. Preformed antibodies are the first line of defense in humoral immunity and a major culprit for the AMR immediately after ABO-I LT. As spleen is the site for maturation of B cells, splenectomy has been a part of protocol for ABO-I LT. However, spleen apart from an antibody production; has many other hemato-immunological functions like a filtration and storage of blood, phagocytosis and destruction of erythrocytes, antigen uptake, and potential hemopoiesis. Post splenectomy infections were precipitated by an aggressive immunosuppression used in ABO-I LT. In this era of ABO-I LT with rituximab, role of the splenectomy needs to be re-evaluated. Spleen comprises only about 25% of the total lymphoid tissue of whole body and an antibody production continues in other lymphoid tissue, it is important to examine an antibody response rather than CD-20 markers in spleen after Rituximab therapy. So we investigated the need of splenectomy in LT for ABO-I patients, focusing on anti- ABO antibody titers in recipient until 56 day after transplantation (Annexure:2).

Portal pressure modulation:

Adult-to-adult living donor liver transplantation (AA- LDLT) hasevolved and the right lobe has become the preferred graft. However, right hepatectomy associated with mortality 2-5 per 1000 hepatectomies. Left hepatectomy has less risk to donor as compared to right hepatectomy.At Kyoto University Hospital, left lobe grafts were used in 66.6% of AA-LDLT.

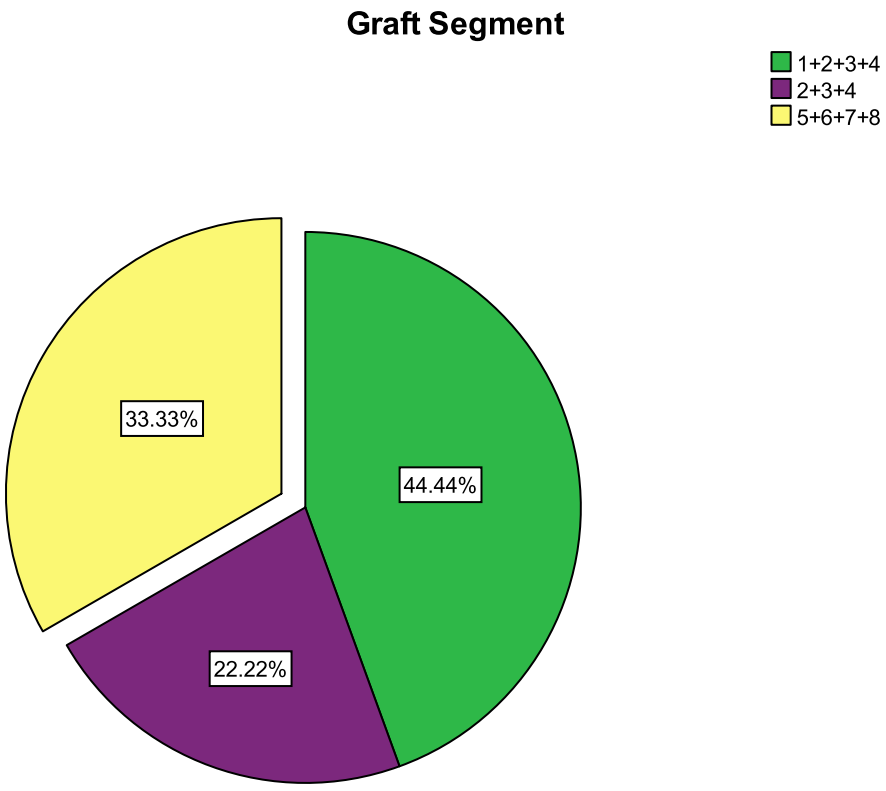

Figure-2 Grafts used in AA-LDLT during September 2009 to September 2010.

Although, Left lobe grafts have an increased risk of development of small-for-size syndrome. At Kyoto University Hospital, portal flow through a liver graft is modulated to prevent excessive and destructive effect of portal pressure. Splenectomy is used as to decease portal pressure below 15 mm Hg and if required porto-systemic shunts are created.As shown in Figure-3, with portal pressure below 15 mm of Hg, accepted GRWR for AA-LDLT at Kyoto University Hospital much below the standard 0.8.

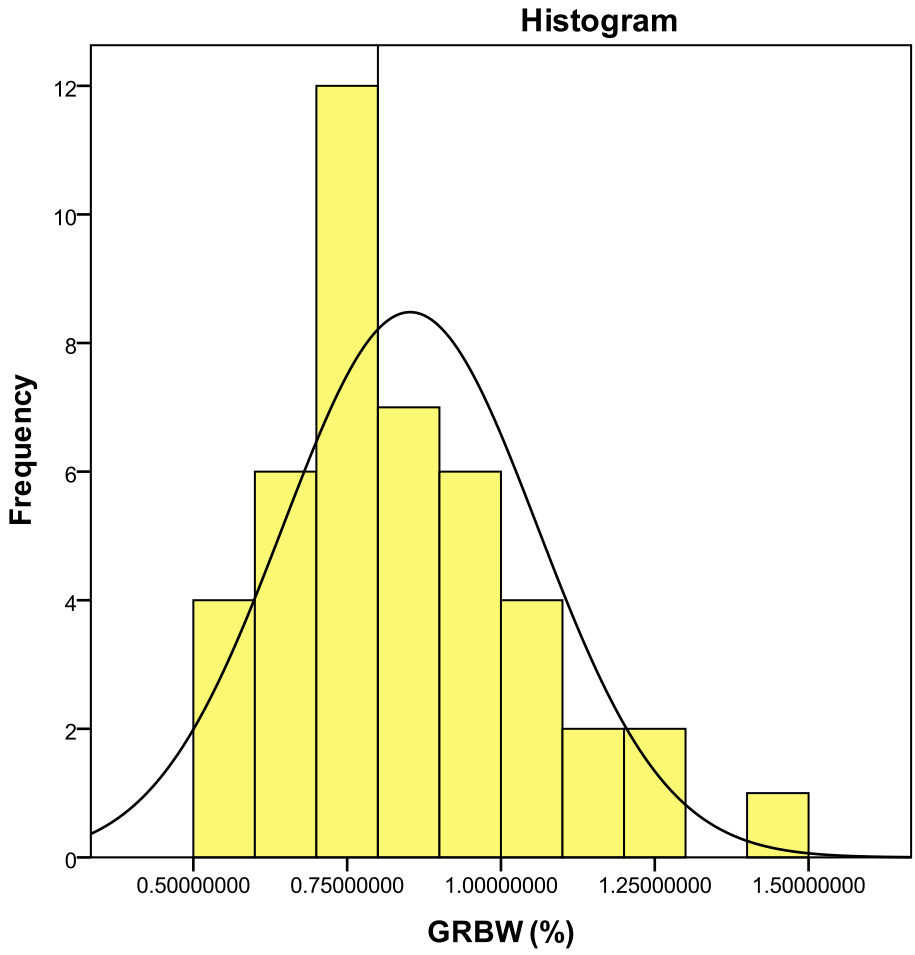

Figure:3 GRWR for AA-LDLT performed during September 2009 to September 2010

Kyoto Criteria for Liver Transplantation for HCC:

In LDLT, which uses a precious but not scarce resource, the value of treatment for the patient and donor is judged based on donor safety, risk of recurrence and probable outcomes without transplantation. Therefore, a Kyoto University Hospital transplantation for HCC is performed beyond Milan criteria.

Kyoto Criteria for transplantation for HCC are:

- HCC with ≤ 10 tumors
- ≤ 5 cm in diameter
- PIVKA-II ≤ 400 mAU/ml.

The reported 5-year recurrence ratein patients who met the Kyoto criteria is 3% and 5-year survival is 87%.

Annexure: 1

Management of ABO-incompatible Living Donor Liver Transplantation: Past and Present Trends

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Abstract:

Based on the concept that the liver is a 'privileged organ', which resists acute rejection, Professor Thomas Starzl introduced liver transplantation across the ABO blood group. However, with improved survival after liver transplantation came reports of an increased incidence of acute rejection, biliary and vascular complications, and decreased survival after ABO-incompatible liver transplantation. As a result, ABO- incompatible liver transplantations are performed only in emergencies when ABO-compatible grafts are unavailable. In living-donor liver transplantation (LDLT), donors are restricted to family members; therefore, breaking ABO blood group barriers becomes inevitable. This inevitable situation has forced liver transplant surgeons to exploit many innovative techniques to overcome the challenges of ABO-incompatible liver transplantation. This review looks at the history and current practices of ABO-incompatible LDLT to provide insight so that the protocol can be improved further.

Introduction: "Organ demand versus supply" is the greatest obstacle to increasing the frequency of liver transplantation, which has been one of the medical breakthroughs in recent times(1). Transplantation across the ABO blood groups is discouraged because of the risk of acute rejection, graft loss, and a poor outcome; thus, it is generally used only in emergency situations. In living-donor liver transplantation (LDLT), donor selection is restricted among family members, and ABO-incompatible (ABO-I) liver transplantation becomes inevitable to breach this obstacle. This has compelled transplant surgeons to devise innovative strategies, such as local infusion therapy and rituximab, to prevent complications in ABO-I liver transplantation. However, with an increased risk of infection, antibody mediated rejection, and consequent vascular and biliary complications, ABO-I liver transplantation continuous to be the formidable challenge in LDLT. In this review, we study the past and current immune strategies adopted by centers across the world, for ABO-I LDLT, to provide insight for change or modification to improve outcomes and reduce ABO incompatibility-related complications in LDLT.

History of ABO-I Liver Transplantation:

Since its inception by Professor Thomas Starzl, ABO-I liver transplantation has evolved through an era of controversy and immunological violation to the current inevitable phase.

Privileged Phase (1969 to 1987): Although ABO-I liver transplantation has been influenced primarily by ABO-I renal transplantation, the initial animal experiments conducted by Professor Starzl(2) demonstrated that the liver is "a privileged organ" with much greater resistance to acute rejection than the kidney or heart. With this understanding, Professor Starzl breached ABO blood group barriers, particularly in the emergency situations when given no choice but to proceed with first available organ. In

1979, Professor Starzl reported 11 human ABO-I liver transplantations without evidence of acute rejection(3). During this period, ABO-incompatibility was not considered a contraindication to liver transplantation. In fact, ABO-I grafts were used in children because of the difficulty of finding compatible small grafts, and in adults during emergencies. In 1986, Robert Gordon (4) reported 31 ABO-I liver transplants, carried out using cyclosporine and prednisolone for immunosuppression, and found that graft survival in the ABO identical group was significantly better than that in the ABO compatible and incompatible groups. In children, he used ABO-I grafts in emergency as well as an elective conditions because of the shortage of small grafts. As the 1-year graft survival rate in adults was acceptable, he advocated the use of ABO-I grafts in adults only in emergency situations.

Phase of Disastrous Outcomes (1987-1992): In 1987, Rego(5) reported hyper-acute rejection after ABO-I liver transplant, despite the "privileged" status of the liver. In 1989, Gugenhein(6-7) confirmed lower graft survival and hyper-acute rejection in ABO-I liver transplantation. In his series of 17 ABO-I liver transplants, Gugenhein postulated immunological damage as the cause of low graft survival and reported antibody-mediated rejection as a cause of graft failure in six patients. He also acknowledged an increased incidence of arterial thrombosis and progressive cholangitis in ABO-I grafts. The debate continued about the increased incidence of complications of ABO-I liver transplants. In a control matched study including 15 ABO-I liver transplants, Sanchez-Urdazpal(8) confirmed an increased incidence of cholangitis, bile leak, cellular rejection, and hepatic artery thrombosis in ABO-I group. Because of the high incidence of complications, ABO-compatible liver transplantation became unpopular and were reserved for emergency transplant surgery only.

Phase of Inevitable Alternatives (1992 onwards): Since the donor of an LDLT is usually a first-degree relative, limiting choice, the use of grafts across the ABO blood groups is often inevitable. This has forced transplant surgeons to adopt various innovative methods to prevent the complications associated with ABO-I liver transplantation. In the early 1990s, Yukihiko Tokunaga (9), Renard(10) and Dunn S P(11) improved the results of ABO-I liver transplantation in children by using pre and post-operative plasma exchange and OKT-3. We have learned much from the experience of ABO-I kidney transplant surgeons who used peri-operative plasma exchange, splenectomy, and high dose immunosuppressive drugs to ensure the success of ABO-I transplantation. Anti-donor antibody-induced compliment fixation and endothelial damage leading to hemorrhagic necrosis by the formation of micro-thrombi in the graft vasculature is a major cause of early graft failure(12). Diffuse intra-organ coagulation can be confirmed by C4D immunofluorescent staining. To overcome this "single organ DIC," Tanabe from Keio University, Japan endorsed portal vein infusion with prostaglandin E1, methyl prednisolone, and gabexatemesilate(13). Prostaglandin E1 improves microcirculation through vasodilatation and the prevention of platelet thrombi. Gabexatemesilate is a protease inhibitor that inhibits platelet aggregation and coagulation factors(14). Nakamura (15) used a hepatic artery infusion of prostaglandin E1 to prevent biliary complications and improve the bile duct blood supply. In 2003, Monteiro(16) gave rituximab, CD 20 monoclonal antibody to a 15-year-old boy undergoing emergency ABO-I liver transplantation for resistant B cell lymphoma, to reduce anti-donor antibody -producing B cells. Kawagishi(17)and Usuda(18) reported similar results with rituximab in Japan.

Current Strategies for ABO Incompatibility in LDLT Worldwide:

Using PubMed, we searched the literature in English and found a total of 332 articles published on ABO-I LDLTs worldwide between 2006 and 2009 (Table 1). With limited access to cadaveric organs, and family restrictions limiting the choice of donor in LDLT, ABO incompatible LT is common in Japan. Fig. 1 shows the total number of LDLTs and Fig. 2 shows the evolving trend of ABO-I LDLTs in Japan.

The outcome of ABO-I LDLT is dependent on the perioperative anti-ABO antibody titers. Therefore, ABO-I LDLT strategies are directed at eliminating or reducing the anti-ABO antibody. Apart from the routine immunosuppression given to all liver transplantation patients, the following methods are also used in ABO-I LDLT:

Plasmapheresis: Anti-ABO antibodies are the trigger for antibody-mediated rejection after ABO-I LDLT. Thus, the anti-ABO antibody titers are reduced preoperatively by plasma exchange, plasma filtration, or immune adsorption in most centers across the world, aiming for Ig M and Ig G titers below 1:16 at the time of transplantation to prevent antibody-mediated rejection. These titers are maintained at these values as increasing antibody titers in the early postoperative period are associated with rejection. Plasmapheresis is the most effective way to control humoral antibody response to prevent rejection(19).

Splenectomy: The spleen is the site of maturation of the B-lymphocytes. Thus, the aim of splenectomy in ABO-I LDLT is to deplete the source of new antibody formation against the ABO antigens. However, the spleen represents only 25% of the peripheral lymphoid tissue(20) and splenectomy compromises the other immune functions of the spleen, as well as increasing the risk of infection in recipients(21). The age of the recipient is also an important limitation, as in all cases of splenectomy. Since the

introduction of rituximab, the need for splenectomy in ABO-I LDLT is questionable. Rituximab depletes CD-20 receptor-marked B cells from the spleen, although splenectomy is required to remove CD-20 negative B cells from spleen. The anti-ABO antibody response is the end-point of rituximab treatment. Our initial unpublished study found no significant difference in anti ABO-antibody response between a splenectomy group and a non-splenectomy group. Moreover, studies from Italy and Korea did not include splenectomy in their protocol.

Local Infusion: Antibody-mediated rejection results from diffuse intravascular coagulation leading to multiple vascular thrombus and necrosis. Preventing local intravascular coagulation in the liver is the primary intention of a local infusion administered through either the hepatic artery or portal vein.

The Keio group advocated the local infusion of prostaglandin E1, 0.01μg/kg/min for 3 weeks; methylprednisolone, 125mg /day for 7 days; and Gabexatemesilate, 1mg/kg/day for 3 weeks, through the portal vein, via a catheter placed through a branch of the superior mesenteric vein. A high bolus dose of methylprednisolone can be administered via this route without any systemic side effects. Prostaglandin E1 causes vasodilatation and prevents platelet aggregation. Gabexatemesilate is a protease inhibitor, which inhibits platelet aggregation, and coagulation factors. Although portal vein infusion resulted in an increase in survival from 22% to 60%, it is frequently complicated by portal vein thrombosis secondary to reaction to the catheter. Portal vein thrombosis increases when PVI is combined with splenectomy due to thrombocytosis and decreased venous return (22). To overcome portal vein thrombosis, in 2005, Kyoto University hospital introduced hepatic artery infusion without splenectomy, placing the catheter through one of the branches of the hepatic artery after anastomosis. Prostaglandin E1 is infused through the hepatic artery catheter at dose of 0.01μg/kg/min for 3 weeks with

methyl prednisolone 125mg /day for 7 days. This protocol improved the 1-year survival after ABO-I LT to 85%, with a decrease in portal vein thrombosis (22). However, severe bleeding can occur if the catheter dislodges, either spontaneously or during its removal (22). Prostaglandin E1 infusion exacerbates the bleeding by preventing platelet aggregation.

Rituximab: Rituximab is the monoclonal chimeric human anti CD-20 antibody that revolutionized ABO-I LDLT. CD 20 is expressed in most of stages of B cell development but not in plasma cells or stem cells. Rituximab was approved for resistant B cell lymphoma at a dose of 375/m^2 weekly for 4 weeks. To deplete normal B cells in an ABO-I recipient, a single dose of rituximab is considered enough. In ABO-I liver transplantation, the timing of giving rituximab varies among centers from 7-15 days preoperatively (21, 22). Today, most knowledge of the pharmacodynamics of rituximab comes from its use in B cell lymphoma. However, Genberg(23) and colleagues recently studied the pharmacodynamics of rituximab in a renal transplant recipient. They found that a single dose of rituximab (375mg /m^2) was sufficient to completely eliminate B cells from the peripheral blood. Although reduced numbers of B cells were seen in the peripheral blood as early as 3 days after rituximab administration, complete elimination was only seen after 3 weeks. Nevertheless, a single dose of rituximab is not enough to completely eliminate B cells from the lymph node. These remnant cells becameactivated after antigen exposure from the graft and produced the anti-ABO antibody. The value of the complete elimination of B cells needs to bebalanced against the need for 2-3 years of prolonged immunosuppression caused by 375 mg/m^2 of rituximab. Conversely, the initial 4-6-weeks is critical for antibody-mediated rejection, and B cell suppression is only required for this period. Therefore, the ideal dose of rituximab remains unresolved.

Mycophenolate Mofetil: Mycophenolate mofetil is a functionally selective drug that is cytotoxic to B and T lymphocytes. Since rituximab is ineffective against the plasma cells with active B-cell producing antibody, mycophenolate mofetil has been incorporated in a protocol used by groups from Chicago (22), Tohoku (16), Tokyo (23), Yokohama (20), and Italy (18). The preoperative administration of mycophenolate mofetil reduces plasma cells in the circulation.

Apart form these conventional therapies used for ABO-I LDLT, investigators have also reported giving intravenous immunoglobulin (IVIG) postoperatively (22, 30). IVIG causes FC-receptor dependent B cell apoptosis and inhibits compliment and T cell-mediated allograft injury. A recent trial at Kyushu University (30), Japan showed the efficacy of IVIG given with rituximab and plasma exchange without local infusion therapy. IVIG is very promising in emergency ABO-I LDLT, when there is insufficient time for the action of rituximab. Cost is the major limiting factor in IVIG treatment. The current protocol for ABO-I LT at Kyoto University, Japan, is rituximab 300mg, 3 weeks prior to transplantation, and plasma exchange before transplantation to keep the anti ABO-antibody below 1:16. IVIG is used as rescue therapy in the case of severe antibody-mediated rejection after ABO-I LT.

Conclusion

Through learning from ABO incompatibility in kidney transplantation, the barriers in ABO-I LDLT are slowly dissolving. As a result, many centers are now selecting ABO-I LDLT to overcome organ shortage. Plasma exchange is an important tool for reducing the anti donor antibody pre- and postoperatively. Rituximab is another promising immune modulation used by most centers, although consensus needs to

reached on it optimal dose and timing. The value of splenectomy and local infusion therapy needs further investigation **(Fig. 3).** Ultimately, emphasis needs to be placed on achieving immunological victory for ABO-I liver transplantation to become a safer alternative.

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Table :1 World wide experience of ABO incompatible living donor liver transplantation

University, Country	**Author**	**Year**	**Patients (n)**	**HAI**	**PVI**	**Rituximab**	**Splenectomy**	**AMR**	**ACR**	**Outcomes**
Belgium, Italy	Troisi(18)	2006	5	0	0	0	0	0	1	100% survival wit
Keio, Japan	Yamada(19)	2006	6	0	3	3	3	0	2	100% survival
Yakohama,Japan	Morioka(20)	2006	6	4	2	5	3	5	0	100% survival
Suwon, S. Korea	Kim(21)	2008	3	3	0	3	0	1	0	1 patient died of of septicemia
Chicago,USA	Testa(22)	2008	5	0	0	3	5	1	4	80% survival (me
Tokyo,Japan	Matsuno(23)	2008	8	7	1	5	6	0	0	75% survival
Japan	Egawa(24)	2008	291	68	41	49	97	52	79	61% 3 year surviv
Tohoku, Japan	Kawagishi(16)	2009	11	0	0	3	2	5	3	81.8% survival (1 yr)

Figure Legends

Fig. 1. Liver transplantations in Japan. ABO-incompatible liver transplantations constitute 10% of the total 5188 liver transplants (source: Japanese Liver Transplantation Society).

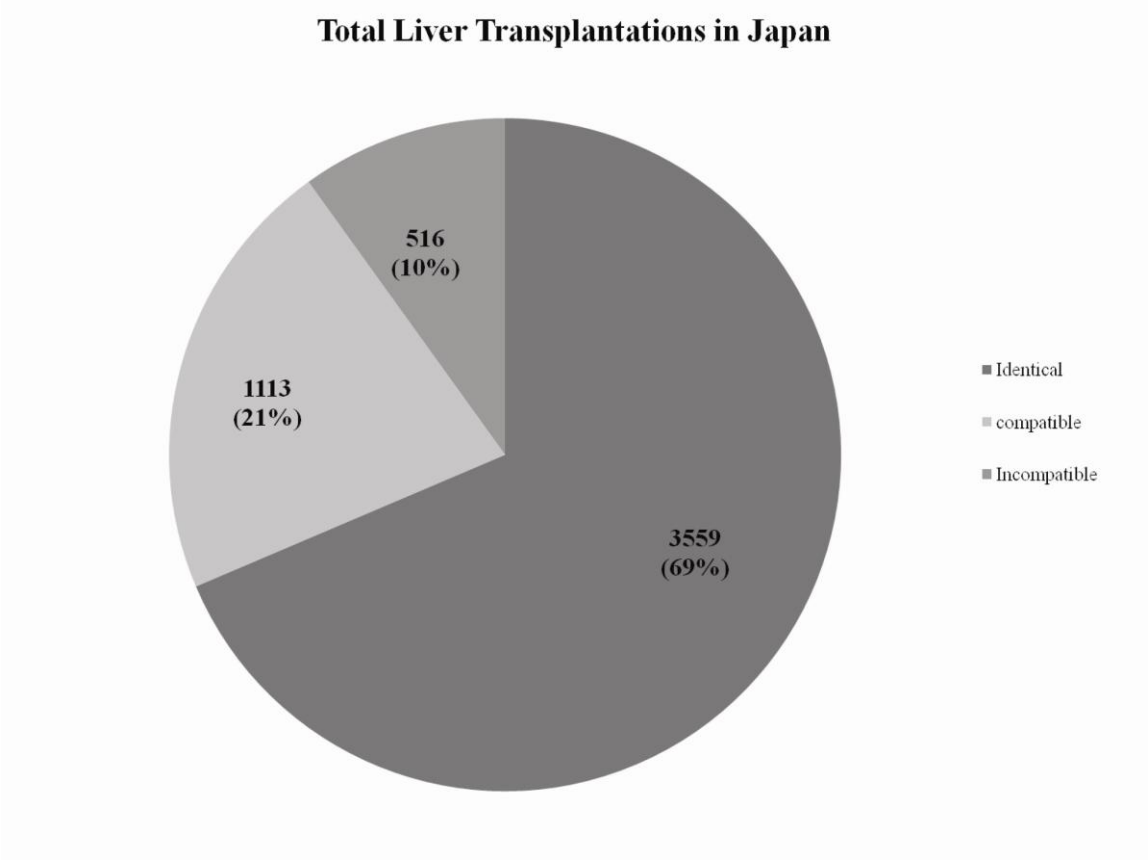


Fig. 2. ABO-incompatible liver transplantations in Japan. The number of ABO-incompatible transplantations, particularly in adults, increased after the introduction of portal vein infusion in 2001 and rituximab in 2005 (source: Japanese Liver Transplantation Society).

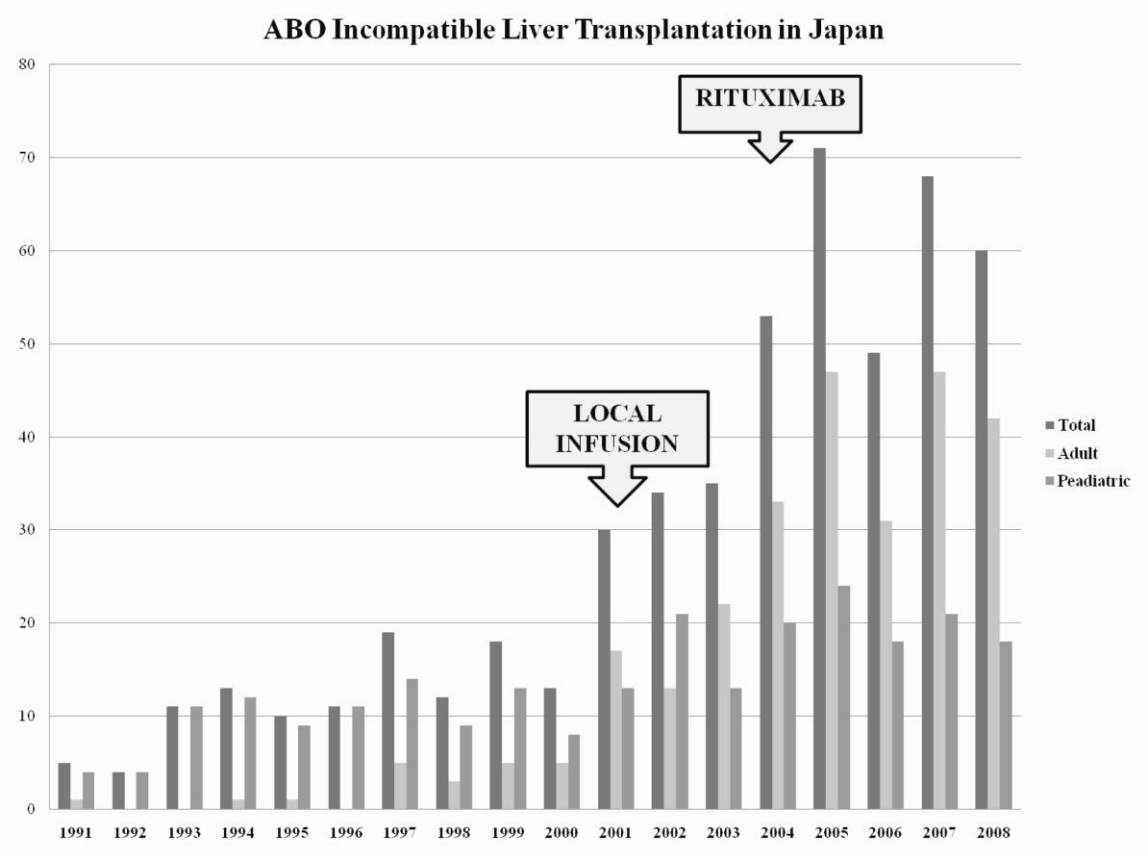
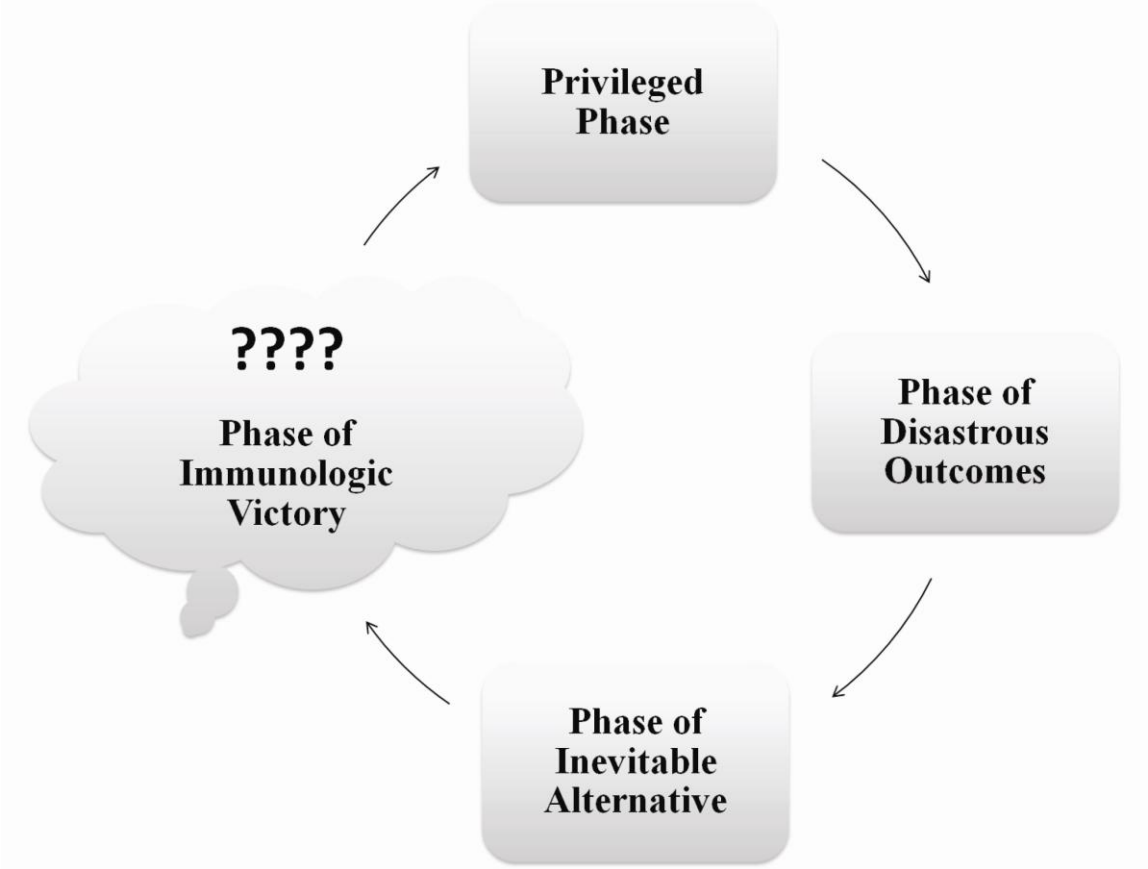


Fig. 3. Transition of ABO-incompatible liver transplantation since its inception. The major challenge today is to gain immunological victory to finally achieve safe ABO-incompatible liver transplantation.



Annexure: 2

Title page

Title: Is splenectomy necessary for ABO-incompatible liver transplantation in the era of Rituximab**?**

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Running Title: Splenectomy in ABO incompatible LT with rituximab

Keywords: Anti-ABO Antibody,

Living Donor Liver Transplantation,

CD-20

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Abbreviations:

LT, Liver Transplantation;

AMR, antibody-mediated rejection;

ABO-I, ABO-incompatible;

LDLT, Living Donor Liver Transplantation;

IgM, Immunoglobulin M;

IgG, Immunoglobulin G;

POD, Post Operative Day;

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Abstract:

Objectives: In ABO-incompatible (ABO-I) living donor liver transplantation, preformed antibodies to donor-blood group antigen induce antibody-mediated rejection, leading to lethal hepatic necrosis and biliary complication. To overcome these complications, the introduction of pre-operative plasma exchange followed by splenectomy and local infusion therapy moderately ameliorated patient survival in adult ABO-I living donor liver transplantation. Thereafter, rituximab prophylaxis has been widely used to deplete CD-20 antigen positive B cells, which produce the anti-donor blood group antibody resulted in dramatic improvement in patient survival. However, the necessity of splenectomy in patients with rituximab prophylaxis is unclear. Therefore, we investigated the need of splenectomy in ABO-I living donor liver transplantation, focusing on anti- ABO antibody response in recipient.

Methods: Retrospective data of thirty-seven patients who underwent ABO-I living donor liver transplantation during May 2006 to July 2009, at Kyoto University Hospital analyzed. 27 patients with splenectomy (splenectomy group) received 329.63±85.77mg Rituximab 17.67±11.86 days prior to LDLT and 10 patients without splenectomy (non-splenectomy group) received 420±103.28 mg Rituximab 26.6 ±21.26 days prior to transplantation. All patients received post-transplant local infusion therapy via hepatic artery. Peri-operative anti-ABO IgM and IgG antibody titers, rejection, biliary complication, infections and survival compared between two groups.

Results: There were no significant differences in anti-ABO IgM as well as IgG antibody titers between ‘splenectomy’ and ‘non-splenectomy group’. Clinical outcomes including the incidence of antibody-mediated rejection, biliary complications, and patient survival did not differ between splenectomy and non-splenectomy group.

Conclusions: Pre-operative Single dose of rituximab effectively reduced anti-ABO titers, sufficiently low level to prevent antibody mediated rejection irrespective of splenectomy.

Introduction

Liver Transplantation (LT) across the ABO blood-type barrier generally considered as a contraindication or exceptionally performed only as a rescue option in an emergent situation because of the lethal risks of antibody-mediated rejection (AMR) (1-3). To overcome donor shortage, learning lessons from ABO-incompatible (ABO-I) kidney transplantation, liver transplant surgeons took many innovative approaches to defeat challenges of ABO-I LT complications including AMR (4-5).With pre-operative plasma exchange, splenectomy and advanced immunosuppression some transplantation centers have reported ABO-I LT results comparable to those of ABO-compatible LT (6-9). Preformed antibodies are the first line of defense in humoral immunity and a major culprit for the AMR immediately after ABO-I LT. As spleen is the site for maturation of B cells, splenectomy has been a part of protocol for ABO-I LT (6, 8-12). However, spleen apart from an antibody production; has many other hemato-immunological functions like a filtration and storage of blood, phagocytosis and destruction of erythrocytes, antigen uptake, and potential hemopoiesis. Post splenectomy infections were precipitated by an aggressive immunosuppression used in ABO-I LT. Rituximab; an anti CD-20 monoclonal antibody used for a resistant B cell lymphoma, was introduced for ABO-I LT by Monteiro(13) in 2003. B cells express CD-20 antigen in the all stages of development except a stage of maturation. Rituximab depletes the CD-20 positive B cells from circulation and lymphoid tissues and this is described as a "chemical splenectomy." In this era of ABO-I LT with rituximab, role of the splenectomy needs to be re-evaluated. Although some reports insist the need of splenectomy for elimination of a subpopulation of B cells those do not express the CD-20 antigen(14) and there are studies showing persistent CD20 markers in a spleen after rituximab therapy(15). Spleen comprises only about 25% of the total lymphoid tissue of whole body (16) and an antibody production continues in other lymphoid tissue, it is important to examine an antibody response rather than CD-20 markers in spleen after Rituximab therapy. In

this article, we investigated the need of splenectomy in LT for ABO-I patients, focusing on anti-ABO antibody titers in recipient until 56 day after transplantation.

Patients and Methods:

During May 2006 to July 2009, 225 living donor liver transplantations (LDLT) performed at Kyoto university hospital. 126 were ABO identical, 42 were compatible, and 57 were ABO incompatible. Outcome of ABO-I LDLT is unaltered in pediatric age group; therefore, we don't use Rituximab or splenectomy and 16 pediatric ABO-I LDLTs excluded from this study. Three LDLT performed without Rituximab prophylaxis are excluded form this study. One patient suffered form cerebral hemorrhage on POD 3 and died on 14^{th} day after transplantation also excluded from this study since their survival was shorter than the observation period of 56 days. Thirty-seven patients with ABO-I LDLT with pre-operative prophylaxis of Rituximab are considered for this study. At Kyoto University Hospital, indications for splenectomy are intra-operative portal vein pressure after reflow above 15mmHg(17) and hepatitis C infection(18) to prevent interferon induced thrombocytopenia expected during treatment of HCV recurrence. 27 patients who underwent splenectomy were grouped as 'splenectomy group' and splenectomy not performed in 10 patients grouped as 'non-splenectomy group'. After permission from Kyoto University ethics committee, data of demography, MELD scores, CD-19 antigen assay, blood group of recipient, operative records including duration of surgery, blood loss, graft-recipient-weight ratio, post-operative histo-pathological data including antibody mediated rejection, acute cellular rejection, were collected. Anti-ABO IgM and IgG antibody titers in recipients measured at admission, transplantation, and on day 3, 6, 9, 12, 15, 18, 21, 28, 35, 42, 49, and day 56 after transplantation. An operative procedure of LDLT and splenectomy at Kyoto University Hospital described in detail elsewhere (19).

Antibody mediated rejection is diagnosed histologically by peri-portal edema and endothelial C_4D staining (20-21) clinically correlating with increased Anti-ABO antibody titers. ACR is

diagnosed by Banff criteria (22). Biliary complications suspected clinically and histologically were confirmed by cholangiogram.

Immunosuppression:

Immunosuppression protocol for ABO-I LDLT at Kyoto University Hospital between May 2006 and July 2009 includes rituximab, plasma exchange, and hepatic artery infusion with prostaglandin E1 and methyl prednisolone. 13 Patients till October 2007 received 500 mg Rituximab. As rituximab is not covered under insurance in Japan, we reduced dose of Rituximab to 300 mg thereafter to cut down the cost. We confirmed elimination of B-cells from circulation by the study of CD-20 and CD-19 marker at time of LDLT. Plasma exchange with blood group AB plasma performed one to three times depending upon anti-ABO antibody titers before transplantation; at dose of 1 unit /kg body weight to reduce anti-ABO antibody titer less than 1:16. Hepatic artery infusion starts during operation after the reconstruction of hepatic artery, using prostaglandin E1 at the initial dose of 0.005 µg/kg/min and the maintenance dose of 0.01 µg/kg/min from postoperative day (POD) 1 to POD 21 and 125mg/day methyl-prednisolone for first 7 days. Cyclophosphamide 100mg/day administrated intra venous systemically for 7 days followed by oral mycophenolate mofetil 500mg/ twice a day. Routine immunosuppression for LDLT at Kyoto University Hospital includes tacrolimus, and steroid (23). Tacrolimus trough maintained between 10-15 ng/ml in first 2 weeks, 7-10 ng/ml between 2-8 weeks, 5-7ng/ml until 6 months and below 5ng/ml thereafter. Oral prednisolone started from POD 8 at dose of 0.3mg/kg then tapered to 0.1mg/kg from 4^{th} week and stopped after 3 moths.

Statistical Method

Patient characteristics between ‘splenectomy group’ and ‘non-splenectomy group’ were compared using Levene's Test for Equality of Variances and Pearson Chi-Square by SPSS statistical software. General linier model with repeated measure used to compare the mean anti-ABO titer between two groups. p value <0.05 was considered as significant. Kaplan-Meier method is used to compare survival between two groups.

Results:

Thirty-seven ABO-I LDLTs were performed in 16 males and 21 in females. Table: 1a describes sex, primary disease for transplantation, relationship of recipient with donor, donor and recipient blood groups, graft type between 'splenectomy' and 'non splenectomy' group. Age, body weight, MELD scores of recipient before transplantation, Rituximab dose and time of administration, CD-19 antigen assay, Graft-recipient-weight-ratio, duration of operation, blood loss during operation, and hospital stay of recipients between two groups are compared in Table 1b. Table: 2 shows blood groups of recipient and donor.

Anti-ABO Antibody Titers in 'splenectomy group' and 'non splenectomy group':

Anti-ABO antibody titers at admission, at LDLT and POD 3, 9, 12, 15, 18, 21, 28, 35, 42, 49, and 56compared between 'splenectomy group' and 'non-splenectomy group'. Rituximab effectively reduced anti-ABO antibody titers in both group. There was no significant difference in post-operative anti-ABO IgM antibody titers in 'splenectomy group' and 'non-splenectomy group' (Figure-1; p=0.920). Although anti-ABO IgG antibody titers remained high in 'splenectomy group' group but the difference was statistically insignificant (Figure-2; p=0.991).

We observed variation in Anti-ABO antibody titers across the ABO blood groups. Recipient with O blood groups had high titer of IgG level at admission as compared to recipient of blood group A or B. Moreover, Anti-ABO IgG titer in recipients with blood group O remained elevated even after treatment with rituximab as compared to that of recipients with blood group A and B (Figure-3;p=0.000).

One patient in each group suffered with AMR. AMR treated with increasing trough of tacrolimus, intravenous steroid therapy, and plasma-pheresis. However, AMR in patient from 'non-splenectomy group' was not responded and this patient received re-transplantation on POD 22.

Difference in AMR, ACR, cholangitis, intra-hepatic bile duct strictures, viral and bacterial infections between 'splenectomy group' and 'non-splenectomy group' shown in table 3:

Survival difference: Four patients in each 'splenectomy group' and 'non-splenectomy group' died during the fellow up period (p=0.257). 1 and 3 year survival rates after ABO-I LDLT (Figure: 4) in 'splenectomy group' were 88.9% and 84% respectively. One and 3 year's survival in 'non-splenectomy' group was 60% and 60%. Cause of death for patients in 'non-splenectomy' group is related with hepatic artery catheter displacement and bleeding, perforation of bowel, CRF, and graft failure. Cause of death in 'splenectomy group' is related to HCV re-infection, septic shock, and graft failure.

Discussion:

Preformed anti-ABO antibodies in recipient are the major culprit for complications of ABO-I LT including AMR. Current protocols for ABO-I LT including plasma exchange, immunoglobulin, and splenectomy and mycophenolate mofetil targeted towards the reducing anti-ABO antibody titers. Spleen is considered a major site for antibody production. In 1985, Alexandre et-al proposed splenectomy as a pre-requisite for successful ABO-I kidney transplantation(24). This knowledge encouraged the liver transplant surgeons to utilize splenectomy along with plasma-pheresis and OKT-3 to overcome the challenge of ABO-I LT(5). Splenectomy in ABO-I LT always has been criticized for a post splenectomy overwhelming infection added by the strong immunosuppression used for ABO-I LT(25). Furthermore, with the introduction of rituximab for desensitization ABO-I LT promoted us to re-evaluate the necessity of splenectomy for ABO-I LT.

Results of our study demonstrate no statistically significant difference in anti ABO antibody titers between patients of 'splenectomy group' and 'non-splenectomy group' who were observed for 2 months after transplantation with 300 mg rituximab administered before transplantation. Rituximab sufficiently deplete B cells from peripheral circulation and from the

lymphoid tissue as confirmed by decreased CD-19 and CD-20 B cell markers before liver transplantation (21). However, Rituximab does not completely eliminate B cells from the lymphoid tissue (15, 26). Furthermore, mature B cells and plasma cells, which do not have CD-20 escape fromthe action of Rituximab.

In our study, anti-ABO antibody titer was similar in a 'splenectomy group' and 'non-splenectomy group', which implies that B cells in the lymphoid tissuereduced effectively by a single dose of rituximab, to keep anti-ABO titers sufficiently lowto prevent AMR in most of patients. Spleen represents only 25 % of total peripheral lymphoid tissue(16) and the antibody production continues in the rest of lymphoid tissue even after splenectomy. Therefore, anti-ABO antibody response was similar in a 'splenectomy group' and 'non-splenectomy group'. Thus, our study confirms no advantage of the splenectomy in ABO-I LT with a pre-operative use of rituximab. We observed thatpreoperative anti-ABO IgG titer in 'splenectomy group' remained elevated during 2-months observation period; this may be because of more O group recipients in 'splenectomy group' Also, high preoperative Anti-ABO IgG titer seen in recipient of O blood group patient. 'Splenectomy group' has more number of the O group recipients and high IgG titer reflected in high post- transplant IgG titer in 'splenectomy group'. This high antibody titer may be the cause of higher incidence of biliary complications observed in 'splenectomy group'. However, in this study the sample size is too small to demonstrate statistically significant relationship between recipient blood group and AMR.

Single administration of rituximab at dose of 375mg/m^2 leads to a complete depletion of CD-20 positive B cell from peripheral circulation. B cell reduction starts as early as third day after a rituximab administration but complete depletion seen only after the 3 weeks(26). However, B cells from lymph nodes can not be completely depleted(26). Sawada T et al had shown B cell also can not be depleted from spleen with a single dose of Rituximab(14). At the same time, a complete elimination of B cell needs to be evaluated for the fear of the prolonged immune-compromised state induced by a rituximab. After single 375/m^2 dose of rituximab CD-20 positive B cells are depleted from circulation for almost 8-12 months(26), despite only the

first 3 months are critical after ABO-I LT for antibody mediated rejection. Rituximab was introduced for treatment of resistant B cell leukemia at a dose of 375/m^2 weekly for four weeks. However, such high dose is not desirable for normal B cells in ABO-I LT recipients. Toki D et al demonstrated that the dose as low as15 mg/m^2 is sufficient to eliminate CD-20 positive B cell for the peripheral blood. In addition, 15mg/m^2 single dose depletes these cells for 3 months and recovery is seen after 3-6 months (15).Mycophenolate mofetila cyto-toxic drug to the B cells including plasma cells is important adjuvant to Rituximab therapy in ABO-I LT. To achieve the maximum response it is important to deplete plasma cells at time of transplantation by staring mycophenolate mofetil before transplantation. Thus, we believe it is more important to standardize the dosage and frequency of administration of Rituximab to achieve maximum outcome in ABO-I LT.

With analyzing the anti-ABO antibody titer in 'splenectomy group' and 'non-splenectomy group', our study showed no statistically significant advantage of splenectomy. Therefore, in current scenario, low dose of preoperative Rituximab with mycophenolate mofetil for maximal effect of B cell depletion followed by plasma exchange for removal of pre-formed antibodies would be substantially more important treatment than splenectomy in ABO-I LT.

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Table: 1a- Characteristics of patient between 'splenectomy' and 'non-splenectomy' group.

Patient Characteristics		Without Splenectomy Group (n=10)	splenectomy Group (n=27)	Significance
Recipients Sex	Female	6	15	0.555
	Male	4	12	
Primary Disease	Cholestasis	0	3	0.656
	Graft failure	1	2	
	HBV	1	1	
	HCC	2	7	
	HCV	3	6	
	LC	1	3	
	PBC	2	5	
Relationship of Donor	Daughter	0	4	0.148
	Father	0	2	
	Husband	5	6	
	Mother	0	5	
	Sibling	2	3	
	Son	0	4	
	Wife	3	3	
Recipient Blood Group	A	2	9	0.048
	B	6	5	
	O	2	13	
Donor Blood Group	A	2	10	0.610
	AB	4	8	
	B	4	9	
Graft Segment	1+2+3+4	3	8	0.626
	2+3+4	1	0	
	5+6+7+8	6	18	
	6+7	0	1	

Table: 1a- Characteristics of patient between 'splenectomy' and 'non-splenectomy' group.

Patient Characteristics	Without Splenectomy Group (mean ± SD)	splenectomy Group (mean ± SD)	Levene's Test for Equality of Variances Significance
Age (Years)	48.66±8.19	47.91± 14.52	0.068
Body weight (kg)	62.23± 13.10	63.55±14.56	0.730
MELD Score	19.51±6.99	20.68±8.45	0.897
Rituximab Administered before LDLT(Days)	26.60±21.26	17.67±11.86	0.086
Rituximab Dose (mg)	420±103.28	329.63±85.77	0.084
CD19 at Admission	13.91±7.23	20.26±11.46	0.129
CD 19 at LDLT	0.60±0.751	1.246±3.47	0.307
Graft-Recipient-Weight- Ratio	0.996±0.198	0.903±0.22	0.855
Duration of Surgery (Hours)	12.17± 1.6	14.19± 2.46	0.190
Blood loss (Liter)	9.517± 6.927	7.632±6.393	0.800
Hospital Stay (Days)	78.56±44.92	61.09±28.98	0.176

Table: 2 Blood groups of donor and recipient.

		Donor Blood Group			Total
		A	AB	B	
Recipient Blood Group	A	0	3	8	11
	B	4	7	0	11
	O	8	2	5	15
Total		12	12	13	37

Table: 3 Difference in clinical complications between 'case' and 'non-splenectomy group'

Conditions	Non-splenectomy group (n=10)	splenectomy group (n=27)	Levene's Test for Equality of Variances Significance
AMR	1(10%)	1 (3.7%)	0.473
ACR	6(60%)	12(44.4%)	0.319
Cholangitis	6(60%)	16(59.5%)	0.635
Intra-hepatic bile duct strictures	0	2 (7.4%)	0.528
Viral Infections	2 (20%)	12 (44.4%)	0.164
Bacterial Infections	7 (70%)	14 (51.9%)	0.272

Figure :

Figure -1 shows a graphical representation of the mean anti ABO IgM antibody titers in 'splenectomy group' and 'non-splenectomy group' at admission, transplantation, and initial 56days after ABO-I LDLT (p=0.709).

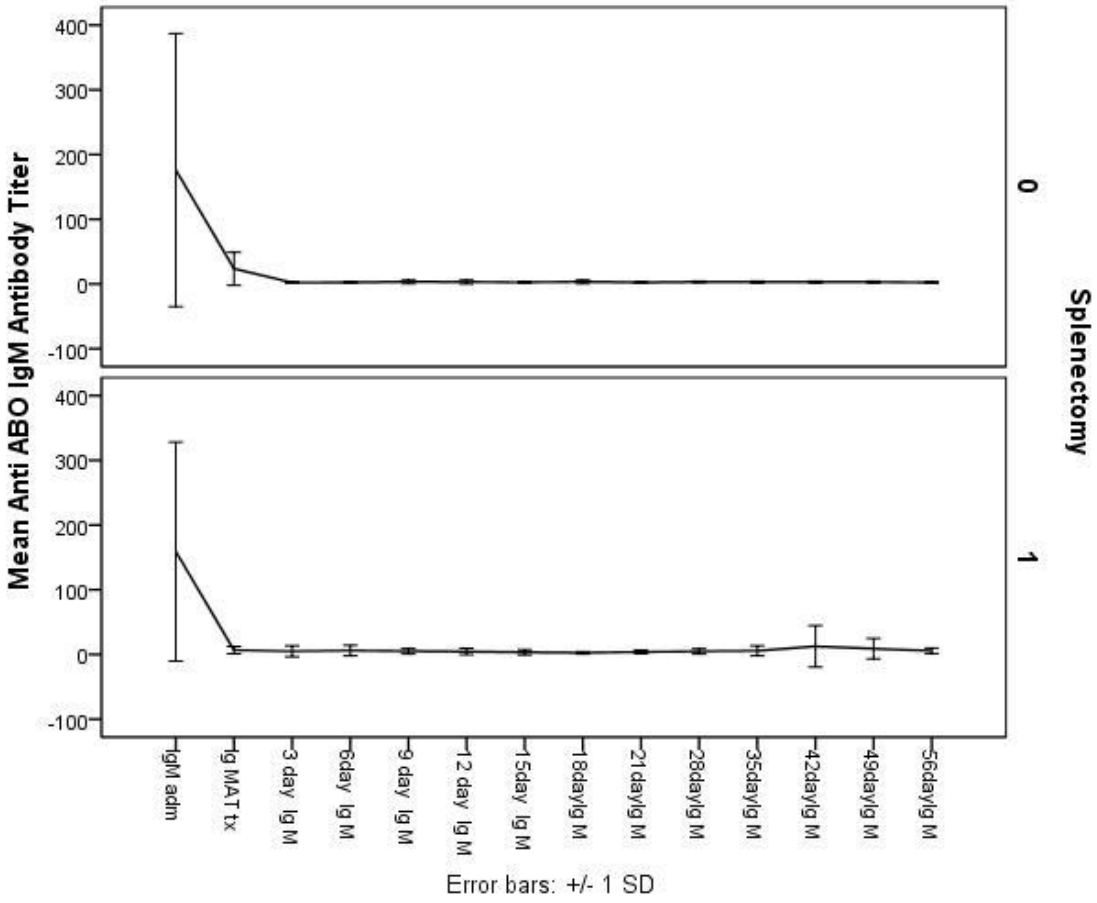

Figure-2 shows graphical representation of mean anti-ABO IgG antibody titer in 'splenectomy group' and 'non-splenectomy group' group at admission, transplantation, and initial 56 days after ABO-I LDLT (p=0.924).

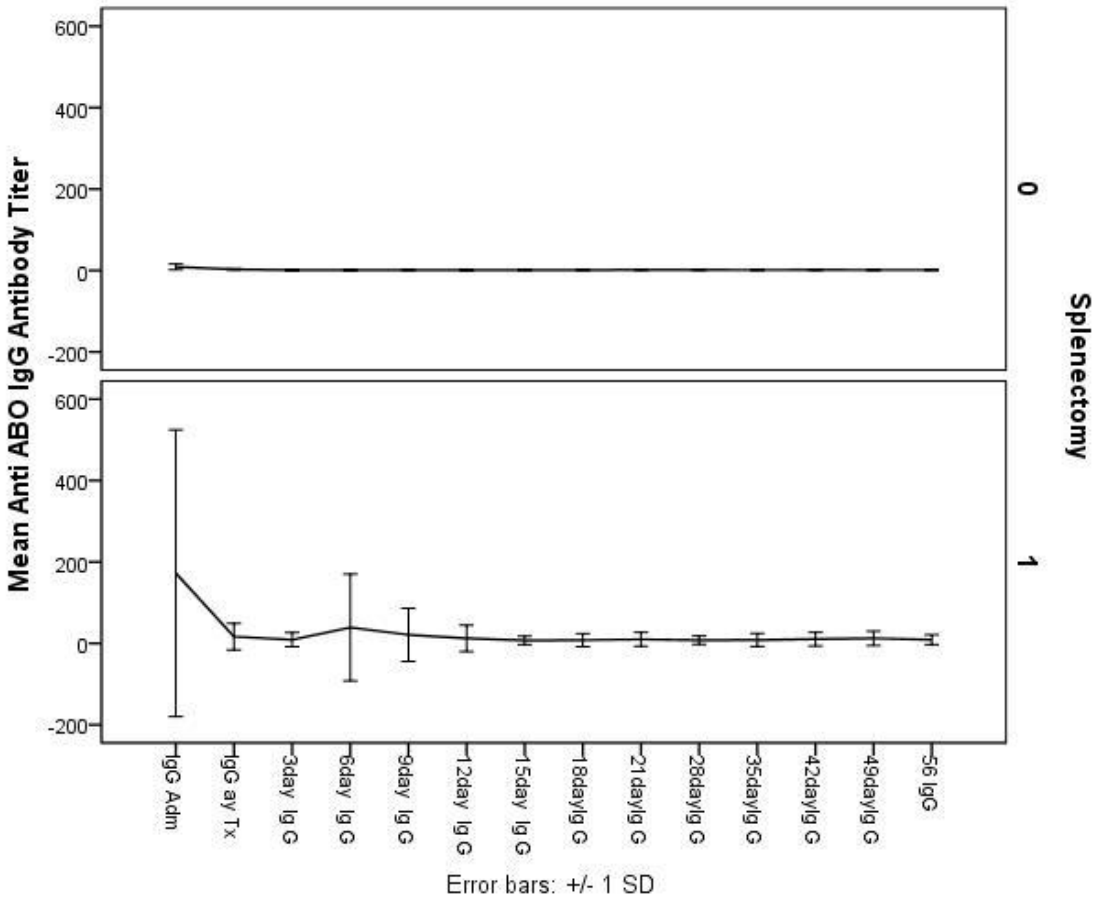

Figure-3 shows mean anti-ABO IgG and IgM titers across ABO blood group recipients.

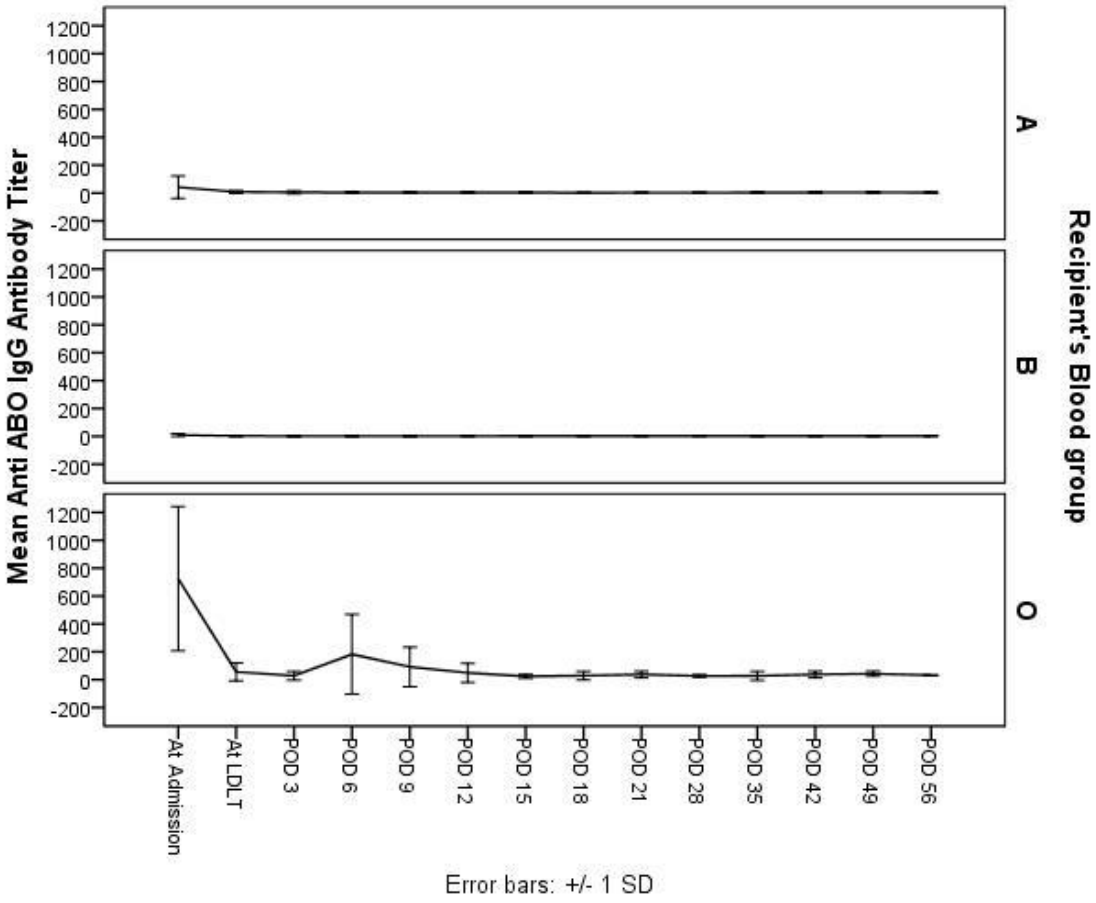

Figure-4 shows survival difference in 'splenectomy group' and 'non-splenectomy group' (p=0.274).