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REVIEW

An update on ABO-incompatible kidney transplantation

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Summary

ABO-incompatible kidney transplantation is nowadays a well-established procedure to expand living donor transplantation to blood group incompatible donor/recipient constellations. In the last two decades, transplantation protocols evolved to more specific isohaemagglutinin elimination techniques and established competent antirejection protection protocols without the need of splenectomy. ABOi kidney transplantation associated accommodation despite isohaemagglutinin reappearance, C4d positivity of peritubular capillaries as well as the increased incidence of bleeding complications is currently under intense investigation. However, most recent data show excellent graft survival rates equivalent to ABO-compatible kidney transplantation outcome.

Introduction

With the increasing unmet need of available organs for kidney transplantation, various efforts have been made to enlarge the pool of possible kidney graft donors: In deceased donation programmes, marginal grafts are being increasingly accepted [1,2]. More importantly, living donor programmes have been systematically developed to expand transplantation options with the major advantage of allowing timed or pre-emptive transplantation with excellent outcome [3–6]. Furthermore, new protocols have been established to broaden the eligibility criteria for living donor programmes such as positive cross-match constellations, the presence of donor-specific antibodies or incompatibility of the ABO blood groups [7,8].

One successful and cost-effective approach to circumvent these constellations is kidney paired donation (KPD). Here, two or more living kidney donor/recipient pairs exchange the donor kidneys in such way that recipients receive compatible kidneys. Matching success increases

with the size of the pool of pairs. Therefore, national registries have been implemented and even international exchange has been performed. Challenges of KPD are travelling distances between involved centres, highly sensitized patients accumulating in KPD registries and a 5% decline of donation by donors who's co-registered recipient already received a transplant. Altruistic donation or ABO-incompatible kidney transplantations (ABOi KTx) are then a possible solution to help these patients [9,10].

Today, graft survival times of ABO-incompatible graft recipients match those of ABO-compatible graft recipients [11–13]. However, this result might be due to selection effects of ABOi pairs, depending, for example, on the availability of alternatives as kidney paired donation programme. Still, this marks a great advance compared to initial reports of ABOi KTx in the 1950s and early 1960s, where hyperacute rejection was a significant issue [14–16].

A successful programme with blood group A2 donors and blood group O recipients stressed the importance of low isohaemagglutinin titres [17]. Elimination of ABO antibodies was subsequently introduced and successfully implemented in a protocol including plasmapheresis, splenectomy, infusion of donor thrombocytes and infusion of A- or B-trisaccharides [18]. Transplantation centres in Japan increasingly performed ABOi KTx since 1989, urged by the fact that almost exclusively living donor transplantations are conducted for cultural and religious reasons [19]. Since the mid-1990s, ABOi KTx protocols were established in the United States and Europe, respectively [20,21].

This review focusses on the recent results of ABOi KTx including our own experience of 10 years and nearly 100 ABOi KTxs at Freiburg, Germany. We discuss the pathophysiological background, protocols and future challenges of ABOi KTx.

ABO blood group system

In 1900, Karl Landsteiner discovered that patient sera showed an inconstant haemagglutination reaction with saline-washed erythrocytes and explained this phenomenon by individual differences. This was the first scientific report on human blood groups A, B and O and the corresponding isohaemagglutinins [22]. These antibodies most likely develop through cross-reactivities with gut flora in infancy and are directed against glycosylated antigens A and B. As primary substrate for this blood group, relevant glycosylation Antigen H was identified, which in its nonglycosylated form corresponds to blood group O. Genetically, A and B are co-dominantly inherited. Thus, there are four blood groups (A, B, AB and O) with corresponding isohaemagglutinins against the missing antigens [23]. This pathophysiological fundament defines today's compatible and incompatible transplantation scenarios (Fig. 1). Recipient antibodies

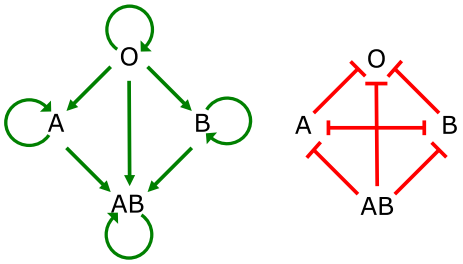

Figure 1 Compatible and incompatible transplantation scenarios based on Karl Landsteiner's ABO blood group system.

against blood subgroup A1 and group B are regarded as major incompatibilities. The glycosyltransferase of blood subgroup A2 can only process Antigen H type 1 and 2 (of 1–4); therefore, blood group A2 antigen is expressed at a much lower density on erythrocytes and other tissues. That is why minor incompatibility constellations against A2 antigen have been safely transplanted without desensitisation protocols [24]. However, a pretransplantation anti-A titre of ≤8 of the recipient seems to be the key to satisfying long-term results of A2 donor grafts [25].

Protocols

ABOi KTx donor candidates are extensively screened to confirm normal renal function and to rule out kidney function deterioration risk factors. Exclusion of cancer, cardiovascular disease and chronic infectious diseases is in major focus of ABOi KTx recipients' pretransplantation work-up. In addition, human leucocyte antigen (HLA) typing, donor-specific antibody screen, and complement-dependent cytotoxicity cross-match tests are performed with blood samples of living donor pairs.

ABOi KTx protocols are (with slight variations) based on preconditioning of B-cell response, extracorporeal elimination of isohaemagglutinins and intensified immunosuppression pre- and postoperatively. Exemplary, a Japanese, American and European protocol scheme is depicted in Fig. 2a–c [11,26,27]. Differences in these protocols include timing and dosage of rituximab, induction therapies and continuous immunosuppression, isohaemagglutinin reduction techniques and surveillance kidney graft biopsy.

From here, the following main questions towards an ideal protocol arise and are subject of current discussions: (i) Isohaemagglutinins – what is the optimal procedure to measure them and to withdraw them from plasma? (ii) Does administration of intravenous immunoglobulin (IVIG) influence ABOi KTx results? (iii) What is the necessary dose and point in time of rituximab application? Is the routine assignment of rituximab mandatory? (iv) What is the mechanism of immunologic accommodation in ABOi KTx and how does kidney transplant histology reflect on

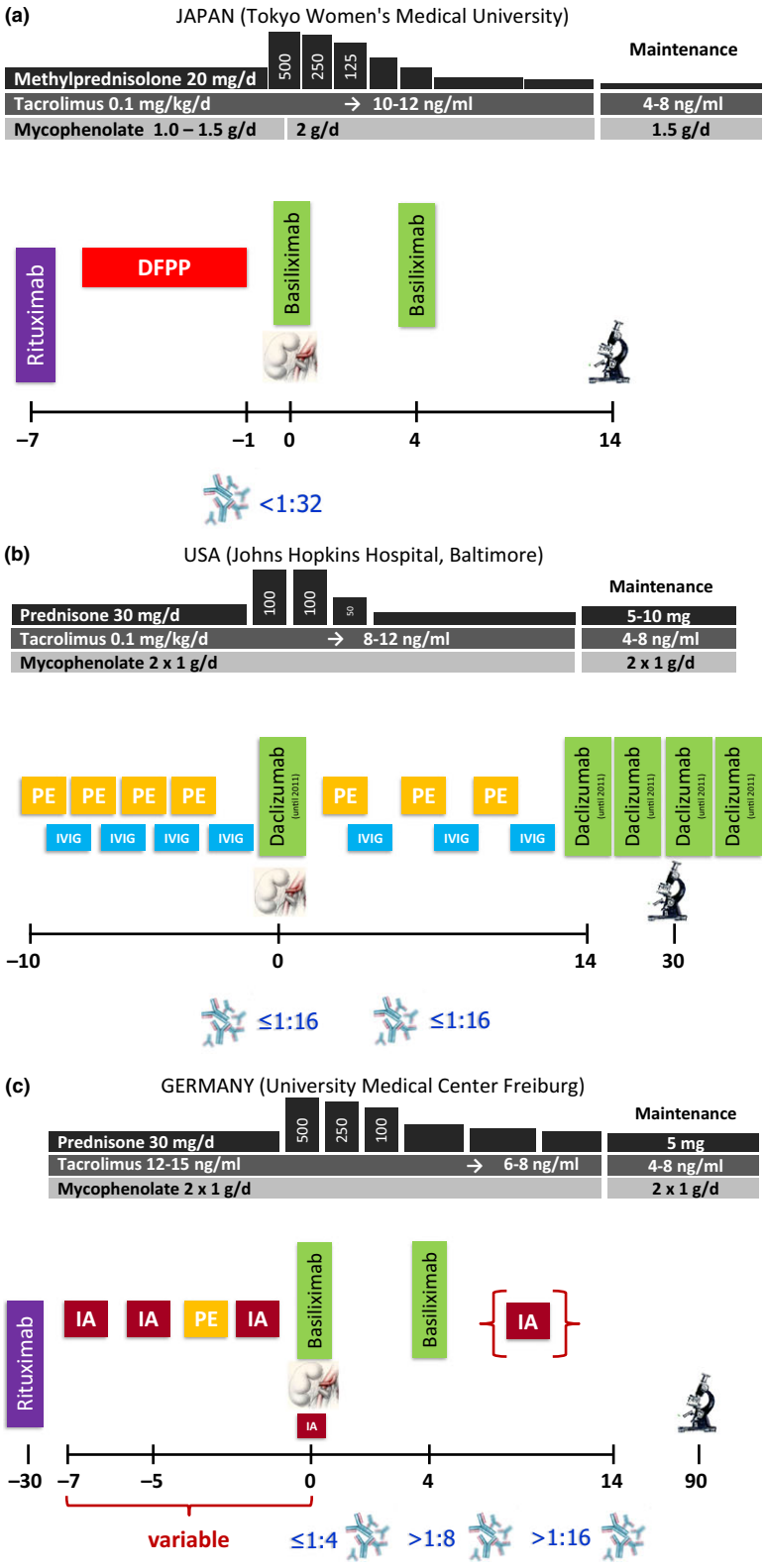

Figure 2 (a–c) Examples of a Japanese, American and European ABOi KT protocol. Differences between these protocols include timing and dosage of rituximab, induction therapies and continuous immunosuppression, isohaemagglutinin reduction techniques and surveillance kidney graft biopsy. DFPP, double-filtration plasmapheresis; PE, plasma exchange; IVIG, intravenous immunoglobulins; IA, immunoadsorption.

this phenomenon? And (v) is there a significant difference in surgical complications in ABOi KTx?

Isohaemagglutinins – quantification methods

Various methods have been established for quantification of isohaemagglutinins. The haemagglutination method was the first method and is still commonly used, with initial tube centrifugation having been replaced by modern gel centrifugation. Despite the reliable and easy-to-establish materials, there are still result variations caused by individual use of diluent, incubation time, temperature, plasma/erythrocytes ratio, concentration of erythrocytes, donor erythrocytes versus pooled erythrocytes and subjective optical result determination. Indeed, there have been reports of significantly divergent results of the same patient samples among different laboratories [28,29]. To tackle this problem, standardization efforts have been made and alternative techniques as flow cytometry have been studied and proved to be more reliable and reproducible [30,31]. Therefore, many centres have their own pre-operation cut-off for conventionally measured isohaemagglutinin titres. In our centre, we started with conventional tube centrifugation haemagglutination test. In the years 2006/2007, we established gel centrifugation haemagglutination tests with the use of donor erythrocytes: This grants a stable antigen density, but whether this antigen density on the erythrocytes correlates with the one in the renal tissue remains elusive. This assay allows the discrimination of IgM and IgG isohaemagglutinins which may arise in certain individuals. For higher reliability, the previous sample is always retested for comparison. If there is no previous sample available, pooled plasma of ten random donors serves as quality control.

Isohaemagglutinins – elimination techniques

Traditionally, there have been different approaches to reduce isohaemagglutinins before transplantation regarding to centre expertise and resources. All protocols aim to lower titres to a minimum at transplantation day [32].

In Japan, conventional plasmapheresis and later double-filtration plasmapheresis have been used as standard procedure [33]. In the United States, conventional plasmapheresis is the mainly used elimination technique [27,34]. Although nonspecific, it very effectively lowers isohaemagglutinin titres. Downsides are the need of plasma transfusion, bleeding complications and potential infectious complications [35]. The Karolinska University Hospital introduced ABOi KTx to Europe using A or B antigen-specific adsorbing columns (Glycosorb AB™, Glycorex, Lund, Sweden) [36–38]. These columns allow elimination of isohaemagglutinins with little alteration of the concentration of other immunoglobulins and coagulation factors.

However, specific immunoadsorption skyrockets costs of ABOi KT: The company designed Glycosorb AB™ columns for single use only, which implies a large financial burden to transplantation programmes. Therefore, less specific but more cost-effective alternatives were investigated. Reusable columns for the same patient such as protein A-based columns (e.g. Immunosorba™, Fresenius Medical Care, Bad Homburg, Germany) or synthetic peptide-based columns (e.g. Globaffin™, Fresenius Medical Care, Bad Homburg, Germany), and polyclonal sheep-anti-human IgG-antibody coated columns (Therasorb™, TheaMed, Bsalim, Lebanon) were successfully tried and implemented in protocols [39–41]. However, there are concerns because of limited adsorption of isohaemagglutinin IgM subclass [42]. Our and other centres combine intercurrent conventional plasmapheresis with unspecific immunoadsorption to tackle this problem. An interesting recent trial combined nonselective immunoadsorption with membrane filtration and achieved impressively improved IgM reduction rates in 14 patients treated for indications (autoimmune disease) other than isohaemagglutinin removal [43]. The benefit of preemptive immunoadsorption after transplantation has not been documented by larger trials [44]; therefore, many centres pursue an on-demand strategy accompanied by closely monitored isohaemagglutinin titres.

Intravenous immunoglobulins

Intravenous immunoglobulins (IVIG) purified from human plasma donors effect immune-modulating pathways: The constant fragments of IVIG interact with Fc receptors of phagocytes and B-cells inhibiting further differentiation and T-cell stimulation. The variable fragments of IVIG prevent binding of autoantibodies to their specific receptors [45,46]. Furthermore, IVIG are able to induce secretion of anti-inflammatory cytokines and act as 'blocking' antibodies in cross-match tests in vitro as well as in clinical observation of immediate HLA-antibody decrease after infusion [47–49]. These potent properties come with relatively mild adverse effects: headaches, nausea, fatigue, myalgia, arthralgia, chills, chest pain, back pain and hypertension [50]. Osmotic renal failure was observed in IVIG formulations containing sucrose [50]. High dose IVIG application might lead to haemolysis due to isohaemagglutinins naturally contained in IVIG [51].

Intravenous immunoglobulins are routinely used in highly immunized patients with anti-HLA antibodies [52] and/or positive cross-match constellation [53]. In ABOi KTx, IVIG were successfully tested as isohaemagglutinin desensitizers [20,27,54,55] and are still an inherent part of many ABOi KTx protocols [56]. In our centre, we had concerns with IVIGs, as we realized higher postoperative bleeding events in ABOi KTx compared with ABOc KTx;

however, the context is not clarified [57]. Our centre discontinued the routine use of IVIG and turned to an on-demand administration monitoring immunoglobulin levels in plasma during isohaemagglutinin removal.

Rituximab

The chimeric mouse/human anti-CD20 antibody rituximab very sufficiently depletes CD20-positive B cells and was approved by the FDA in 1997 as the first therapeutic antibody in cancer therapy. Its first successful application in ABOi KTx was published by Sawada *et al.* from Tokyo Women′s University Hospital [58]. Since then, splenectomy was abandoned from all ABOi KTx protocols worldwide [27,59,60]. However, point in time and dosage of rituximab administration is still a matter of debate. We adopted Karolinska University Hospital protocol and administer rituximab 4 weeks before the scheduled transplantation [57]. The dosage of 375 mg/m² body surface (lymphoma therapy protocols) was proven to be safe and efficient [61]. The effect of lower rituximab doses was tested sufficiently on splenic B cells [62] and low-dose protocols have been successfully used [63–65]. Still, there is a lack of a prospective randomized clinical trial comparing different dosage of rituximab in transplant outcome and adverse effects.

More recent reports now challenge the assumption that B-cell depletion is essential to prevent antibody-mediated rejection [66–69], for example some centres started to routinely perform ABOi KTx without rituximab [20]. However, there has been evidence that there is less chronic antibody-mediated rejection (AMR) when rituximab is being used [70]. Reports on infectious complications on rituximab protocols have been showing heterogeneous results [11,60,71–74]. In our centre experience, we did not recognize a trend towards more infectious complications in 94 ABOi KTx compared with 239 ABOc KTx (unpublished data). In addition, there seems to be no additional risk of malignancies in ABOi KTx rituximab containing protocols [75,76].

Accommodation

Accommodation is a phenomenon characterized by lack of antigen–antibody reaction despite the presence of specific antibodies against donor tissue in the graft recipient [77]. Soon after ABOi KTx, isohaemagglutinins will rise to levels, which are usually thought to cause a hyperacute rejection and kidney biopsies demonstrate the persistence of ABO antigens [78]. However, within the first 2 weeks after transplantation, adaptive changes of the immune system occur. The mechanism of accommodation might be explained by decreased ABO antigen expression and the disappearance of an ABO antigen donor-recipient chimerism [26,79]. Interestingly, the appearance of protective cell surface molecules that inhibit transcription factor NF-kappa B might contribute to prevention of T-cell response and terminal complement membrane attack complexes [80]. Additional hints to explain accommodation were found in upregulation of complement-inhibiting proteins [81]. In this context, complement inhibitors have been demonstrated to facilitate accommodation in rhesus monkeys [82].

C4d positivity and other histological findings

The C4 complex is critical for antibody-dependent classical complement pathway as well as the lectin pathway and helps to recruit the C3 complex to the cellular membrane [83–85]. C4d remains covalently bound to the cellular membrane after cleavage of C4b [86–88] and therefore is considered being a more stable readout of antibody-dependent complement activation [89]. In contrast to ABO compatible kidney transplantations [90–92], C4d staining in peritubular capillaries however is no sign of antibody-mediated rejection [93,94]. Electron microscopic evaluations showed no significant difference between C4d positive ABOi KTx and controls (C4d negative ABOc KTx), regarding acute or chronic damage of glomerular and peritubular capillary endothelia and basement membranes [95]. C4d positivity occurs in about 16–57% of patients after 1 h in ABOi patients [13,96] whereas weeks to months after transplantation 70% to >95% of the patients show C4d positivity in peritubular capillaries [13,93,94,97]. Further observations indicated that lack of C4d staining correlates with graft failure due to chronic rejection events, indicating that C4d may be a protective factor [93,98]. Linear, nongranular staining of C4d in glomeruli can be found in virtually all kidneys already in pretransplant biopsies without further signs of glomerular damage and/or inflammation [99]. Together these data raise the question whether C4d is generally only a marker of antibody-dependent complement activation or perhaps could be also a negative regulator of antibody or complement driven damage in ABOi KTx.

C3 is key component of the alternative and classical complement pathway, able to bind directly to bacterial membranes [83,100–102]. The cleavage product C3d enhances B-cell activation via CD21, thereby enhancing humoral responses [103]. The role of C3 at the convergence of the different complement pathways could make it an ideal readout of complement activation in the ABO incompatible environment. In fact, C3d positivity correlated with antibody-mediated rejection in ABO incompatible grafts in a study with small patient numbers [93]. However, further studies will be needed to clarify the role

of C3d as a biomarker and activity marker for transplant rejections.

Surgical complications

Compared with ABO-compatible living donor kidney transplantation, there is no difference in surgical techniques and spectrum of surgical complications (as classified in Table 1) [11,104–114]. However, one study reported a nonsignificant trend to a higher incidence (25%) of immediate postoperative bleeding and a more frequent surgical revision rate [11]. In the majority of the cases, diffuse retroperitoneal haemorrhage occurred. Whether plasmapheresis or immunoadsorption eventually contributed to impaired coagulation in these cases remains unclear [115]. Furthermore, uraemic thrombocyte dysfunction might trigger postoperative haemorrhage, although this is not specific to ABOi KTx [116]. Another explanation might be the commonly used higher doses of perioperative heparin in ABOi KTx, which was indirectly supported by investigations of lower postoperative bleedings with the use of less heparin in the analysis of Renner *et al.* [115].

In ABOi KTx, in 25% (n = 96) of our patients, surgical revision is required based on a lymphocele. It is conceivable to speculate that the increase is due to the pre-operative application of immunosuppressive medication in ABOi KTx [111]. Generally, the symptomatic lymphoceles can be treated minimal-invasively in most of the cases.

Current experience and results

Since 1989, about 2000 ABOi KTx were performed in Japan [13]. Three-years graft survival rate of rituximab-based protocols were as high as 95.8% and have been proofed to be comparable to a historic splenectomy cohort [117].

Table 1. Spectrum of surgical complications in living donor kidney transplantation.

Category	Incidence
1. Vascular	0.2–30% [102–107]
-Bleeding	
-Arterial stenosis	
-Arterial obstruction	
2. Urological	2–10% [9,103,108,109]
-Urinary leakage	
-Ureteral obstruction	
-Urinary retention	
3. Fluid collection	2–18% [107,109–112]
-Seroma	
-Lymphocele	
-Lymph fistula	
4. Local infections	3–5% [110]
-Wound infection	
-Abscess	
-Impaired wound healing	

In the United States, 738 ABOi KTx were analysed from 280 transplant centres from 1995–2009. Graft survival rate was 94.1%, 89.6%, 82.6% and 72.9% at 1, 3, 5 and 10 years of follow-up, respectively [12]. Overall graft survival rates improved towards more recent years of the study period. In a study from 1999–2007 at John Hopkins Medical Institutions, 28 of 60 patients did neither receive rituximab nor splenectomy, as the protocol was changed during the follow-up. AMR and graft loss rate did not increase according to these data [20].

The Swedish protocol was the first to establish rituximab in 2001 and the first which used specific isohaemagglutinin elimination. Results proofed comparability to compatible living donor programmes [60]. Safety and efficacy was investigated in 274 patients in a combined analysis of three centres (Stockholm, Uppsala, Freiburg). Graft survival was excellent with 97% in ABOi KTx – compared with 95% in ABOc KTx control cohort [118].

In Freiburg, we adopted the Swedish protocol in 2004 with three variations, namely on-demand isohaemagglutinin elimination after transplantation, higher pre-operative isohaemagglutinin titre cut-offs and a standard administration of basiliximab on day 0 and 4 after transplantation as induction therapy. All ABOi pairs were intensively screened for cardiovascular morbidity, cancer and infectious burden. Median pre-immunoadsorption titre was 64 [0-2048]. Since 2004, eight patients did not reach pre-operative isohaemagglutinin cut-off titre and therefore were not transplanted. Kidney paired donation ('cross-over') is available in Germany, but not frequently used, thus there was no additional selection. Death censored graft survival rates were as high as 100% at 1, 3 and 5 years of follow-up, respectively [11]. Most recent analysis of 10 years data of our 95 ABOi KTx recipients cohort confirmed excellent median graft survival of 94% which was not significantly different to our 245 ABOc KTx recipients cohort which had 89% graft survival in the same time period (log-rank test p = 0.53) (unpublished data). These results are supported by the latest data of the Collaborative Transplant Study (CTS) showing three-year outcomes for 1420 ABO-incompatible kidney transplantations of 101 centres: the outcome is comparable to the ABO-compatible control group [119].

Conclusion

ABOi KTx is an emerging procedure to compensate for ABOc kidney donor shortness. Various efforts have been made to establish standardized protocols and recent data evidence excellent graft survival rates equivalent to ABO-compatible kidney transplantation outcome.

Authorship

All authors collected data, analysed data and wrote the paper.

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