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ORIGINAL ARTICLE

HLA matching and rabbit antithymocyte globulin as induction therapy to avoid multiple forms of rejection after a third liver transplantation

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Abstract
Background. — Despite immunosuppressive drug regimens, T cell-mediated rejection, antibody-mediated rejection with donor-specific antibodies, and chronic rejection occur after liver transplantation (LTx). Rejection may significantly impact allograft survival and often a standard re-LTx is required. However, in some cases rejection recurs. Little is known on how to approach this and which aspects to consider.
Case. — Here we describe a case in which two successive liver grafts where lost due to T cell-mediated rejection, possible antibody-mediated rejection with de novo donor-specific antibody formation, and chronic rejection that occurred within a month. In an attempt to avoid recurrence with the third graft, we decided to administer a more rigorous immunosuppressive drug induction regimen with rabbit antithymocyte globulin, while applying HLA matching between recipient and donor. This resulted in rejection free survival for 337 days until a mild T cell-mediated rejection occurred, which could then be easily treated with high dose steroids. Graft survival is now at least 683 days without chronic rejection, antibody-mediated rejection or de novo donor-specific antibody formation.

Abbreviations: AMR, antibody-mediated rejection; CR, chronic rejection; DBD, donation after brain death; DSAs, donor-specific antibodies; HLA, human leukocyte antigen; h-score, histopathology-score; IS, immunosuppressive drugs; LTx, liver transplantation; RAI, rejection activity index; rATG, rabbit antithymocyte globulin; re-LTx, retransplantation; TCMR, T-cell-mediated rejection.

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Conclusion. — In conclusion, when a liver graft is lost due to multiple forms of rejection short after LTx, the combination applied in this case could be considered as a viable option to improve graft and patient survival instead of a standard re-LTx.
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Introduction

Liver transplantation (LTx) is the only treatment for end stage liver disease. Prophylactic induction treatment is administered around the time of LTx in order to prevent severe rejection during the first few weeks after transplantation. Since there is no consensus on a specific induction treatment worldwide, multiple treatment approaches exist, such as lymphocyte depleting agents alemtuzumab and rabbit antithymocyte globulin (rATG) and non-depleting agents basiliximab and methylprednisolone [1].

Despite induction and maintenance immunosuppressive drug (IS) regimens, T-cell-mediated rejection (TCMR), antibody-mediated rejection (AMR) and chronic rejection (CR) occur after transplantation. The formation of donor-specific antibodies (DSAs) against human leukocyte antigen (HLA) types of the donor graft is common across all solid organ transplantations [2]. Unlike in other types of solid organ transplantation, many DSA positive LTx recipients do not experience clear symptoms of acute or chronic rejection. Nevertheless, both preformed and de novo DSAs have been associated with lower graft survival [3] and an increased risk of acute and chronic rejection (CR) [4], including AMR [5]. Acute AMR diagnosis requires four criteria and according to the BANFF scoring system this indicates a probability of AMR [6]. TCMR is quantitatively estimated by the Rejection Activity Index (RAI) score that includes portal, bile duct and venous endothelial inflammation. CR is a severe deleterious complication after LTx, characterized by foamy arteriopathy and progressive bile duct loss (ductopenia). In contrast to TCMR, CR does traditionally not respond to IS treatment. [6]

In only a minority of LTx recipients severe acute or chronic rejection after transplantation due to alloreactivity against donor HLA, leads to graft failure. For these patients, a retransplantation (re-LTx) is the only treatment available [7,8]. Nonetheless, in some cases a standard re-LTx is not sufficient and recurrence of rejection occurs. Until now little is known on how to approach this complex situation. Here we present a case where a third transplantation with HLA matching between recipient and donor in combination with a change in induction regimen was necessary to circumvent the severe TCMR, AMR, de novo DSA formation, and CR that arose after the first two liver transplantations.

Case

A 35-year old Caucasian male with a history of several cholestatic complications due to primary sclerosing cholangitis (PSC), was transplanted with a ABO-compatible donation after brain death (DBD) split liver in September 2017 at our institution. The donor liver had five HLA mismatches with the recipient (Table 1). As our standard protocol indicates, methylprednisolone 500 mg was administered during implantation and basiliximab 20 mg i.v. was administered within 6 hours after reperfusion, and repeated at day 4 after LTx. The following maintenance IS regimen was administered after LTx: daily oral prednisolone 20 mg and mycophenolate mofetil 2 x 1000 mg; at day 5 tacrolimus at a dose of 0.1 mg kg (2 x 4 mg) was added; at day 7 prednisolone was reduced to 10 mg, which was continued for 3 months. After two adequate tacrolimus trough levels (8-15 μg/L), mycophenolate mofetil was discontinued. After 25 days the patient experienced symptoms of rejection, including a fever and substantially increasing liver enzyme abnormalities. A liver biopsy was evaluated by a liver pathologist and a severe acute TCMR and CR (Fig. 1 A; Table 2) were diagnosed. As a rescue treatment 1000 mg of methylprednisolone was administered for three consecutive days. Unfortunately, liver enzyme abnormalities did not decrease and a follow-up liver biopsy indicated that the rejection episode was not resolved (Fig. 1B; Table 2). As a consequence, a second liver transplantation was needed. In the explant (Fig. 1C; Table 2) clear TCMR with central perivenulitis and CR with ductopenia were present. There was also a suspicion of acute AMR [6] because of a combined C4d-score + h-score of 3 in the explant (Supplementary Fig. 1; Table 2) and presence of de novo DSAs against donor HLA (Table 1) in the blood of the recipient.

Fifty-five days after the first LTx, a second LTx was performed with a ABO-compatible DBD donor liver. It is common practice for LTx not to consider the donor HLA type in combination with the recipient's. The second donor liver had four HLA mismatches with the recipient (Table 1). Of these four, three HLA mismatches were similar to those of the first donor liver. Similar induction treatment and maintenance IS regimen were given as described above. Again, the IS trough levels were adequate (6-15 μg/L). Two weeks after the second LTx, the patient again experienced increasing liver enzyme abnormalities. A liver biopsy (Fig. 1D; Table 2) indicated again TCMR and CR. An AMR was also considered, because of DSA positivity (Table 1). As a rescue treatment methylprednisolone was administered as described above and an additional 65 g of i.v. immunoglobulins for two consecutive days thereafter. The patient responded to some degree to the rescue treatment according to the RAI score in the follow-up biopsy, but the CR episode and central perivenulitis did not resolve (Fig. 1E; Table 2). Eventually, the graft could not be saved. In the explant (Fig. 1F; Table 2) clear TCMR and CR with ductopenia and foamy arteriopathy were present. Despite a C4d score of 3 in the explant (Supplementary Fig. 1; Table 2) and DSA positivity, acute AMR could not be confirmed due to a zero h-score. [6]

To save the patient's life, a different approach seemed to be needed. After a literature search, and multidisciplinary meetings with experts in the area of transplantation

Table 1 The HLA types of the recipient and the three liver donors, their HLA mismatches and the DSAs present.

Recipient	A1 A2 B7 B8 DR2 (DR15) DR6 (DR13) DQ1 DQ6	HLA mismatch with recipient				
		A	B	DR	DQ	Total
Donor 1	A1 **A19 (A32)** B7 B8 **DR3 (DR17) DR4 DQ2***‡ **DQ3***‡ **(DQ8)**	1	0	2	2	5
Donor 2	A2 **A19 (A32)** B7 **B15 (B62)** DR2 (DR15) **DR4** DQ1 (DQ6) **DQ3***§ **(DQ8)**	1	1	1	1	4
Donor 3	A2 B7 **B16 (B38)** DR2 (DR15) DR6 (DR13) DQ1 DQ6	0	1	0	0	1

In bold the HLA mismatches of donor with recipient are indicated. *de novo DSAs against the HLA type of the donor present in the recipient after LTx. ‡Mean fluorescence intensity measured: 6400 for both. §Mean fluorescence intensity measured: 1000. DSAs were measured in pre- and post-LTx serum samples by a standardized Luminex single antigen test.

Table 2 Histology of biopsies and explants.

Indicative of	TCMR	Transition of AR to CR	CR	AMR	
Biopsy (B) or explant (E)	RAI score	Central Perivenulitis	Ductopenia (% of portal field)	C4d score§	h-score
B1a	5*	+++	Yes (90%)	ND	1
B1b	3*	+++	Yes (90%)	0-1	1
E1	7	+++	Yes (80-90%)	2	1
B2a	5*	++(+)	Yes (90%)	0	1
B2b	1*	++(+)	Yes (90%)	2-3	0
E2	5*	+++	Yes (>95%)‡	3	0
B3a	7	+(+)	No	0	1
B3b	2	-	No	0	0

Biopsies after 1st (B1), 2nd (B2) and 3rd (B3) LTx, before (a) and after (b) rescue treatment, and explants after 1st (E1) and 2nd (E2) LTx are depicted. *Bile duct inflammation could not be determined due to ductopenia. Therefore, RAI scores are without bile duct inflammation score. ‡Foamy arteriopathy present towards hilus. §C4d was determined in formalin-fixed paraffin-embedded biopsies by immunohistochemistry with a monoclonal antibody. Due to a shortage of biopsy tissue before rescue treatment after first LTx, C4d staining could not be performed. — none, + mild, ++ moderate, +++ severe. AMR, antibody-mediated rejection; AR, acute rejection; CR, chronic rejection; h-score, histopathology-score; ND, not determined; RAI, rejection activity index; TCMR, T-cell-mediated rejection.

and rejection, an alternative protocol was developed. In order to minimize the possibility of developing TCMR, CR and possible AMR due to pre-existing DSAs or de novo DSAs, it was decided to accept only a donor liver with minimal HLA mismatches with the recipient. Fortunately, a suitable ABO-compatible DBD donor liver, with only one HLA mismatch with the recipient (Table 1), was available 189 days after the second transplantation. The second change was the type of induction therapy given around the time of LTx. A case report by Yamada et al. [9] indicated that rATG induction therapy resulted in minimal TCMR and no AMR after the second (non-HLA-matched) transplantation, while the first liver transplant was lost due to severe AMR. A study performed by Kubal et al. [10] indicated that with the use of rATG lower rejection rates were observed compared to other induction therapies. Therefore, instead of administering methylprednisolone and basiliximab, 1.5 mg/kg i.v. rATG (as used in the case report of Yamada et al.) was administered on day 1 and 3 after LTx. Maintenance IS regimen was administered as described above. With this adjusted protocol the patient did not develop CR, nor de novo DSAs and AMR after the third LTx (Fig. 1G; Table 2). Unfortunately, a mild acute TCMR developed after 337 days, which quickly and fully resolved after administering standard 1000 mg methylprednisolone for three consecutive days, as was confirmed by a follow-up liver biopsy (Fig. 1H; Table 2). Maintenance IS regimen was set to tacrolimus 5 mg and prednisolone 7.5 mg. For at least 346 days thereafter (683 days in total), the patient is stable with the third donor liver graft.

Discussion

Here we describe a young patient with PSC in which the two first liver transplants led to TCMR, CR and possible AMR after 25 and 13 days respectively, including de novo DSA formation. The allograft response against donor HLA was probably the cause of severe rejection and graft loss after both LTx. There is no clear protocol and/or study in the literature that describes how to approach and resolve such difficult situations. Based on available information and experience in our transplant unit, we chose to match the HLA type of the donor as closely as possible to the HLA of the recipient, and to apply another induction regimen post-operatively. In this way, the allograft response against donor HLA was limited after the third LTx. The adjusted protocol led to rejection-free survival for up to 337 days, absence of DSA formation and graft survival for at least 683 days. At day 337 a TCMR was diagnosed, but the patient responded well to the standard rescue treatment, in contrast to the earlier episodes of rejection observed after the first and second LTx. We speculate that the extent of the rejection after the third LTx

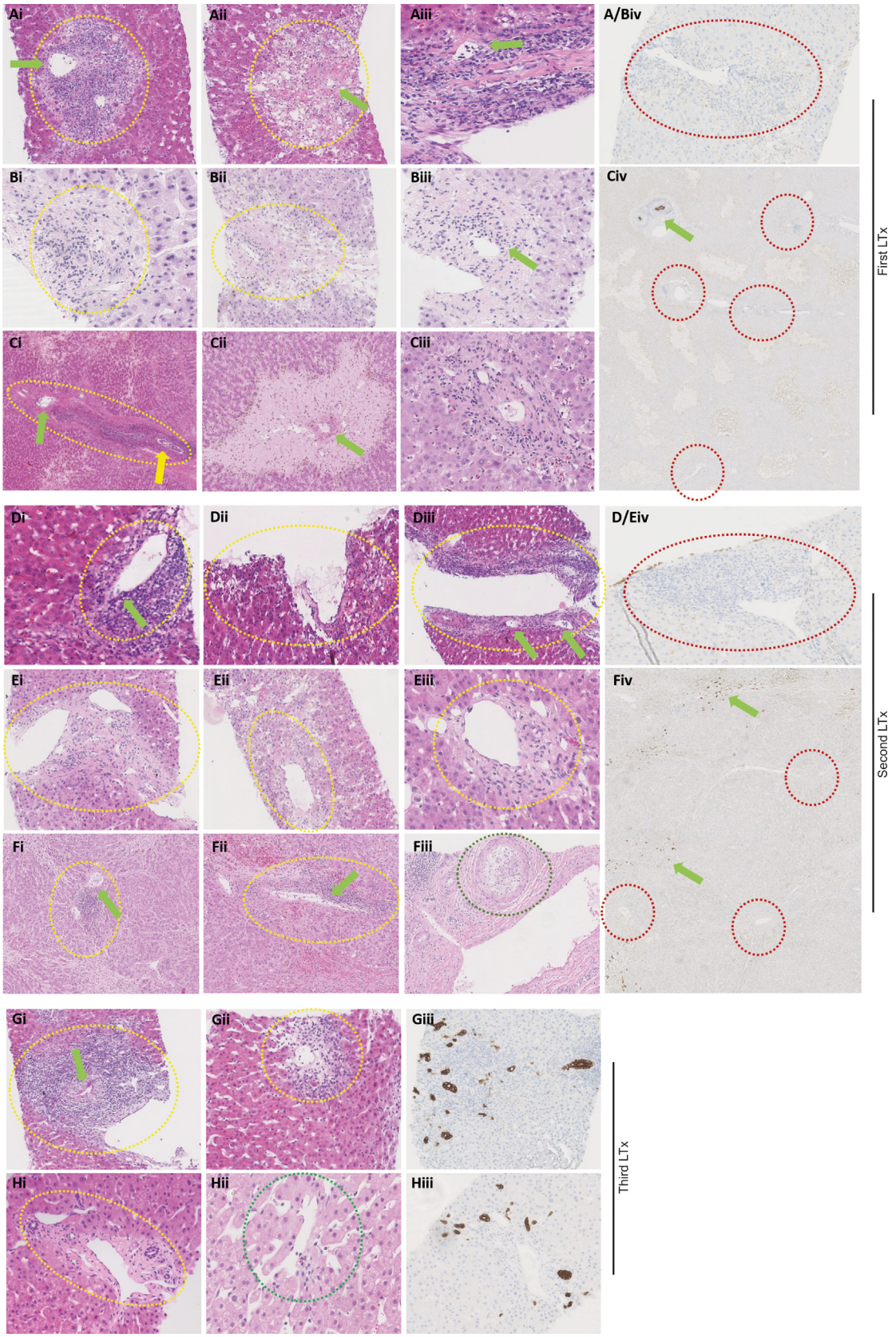

Figure 1 Biopsies and explants after first and second LTx indicate multiple forms of rejection, whereas biopsies after third LTx indicate TCMR only.

was limited due to HLA matching, as is supported by the absence of DSA formation and CR, and that this contributed to a rapid clinical response after rescue treatment with methylprednisolone.

Instead of the standard induction treatment of methylprednisolone and basiliximab, rATG was used around the third LTx. Although rare in liver transplantation, rATG is a lymphocyte-depleting polyclonal antibody commonly used and indicated for the (induction) treatment of acute renal allograft rejection. It causes a persistent increase of regulatory T-cells and a prolonged reduction in effector CD4+ T-cells and B-cells [11]. On the other hand, methylprednisolone works by preventing overall inflammation, whereas basiliximab prevents activation of T-cells. Yamada et al. [9], described that with the use of rATG as induction regimen, an AMR and subsequent second liver graft loss was prevented. In addition, several other studies in liver [10] and kidney [12] transplantation have indicated that with rATG lower rejection rates were observed compared to other induction regimens. However, for some sensitized kidney transplant recipients rATG (with rituximab) induction treatment alone was not sufficient to avoid an AMR [13]. Therefore, matching of HLA between recipient and donor was added to our adjusted protocol. We believe that this rigorous method of induction therapy, aside from HLA matching, was needed to avoid de novo DSA formation with AMR and CR and to succeed with a re-LTx.

Although definite criteria have been described for the diagnosis of acute AMR after LTx [6], its lower incidence compared to other solid organ transplants, the (technical) difficulties in unmasking a C4d staining and the relatively often patchy expression of C4d (C4d score 2 and 3 show only minimal of focal staining), make a confident histopathological diagnosis on a liver biopsy challenging [14]. On the other hand, CR with absolute ductopenia within a month after both LTx, was very striking in this case. Other etiologies/diseases leading to vanishing bile ducts were considered in the differential diagnosis, but both the lack of ductular reaction and an appropriate clinical history made them incompatible with this case. Moreover, retrospectively the identification of foamy arteriopathy in the big (peri)hilar arterial branches in the second explant specimen, also pointed towards CR. Having a multidisciplinary team with experts in early recognition and application of a suitable treatment is paramount for solving such complicated cases with different types and severity of rejection.

From this case we can conclude that whenever a liver graft is (repeatedly) lost due to severe acute TCMR, possible AMR, DSA formation and chronic rejection short after LTx, HLA matching between donor and recipient in combination with a more rigorous IS regimen should be considered as a primary treatment option instead of a standard re-LTx, in order to improve graft and patient survival.

Author contributions

AAD participated in research design and data extraction, performance of the research and prepared the first and subsequent drafts of the manuscript for submission. MD participated in data extraction, data analysis, provided critical input for writing of the manuscript and critical insight of the research. MGHB provided critical input for writing of the manuscript and critical insight of the research. TPPB participated in data extraction and data analysis. SDM provided the unique case and critical input for writing of the manuscript and critical insight of the research. NHRL obtained funding, provided critical input for writing of the manuscript and critical insight of the research. DS participated in research design, provided critical input for writing of the manuscript and critical insight of the research. JK obtained funding, participated in research design, provided critical input for writing of the manuscript and critical insight of the research. All authors approved the final version of the manuscript.

Biopsies before **(A)** and after **(B)** rescue treatment and explant **(C)** after first LTx. **A, B, C. i:** TCMR with prominent portal inflammatory cell infiltrates. Yellow dotted circle: portal triad, Green arrow: endotheliitis, Yellow arrow: bile duct inflammation and damage. **A, B, C. ii:** TCMR with central perivenulitis, obvious hepatocyte dropout and mild/moderate inflammatory infiltrates. Yellow dotted circle: perivenular area, Green arrow: central vein. **A, B. iii:** Focal microvasculitis (green arrow), suggestive of AMR. **C. iii:** Portal area without recognizable bile duct, indicative of CR. **A/B, C. iv:** Keratin 7 (#790-4462, Clone: SP52, Ventana Medical Systems) immunostaining indicates obvious loss of bile ducts and the lack of ductular reaction in the portal areas (red dotted circles), indicative of CR. Green arrow: a single portal field in the explant liver with native bile duct present. Biopsies before **(D)** and after **(E)** rescue treatment and explant **(F)** after second LTx. **D, E, F. i:** TCMR with prominent **(D** and **F)** and mild **(E)** portal inflammatory cell infiltrates. Yellow dotted circle: portal triad, Green arrow: endotheliitis. **D, E, F. ii:** TCMR with central perivenulitis, hepatocyte dropout and moderate inflammatory infiltrates. Yellow dotted circle: perivenular area, Green arrow: central vein endotheliitis. **D, E. iii:** Focal microvasculitis (**D**, green arrow), suggestive of AMR. In **E** no recognizable microvasculitis. Yellow dotted circle: perivenular area. **F. iii:** Portal area with foamy arteriopathy (green dotted circle), indicative of CR. **D/E, F. iv:** Keratin 7 immunostaining indicates the obvious loss of bile ducts and the lack of ductular reaction in the portal areas (red dotted circles), indicative of CR. Green arrow: areas with ductular metaplasia. Biopsies before **(G)** and after **(H)** rescue treatment after third LTx. **G. i:** TCMR with prominent portal inflammatory cell infiltrates. Yellow dotted circle: portal triad, Green arrow: bile duct inflammation and damage. **G. ii:** TCMR with central perivenulitis and mild/moderate inflammatory infiltrates. Yellow dotted circle: perivenular area. **H. i, ii:** No signs of rejection in the portal (dotted yellow circle) and pericentral area (green dotted circle). **G, H. iii:** Keratin 7 immunostaining highlights the presence of the original bile ducts. Abbreviations: AMR, antibody-mediated rejection; CR, chronic rejection; LTx, liver transplantation; TCMR, T-cell-mediated rejection.

Conflict of interest

The authors of this manuscript have no conflicts or competing of interest to disclose.

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Data availability

All available data is shared within this article.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.clinre.2020.08.014.

Références

[1] Rostaing L, Saliba F, Calmus Y, Dharancy S, Boillot O. Review article: use of induction therapy in liver transplantation. Transplant Rev (Orlando) 2012;26(Oct):246—60.
[2] Cillo U, Bechstein WO, Berlakovich G, Dutkowski P, Lehner F, Nadalin S, et al. Identifying risk profiles in liver transplant candidates and implications for induction immunosuppression. Transplant Rev (Orlando) 2018;32(Jul):142—50.
[3] Legaz I, Boix F, Lopez M, Alfaro R, Galian JA, Llorente S, et al. Influence of Preformed Antibodies in Liver Transplantation. J Clin Med 2020;9(Mar 5).
[4] O'Leary JG, Klintmalm GB. Impact of donor-specific antibodies on results of liver transplantation. Curr Opin Organ Transplant 2013;18(Jun):279—84.
[5] Kozlowski T, Rubinas T, Nickeleit V, Woosley J, Schmitz J, Collins D, et al. Liver allograft antibody-mediated rejection with demonstration of sinusoidal C4d staining and circulating donor-specific antibodies. Liver Transpl 2011;17(Apr):357—68.
[6] Demetris AJ, Bellamy C, Hubscher SG, O'Leary J, Randhawa PS, Feng S, et al. 2016 Comprehensive update of the Banff Working Group on liver allograft pathology: introduction of antibody-mediated rejection. Am J Transplant 2016;16(Oct):2816—35.
[7] Ho MH, Wu SY, Ou KW, Su TF, Hsieh CB. Retransplant as rescue treatment for ABO-compatible living-donor liver transplant related antibody-mediated rejection: a case report. Exp Clin Transplant 2018;16(Apr):222—6.
[8] Yoo PS, Umman V, Rodriguez-Davalos MI, Emre SH. Retransplantation of the liver: review of current literature for decision making and technical considerations. Transplant Proc 2013;45(Apr):854—9.
[9] Yamada Y, Hoshino K, Mori T, Kawaida M, Abe K, Ishihama H, et al. Successful living donor liver retransplantation for graft failure within 7 days due to acute de novo donor-specific anti-human leukocyte antigen antibody-mediated rejection. Hepatol Res 2018;48(Feb):E360—6.
[10] Kubal CA, Mangus RS, Saxena R, Lobashevsky A, Higgins N, Agarwal A, et al. Crossmatch-positive liver transplantation in patients receiving thymoglobulin-rituximab induction. Transplantation 2014;97(Jan 15):56—63.
[11] Ippoliti G, Lucioni M, Leonardi G, Paulli M. Immunomodulation with rabbit anti-thymocyte globulin in solid organ transplantation. World J Transplant 2015;5(Dec 24):261—6.
[12] Alloway RR, Woodle ES, Abramowicz D, Segev DL, Castan R, Ilsley JN, et al. Rabbit anti-thymocyte globulin for the prevention of acute rejection in kidney transplantation. Am J Transplant 2019;19(Aug):2252—61.
[13] Lee KW, Park JB, Cho CW, Lee N, Yoo H, Kim K, et al. The Impact of donor-specific anti-human leukocyte antigen (HLA) antibody rebound on the risk of antibody mediated rejection in sensitized kidney transplant recipients. Ann Transplant 2017;22(Mar 28):166—76.
[14] Muro M, Moya-Quiles MR, Mrowiec A. Humoral response in liver allograft transplantation: a review of the role of anti-human leukocyte antigen (HLA) antibodies. Curr Protein Pept Sci 2016;17:776—84.