Treatment Update of Sensitized Pediatric Kidney Transplant Recipients: A Review

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Abstract

Sensitization of recipients is an increasing problem in children. Some case series in children exist comparing the diverse desensitizing protocols. These protocols include intravenous immunoglobulin, cytomegalovirus immune globulin, plasmapheresis, and some adjunctive therapies such as rituximab. Desensitizing protocols have advantages and disadvantages.

Clinical trials are required to determine suitable protocols for sensitized pediatric recipients. We performed a systematic review of these protocols in children. A massive search was done in PubMed, Embase, and the Cochrane library system. The results of these studies are compared.

Key words: *Renal transplant, Highly sensitized recipients, Children*

Introduction

All candidates for renal transplant should be screened for anti-HLA antibodies before transplant. The presence of these antibodies for anti-donor HLA antibodies may lead to hyperacute or acute humeral rejections and ultimately, to chronic graft dysfunction and loss. In these cases, antibody-mediated rejection can be subclinical with long-term chronic graft microvascular damage, interstitial fibrosis, and tubular atrophy. Thus, presence of donor-specific antibodies (DSA) is an contraindication to transplant. Sensitization usually occurs after transfusion and previous transplant.[1] Sensitized patients have a long wait.

Sensitization is an increasing problem in children. Approximately 3% of children who are candidates for transplant have sensitization and panel reactive antibodies (PRA) more than 80%.[2] Desensitizing protocols with aim of antibody lowering and preventing more antibody production may result in a successful transplant. In this review, we discuss treatment methods of sensitized pediatric recipients.

Sensitization of patients

The presence of anti-donor antibodies causes recipients to be sensitized. We consider recipients as highly sensitized when their PRA is at least 30% to 50%. Unsensitized recipients are considered when their PRA is 0% to 10%. However, it seems that the presence of specific anti-donor antibodies is the real definition of sensitization. These antibodies are produced when a nonimmunosuppressed recipient is exposed to foreign antigens and include antibodies against ABO antigens, HLA class I and II, and perhaps endothelial monocyte antigens. The reasons for long-term persistence of these antibodies are the persistence of donor proteins in the recipient follicular dendritic cells and chimerism or microchimerism (presence of donor stem cells in the recipient).[3]

The main anti-donor antibodies are anti-HLA antibodies. These antibodies can be detected by PRA or crossmatch. The limitations of PRA are the detection of anti-HLA I not anti-HLA II and the lack of the specificity of anti-HLA antibodies.[4] All patients with positive PRA are not at risk of graft rejection and/or lost. Antibodies against HLA without reactions with donor HLA antigens cannot damage a graft. In addition, PRA cannot truly indicate the anti-HLA antibodies level.

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Acknowledgements: *This study was sponsored by Tehran University of Medical Science.*

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Experimental and Clinical Transplantation (2012) 6: 523-530

DOI: 10.6002/ect.2012.0124

Anti-HLA antibodies also can be detected by other laboratory methods. The standard complement dependent cytotoxicity (CDC crossmatch = CDCXM) is the basic method for anti-HLA antibody detection. The negative CDCXM usually eliminates the hyperacute rejection after transplant. The sensitivity of this method is enhanced by antihuman globulin (AHG) antibody (enhanced or modified CDC). Flow cytometry cell-based assay or crossmatch is a more-sensitive test that detects low titer and noncytotoxic antibodies.[4] This assay is sensitive but not specific. Recently, flow PRA and flow single antigen beads are suggested to increase the specificity of flow cytometry crossmatch. The single-antigen bead method is a sensitive and specific assay. However, controversies remain regarding the association between the results of this test and the outcome of transplant.[5] It is thought that CDC-AHG is sufficient for predicting hyperacute rejection. Patients with low titer anti-HLA antibodies with negative CDC and positive flow cytometry may be involved by acute cellular and/or humeral rejection but not involved by hyperacute rejection.

Recent studies show that the antibodies against non-HLA antigens also are associated with antibody-mediated rejection after solid-organ transplant. The association between non-HLA antibodies and immunologic damage of graft can be better explained by observations that immunologic damage occurred despite identical HLA renal transplant. These non-HLA antigens include polymorphic antigens of endothelial cells, major histocompatibility complex class I-related chain A (MICA). Major histocompatibility complex (MHC) class I-related chain A antigens are endothelial cell surface antigens. Anti-MHC class I-related chain A antibodies are found in sensitized recipients.

Some studies have shown that anti-MICA antibodies may play some roles in immunologic damage of vascularized grafts. Because MICA is not expressed on the lymphocytes, recent crossmatch assays cannot detect anti-MICA antibodies, and we cannot recognize sensitized recipients with these antibodies by current laboratory methods.[6] There is also cellular sensitization (donor reactive T-cell sensitization) in addition to humeral sensitization. The importance of this sensitization is unclear.

Preventive protocols

Prevention of sensitization in children who are candidates for renal transplant is important. Avoiding transfusions, particularly multiple transfusions, is suggested. Some authors have suggested transfusions be performed under cyclosporine cover if necessary.[7] This idea is not for widespread use because most candidates have severe renal failure with high serum potassium levels which limit cyclosporine use. Although many centers suggest using leukocyte poor blood in transplant candidates when necessary, the effect of leukoreduction procedures on transfused blood and on sensitization rates is unknown. Many physicians also advise their sensitized patients that their PRA may decrease spontaneously with time; thus, they can wait for a deceased-donor transplant. Meanwhile, we can use desensitizing protocols if sensitized patients have living donors or persistent high PRA with time.

Desensitizing protocols

Successful renal transplant is the main goal in sensitized patients. This is achieved by HLA-matched donor selection and the use of desensitizing protocols. The aim of desensitizing protocols is to reduce DSA levels to those associated less with the immunologic damage, and to maintain this reduced level of antibodies for the first several months after transplant. Accommodation can be due to some protective genes that protect the graft from antibody-mediated graft injury after several months of transplant.

Some protocols exist to achieve these therapeutic aims. These desensitizing methods include high-dose intravenous immunoglobulin (IVIG),[8-11] plasmapheresis alone, plasmapheresis/low-dose IVIG,[12-14] plasmapheresis/rituximab, plasmapheresis/immunosuppressive agents,[15] immunoadsorption, immunosuppressive medicines such as alkylating agents, and infusion of rituximab.[13,14,16] Each desensitizing protocol has some strengths and weaknesses. Infections and rebound of anti-donor antibodies (after cessation of desensitizing treatment) are major problems of these protocols. The endpoint of desensitizing treatments is elimination of DSAs. At this point, transplant can be performed. If the transplant cannot be performed at this time, the sensitization protocol should be continued until the transplant time.

Studies on the effects and complications of desensitizing protocols are rare in children. This can be due to the lower numbers of sensitized recipients

in the pediatric age group and higher complications of some desensitizing methods such as splenectomy and plasmapheresis in them.

Intravenous immunoglobulin

Intravenous immunoglobulin is prepared by isolating polyclonal IgG from healthy donors. It can lower PRA and/or convert the positive crossmatch to the negative form. The main mechanism of IVIG is blocking the anti-HLA antibodies effects. Other mechanisms of IVIG are down-regulating B cell differentiation, B cell apoptosis, and ultimately reducing antibody production, inhibiting T cell proliferation, and activating and inhibiting ϒ-IFN, complement system inhibition, suppression of dendritic cells, and anticytokine activity.[17,18] Intravenous immunoglobulin also can inhibit memory cells. The suppressive effect of IVIG on antibody production persists long term.

Pradhan and associates showed that IVIG was not effective in reducing panel reactive antibodies. They used high-dose IVIG (2 g/kg) every 4 weeks (3 doses each week) but it was not effective.[15] In contrast, Al-Uzri and associates have shown that long-term high-dose IVIG use can change the PRA to zero.[19] They used 500 mg/kg IVIG weekly for 3 consecutive weeks every 12 weeks. The PRA activity fell to zero after almost 40 months.[19]

Tyan and associates used IVIG to reduce panel reactive antibodies in vitro and in vivo in a 13-year-old recipient awaiting retransplant and showed a reduction of PRA from 95% to 15% and a successful retransplant.[8] The results of IVIG therapy alone in sensitized adults are inconsistent. Sometimes sensitized patients had only a partial response or none at all. After achieving inconsistent results from adult studies focusing IVIG use in sensitized recipients they used the combination of IVIG and other medicines to increase the efficacy.

The major complications of IVIG are infusion-related reactions, renal tubular toxicity, and thrombotic events. To prevent acute renal failure, we must use types with lower sucrose concentrations. The use of higher sucrose IVIG products may result in acute renal tubular damage, whereas the lower sucrose concentration may lead to volume overload. Administration of 5% IVIG solution (with lower osmolality) instead of 10% form also helps reduce thrombotic events and graft loss after transplant.[11, 20, 21]

Some centers have used *Cytomegalovirus*-Ig (CMVIg) in place of IVIG.[16] *Cytomegalovirus*-Ig has an additional anti-CMV effect. There are no well-controlled trials comparing the efficacy of these products, and the idea that administration of CMVIg has additional immunomodulatory effects.

Plasmapheresis

Plasmapheresis can be used in sensitized patients alone or with other treatments such as rituximab and CMVIg. Plasmapheresis is performed until negative PRA and DSAs are achieved. It is important for all patients to continue the plasmapheresis treatments until after transplant. The numbers of these treatments before and after transplant depends on the DSA titer at initiation. Patients with higher starting DSA levels have a higher risk of antibody-mediated rejection and consequently need more desensitizing treatments before and after transplant. Montgomery and associates determined the numbers of plasmapheresis/CMVIg treatments before and after transplant based on starting DSA titer (Table 1).[16] Plasmapheresis has a transient effect on the DSA titer, and a rebound of antibodies occurs after its discontinuation. Thus, plasmapheresis is not suggested for desensitization of recipients with deceased-donor transplant.

Concurrent use of IVIG or rituximab maintains the antibody lowering effects of plasmapheresis and the combination of plasmapheresis and these modalities can be used in deceased-donor transplant. Plasmapheresis has problems. It needs a vascular

Table 1. Number of PP/CMVIg Treatments Based on Starting DSA Titer[16]

DSA Titer on AHG CDCXM	No. of Pretransplant Treatments	No. of Posttransplant Treatments
(+) flow, (-) AHG	2	2
1:1-1:4	3	2
1:8-1:16	4	3
1:32-1:64	5	3
1:128	6-7	4
1:256	8-10	4
1:512	11-15	5
> 1:512	≥ 20	5

Abbreviations: AHG, antihuman globulin; CDCXM, complement-dependent cytotoxicity crossmatch; CMVIg, *Cytomegalovirus*-Ig; DSA, donor-specific antibodies; PP, plasmapheresis

access. Thus, its administration is difficult in patients with peritoneal dialysis. Plasmapheresis can lead to complications including infection, reduction in platelets, hemoglobin and coagulation factors, and consequently bleeding tendencies. We avoid the plasmapheresis on the day of transplant or hemodialysis. We also must perform coagulation tests before transplant and administer fresh frozen plasma if needed.

Protein A immunoadsorption

This modality as plasmapheresis can rapidly reduce the serum level of alloantibodies. This modality also can be used in combination with other modalities. There is no report regarding the use of this modality in sensitized pediatric recipients.

Rituximab

Rituximab is monoclonal antibodies against CD20 "a pan B cell marker." This marker is present on the surface of premature and mature B cells but not on the surface of plasma cells. Thus, rituximab inhibits proliferation and differentiation of B cells but not plasma cells. For this reason, this medication cannot reduce the serum anti-HLA antibody level, but it can prevent clonal B cell expansion and consequently DSA production. In this regard, rituximab should be administered in combination with other modalities such as plasmapheresis/CMVIg and splenectomy as adjunctive therapy. In these states, rituximab maintains low levels of antibodies achieved by other modalities such as plasmapheresis.

It appears that rituximab therapy does not result in severe infections in sensitized patients[22] and is well tolerated. The effects of rituximab can be up to 6 months. Montgomery and associates suggested a desensitizing protocol including rituximab and plasmapheresis/CMVIg (Figure 1).[16] The use of rituximab in this protocol can reduce the risk of immunologic complications during transplant and the number of treatments before or at the time of transplant.[16]

Mycophenolate mofetil

Mycophenolate mofetil (MMF) inhibits proliferation of T and B cells, and consequently antibody production. Mycophenolate mofetil has been shown to decrease PRA in solid-organ transplant by some studies.[23-25] Wong and associates have suggested that MMF starting early after exposure is a good prophylaxis for anti-HLA antibodies suppression in a 4-year-old sensitized recipient. The recommended dosage of MMF is 390 to 500 mg/m^2/d divided twice daily.[26]

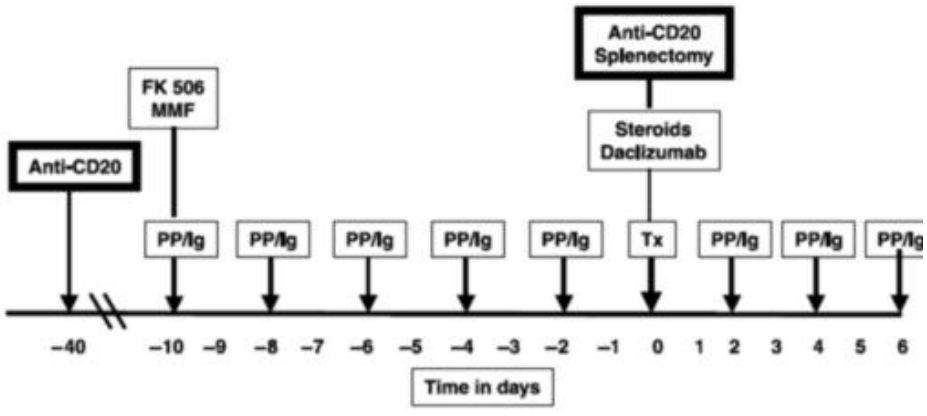
Splenectomy

Splenectomy may remove many B cells and consequently reduce antibody production. The most important problem with this is its permanent effect on the immune system and increase in the risk of sepsis with encapsulated microorganisms especially in children. Splenectomy is sometimes suggested in ABO incompatible renal transplant and/or high-risk sensitized patients.

Future treatments

New treatments include complement blockers and proteasome inhibitor-mediated plasma cell depletion (bortezomib). Alloantibodies bind to endothelial cells of the graft and stimulate the complement pathway. The complement system activation is the key event in this antibody-mediated graft damage. The blockade

Figure 1. Desensitization Protocol in John Hopkins University[22]



of complement system such as soluble CD35+ or eculizumab (an inhibitor of the terminal complement protein C5) can be an important medicine for both prevention and treatment of antibody-mediated rejection.[27,28] Wahrmann performed a pilot study and administered bortezomib alone to 2 sensitized patients. The results of this study demonstrated no effect or little effect of bortezomib on the sensitization status of recipients.[28] However, it appears that the combination of bortezomib and plasmapheresis is a better desensitizing treatment than IVIG-based desensitization protocols. This idea needs to be confirmed by more studies.[29]

Clinical trials of desensitizing protocols

To our knowledge, there are no clinical trials in children focusing on desensitizing protocols in sensitized recipients. Adult studies are also low in numbers. The IGO2 study is a double-blind controlled clinical trial regarding the comparison the 2 groups of sensitized adult patients who are treated with IVIG or placebo.

The results of this study showed the superiority of IVIG when compared with placebo to reduce the anti-HLA antibodies titer.[10] Recently, a clinical trial showed promising results about the use of high-dose IVIG and rituximab combination in sensitized adults.[30] Now, the first clinical trial comparing high-dose IVIG alone and combined rituximab and IVIG is being done in pediatric sensitized patients at Stanford University, but its results are not available.

Recently Montgomery and associates[31] performed a clinical trial by comparing a group of sensitized patients treated with plasmapheresis and low-dose IVIG with a control group. Incompatible HLA transplant was performed in patients under desensitizing treatment after reaching negative crossmatch. Compatible transplant or dialysis was performed for control. The patients showed better survival rate compared with controls during 11-year study.[31] In another randomized clinical trial, thymoglobulin and daclizumab were compared as induction therapy in sensitized patients. The results of this study showed the superiority of thymoglobulin compared with daclizumab for preventing biopsy-proven acute rejection. However, graft and patient survival at 1-year posttransplant were similar in both groups.[32]

Which sensitized recipients are more susceptible to immunologic complications after transplant?

We must recognize sensitized recipients who are more susceptible to antibody-mediated rejection and graft loss after transplant. This immunologic risk determination should be performed in all recipients with a positive crossmatch. First, we must rule out false-positive crossmatches (autoantibodies in donor or recipients and non-HLA specific antibodies). Then, for immunologic risk assessment, we should evaluate the titer of these antibodies. Patients with higher starting titers have a higher risk of immunologic reactions after transplant. Other factors also increase the risk of immunologic damage. The presence of multiple risk factors causes early antibody-mediated rejection even in the presence of low titer of DSA. In these patients, although the low numbers of desensitization protocols convert positive crossmatch to a negative form, they remain at high risk of antibody-mediated rejection after transplant. Thus, we must know these risk factors, including previous early graft loss, multiple donor reactive antibodies, rising DSA titer at the time of desensitization starting, rebound DSA titer between treatments, multiple sensitizing events, and high-risk transplants (eg, husband to wife and child to mother transplants).[16] The presence of DSA reactive to DRW52 and DRW53 HLA antigens causes the resistance to desensitizing protocols.[8, 33] Additionally, comorbid conditions in other organs such as heart and liver should be recognized, because these conditions can result in complications during desensitization protocols such as plasmapheresis.

Who needs desensitization treatment?

It seems that every patient with highly positive panel reactive antibody should be assessed for DSA. We must determine if the positive PRA is due to DSA or not.

It appears that the patients with anti-HLA DSA need desensitizing treatments (preconditioning). We do not know what to do regarding the desensitizing treatment of patients with non-HLA antibodies. There also are controversies treating patients with high anti-HLA antibodies titers. Some transplant centers do not perform desensitization for patients with AHA-CDC > 1/16.[15,34,35]

There are also some problems in treating patients with positive crossmatch reported by different assays

(complement-dependent cytotoxicity assay vs flow cytometry). Should we start desensitization treatment in patients with positive flow-cytometric crossmatching as patients with positive complement-dependent cytotoxicity assay? Are the treatment protocols similar in patients with positive flow cytometry or positive CDCXM? Should we continue treatment until achieving negative flow cystometry results, or several treatments are enough? What about B-cell crossmatch? Are treatments needed in patients with positive B-cell crossmatch?

Most centers believe that they should check DSA in recipients with positive flow cytometry crossmatch to increase the specificity of this test by other assays such as single antigen beads. Maryland University recommends the desensitizing treatment in patients with negative AHG and positive flow cytometry which anti–DSA are positive. Plasmapheresis, IVIG, and immunosuppressive medications have been suggested in these patients. The degree of positivity of the flow crossmatch is also an important factor in determining the type of treatment. Meanwhile, they do not insist on converting positive flow cytometry to negative before transplant.

Additionally there is no consensus regarding the association between the positive single antigen beads and graft outcome. Some small studies have been performed in children and the results are inconsistent.[5,35,36]

Which protocol is better?

As mentioned before, each protocol has some advantages and some disadvantages. There are no clinical trials comparing methods of desensitization in children. We do not have any therapeutic approach in sensitized pediatric recipients. Another problem is to decide which protocol is suitable for which patients. Ultimately, selection of each protocol depends on the transplant center, cost of treatment, living compared with deceased transplant, and the degree of sensitization of patients. Figure 1 shows the desensitization protocol of John Hopkins University.

Table 2. Studies on Desensitization Protocols in Children

Study	n	Age	Protocol	Result
Pradhan et al[15]	1	11	High-dose IVIG (2 g/kg) every 4 week for 3 doses	Without effect
Pradhan et al[15]	1	11	Plasmapheresis every other day × 5 + rituximab 375 mg/m^2	Change PRA to 0 and increase again after 4 mo, No B-cell detection until 14 mo
Pradhan et al[15]	1	11	Plasmapheresis 3/wk for 2 wk. Then 2/wk until transplant, plasmapheresis immediately before Tx, at day 1 and 4 post-Tx + single dose IVIG 100 mg/kg at day 1	Successful Cr=0.6 at 17 mo post-Tx without rejection, sustained increase of anti-HLA Ab
Al-Uzri[19]	1	7	500 mg/kg IVIG weekly for 3 consecutive weeks every 12 weeks, IVIG 1 g/kg before and at day 4 post-Tx	Successful deceased Tx Cr=0.5 at 10 mo post-Tx without rejection
Valentini[37]	2	11, 13	IVIG 10% 2 g/kg monthly for 4 doses and then yearly, IVIG 5% on the day of Tx and 1 mo after Tx and HLA-matched donor selection	Successful deceased Tx (Cr=0.8 at 19 mo and Cr=1 at 15 mo post-Tx
Shapiro et al[38]	1	10	Alemtuzumab 0.4-0.5 mg/kg single dose after induction of anesthesia over 2 to 3 h, 10 to 20 mg/kg methylprednisolone before Tx and during arterial anastomosis with tacrolimus after Tx	Successful transplant without rejection after 2 years
Montgomery et al[16] (Figure 1)			Rituximab 375 mg/m^2 one month before PP and at the day of Tx, every other day PP followed immediately by 100 mg/kg CMVIg until negative DSA and continue post-Tx, FK 506: 0.1 mg/kg/d (target level, 10-12 ng/dL) and MMF at the time of PP initiation, methylprednisolone and daclizumab at the day of Tx, 4 doses daclizumab every 2 weeks	1- to 3-y graft survival rate similar to unsensitized recipients
Tydén et al[39]	1	10	Antigen-specific immunoadsorption and rituximab in ABO-incompatible kidney Tx	Comparable graft function with ABO-compatible kidney Tx

Abbreviations: CMVIg, *cytomegalovirus*-immunoglobulin; Cr, creatinine; FK 506, tacrolimus; HLA, human leukocyte antigen; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; PP, plasmapheresis; PRA, panel reactive antibody; Tx, transplant

Some treatment protocols are considered the main desensitizing treatment such as plasmapheresis and/or IVIG/CMVIg and other protocols are adjunctive or enhancer treatments such as rituximab and splenectomy.

Regarding the comparison between high-dose IVIG and plasmapheresis, plasmapheresis removes anti-HLA antibodies more potently and more rapidly than does high-dose IVIG, and DSA monitoring is more easily performed in patients on the plasmapheresis than those on the IVIG treatment. Thus, plasmapheresis is considered a suitable method for antibody removal in patients with high titer DSA preparing for living transplant. Plasmapheresis also is expensive and the rebound of DSA when plasmapheresis is discontinued. Thus, this modality must continue until transplant and can be used in a living-donor transplant, not a deceased transplant, where waiting is unpredictable. In some articles, continuation of immunosuppression is suggested after plasmapheresis discontinuation to prevent rebound antibodies.

In contrast to plasmapheresis, IVIG is less expensive and is easier to perform. This desensitizing protocol can be used in both living and deceased transplants. Intravenous immunoglobulin also has some disadvantages. The DSA monitoring is difficult and the antibody removal is slow in patients receiving IVIG. Table 2 shows the sensitizing protocols that have been reported in children.

How does one treat and monitor patients after transplant?

Lymphocyte depleting antibodies are suggested as induction therapy in sensitized patients to prevent re-formation of alloantibodies and cellular rejection. In some studies, daclizumab is suggested as an induction therapy.[16] The superiority of thymoglobulin was shown in a recent clinical trial in adults. In most studies, triple immunosuppressive therapy is used in sensitized patients including MMF, steroids, and tacrolimus. It appears that the patients should be treated with several additional plasmapheresis, IVIG, and/or CMVIg after transplant. The type and number of treatments before and after transplant depends on the DSA titer at initiation and the risk of AMR after transplant shows the number of PP/CMVIg treatments after transplant based on starting DSA titer as explained in the John Hopkins protocol.[16]

It is necessary to follow the DSA titer and graft function closely after transplant, especially in patients undergoing plasmapheresis because of rapid rebound of antibodies. The John Hopkins University has a protocol for monitoring of sensitized recipients after transplant. This protocol includes protocol biopsies at 1, 3, and 12 months posttransplant, antibody titer measurement and crossmatch before and after each posttransplant plasmapheresis, 72 hours after the last plasmapheresis, and at 2, 3, 6, and 12 months after transplant. Additionally, close monitoring of antibody-mediated rejection should be done. It is important to know that the risk of antibody-mediated rejection is highest in the first days of transplant whereas its occurrence is rare after 1 month posttransplant. In patients involved with acute mediated rejection, the endpoint of AMR treatment is achieving negative cytotoxicity and/or flow cytometry crossmatch.

Conclusions

Sensitization is a problem with increasing incidence in children, but our information about definition, approach, and treatment in sensitized children is lacking. We also do not know what amount DSA increases the risk of acute and subclinical antibody-mediated rejection. We do not know how to assess and monitor non-HLA antibodies and their exact roles in immunologic damage after transplant.

There are some case series in sensitized pediatric patients to suggest some treatment protocols for crossmatch conversion from positive to negative. However, the results of these studies are controversial. Desensitizing procedures are expensive and invasive. To clarify and determine the best desensitizing methods in this age group, we must have clinical trials with larger numbers of pediatric patients and longer follow-ups.

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