14 Recent Advances in the Diagnosis and Treatment of Antibody-Mediated Rejection in Pediatric Kidney Transplants

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14.1 Diagnosis

14.2 Detection of Donor-Specific Antibodies

Cell-based techniques were first described by Terasaki and Patel (Fig. 14.1), when they showed immediate graft failure in 80% of the patients with circulating donor-specific antibodies (DSA) identified by the complement-dependent cytotoxicity (CDC) assay [1]. This test only tells you that there are antibodies present that are activating complement; it does not tell you which antibodies are present. At the time that this assay was developed, the assumption was that positive crossmatches always represented clinically relevant human leukocyte antigen (HLA) antibodies and that a negative crossmatch would ensure long-term graft survival, which is now known not always to be the case.

This test is performed by incubating the recipient's serum with donor lymphocytes. If the recipient's serum has complement-fixing antibodies directed toward the donor HLA antigens, then addition of complement (typically rabbit) will result in cell death/lysis. The more complement-fixing antibodies present, the more cells that die, leading to a strong crossmatch and a higher concern for subsequent ABMR. A score of 0 means no reaction (little risk) and a score of 8 is the strongest score (highest risk), providing the clinician with a semiquantitative result. You can also perform a "titered crossmatch." In this test, the serum of the recipient is serially diluted to 1,2,4,8,16,32,64,128, etc. The result is reported as the lowest dilution that gives you a negative reaction (e.g., 1:128). The main advantage of the CDC assay is that it specifically picks up complement-fixing antibodies that are known to pose a risk to

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Fig. 14.1 Cell based Assays (**a**) CDC Cross Match (**b**) Flow Cross Match. Recipient's sera containing anti-HLA donor specific antibodies. Donor lymphocytes+ complement or fluorescent-conjugated antihuman globulin

the allograft. One of the disadvantages is that complement-fixing antibodies that are present at low titers or potentially clinically relevant weak IgG HLA-specific antibodies that may be rendered negative during the preparation may not be picked up by this assay. In addition, CDC assay may be positive in the setting of antibodies directed toward non-HLA antigens (autoreactive antibodies).

The next tests that were developed were the solid-phase antibody detection systems (Fig. 14.2), and they include enzyme-linked immunosorbent assay (ELISA), flow cytometry, and Luminex® (Luminex Corporation, Austin, TX). As Gebel and Bray summarized in their paper titled "HLA Antibody Detection With Solid Phase Assays: Great Expectations or Expectations Too Great?" [2], they have changed the field for better and/or for worse. The main advantage of these assays is that they have allowed for the determination of specific anti-HLA antibodies.

Flow cytometry (lymphocyte crossmatch) is currently considered the gold standard for identifying the presence of HLA donor-derived antibodies [3]. It uses microparticles coated with purified HLA class 1 and class 2 antigens [4]. The process usually starts by using multiple antigen beads that delineate between the presence of antibodies directed toward HLA class 1 or class 2, and the intensity of these antibodies in flow cytometry screen is expressed as mean channel shift (MCS) [5]. Once there is a positive flow screen, then single bead testing can be performed to identify the specific antibodies. This is typically done by flow cytometry or Luminex®. The main difference between these two tests is that with Luminex® there are fluorescence beads, with antibody binding to antigen beads on a plate. With flow, the reaction takes place in suspension. The main disadvantage of these tests is that there is no information as to whether the antibodies detected are able to activate complement. The main advantage of these tests is that once the specificities of the recipient HLA antibodies have been determined, the crossmatch can be more accurately interpreted, making up for the main disadvantage. For example, if recipient solid-phase testing does not show any donor-derived HLA antibodies, then the

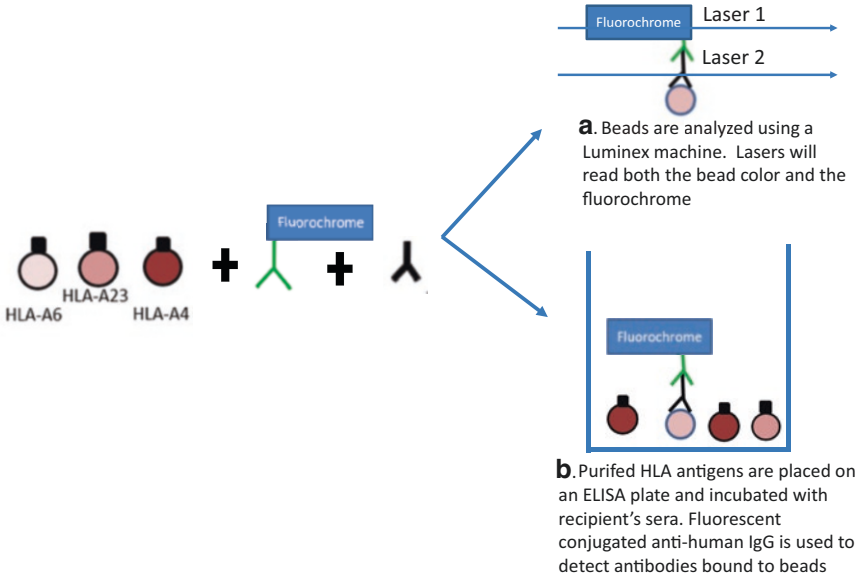

Fig. 14.2 Solid Phase Assays (**a**) Luminex (**b**) ELISA. Internally dyed color coded microsphere beads are coated with a single HLA class I or class II molecules. The recipient's sera is incubated with the beads and fluorescent conjugated anti-human IgG. (**a**) Beads are analyzed using a Luminex machine. Lasers will read both the bead color and the fluorochrome (**b**) Purifed HLA antigens are placed on an ELISA plate and incubated with recipient's sera. Fluorescent conjugated anti-human IgG is used to detect antibodies bound to beads

lymphocyte crossmatch would be predicted to be negative. But if the solid-phase testing is positive, a positive lymphocyte crossmatch could be interpreted as not due to HLA antibodies [6].

With flow and Luminex®, the clinician obtains a semiquantitative measure of the amount of antibodies present expressed in terms of median fluorescence intensity (MFI) or molecules of equivalent soluble fluorochrome (MESF). While studies have demonstrated an association between the strength of DSA and the risk of development of ABMR, response to treatment of ABMR, and subsequent allograft survival [5], one of the main problems is that there is currently no way to develop a consensus on the cutoff strength of DSA that is clinically relevant. Even when protocols and reagents are exactly identical, there is still around 20–25% variability in the level of antibody activity reported by laboratories. There are several reasons for this. It is difficult to standardize flow due to variability in cytometers, fluorochromes, antiglobulin reagents, and cell-to-serum ratios [7]. Luminex® is affected by several factors, including antibody concentration in the serum, density, conformation, and orientation of the antigen, as well as by the antibody avidity toward the respective antigen [8]. Currently, it is recommended that each lab make their own cutoffs and always test subsequent samples in the same lab. More recent analyses have suggested a consensus significance of an MFI of 1400 or greater.

The reporting of MFIs or MESFs has led clinicians to believe that those values represent the strength of the antibodies, but this is not always the case. We now know that all HLA antigens are not the same, and this has led to some labs to make some MFI thresholds more locus specific. Sullivan et al. described their practice with antibodies against C-locus [9]. They noted that antibodies against C-locus specificities do not tend to be clinically significant until they reach higher thresholds (5000 MFI) compared to other HLA class 1 loci (2000 MFI), as antigen cell surface expression for the C-locus is lower [10]. More recently, epitope and eplet matching has come to the forefront and is gaining traction in the field of transplantation. An epitope is the sequence of amino acids on an antigen where an antibody can bind, and each HLA antigen can be composed of multiple overlapping epitopes. An eplet is when amino acids are not in sequence but are in close enough proximity in the quaternary structure to allow for antibody binding. A single antibody to a shared amino acid sequence (epitope or eplet) can react with multiple antigens**.** Online tools that assist with the identification of shared epitopes have been developed and include HLAMatchmaker (http://www.epitopes.net) and the HLA Epitope Registry (http://www.epregistry.com.br/terms/index).

It has become clear that de novo DSA (dnDSA) development is one of the biggest risk factors in developing ABMR post renal transplantation [11], underscoring the need for the best possible matches. A single antigen can have multiple epitopes that can be pathogenic and just like antigens, not all epitopes are the same. It has now been shown that less dnDSA developed when matching was done with HLA class II antigen and eplet matching only compared to antigen matching alone [12]. There are minimal data on the use of this new technology in pediatrics [13, 14], and none of the long-term outcomes of pediatric kidney transplant patients that were matched by epitopes or eplets have been reported to date. There is still a great deal to learn about this technology and how it will apply to children.

14.3 Surveillance DSA Monitoring

The routine monitoring for dnDSA development post kidney transplantation has not been universally adopted by the pediatric kidney transplant community, but it is becoming more common. There are arguments for and against this practice. Ginevri et al. in showed that the development of dnDSA preceded the development of ABMR by a median time of 1 year in pediatric kidney transplant patients [15] and that the patients who developed dnDSA were at a higher risk of developing ABMR, renal dysfunction, and graft loss. Chaudhuri et al. showed that the presence of de novo antibodies (HLA and MHC class 1-related chain A) was associated with significantly higher rates of acute rejection, chronic graft injury, and decline in graft function, but not all patients who developed dnDSA had rejection [16]. This is partly why the presence of isolated dnDSA without histologic changes suggestive of ABMR continues to be a point of debate in terms of treatment approach.

14.4 Histology

Performing a kidney biopsy is still a key component in the diagnosis of ABMR in children, and the histologic diagnosis of ABMR has changed recently. Historically, the diagnosis consisted of features that showed the evolution of events during ABMR as we understood them at the time: presence of circulating DSA, evidence of complement activation (deposition of C4d along the peritubular capillaries) with histologic evidence of tissue injury, and acute kidney injury characterized by elevation in serum creatinine [17–19]. We now recognize that ABMR can occur in the absence of C4d positivity, and we now have criteria for the recognition of increased expression of gene transcripts/classifiers in the biopsy tissue that have been validated and strongly associated with ABMR. The definition for DSA has also been expanded to include nonhuman leukocyte (HLA) antibodies (angiotensin type 1 receptor (AT1R) antibodies, vimentin antibodies, etc.).

14.4.1 Detection of C4d by Immunostaining

C4d staining has come full circle from being required as a diagnostic criterion for antibody-mediated rejection in kidney allografts in 2003 [20] to being removed as a required criterion in 2014 with the acceptance of C4d-negative ABMR [21]. The presence of C4d indicates complement has been activated once an antibody/antigen interaction has occurred. C3a and C5a are also generated, but they mainly serve as anaphylatoxins that signal recruitment of other inflammatory cells [22]. While it is possible to stain for C3 as well as other complement components, C4d forms covalent bonds with the tissue that allows it to have a longer half-life to remain at the site of complement activation longer [22] and withstand tissue processing. Thus, it serves as a footprint of ABMR picked up by immunohistochemistry or immunofluorescence much more reliably [22].

The use of C4d in diagnosing rejection is not perfect, as it is not always associated with rejection. Occasionally, C4d staining may be observed in organs years after transplantation without other evidence of rejection. It has also been shown that biopsies with histological features of ABMR such as capillaritis, glomerulitis, interstitial fibrosis, and tubular atrophy, without C4d staining when found with circulating DSA, were found to lead to transplant glomerulopathy [23] and have poor long-term outcomes [22]. Treatment of these patients appears to prevent or at least delay the occurrence of transplant glomerulopathy [24]. However, the presence of C4d staining in an allograft may not always be pathologic. In fact, diffuse C4d staining is often found in ABO-incompatible allografts without evidence of allograft dysfunction and is thought to be more associated with accommodation rather than rejection [22, 25].

14.5 Histologic Changes of Tissue Injury

A biopsy of the renal cortex stained with hematoxylin-eosin (H&E) and periodic acid Schiff (PAS) stains will demonstrate an array of histologic changes. The histologic features can vary depending on the timing of the biopsy starting from margination of neutrophils and mononuclear leukocytes and later on monocytes and macrophages in peritubular and glomerular capillaries, thrombotic microangiopathy, and in severe cases, necrotizing arteritis [24]. It is now recognized that intimal arteritis may also occur in ABMR, perhaps as frequently as it does in cellular rejection [23]. It is not uncommon to find concurrent changes of cellular and antibody-mediated rejection in one specimen.

With the recognition of C4d-negative ABMR, there has been a focus on other histologic findings in an attempt to better define the presence of early antibody-mediated renal allograft injury. Adult studies on protocol and for cause biopsies have compelling evidence that the presence of microvascular injury (glomerulitis and/or peritubular capillaritis) is a better indicator of graft survival rather than C4d staining [26–28]. The Banff 11th meeting recognized that microvascular injury can be seen in early protocol biopsies and correlates with an increased risk for the developmental of transplant glomerulopathy [17]. Future studies in children will need to be done to confirm these findings.

14.6 On the Horizon

Current diagnostic testing is not perfect. To do a renal allograft biopsy on a child requires sedation and sometimes an admission which are time-consuming and costly [29]. Children have a small body surface area compared to the large kidney allograft, and a great deal of damage can be done before there is a change in creatinine [30], underscoring the need for detection of damage earlier. Most concerning is that children with lower body surface areas had higher fibrosis scores over time, possibly related to undetected acute rejections [31–33].

Recently, there has been new technology developed which is donor, i.g. graft, derived cell-free DNA (dd-cfDNA) that can be found in the plasma of the recipient. While there are currently no studies in pediatric kidney patients on this test, it is definitely a promising technology.

Children typically obtain disproportionately large renal grafts compared to adults, and this can potentially be problematic with dd-cfDNA testing in children. Studies done on other organs have shown that size does matter when comparing levels of dd-cfDNA; liver and lung recipients have higher levels than kidney and cardiac recipients [34, 35]. This suggests that smaller children could have potentially have higher levels than older children, where the graft size is more proportionate to the recipient's body, but this is not known. This therapy needs to undergo rigorous testing in all children before it can be put into routine practice.

14.7 Treatment of Antibody-Mediated Injury

The optimal therapy for ABMR is not well defined in children or adults. There are variable reported treatment options in the literature, but the data on children treated for ABMR are rare. Table 14.1 gives some of the most commonly used treatments,

Table 14.1 Dosing, duration and side effects of common medications used in antibody mediated rejection treatment

Drug	Dose	Duration	Common Adverse Side Effects
Prednisone	1–30 mg/kg/dose	Used as either a premedication for other drugs or as multiple standalone single doses	Obesity, hyperactivity, insomnia, hyperglycemia, acne, hypertension among others
IVIgG	1–2 g/kg total cumulative dose	Can be given at alone either at the beginning and/or end of treatment, but 100 mg/kg can be given after each pheresis session.	Aseptic meningitis, acute renal failure, thrombotic events, anaphylactic reactions, fever, chills
SQIgG	0.5 mg/kg divided twice weekly over a month	Unknown	Injection site reactions
Rituximab	375 mg/m^2/dose or 750 mg/m2/dose	Anywhere from 1–4 doses	Fever, chills, infection, hypotension during infusion, asthenia, progressive multifocal leukoencephalopathy, and activation of hepatitis B
Bortezomib	1.3 mg/m^2/dose	4 doses every 72 h	Diarrhea, vomiting, thrombocytopenia, hypercalcemia, paresthesias
Eculizumab	5–20 kg = 300 mg/dose 20-40 kg = 600 mg/dose >40 kg = 900 mg/dose	Weekly for 1–4 weeks	Neisseria meningitidis infections
TPE	1–1.5 volume exchange with either FFP, 5%albumin, or IVIgG replacements	Every 48-74 h for 5 treatments	Bleeding, infection, hypocalcemia, hypotension, nausea, dizziness, chills
Anti-thymocyte or anti-lymphocyte globulin	1–1.5 mg/kg/dose	1–7 treatments Q24-48 h	Chills, nausea, leukopenia, fever, nausea

Kg kilograms, *IVIgG* intravenous immunoglobulin, *mg* milligrams, *m*^{2} meters squared, *TPE* therapeutic plasma exchange, *SQ* subcutaneous

doses, duration, and side effects of these treatments, but this can be variable depending on the biopsy finding as well as other treatments that are being given. Children also have naive immune systems compared to adults, making infections a significant concern when treating ABMR [36–38]. There is not one single medication or therapy available at this time to treat pediatric ABMR, but use of these medications in combination is more likely to have better results. The big question that remains unanswered is which combination is most beneficial.

14.8 Removal/Neutralization of Antibody

Intravenous immunoglobulin G (IVIgG) and therapeutic plasma exchange (TPE) were two of the first and are still two of the most widely used therapies in the treatment of ABMR. TPE was first reported in the treatment of ABMR in the early 1980s as it is known to remove circulating antibodies. One of the first case reports for TPE use in treating ABMR was in 1983 by Soulillou et al., and not surprisingly, they did not find a benefit when TPE treatment was used alone [39]. This underscores the concept that it is not enough to just remove the circulating antibodies, but it is also necessary to stop the production of more antibodies.

The benefit of TPE depends on several factors: [40] the tissue compartments in which each immunoglobulin subclass resides and [41] the type of immunoglobulin being targeted. Different types of immunoglobulins have different characteristics. For example, IgM is found in the intravascular space and is easily removed in large quantities; therefore, it does not repopulate by re-equilibration following TPE. IgG and IgA on the other hand are both intravascular and extravascular and re-equilibrate into the intravascular space between TPE treatments, therefore requiring multiple TPE treatments to remove a significant amount of total body antibody [42–44].

The exact mechanisms of action of IVIgG are not entirely clear, although IVIgG is thought to have immunomodulatory as well as anti-inflammatory actions. One of the more well-known mechanisms of IVIgG is its ability to inhibit complement activation, which can be a crucial step in ABMR allograft dysfunction. Other mechanisms include inhibition of costimulatory molecule CD80/86 expression and suppression of HLA class I/II expression [45]. IVIgG is also thought to decrease the secretion of interleukin (IL)-12 and increase the secretion of IL-10, suggesting that treatment started at the time of antigen presentation could potentially induce a beneficial regulatory rather than damaging inflammatory pathway. Lastly, IVIgG is thought to induce significant B-cell apoptosis in vitro through Fc receptor-dependent mechanisms [46].

Jordan et al. first reported the beneficial effects of IVIgG in the treatment of ABMR in 1998 [47]. This led to the development of subsequent protocols that included either high-dose IVIgG alone or a combination of TPE and low-dose IVIgG [48–50]. However, this alone is not usually enough to stop the injury. More recently, there has been the development of subcutaneous IgG (SQIgG) that is being used off label for the treatment of chronic ABMR. SQIgG infusions are typically administered biweekly, resulting in more constant steady-state concentrations.

These infusions can be done at home and for extended periods of time. To date, there are limited to no pediatric data on this treatment in pediatric renal ABMR.

14.9 B-Cell Depletion

Anti-thymocyte globulin (ATG) and antilymphocyte globulin (ALG) also have some B-cell activity [51–53] and have had varying success in the treatment of ABMR. ATG is made by taking pediatric human thymus tissues that are removed routinely during pediatric cardiac surgery. The predominant cell population that is harvested is CD3+ T cells [51], but there is some B-cell lymphopoiesis that occurs in the human thymus, so it is not unexpected that there are CD20+ as well as CD138+ cells in these preparations [52, 54]. Both ATG and ALG have been shown to induce apoptosis in naive and activated human B cells and plasma cells. ATG has also been shown to increase the number of T-regulatory cells in vitro and in vivo [55–58]. Furth et al. published one of the first successful pediatric case reports using TPE, cytomegalovirus-specific IVIgG, and ALG in 1999 [59], followed by Shah et al. demonstrating that ATG with TPE to effectively treat acute ABMR [60]. [*The usefulness of ATG in ABMR is not very high*.]

Rituximab is a chimeric monoclonal anti-CD20 antibody. CD20 is found on the surface of most B cells, but it is not found on mature plasma cells [61, 62]. It has been used in the treatment of ABMR with varying degrees of success [63–66]. Through antibody-dependent cell-mediated and complement-dependent cytotoxicity in addition to direct signaling that leads to apoptosis, rituximab ultimately leads to less CD20+ cells than can turn into antibody-producing plasma cells [67–69].

Reports of successful rituximab therapy in pediatric renal transplant recipients with ABMR are found in the literature with varying degrees of success. Billing et al. treated six children with chronic antibody-mediated rejection with IVIgG and rituximab which led to an improvement in GFR within 12 months [70, 71]. Others have used rituximab in combination with steroid pulses, IVIgG, and/or PP in the treatment of AMR in children [67]. Unfortunately, rituximab did not have a significant effect on antibody intensity [63]. This is concerning when used also as failure to significantly reduce or remove the antibodies can lead to chronic allograft injury. Rituximab, like TPE and IVIgG, is likely not an effective therapy when used alone.

14.10 Depletion of Plasma Cells

Mature plasma cells are the main cells that produce DSA, which is why targeting them is so attractive [56]. The proteasome inhibitor bortezomib was approved in 2003 for the treatment of multiple myeloma, and now there are reports of its use in the treatment of ABMR. The power of these mature plasma cells is significant, as evidenced by the production of antibodies at a rate of several thousand per second. These antibodies can appear as early as 1 week after antigen presentation and persist for months [72, 73]. The process of antibody production leads to increased protein

synthesis and accumulation of unfolded proteins in the endoplasmic reticulum of the plasma cells, and proteasome inhibitors prevent the clearance of these unfolded proteins which ultimately leads to plasma cell death [74, 75].

Everly and associates were the first to report the beneficial effects of bortezomib treatment in patients with refractory acute ABMR [76]. More recently, there have been published data on pediatric cases. Twombley et al. were the first to describe its use in pediatric kidney patients. The most important finding of this paper was that there were no reported serious side effects and no infections 2 years posttreatment [77]. Subsequently, Pearl et al. showed stabilization of estimated glomerular filtration rate 1 year after treatment with bortezomib [78]. A multicenter retrospective study showed that the use of bortezomib led to a 25% reduction in the MFI levels of the immune-dominant DSA in 56% of the patients 1–3 months posttreatment [79]. There is still much to be learned about the potential benefits and long-term outcomes of bortezomib use in the treatment of pediatric renal ABMR. [You might want to mention that bortezomib is used in conjunction with pheresis.]

14.11 Complement Inhibition

Eculizumab is a humanized monoclonal antibody that blocks the cleavage of human complement component 5 and prevents the formation of the membrane attack complex (MAC) [80]. It has been successfully used to prevent posttransplantation recurrence of atypical hemolytic uremic syndrome after kidney transplantation [81, 82]. Some have now started to use it as not only a treatment of ABMR but to also potentially prevent ABMR in highly sensitized patients. Stegall et al. have reported their experience with eculizumab in the prevention of ABMR. Despite avoiding ABMR with eculizumab use, some patients still had evidence of chronic humoral injury with eculizumab use [83]. Also published was a case of biopsy-proven severe ABMR despite adequate levels of eculizumab and C5 blockade [84]. These reports suggest that ABMR might involve more proximal components of the complement pathway (e.g., C3a anaphylatoxin) or that some ABMR episodes might be completely complement independent in some patients.

14.12 Summary

There have been many advances in the field of transplantation, but little has changed in the treatment outcomes of ABMR. There is still little consensus on the treatment of ABMR. There are promising new techniques in the area of prevention of DSA with epitope or eplet matching that will hopefully lead to progress. With these newer advancements, some progress is in sight, but we still have a long way to go.

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