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EDUCATIONAL REVIEW

Acute and chronic antibody-mediated rejection in pediatric kidney transplantation

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Abstract Acute antibody-mediated rejection is a diagnostic challenge in renal transplantation medicine. However, it is an important diagnosis to make, since chronic antibody-mediated rejection (CAMR) is the main cause of long-term graft loss. Antibody-mediated rejection is diagnosed by detecting donor-specific antibodies (DSAs) in the blood in combination with observing typical histomorphological signs in kidney biopsy, as described in the Banff classification. Therapy is based on the removal of DSAs by administering intravenous immunoglobulins (IVIGs), plasmapheresis, or immunoadsorption. Reoccurrence of antibodies is diminished by the use of rituximab, increased immunosuppression, and in some cases additional experimental substances. A combination of these techniques has been shown to be successful in the majority of cases of acute and chronic antibody-mediated rejection. Routine DSA monitoring is warranted for early detection of antibody-mediated rejection.

Keywords Antibody-mediated rejection · Donor-specific antibodies · Banff classification · Immunoadsorption · Rituximab

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Introduction

After pediatric kidney transplantation, it is important to maintain the right balance between over- and under-immunosuppression. Over-immunosuppression can lead to severe infections and immunosuppressant side effects, such as nephrotoxicity, hirsutism, hypercholesterolemia, and proteinuria, whereas under-immunosuppression can be followed by episodes of acute or chronic antibody-mediated and cellular rejection. The last decades have been very successful regarding the prevention and treatment of acute cellular rejection. However, chronic antibody-mediated rejection (CAMR) remains a leading cause of the late loss of kidney transplants in adults [1]. The prevalence of CAMR could even be higher in pediatric nephrology. Since the prognosis for transplant survival is limited once transplant glomerulopathy is present—the main manifestation of CAMR [2, 3]—early diagnosis is warranted. Thus, the goal should be to prevent or at least detect acute antibody-mediated rejection early before irreversible damage has occurred.

Histopathological diagnosis of acute antibody-mediated rejection

According to the Banff classification, the most widely used classification scheme for kidney allograft pathology, the diagnosis of acute antibody-mediated rejection is made by a combination of histological findings and the presence of donor specific antibodies (DSAs), none of which is entirely specific in itself [4].

Typical histological features for the diagnosis of acute antibody-mediated rejection according to the current Banff guidelines are shown in Figs. 1 and 2. They include arteritis, as defined by the Banff component v, transplant glomerulitis (Banff component g), peritubular capillaritis (Banff component ptc), microthrombosis without any other cause and C4d positivity of the peritubular capillary endothelium (Banff component C4d)

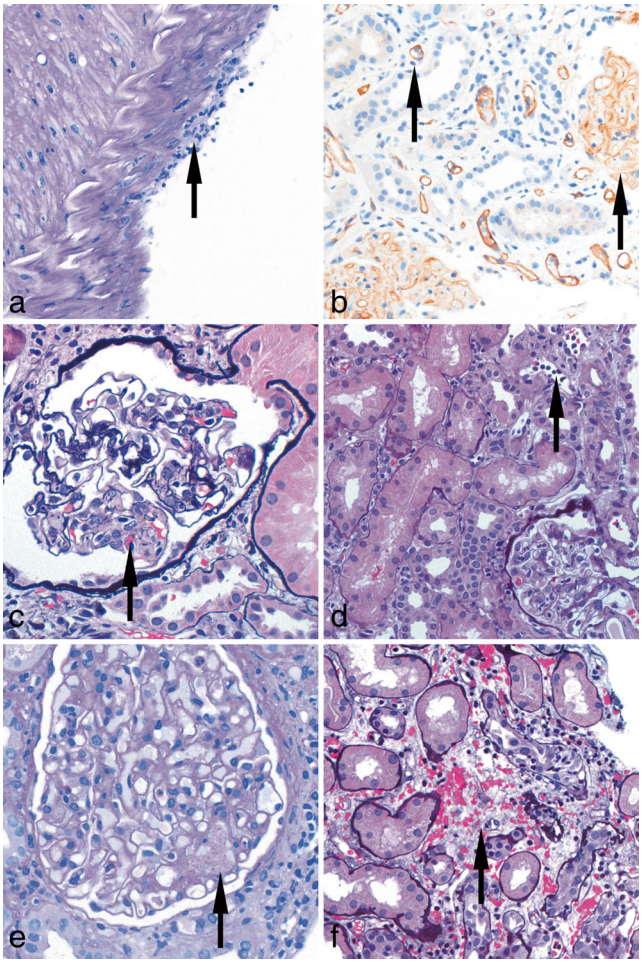

Fig. 1 Histomorphological indicators of acute humoral rejection. **a** Arterial endothelialitis at the *arrow* with lymphoid infiltrates underneath swollen endothelium (Banff component v), PAS, original magnification ×400. **b** C4d-positive endothelium (brown, *left arrow*) in peritubular capillaries (Banff component C4d) and in glomeruli (*right arrow*). The latter is currently not considered in the Banff classification. Immunoperoxidase on paraffin-embedded tissue, original magnification ×400. **c** Transplant glomerulitis with increased content of intracapillary mononuclear cells and swollen endothelium, particularly at the segment marked with an *arrow*. Jones silver stain, original magnification ×400. **d** Increased content of mononuclear cells in peritubular capillaries (*arrow*). Jones silver stain, original magnification ×400. **e** Glomerular microthrombus (*arrow*). This particular example consists predominantly of thrombocytes, fibrin-rich forms, and mixed forms can also be observed. PAS, original magnification ×600. **f** Acute tubulointerstitial hemorrhage (*arrow*), most likely due to ruptured peritubular capillaries. Jones silver stain, original magnification ×400

[5]. The addition of arteritis, or vascular rejection, to the criteria for acute antibody-mediated rejection was stimulated by a recent cluster analysis in a large cohort of patients from France that identified a humoral component in about two-thirds of patients with arteritis [6]. This and also the recognition of C4d-negative acute antibody-mediated rejection in about 20–40 % of cases remain about the only merits of the new Banff guidelines [5]. The exact definition of the Banff component g (transplant glomerulitis), although long overdue, has been designed to

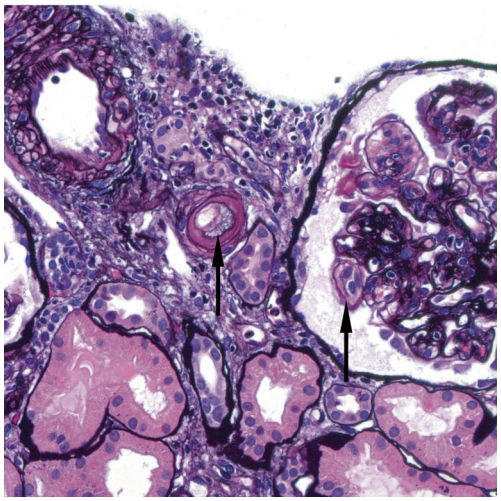

Fig. 2 Histomorphological indicators of chronic humoral rejection. The *left arrow* highlights intimal expansion (Banff component cv) with foam cells indicative of transplant vasculopathy, the *right arrow* split glomerular basement membranes (Banff component cg) indicative of transplant glomerulopathy. Jones silver stain, original magnification ×400

maximize reproducibility and correlation with mRNA transcriptome changes closely linked with tubulointerstitial rejection on only 47 biopsies; a correlation with outcome was not sought [5]. This and also the change of the threshold between g2 and g3 from previously 75 % [7, 8] to now 50 % involved glomeruli without announcement is problematic in our view. However, the new consensus guidelines should be used in routine assessment. The excellent work of Randhawa's group [9] for the definition of transplant glomerulitis can still be recommended. Another new and debatable addition to the criteria for acute antibody-mediated rejection is mRNA transcriptome expression analysis [5]. While the addition of novel techniques is certainly desirable, these methods are not available to the entire transplantation community and should prove their usefulness in multi-center studies. Another histological sign of acute antibody-mediated rejection is tubulointerstitial hemorrhage. It was briefly mentioned in the Banff 1997 meeting report and is coded as v* [7]. It is currently not listed in the defining features of the current Banff guidelines [5]. In the authors' view, it is a rare but specific feature of acute antibody-mediated rejection.

The second cornerstone of the diagnosis of acute antibody-mediated rejection is solid-phase serologic testing for DSAs (see below). According to the present Banff guidelines, the serologic and histologic findings should then be taken into consideration to reach a diagnosis of acute antibody-mediated rejection [5]. Details for this reasoning are too complicated to be included in this review.

In summary, the current criteria for the diagnosis of acute antibody-mediated rejection according to the latest update of the Banff classification are still a work in progress and are far from perfect. Future changes are likely and desirable, for example a simplified approach with a scoring system combining histologic and serologic findings of acute antibody-mediated rejection.

General principles of antibody-mediated rejection treatment

Treatment of antibody-mediated rejection (acute and chronic) is mainly based on two principles: the elimination of the antibodies that cause antibody-mediated rejection and the modulation of adaptive and/or innate immunity to decrease future production of new antibodies. Primarily, intravenous immunoglobulin G (IVIG) is used as a non-invasive therapy to bind and remove DSAs administered as 1 g/kg body weight once weekly for 4 weeks [10], if there is no fast deterioration of renal function. If IVIGs are not effective, plasmapheresis or immunoadsorption are used to remove antibodies directly. Immunoadsorption alone has proven to be a very efficacious method [11] that is most often carried out six times every second day. Before this technique is performed, a Shaldon catheter must be placed. Therefore, the indication has to be set carefully, especially in small children who require anesthesia for this procedure.

Additionally, the anti-CD20 monoclonal antibody rituximab is often administered in a dose of 375 mg/m^2 to deplete B cells and B-cell precursors and thereby significantly reduce antibody production through complement-dependent and independent mechanisms [12]. It has to be taken into account that rituximab does not directly target plasma cells that are CD20 negative. The frequency of rituximab courses ranges from once to four times every 4 weeks. Rituximab also directly targets CD20-positive cells in the graft [13]. According to our experience, the density or extent of CD20-positive infiltrates as detectable by immunohistochemistry in the index biopsy is not predictive of response to rituximab (manuscript submitted). It has been shown that rituximab alone does not reduce DSA titers [14], whereas rituximab in combination with IVIGs is effective in treating antibody-mediated rejection [15], despite the fact that the complete mechanism of IVIG action is not well understood [16]. In most cases, immunosuppressive maintenance therapy is increased, i.e., by a change from cyclosporine A to tacrolimus, addition of MMF or mammalian target of rapamycin (mTOR)-inhibitors (eventually as a quadruple therapy), or simple dose increases of the calcineurin-inhibitors.

In difficult cases that do not improve under the therapy described above, experimental therapies have been used with conflicting results. For instance, bortezomib, a proteasome inhibitor, directly inhibits the production of antibodies in plasma cells in vitro [17], which leads to apoptosis of alloantibody-producing plasma cells and consequently should also reduce DSAs at a dose of 1.3 mg/m^2. However, one study found that bortezomib alone did not decrease DSA levels [18]. These results are conflicting, and it remains unclear if bortezomib used in combination therapy really leads to a significant decrease in DSAs [19, 20].

Upon binding of DSAs to target cells, the complement system plays an important role. It has been hypothesized that inhibition of the terminal complement complex might also reduce antibody-mediated rejection. Initial reports on the use of the C5-complement-inhibitor eculizumab to treat acute antibody-mediated rejection are promising [21]; however, the results of prospective randomized trials that are currently being carried out are still awaited. Pediatric dosage for this indication is not known, but might be paralleled to treatment of atypical hemolytic uremic syndrome (10-40 kg: 600 mg, >40 kg: 900 mg). Eculizumab is even speculated to "revolutionize" the treatment of acute and chronic antibody-mediated rejection [22]. However, given the immense cost of treatment that potentially has to be continued for the entire lifespan of the transplant, this "revolution" might become quite costly. These main treatment principles are summarized in Table 1.

Detection of HLA antibodies

Although donor-specific HLA- and non-HLA antibodies (DSAs) play a critical role in the pathogenesis of antibody-mediated rejection, detection and identification of clinically relevant antibodies is currently intensely discussed. This discussion is primarily driven by the introduction of novel solid-phase assay (SPA)-based techniques for serological antibody detection. The complement-dependent lymphocytotoxicity (CDC) assay, which has been the "gold standard" method for HLA antibody detection in kidney transplantation for more than 40 years, has turned out to have major limitations [23, 24]. These limitations include failure to detect non-complement fixing antibodies, difficulties in distinguishing IgG from IgM antibody isotypes, and the imprecision in determining DSAs. In contrast, SPA-based techniques, in particular the Luminex test, are markedly more sensitive in the detection of HLA antibodies in comparison to the CDC [23]. The recent widespread application of SPA-based tests in clinical practice has confirmed the higher sensitivity of these diagnostic methods. Conflictingly, however, a growing number of studies in kidney transplantation patients have also shown that only a portion of Luminex-detectable HLA antibodies appear to cause antibody-mediated rejection [25–27]. This differentiation between clinically relevant and irrelevant DSAs is a major challenge, both before and after kidney

Table 1 Two treatment principles of antibody-mediated rejection and treatment options in each category

Antibody depletion	Decrease antibody production/ prevent antibody-mediated rejection
IVIG	Change basic immunosuppressive therapy (i.e., tacrolimus instead of CsA, addition of mTOR-inhibitors or MMF, dose increases)
Plasmapheresis	Rituximab
Immunoadsorption	Bortezomib (?)
	Eculizumab (?)

IVIG intravenous immunoglobulins

transplantation [24, 27, 28]. For example, the pathogenetic roles of different Ig subclasses or those of complement- and non-complement-fixing DSAs in antibody-mediated rejection are controversially discussed [29]. On the one hand, complement-fixation is considered to be a major criterion for the pathogenicity of HLA antibodies in antibody-mediated rejection and has recently been confirmed in a large study with more than 1,000 patients [30]. On the other hand, accumulating evidence indicates that non-complement-fixing antibodies are also crucially involved in graft rejection via direct endothelial cell activation [31]. Independently, non-HLA antibodies have been demonstrated to play a major role in the pathogenesis of antibody-mediated rejection [32–35]. In particular, non-HLA antibodies against endothelial antigens such as the major histocompatibility complex class I-related chain A (MICA) cause antibody-mediated rejection in transplantation patients [36, 37]. Moreover, anti-endothelial cell antibodies against other known endothelial surface antigens such as the angiotensin II type 1-receptor, vimentin and collagen V or against unknown antigens have been implicated in antibody-mediated rejection after kidney transplantation [32, 34]. For a comprehensive overview of the role of antibodies in clinical decision-making for kidney transplantation, we refer to recently published consensus guidelines, which summarize various critical aspects in HLA and non-HLA antibody detection [38]. It is also important to note that strong efforts are currently underway to standardize the detection methods for antibodies by SPA-based methods for a better comparibility of antibody testing results in different laboratories [39].

HLA antibodies are predominantly detected by LABScreen Mixed assays and the LABScreen Single Antigen assay. As a screening test, mixed antigen beads are used in many centers. In the case of positive results, the single antigen test should be performed to confirm the development of DSAs. Other centers only carry out the more expensive single antigen test. The amount of DSAs is detected as the mean fluorescence intensity (MFI). There is an ongoing discussion about the valid threshold levels that define antibody-mediated rejection. Most often, a mean fluorescence intensity (MFI) >1,000 is considered as positive for HLA antibodies [40]. The British Society of Transplantation has even suggested testing for DSAs every 3 months after kidney and pancreas transplantation [40]. The rates of DSAs in routine monitoring have been published to be between 2.5 and 24 % [41, 42]. Especially high HLA class II antibody levels are related to an increased risk of developing transplant glomerulopathy and C4d deposits in peritubular capillaries [43]. The diagnostic role of measuring C1q-fixing DSAs is still unclear [44], but might help to distinguish between clinically relevant and non-relevant DSAs. The Transplantation Society has published guidelines for the detection of DSA after kidney transplantation that should be used as a basis for local decisions [45].

Acute antibody-mediated rejection

Acute antibody-mediated rejection in children is most often observed within the first weeks after transplantation. However, it can be observed any time after transplantation, often after periods of non-adherence to immunosuppressive therapy.

In case of an impairment of graft function, a kidney biopsy should be performed in combination with serological detection of DSAs. If acute antibody-mediated rejection is detected, a fast increase in immunosuppressive therapy is advisable. Most often, treatment begins with steroid pulse therapy (six pulses with 300 mg/m^2 daily) followed by antibody removal via immunoadsorption or plasmapheresis six times every second day and rituximab administration once with 375 mg/m^2. Some cumulative case reports published by Kranz et al. demonstrate that this combination therapy is successful in most cases and leads to a reversal of graft dysfunction [46]. In adults, similar cases have been successfully treated with the complement C5 inhibitor eculizumab [21]. Several prospective trials on acute antibody-mediated rejection in adults, who are predominantly presensitized high-risk-patients, are being carried out. The results of these trials are awaited before this therapy can become a standard treatment in children with acute antibody-mediated rejection. Continuous DSA monitoring after the first episode of acute antibody-mediated rejection is a prerequisite for early intervention and prevention of a second episode.

To avoid acute antibody-mediated rejection in the early phase after transplantation, pretransplant desensitization strategies (most often including IVIGs, rituximab, and immunoadsorption) should be considered. These strategies reduce panel reactive antibodies or even "future" DSAs if transplantation against "forbidden antigens" is planned [47]. A special entity is ABO-incompatible pediatric kidney transplantation, where, if special protocols are applied, antibody-mediated rejection can be avoided [48].

Chronic antibody-mediated rejection (CAMR)

Until the BANFF 2005 meeting, chronic changes in transplanted kidneys had been summarized as chronic allograft nephropathy [49], with interstitial fibrosis and tubular atrophy as the surrogate markers in biopsies [50]. However, it took some time before this diagnosis was integrated into routine care. In 2009, El-Zoghby et al. differentiated the reasons for chronic transplant loss. These authors identified CAMR as a main cause in adults [1]. The same has been shown in children [51].

After kidney transplantation, interstitial fibrosis and tubular atrophy (IF/TA) develop within the first 12 months as early changes [52]. Late changes, such as microvascular and glomerular damage, most often occur in the following years [49]. In 2011, Sellares et al. showed that IF/TA alone is not a risk factor for future loss of graft function. In late allograft biopsies,

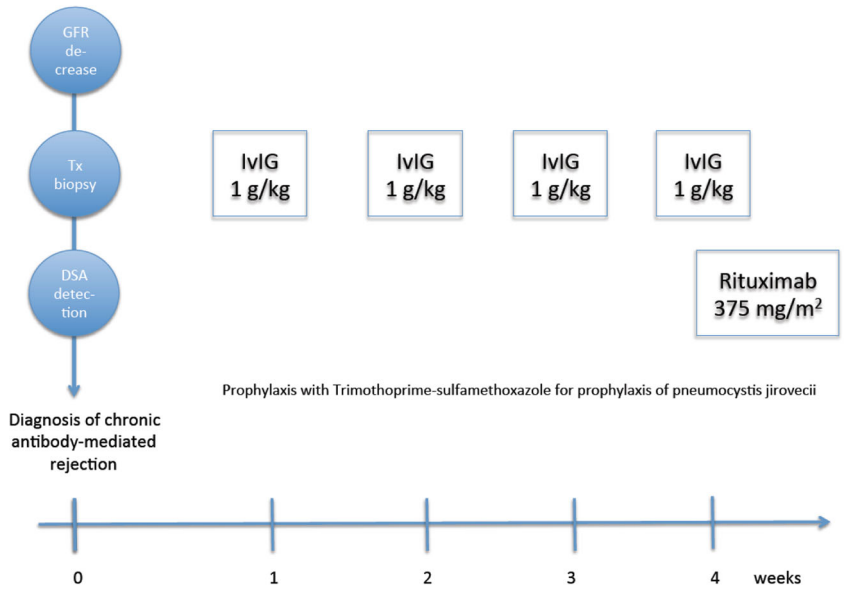

Fig. 3 Algorithm for diagnosis and treatment of chronic antibody-mediated rejection according to Billing et al. [63]

infiltrates were mainly associated with future graft loss as a surrogate marker of inflammation [53]. Park et al. demonstrated that neither IF/TA nor inflammation alone is a convincing predictor of future graft survival, but in patients with a combination of both inflammation and fibrosis, the future glomerular filtration rate was significantly lower [54]. These histological findings are now explained by the underlying concept of CAMR.

CAMR is defined by histomorphological signs of transplant glomerulopathy and/or transplant vasculopathy [55]. The detection of transplant glomerulopathy predicts an unfavorable outcome [56]. Graft dysfunction most often occurs later. This continuous process is very heterogeneous and associated with fluctuation of de novo DSA levels with and without C4d deposits in capillaries [5–7]. The negative association between the detection of DSAs and transplant success in adult kidney transplant recipients has been well known for several years [57]. A similar association has now been shown in children, demonstrating that the detection of DSAs is associated with impaired graft survival [58]. Interestingly, Chaudhuri et al. observed that non-HLA antibodies, such as MICA antibodies, were also associated with subsequent worsening graft function [59]. The underlying trial was a steroid-avoidance study, and unexpectedly, the amount of DSAs detected was higher in the steroid group than in the group not receiving steroids. Consequently, steroids do not seem to be protective against developing DSAs in children. In a large trial, Ginevri et al. confirmed that children with early de novo DSA detection are at risk for late antibody-mediated rejection [51]. These findings were only true for de novo DSAs, but not for all DSAs detected.

Unfortunately, the number of studies published on the treatment of CAMR, especially in children, is very low. It has been shown that a fast reduction of DSAs results in better graft survival [60, 61]. The most important trial studying the treatment of CAMR in children was carried out by Toenshoff and Billing. Children with chronic, active antibody-mediated rejection were treated with four weekly doses of 1 g/kg IVIGs followed by administration of a single dose of rituximab of 375 mg/m^2 (Fig. 3). With this regimen, the mean decrease in the glomerular filtration rate (GFR) of 25 ml/min/1.73 m^2 observed in the 6 months before treatment could be reversed to an increase of 21 ml/min/1.73 m^2 in the following half year. Only two of the six patients did not respond to this regimen [62]. In a larger trial with 20 patients and a 2-year follow-up, the researchers confirmed that the decrease of GFR could be significantly reduced over 2 years by this combination treatment, and the response rate was 70 %. This result was associated with a 61 % decrease in HLA class I antibodies and a 63 % decrease in HLA class II antibodies [63]. A study in adults showed that a combination of this regimen with plasmapheresis therapy can further improve these results [64]. There are conflicting reports on whether additional therapy with the proteasome inhibitor bortezomib provides a further beneficial effect [19]. However, bortezomib alone was not effective in treating CAMR [20].

Conclusions

Acute antibody-mediated rejection is an entity that can occur at any point in time after transplantation. The diagnosis can be made by renal biopsy and the detection of DSAs in blood. The treatment of acute antibody-mediated rejection is straightforward with immunoadsorption/plasmapheresis to remove antibodies in combination with IVIGs, rituximab, and an increase in basic immunosuppression. If detected and treated early enough, then the prognosis is good.

CAMR is the main cause of long-term graft loss. The diagnosis with transplant glomerulopathy and/or transplant vasculopathy as criteria is straightforward. Nevertheless, many cases are a therapeutic dilemma, because signs of activity (DSAs, C4d-positivity, transplant glomerulitis, or peritubular capillaritis) are often scarce or absent. Moreover, when CAMR is diagnosed, it is often quite late for treatment, because the prognosis at this stage is guarded. Nevertheless, treatment with IVIGs and rituximab with or without plasmapheresis/immunoadsorption can halt loss of graft function in the majority of cases. Thus, it is of utmost importance to prevent acute antibody-mediated rejection, which is often caused by non-adherence; early diagnosis can prevent irreversible structural changes in the transplant.

Key summary points

1. Acute antibody-mediated rejection can be diagnosed by detection of DSAs and renal biopsy and is treated with a combination of immunoadsorption/plasmapheresis, rituximab, and IVIGs.
2. DSAs suggest the diagnosis of CAMR.
3. Treatment of CAMR with IVIGs and rituximab improves graft function in >70 % of cases.
4. Under-immunosuppression must be avoided to prevent CAMR.

Multiple-choice questions (answers are provided following the references below)

1. The detection of DSAs in a pediatric kidney recipient

a. is always associated with antibody-mediated rejection
b. is always associated with C4d detection in kidney biopsy
c. should primarily be treated with immunoadsorption/plasmapheresis
d. is suspicious for chronic under-immunosuppression

2. Treatment of chronic antibody-mediated rejection is not performed with

a. IVIGs
b. Bortezomib
c. Methotrexate
d. Rituximab

3. Steroid-free immunosuppression

a. increases the number of patients with DSA detection
b. leads to higher MFI values in case of DSA detection
c. increases the number of patients with chronic humoral rejection
d. is not associated with an increased risk of HLA antibody formation

4. Which is the following is not a histologic feature of antibody-mediated rejection?

a. glomerulitis
b. tubulitis
c. IF/TA
d. hyalinosis of small arteries

5. The worst outcome is associated with

a. IF/TA in kidney biopsy
b. Inflammation in kidney biopsy
c. IF/TA and inflammation in kidney biopsy
d. IF/TA and C4d detection in kidney biopsy

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Answers to the questions:

1d, 2c, 3d, 4b, 5c