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Desensitization protocols for patients undergoing solid organ transplantation

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Abstract

Approximately 30% of patients on transplantation waiting lists are sensitized to HLA antigens as a result of previous exposure through prior transplants, pregnancies or blood transfusions. Besides increasing the waiting time on the list, sensitization to HLA antigens is a major immunologic barrier to solid organ transplantation. Indeed, without proper immunosuppressive strategies, sensitized patients are at high risk of antibody-mediated rejection (AMR) and graft loss. Over the past years, several centers have developed desensitization and immunomodulatory approaches that result in reduction of anti-HLA antibodies with improved rates of successful transplantation.

The aim of the current work was to review current therapeutic practice for sensitized patients awaiting a solid organ transplant. In particular, we were interested in summarizing current data regarding the management of sensitized patients awaiting a heart transplant. To illustrate the topic, we first studied in detail two complex clinical cases. We then performed a systematic review of current literature in the field (since 1997) regarding current immunomodulatory approaches to treat anti-HLA antibodies before transplantation, focusing on cardiac transplantation.

To date, there is no approved therapeutic protocol or consensus on the management of sensitized patients and anti-HLA antibodies before solid organ transplantation. The data are even scarcer regarding thoracic organ transplantation. This study that reviewed current practice may help define best possible approaches for these patients before and after transplantation to ensure better outcomes.

Introduction

Over the last decade, transplant procedures and waiting list candidates have steadily increased for the various solid organs, including kidney and heart (1,2). Kidney and heart transplantation are the best treatment for end-stage renal disease (ESRD) and end-stage heart disease (ESHD), respectively, as transplantation offers better patients survival results than the respective substitution methods that are dialysis or ventricular assist devices (VAD) (3).

The immune status of the recipient at the time of transplantation has a direct impact on the success rate (4). In the setting of allogeneic solid organ transplantation, the main targets of the host's immune response are the major histocompatibility complex (MHC) molecules, human leukocyte antigens (HLA) in humans, which are present on donor grafted tissues and cells. Besides T-cell mediated immune responses against donor alloantigens that result in cellular rejection, anti-HLA alloantibodies from the recipient can initiate an inflammatory process leading to the activation of the complement cascade and acute antibody-mediated rejection (AMR). Allosensitization, and in particular the presence of pre-existing anti-HLA alloantibodies, prior to transplantation has a deleterious impact on graft outcome and represents an important challenge faced in clinical transplantation today (5,6). Indeed, besides the risk of acute AMR, the presence of pre-formed donor-specific anti-HLA antibodies (DSA) prior to transplantation has been correlated with an increased incidence of T-cell mediated graft rejection compared to non-sensitized patients.

In order to avoid acute rejection and preserve optimal graft function, transplantation is usually done only if the transplant candidate does not have DSA or, at least, that these DSA are under a certain threshold at the time of transplantation. Sensitization prior to transplantation is defined by the presence of anti-HLA antibodies with a positive panel reactive antibody (PRA) test. Highly sensitized patients have a reduced possible donor pool and hence are less likely to receive organs, have a longer waiting time-to-transplant, higher morbidity, and increased likelihood of death while awaiting transplantation (7,8). The main sources of HLA-sensitization are exposure to blood products, pregnancies and previous transplantation. For the patients awaiting heart transplantation, more risk factors of sensitization are known, such as prior cardiac surgery, presence of homograft material, history of VAD. According to the United Network for Organ Sharing (UNOS), >20% of the patients on the waiting list for all solid organs in USA are sensitized with PRA values >10% (2). The International Society for Heart & Lung Transplantation (ISHLT) reports that about a third of the patients on list for cardiac transplantation are sensitized (9) and about 18% of the cardiac transplant recipients are sensitized patients with PRA value > 10% (10).

The detection of anti-HLA antibodies has beneficiated from important technological developments within the past 10 years. Serum anti-HLA antibodies can be detected either by complement-dependent lymphocytotoxicity (CDC) assay against a panel of B- and T-cells, flow-cytometry technology or solid phase assay, the last two also providing information about the specificity of the antibodies as well as the titer of each specific antibody. Solid-phase immunoassays with single-antigen beads arrays (Luminex® technology) and flow-cytometry-based crossmatch analyses (FCXM) have a higher sensitivity than the classical CDC-based test. In addition, in many transplantation programs, the first step in the allocation of organs is now based on calculated PRA (cPRA) values (virtual crossmatch), based on the known HLA of the available donor and of known values of anti-HLA antibodies of potential recipients on the waiting list.

Various protocols have been described for desensitization prior to transplantation, mainly regarding kidney transplant recipients. These strategies usually combine extracorporeal purification and pharmacotherapy with immunomodulatory drugs. Serum pre-formed antibodies can be removed mainly by two different techniques: plasmapheresis/plasma exchange (PE) or immunoadsorption (IA), which differ in the selectiveness of the proteins that are removed and in the costs. Until now, there is no consensus about the optimal way to manage hypersensitized patients, and the data are even scarcer in non-renal transplant recipients. As the number of patients needing efficient desensitization prior to transplantation is foreseen to increase in the coming years also for non-renal recipients, we aimed to summarize current used protocols and analyze their outcome. In this work, we first report on two patients who beneficiated from a desensitization process allowing them to be successfully transplanted with a donor heart, and we review current literature in the field.

Aims

The aims of this work were

- to describe two illustrative cases of desensitization of recipients allowing successful heart transplantation
- to review current literature regarding the management of sensitized heart transplant recipients on the waiting list

Material and methods

Case reports

This study describes two cases based on retrospective data collection of 2 adult patients who underwent cardiac transplantation at the Centre Hospitalier Universitaire Vaudois (CHUV). The reports are based on records collected on medical paper files and follow-up charts and completed by documents archived on electronic centralized files of the CHUV (Archimède, Soarian).

Literature review

Eligibility criteria. We included all studies that met the following criteria: (1) original report; (2) published in English; (3) randomized controlled trial or cohort/case series (4) systematic review or guidelines; (5) describing application or comparison of preoperative or perioperative desensitization protocols; (6) pediatric or adult heart transplant recipients; (7) ABO/blood group-incompatible transplantation or HLA-sensitized; (8) publication from year 1997 onward. We excluded (1) bone marrow transplantation studies; (2) postoperative desensitization procedures; (3) treatment of cellular or humoral rejection episodes protocols; (3) reports that included less than 2 patients. Sensitized recipients were defined as those with a positive cross-match (either CDC, FCXM or cPRA-based virtual crossmatch), DSA (any level), or a positive PRA level (threshold varying between the studies).

Literature search. A systematic literature search was performed in PubMed and ScienceDirect. Search terms included keywords and free text terms such as : “desensitization protocol”, “transplantation”, “ABO-incompatible”, “HLA-sensitized”, “bortezomib”, “rituximab”, “intravenous immunoglobulin”, “plasmapheresis”, “plasma exchange”, “immunoadsorption”, “kidney transplantation”, “heart transplantation”. The final date for searches was August 4th, 2016.

Data collection and analysis

The studies/data reviewed included collective summaries of experience with intravenous immunoglobulin (IVIG) at different dosing, B-cell depletion with rituximab, antibody removal with extracorporeal exchange strategies (plasma exchange, plasmapheresis, immunoadsorption), new molecules such as bortezomib, or a combination of the different techniques cited above.

Studies were identified by the first author and year of the first full publication (if available) or published abstract. The following information were classified in a database: type of study, study period, age of the patients, number of patients included, follow-up duration, definition of sensitization, methods used to determine the immune status, desensitization protocol, induction therapy and post-transplantation medication, patient and graft survival, occurrence of adverse events. The study end-points included efficacy of the desensitization protocol, % of patients being transplanted, patient and graft survival, incidence of rejection and safety of the treatment protocols (incidence of adverse events).

Analysis and synthesis of results

This is a descriptive study. No statistical analysis or comparison between the published studies (meta-analysis) could be performed, because of the small amount of available data and the variability in the quality of these data.

Case reports

Case 1

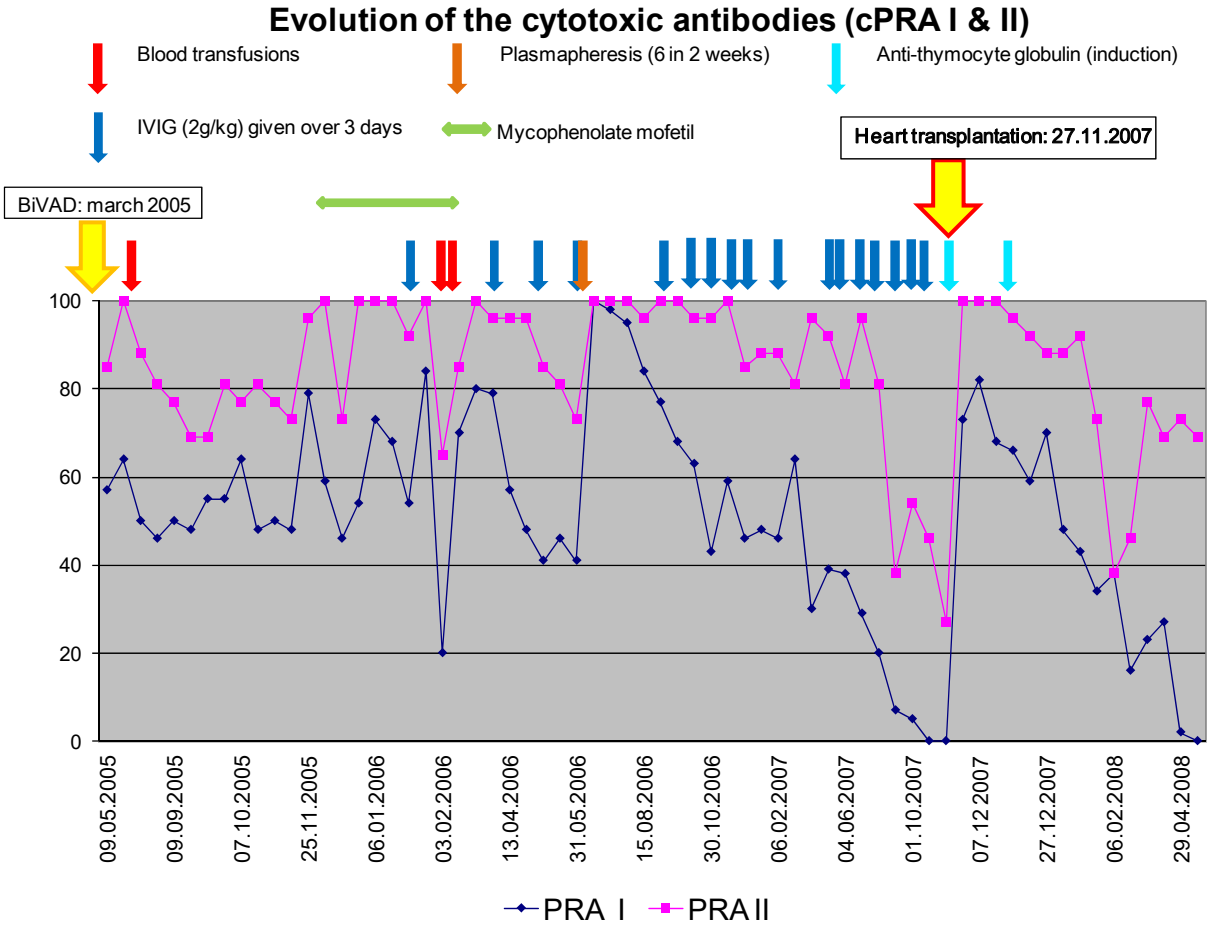
A 48-year-old woman, diagnosed in March 2013 with dilated cardiomyopathy of probable familial origin, was evaluated for heart transplantation. Because of her severe cardiac condition, she was rapidly evaluated to be considered for the transplantation waiting list. The immunology detailed assessment revealed 35 alloantibodies against HLA class I and 3 anti-HLA class II with mean fluorescence index (MFI) levels between 2'000 and 10'000, as determined by solid phase single-antigen beads-based testing (Luminex assay). The reason of her highly sensitized state was probably previous pregnancies and transfusions. As her PRA levels stayed high during follow-up, with multiple positive CDC crossmatches which impaired transplantation, we considered desensitization. Because of multiples infectious episodes, she was not eligible for immunomodulatory approaches until April 2014. From April 2014 to February 2015, the patient received monthly infusions of polyclonal immunoglobulin (IVIG, 2g/kg total dose over 3 consecutive days) for a total of 9 infusions. A unique dose of Rituximab (375mg/m^2 body surface) was also given in August 2014. The treatment then had to be interrupted for infectious reasons, including infection of the heart assistance device, as well because of acute worsening of her cardiac condition requiring monthly levosimendan (Simdax®) treatment from May to July 2015. A second dose of Rituximab was given in September 2015 before resuming her monthly IVIG infusions for 3 more doses (total of 12 doses).

The patient could be successfully transplanted on November 3rd 2015 across the following two class I DSA: anti-HLA B27 (6093 MFI) and anti-HLA A1 (2135 MFI), but with a negative CDC crossmatch. As induction treatment, she received methylprednisolone, eculizumab (Soliris®) 900 mg (single dose), basiliximab (Simulect®) followed by Thymoglobulin® 50mg and cyclosporine, mycophenolate mofetil (MMF) and oral steroids as maintenance immunosuppressive treatment together with antibacterial and antiviral prophylaxis with sulfamethoxazole/trimethoprim and valgancyclovir, respectively. Post-operatively, protocol graft biopsies were performed (every 2-3 weeks) which did not assess any sign of rejection, except on one occasion light acute cellular rejection (Banff grade 1R). Control echocardiography did not reveal any acute cardiac dysfunction. Despite perioperative transfusions of blood products (6 fresh frozen plasma (FFP) and 5 packed red blood cells), we observed spontaneous decrease of the two pre-existing DSA MFI, without occurrence of *de novo* DSA. At the last check-up in December 2016 at 13 months post-transplantation, the patient was clinically well, with a good heart function (LVEF 70%). She was under an immunosuppressive regimen composed of prednisone, mycophenolate mofetil (Cellcept®) and Tacrolimus (Prograf®). There were neither signs of cellular nor humoral rejection despite presence of donor-specific antibodies.

Case 2

A 16-year-old man was diagnosed in May 2004 with heart failure caused by dilated cardiomyopathy of unknown origin (most probably viral). As he had a cardiogenic shock (ejection fraction ≤20%) and was hospitalized in the intensive care unit, he was rapidly evaluated and listed for a heart transplantation. On the waiting list, his condition worsened and by March 2005, his heart failure was terminal. He beneficiated from a VAD implantation (Thoratec®) in March 2005, meant as bridge-to-transplantation. Follow-up immunological screenings before and after VAD implantation revealed progressive sensitization of the patient over time. Indeed, since April 2005 there was a rapid increase in PRA levels, reaching 54% and 87%, for anti-HLA class I and class II, respectively. While the patient received several blood product, the sensitization was possibly mainly due to the VAD and related repeated infectious episodes, as the patient only received leukocyte-depleted packed red blood cells and FFP. In September 2005, the patient started a treatment with MMF (Cellcept® 250mg twice a day) in order to reduce his sensitization status, followed by one infusion of IVIG (2g/kg) in January 2006. By the end of January 2006, the patient developed an abdominal wall abscess at the VAD exit site requiring surgery and repeated antibiotic and antifungal treatments based on regular microbiological assessments. Due to this infectious complication and as the PRA levels continued to increase, MMF was stopped in February 2006. In June 2006, he received a second IVIG infusion together with plasma exchange (2 cycles). Paradoxally, the PRA levels continued to increase (from 41% for PRA I and 73% for PRA II before plasmapheresis to 100% for both PRA I and PRA II). The patient received two other IVIG infusions in September and November 2006. As the PRA levels showed encouraging results, monthly-based IVIG infusions were established. This treatment resulted to a significant and progressive decrease of anti-HLA class I antibodies, with PRA I falling down to 0% in November 2007. The anti-HLA class II antibodies levels also dropped to 27%.

On November 27th 2007, the patient was successfully transplanted with a negative CDC cross-match (PRA I 0%, PRA II 27%). Three DSA were present at low levels at the time of transplantation; A30 (377 MFI), B44 (2052 MFI) and DR13 (1358 MFI). The perioperative immunosuppressive protocol was composed of Thymoglobulin induction with methylprednisolone, followed by one infusion of IVIG and tacrolimus, MMF and oral steroids as maintenance immunosuppression together with antibacterial and antiviral prophylaxis with sulfamethoxazole/trimethoprim and valgancyclovir, respectively. Because of a chronic abdominal wall abscess, additional antibacterial and antifungal treatments were also given initially. While the PRA titers initially rose after transplantation (73% and 100% for PRA I and PRA II, respectively), they fell almost back to pre-operative levels after two months. Post-operatively, protocol myocardial biopsies were performed (every 2-3 weeks) which didn't reveal any sign of acute rejection (Banff grade 0R). By September 2012, 5 years after transplantation, all biopsies were still free of rejection and the cardiac evaluation showed excellent systolic function (LVEF at 78%).


Evolution of the cytotoxic antibodies (cPRA I & II)
Blood transfusions
Plasmapheresis (6 in 2 weeks)
Anti-thymocyte globulin (induction)
IVIG (2g/kg) given over 3 days
Mycophenolate mofetil
Heart transplantation: 27.11.2007
BiVAD: march 2005
100
80
60
40
20
0
09.05.2005
09.09.2005
07.10.2005
25.11.2005
06.01.2006
03.02.2006
13.04.2006
31.05.2006
15.08.2006
30.10.2006
06.02.2007
04.06.2007
01.10.2007
07.12.2007
27.12.2007
06.02.2008
29.04.2008
PRA I
PRA II

Literature review

Study Characteristics

Based on our 2 clinical cases, we focused our research on reports regarding heart transplant recipients. As compared to kidney transplantation where there is much more data available on the type of desensitization protocols used and the outcome of HLA-sensitized patients after transplantation, the literature is scarce when considering sensitized heart transplant patients awaiting transplantation.

Thirty-four records met our eligibility criteria (see material and methods, mainly "describing application or comparison of preoperative or perioperative desensitization protocols", "pediatric or adult heart transplant recipients"): 30 full articles and four abstracts, relating to 30 distinct studies.

We focused on studies related to HLA-sensitized patients and found 20 studies concerning HLA-sensitized transplant recipients. The majority of the reports were made from very small studies and small case series. We identified zero randomized controlled trials (RCTs), 3 prospective studies, 11 retrospective cohort studies and 6 case series/reviews. The majority of the studies reported data on adult heart transplant patients with 9 distinct studies. The remaining consisted of 6 papers discussing pediatric populations and 5 which did not specify the age groups of the described population (**Table 1**).

Table 1. Summary of recorded studies

Study characteristics	ABOi	HLA-sensitized	Total
Records	11	23	34
- Full article	- 11	- 19	- 30
- abstract	- 0	- 4	- 4
Distinct studies	10	20	30
Study design:			
- Prospective, cohort studies	- 1	- 1	- 2
- Prospective, case series	- 0	- 2	- 2
- Retrospective, cohort studies	- 3	- 11	- 14
- Retrospective, case series/reviews	- 6	- 6	- 12
Population			
- Pediatric	- 9	- 6	- 15
- Adult	- 1	- 9	- 10
- NR	- 0	- 5	- 5

1st author Year Type of paper Study design *Study period*	Age	N	Follow-up	Assays used *Definition of sensitization*	Desensitization protocol preHTx	Induction and maintenance treatments	Results: Desensitization MTT HTx rate	Adverse effects	Incidence of rejection after Htx	Survival rate
Itescu S (11) 1998 Full article Retrospective Case series	Adults 51.37 ± 10.46 y	68 HTx at high risk of developing anti-HLA Abs 45 primary HTx with LVAD 23 recipients of a second HTx	>1700 d for patients without Abs	anti-HLA Abs CDC-XM (± DTT) *T-PRA >10%*	NR	CsA, CS, AZA or MMF ACR treated with CS pulses + cytolytic therapy (OKT3 or ATGAM) if refractory	Tx if negative CDC-XM Anti-HLA class I Abs increase waiting time to HTx: 175 d vs. 90 d (p=0.009) MTT not different between bearers and non-bearers of anti-HLA class II Abs (p=NS)	NR	Median time to 1st high-grade ACR 70 d if positive anti-HLA II Abs vs. actuarial freedom from rejection >50% during follow-up if no anti-HLA II Abs Anti-HLA Class II Abs at Tx associated with rejection : shorter delay to 1st high-grade ACR, major risk for ACR, higher cumulative annual rejection rates	NR
John R (12) 1999 Full article Prospective Cohort *1990-1996*	NR	16 HLA-sensitized post-LVAD (*IVIG cohort*) 4 HLA-sensitized post-LVAD (*PP cohort*) 27 non-sensitized post-LVAD (*untreated controls*)	NR	CDC-XM (with DTT) *PRA >10%* If PRA >20% → absolute indication to donor-specific XM before HTx	"*IVIG cohort*" 1-3 monthly cycles CYC 0.5-1.0 g/m^2 (single dose iv) and IVIG 2 g/kg Refractory pats received higher dose: 3 g/kg "*PP cohort*" CYC (see above) PP 2-3x/w for 1-2 mo "*controls*" No treatment	NR	Tx if negative CDC-XM Mean 33% reduction of anti-HLA class I alloreactivity in *IVIG cohort*. Similar reduction in *PP cohort*, but achieved after longer treatment MTT (p=NS): *IVIG cohort*: 3.3 mo (range 0.3-6.2) vs. *controls* 3.1 mo (range 0.3-10.7)	Total of 27 monthly IVIG courses: 1 sepsis, 4 immune complex diseases, 4 reversible renal insufficiency associated with high dose IVIG Total of 6 PP courses: 3 systemic infections, 2 anaphylaxis	NR	NR

Itescu S (13) 2002 Full article Retrospective Cohort *1990-1999*	Adults Mean age: 43 y	23 HLA-sensitized post-LVAD, Treated *(S+T+)* 44 HLA-sensitized post-LVAD, untreated *(S+T-)*	≥12 mo 53.5 mo (range 6-38)	Lymphocytotox. assay *NR*	1-3 monthly cycles CYC 0.5-1.0 g/m^2 (single dose iv) and IVIG 2g/kg (in 4 divided daily doses)	CsA, CS, AZA or MMF *S+T+* had CYC postTx (same dosing) for 4 mo, then MMF	All 23 had HTx, none across a positive T-cell CDC-XM Mean 33% (14-52%) reduction in anti-HLA Class I and II alloreactivity MTT: 75 d (7-143) in non-sensitized, 3.3 mo (0.3-6.2) in *S+T+,* 120 d (23-217) in *S+T-*, 69 d (50-88) in no LVAD	No difference in infections or other complications CMV disease lower in CYC-treated (12% vs.19%) No other systemic viral, bacterial or fungal infections No malignancies	NR	0/23 *S+T+* died while awaiting HTx vs. 14% (6/44) in *S+T-;* p=0.08 Post Tx survival: NR
John R (14) 2003 Full article Retrospective Cohort *Jan 1992-Nov 1999*	Adults Mean age: 50.8 ± 12.7 y	26 HLA-sensitized post-LVAD, treated *(S+T+)* 43 HLA-sensitized post-LVAD, untreated *(S+T-)* 33 non-sensitized with LVAD *(S-)*	53.5 mo (range 6-38)	CDC-XM *PRA >10%* If PRA >20% → negative prospective CDC-XM required before HTx	1-3 monthly cycles CYC 0.5-1.0 g/m^2 and IVIG 2g/kg Multiples courses (but ≤3) if no significant drop in alloreactivity with initial course	CsA, CS, AZA or MMF ACR treated with CS pulses + cytolytic therapy (OKT3 or ATGAM) if refractory	Tx if negative CDC-XM 54% Tx within 2 mo in *S+T+* vs. 33% in *S+T-* Time from LVAD placement to Tx: 3.13 ± 1.7 mo	In treated pats: 22% systemic fungal infections, 11.5% CMV disease No other infections No malignancies 15.4% immune complex disease 15.4% reversible renal insufficiency	Rejection within 12 mo: 22% in *S+T+* vs. 48% in *S+T-* (p=0.04) vs. 27% in *S-* No difference in rejection incidence between *S-* and *S+T+*	at 1 y: 88% *S+T+* vs. 84% *S+T-* vs. 82% *S-;* p=NS at 5 y postTx: 75% in LVAD vs. 72% in non-bridged HTx pats; p=0.53
Patel J (15) 2010 Abstract Case series *2007-2009*	NR	9 HLA-sensitized awaiting HTx	NR	Single-antigen beads solid-phase assay (Luminex®) *cPRA >50% (using*	IVIG 2mg/kg on d1 + d30 and RTX 1g iv on d7 + d21 and BTZ 1.3 mg/m^2 for 2	NR	Mean cPRA post-treatment: 22% 8/9 had significant reduction in cPRA, among them 2 were given BTZ 1 continuing therapy, non-	NR	NR	No report about eventual HTx

				threshold MFI of 7500) Mean baseline cPRA: 67%	refractory pats		responsive			
Kobashigawa JA (16) 2011 Full article Retrospective Single center cohort *Jul 1993-Jul 2003*	Adults Mean age: *S+T+*: 54 y *S+T-*: 53 y *S-*: 55 y	21 treated sensitized, with mean peak PRA 70.5% (range 28-100) *(S+T+)* 74 untreated sensitized, with mean peak PRA 18.8% (range 11-48) *S+T-* 428 non-sensitized controls, with peak PRA <10% *(S-)*	21.6 ± 15.0 mo	CDC-XM FC-XM *PRA >10%*	1-6 cycles of: PP (daily for 5d) +/- IVIG 2g/kg divided over 2d +/- RTX 375 mg/m^2 PostTx PP and IVIG if detection of rise in circulating Abs	No routine induction Thymoglobulin if persistent elevated PRA despite desensitization CsA or TAC, CS, AZA or MMF	PRA 70.5% → 30.9% 100% (21/21) transplanted with negative CDC-XM 28.6% (6/21) had retrospective positive FC-XM MTT: NR	5y freedom from any treated infection: similar in all 3 groups (71.4 vs. 73.8 vs. 71.6 %; p=NS)	1y freedom from treated rejection: 57.1% in *S+T+* vs. 85.1% in *S+T-* vs. 87.4% in *S-*; p=0.013 from AMR: 66.7% in *S+T+* vs. 89.2% in *S+T-* vs. 96.5% in *S-* ; p≤0.01 from ACR: 91 vs.90 vs. 95%; p=NS from CAV at 5y: 74.3 vs. 72.7 vs. 76.2%; p=NS 5-y NF-MACE: 95.2 vs. 90.9 vs. 90.5%; p=NS Rejections with HD compromise: 4.8 vs. 1.4 vs. 1.6%; p=NS	at 5y: 71.4 vs. 81.1 vs. 75.7%; p=NS 6/21 had retrospect. FC-XM+, but similar outcomes compared to XM- pats
Patel J (17) 2011 Full article Case series *Jan 2009-Mar 2010*	Adults Age range: 31-63 y	6 HLA - sensitized with elevated anti-HLA Abs, refractory to treatment with IVIG (2g/kg), RTX (1g, up to 3 times) and PP	>6 mo (range 6-17)	Luminex® →cPRA CDC-XM FC-XM *cPRA >50%, (with MFI threshold >5'000)* mean cPRA: 62% (51-88%)	BTZ 1.3 mg/m^2, up to 5 doses in 2 w PP 2d prior each BTZ session	ATG 1.5 mg/kg for 5 d TAC, MMF, CS +/- PP	Tx if negative FC-XM Mean cPRA after BTZ and PP: 62% → 35% Tx rate: 66.67% (4/6) Tx and alive rate: 50% (3/6, 1 death of sepsis at 1 mo postTx) 1 died while awaiting Tx	1 pats with multiple infections: urinary, lung, C. difficile colitis 2 deaths post sepsis (1 while awaiting HTx and 1 at 1 mo postTx) Anemia (1) Leucopenia (3), Neuropathy (1)	No treated acute rejection during follow-up	Overall patient survival: 66.67% (4/6) HTx recipient survival: 75% (3/4)

Patel J (18) 2015 Abstract (extension of the initial study) Prospective Case series *2010–2014*	Adults	30 highly HLA-sensitized	NR	NR (see above)	BTZ, PP, ≥1 cycle, (see above)	ATG induction TAC, MMF, CS	Tx rate: 73% (22/30) 76.9% decrease in HLA Class I PRA (20/26) 55.6% decrease in Class II (15/27) 51.7% decrease in cPRA (15/29)	Freedom from infection at 1 y: 33.3% Generally well tolerated Neuropathy (4) GI symptoms (2) Rash (1) Thrombopenia (1)	Freedom from treated rejection at 1 y: 73.9% from ACR: 85.2% from AMR: 81.8%	Patient survival: 100% at 1 y for the 22 Htx pats
Patel J (19) 2015 Abstract Case series pilot study evaluating C5 inhibitor (ECZ) *NR*	NR	9 HLA-sensitized awaiting HTx	NR	NR PRA at Tx: 92±5%	ECZ infusion perop and postTx for 3 mo (weekly, then biweekly)	rATG TAC, MMF, CS	8/9 pats transplanted 8/9 pats had retrospective positive T and B FC-XM	No treated infection postTx 1 intraoperative death due to purulent mediastinitis	Freedom from any treated rejection at 1 y: 75% from ACR grade ≥2R: 100% from AMR : 75%	Actuarial survival: 88.9% at 12 mo with preserved graft function
Dowling RD (20) 1998 Full article Case series *NR*	NR	4 LVAD recipients who developed high PRA	NR	AHG-CDC PRA Prospective XM (CDC, FC) Peak PRA: 64, 57, 98, 19% (before LVAD: PRA 0-6%)	IVIG 0.5g/kg, weekly	NR	All pats had significant drop in PRA, shortly after initiating treatment Mean decrease in PRA: 23% at 1 mo 52% at 2 mo 97%±2% at 6 mo	No morbidity related to IVIG 1 LVAD pocket infection 1 stroke 1 chronic drive line exit site infection (	NR	NR
Holt DB (21) 2007 Full article Retrospective Cohort *May 1995-May 2006*	Pediatric Age at Tx: 1-21 y	17 HLA-sensitized Among them: 13 CDC-XM+ 4 CDC-XM-	≥12 mo People alive: 1.5-11 y	Anti-HLA Abs lymphocytotox. screen (CDC) After 1998: ELISA screen *PRA >10%*	Perop PP (1.5x volume exchange) If XM+, PP postTx for 5-7d	ATG,ATGAM, Thymoglobulin induction CsA or TAC, AZA or MMF, CS If XM+, cytolytic therapy postTx for 7-14d, CYC	Donors accepted without prospective XM Retrospective XM immediately after HTx	Cumulative rate of serious infections: not different between the groups in the first 36 mo after HTx Overall infections rate: greater in XM+	Overall freedom from rejection: 7.7% (1/13) with 38.5% having recurrent rejection Rate of rejection significantly higher in XM+ vs. XM- group at 6 mo, but not afterwards HD compromise: 58%	Actuarial survival after CDC-XM+ HTx: 85% at 1 y, 73% at 3y 1 death at 34 mo, RSV pneumonia 1 death at 4.3 y, pulmonary veno-occlusive disease

						1-2 mg/kg/d for 4 w instead of AZA or MMF Treatment of rejection according to hemodynamic conditions: PP, CS pulses, IVIG 0.5g/kg, CYC + cytolytic therapy if steroid-resistant + total lymphoid irradiation if recurrence		group at 6 mo, but not different afterwards .	of the 1st rejection episode Late rejection (>6 mo) was infrequent (occurred in 3 pats)	1 death at 5.2 y, graft failure from recurrent rejection, non-compliance
Robinson JA (22) 1997 Full article Case series *NR*	Adult Mean age: 56.5 y (range 47-62)	4 HLA-sensitized	Mean: 36.5 w (range 22-52)	AHG-CDC CDC-XM (+/- DTT) *PRA >10%* (Peak PRA: 75-100%)	Pre and perop PP, IVIG 20g Empirical apheresis postTx in 1 pat	CsA, AZA, CS	PRA Post-PP: 0-70% PRA PostTx: 0-2% All had positive retrospective donor-specific XM	No reactions during IVIG No significant bacterial or viral infections	Minor to no rejection over range from 5.5-12 mo	Survival: 100%
Pisani BA (23) 1999 Full article Retrospective Cohort *Jul 1994-Mar 1998*	Adults Mean age: 50.2 y	16 HLA-sensitized 102 non sensitized, untreated	21.6 ± 15.0 mo	Retrospective AHG-CDC-XM (+/- DTT) *PRA >10%*	Pre and perop PP, IVIG 20g	CsA, AZA, CS Treatment of rejection: CS pulses, PP, MTX, switch to TAC and MMF	Mean PRA: 55.5% → 34.9%, (mean 20% reduction) with 3 negative (0%) PRAs Positive XM in 12/16 (75%) sensitized vs. 4/102 (4%) of the non-sensitized Time to HTx: 91.8 ± 69.2d	NR	No difference in mild, moderate or severe ACR or AMR No episode of hyperacute rejection in either group	Patient survival: 87% (14/16 sensitized) vs. 82% (84/102 controls); p= NS
Leech SH (24) 2003 Full article Case series *Jul 1993-Jul*	Adults Age range: 26-55 y	4 pre-sensitized, with positive CDC-XM (1 received renal Tx at	Mean: 31.5 mo (range 17-57)	Anti-HLA Abs by Luminex® CDC-XM If PRA >10%	Various combinations of: IVIG, preop and postTx PP, CYC postTx	eATG or BAS induction Standard maintenance therapy	All patients were transplanted through positive prospective XM MTT: mean 13 mo (range 11-17 mo)	NR	ACR: 2 pats AMR: 2 pats	Graft and patient survival: 100%

2003		the same time) 3 had LVAD		→ prospective CDC-XM before HTx						
Leech SH (25) 2006 Full article (extension of the initial study) Retrospective Cohort *1998-2005*	Adult Mean age: 50 y (range 23-67)	35 HLA--sensitized (2 received renal Tx at the same time) 277 non sensitized, controls	Mean: 21.2 mo (range 5-91)	Anti-HLA Abs CDC-XM FC-XM *PRA >10%* If sensitized → CDC-XM prior to HTx If not, retrospective CDC-XM	2 regimens: 1) PP+IVIG 20g 5 cycles 2) PP+IVIG Single cycle at time of surgery	ATG 1.5mg/kg or BAS induction TAC, MMF, CS	After treatment, 15 pats still with anti-HLA class I and 7 with anti-HLA class II Abs Significant declines in class I and II Abs levels in 88.6% (31/35) 10 pats transplanted with positive XM XM prior PP+IVIG: 13 T+B+, 4B+T- XM after PP+IVIG: 8 T+B+, 2T+B-, 0B+T-	NR In sensitized: 4 deaths (2 primary graft nonfunction, 1 sepsis after 6 mo, 1 graft failure after 2y)	Rejection in 34.3% (12/35) of sensitized including 20% (7/35) with characteristics of AMR NR for controls	Patient survival: 88.57% (31/35 sensitized) vs. 58% controls; p=0.04 Graft survival: 32/35 (91% sensitized), NR for controls
Daly KP (26) 2013 Full article Retrospective Single center cohort *Jul 1998-Jan 2011*	Pediatric Mean age: 6.1 y (range 0.4-15.9)	12 CDC-XM+ (10 had DSA, 2 donor-specific T-cell XM+) 122 CDC-XM-, control cohort	1.5-15 y death occurred between 2 w and 7 y post-HTx)	Anti-HLA Abs: AHG-CDC Luminex® with MFI cutoff ≥1000 → cPRA *cPRA or PRA >10%*	Perop PP or PE (1.5-2 volume exchange) If XM positive, postTx PP (4-6 cycles), IVIG (2g/kg) at day 6-10, then every 3-4 w for 6-12 mo	ATG induction since 2005 CsA or TAC, AZA or MMF, CS only if XM+	CDC-XM performed at HTX Tx was done even if XM positive	More treated infections during the 1st y in XM+ (50% vs 16%; p=0.005) Shorter time to 1st infection in XM+ patients (p<0.001) No death from infection	No difference in rejection occurrence All patients with survival > 5 y or still alive at last follow-up had continued decreases in the number and MFI of DSA AMR with HD compromise within 1st y: 50% in XM+ vs. 2% in XM-	No difference in graft survival between XM+ and XM- (p=0.11) Patient survival: 58.3% (7/12 sensitized) 2 sudden death after de novo DSA with AMR 1 death from ACR with diffuse CAV 1 death due to primary graft failure
Jacobs JP (27) 2004 Full article Retrospective Single center cohort *May 1995-Apr*	Pediatric Mean age: 130.5 d (8 neonates <28 d, 40 infants <1 y	8 HLA-sensitized 52 non-sensitized, untreated	Mean: 140 d (295-453) for sensitized group 1376 d (17-2844)	CDC PRA (+DTT) FC PRA *PRA >10%*	IVIG weekly and/or CYC or MMF daily PE or PP pre- and postTx	rATG, CS pulse CsA or TAC, AZA or MMF	No prospective XM All had retrospective XM: 50% positive (4/8) 3 XM- still alive, 1 XM- died (pulmonary vein stenosis) 1XM+ still alive, 3XM+ died	1 XM- and 3 XM+ still alive 3 XM+ and 1 XM- died	Mean 1 rejection episode/patient (treated sensitized) vs. 0.66 (controls); p=NS	30-d survival: 75% (treated sensitized) vs. 92% (controls); p=NS Patient survival: 50% (treated sensitized) vs. 85% (controls);

2003			for non-sensitized group							p=0.04
Asante-Korang A (28) 2015 Full article Retrospective Cohort *Jan 2005-Jul 2013*	Pediatric Mean age : High PRA: 2336.5 d (range 21-6128) Low PRA: 810 d (range 8-7125) (p=NS)	14 high PRA 56 low PRA	High PRA: 967 d (8-3035) Low PRA: 866 d (2-2951) (p=0.7)	Anti-HLA Abs by: Luminex® FC-XM PRA (HLA Class I and II coated beads) Virtual XM FC-XM postTx *PRA >10% subgroups*: PRA 11-50% with low-level anti-HLA Abs MFI <3000, PRA 11-50% MFI 3000-7000, PRA >50% and/or MFI >7000)	Weekly PP or PE + IVIG If MFI >3000, monthly CYC (500-1000 mg/m^2) or RTX (375 mg/m^2) PostTx: Depending on XM positivity and preTx MFI intensity: add PP +/- RTX PRA <10%, treated like the PRA=0%	rATG (0.5-1mg/kg for 3-5d) or BAS, CS (7.5mg/kg twice a day for 4 d), IVIG (0.5g/kg) TAC, MMF	4/14 had positive retrospective FC-XM MTT: High PRA: 56.5 d, Low PRA: 29.5 d; p=NS	50% sensitized had ≥1 episode of significant infection (15 episodes in 7 pats) Vs. 45% non-sensitized (57 episodes in 25 pats) No PTLD in the subgroup treated with RTX	Freedom from AMR and ACR grade ≥ 2R/3A: 71.4% in sensitized vs. 64% in non-sensitized; p=NS Freedom from CAV: 93% in sensitized vs. 91% in non-sensitized;, p=NS Median time to rejection : High PRA: 19 d Low PRA: 297d (p=0.02)	30-day survival: 100% for sensitized vs. 88% for non-sensitized; p=NS Overall survival: 92.9% for sensitized vs. 80.4% for non-sensitized group; p=0.44
Patel PC (29) 2007 Abstract Retrospective Cohort / database review *1988-2005*	NR	36 with LVAD, 3 subgroups : 10 sensitized and treated *(S+T+)* 11 sensitized untreated *(S+T-)* 15 non-sensitized *(S-)*	NR	NR Peak PRA: 79% *(S+T+),* 26% (*S+T-)* (p<0.0001)	CYC, IVIG, MTX, MMF, IA (combinations)	NR	PRA reduction: 65% (S+T+) vs. 13% (S+T-); p<0.0001 MTT: 110 d (S+T-) vs. 290 d (S+T+)	NR	NR	NR 6 patients died before HTx 2 S+T- 1 S+T+ 4 S-

Pollock-BarZiv SM (30) 2007 Full article Retrospective Database review *1990 - 2006*	Pediatric Median age: 7 mo (3.5 mo-15.5 y)	13 HLA-sensitized	Median for 9 survivors: 1.7 y (3 mo-3.7 y)	AHG-CDC PRA Luminex Retrospective XM *PRA (Class I or II) ≥10% or positive T-/B- XM*	IVIG 1g/kg weekly or MMF 20mg/kg/d PE on CPB +/- RTX empirically periop for 3 pats + PP if retrospective XM+	ATG 1.5 mg/kg/d TAC, MMF, CS, +/- sirolimus for renal sparing, +/- CYC earlier in the study If AMR: RTX		No PTLD or other malignancy Infections in 5 pats: respiratory or central-line; but no difference with non-sensitized or non-Tx cardiac surgery population	ACR: 7/13 pats AMR: 9/13 pats No AMR developed after 6mo No CAV in the 9 survivors	Survival: 89% at 3 mo, 71% at 1 y 4 deaths ranging from 11 d to 9 mo
Lick SD (31) 2008 Full article Case series *NR*	3 adults Age: 19 y, 29 y, 54 y	3 HLA-sensitized	13 mo, 16 mo, 26 mo postTx	CDC-XM T-PRA : 70-96% B-PRA : 24-73%	PP on CPB	ATZ 20mg induction CsA, CS MMF started several mo postTx	1st Tx was a false negative prospective XM 2nd and 3rd had positive retrospective XM Time to HTx: 29 d, 94 d, 187 d	NR	All 3 pats presented rejections and were successfully treated	All 3 pats well at last follow-up
Lick SD 2011 (32) Full article (extension of study above) Retrospective Cohort Jan 2006-Feb 2011	Adults Mean age:: 40.3 y (range: 20-55)	8 HLA-sensitized, with LVAD (*S+*) 23 non-sensitized 43% (10/23) with LVAD (*S-*)	2.3 ± 1.6 y (S+) 2.4 ± 1.5 y (S-)	CDC-XM FC-XM *T- or B-PRA >70%* Control group: T-PRA = 2±6 B-PRA = 1±3	PP on CPB	ATZ 20mg or BAS induction CsA or TAC, CS (tapered) MMF or everolimus, started several mo after Tx for *S+* Therapy of rejection: CS pulses If AMR: RTX If HD compromise : ATZ and rATG	No prospective XM done Retrospective XM in *S+* : 4 CDC-XM+, 3 FC-XM+ 1 XM- *S-* had all negative XM	No CMV infection in *S+* vs. 2 in *S-* controls 1 CNS toxoplasmosis in *S+* group (donor-to-recipient transmission) No other clinically significant infection in *S+*	ACR rates at 1y higher in *S+* but p=0.07 NS AMR: 38% in *S+* vs. 4% in *S-*; p=0.04	Survival at 1y: 100% for *S+*, vs. 94% for *S-*; p=NS Actuarial at 2.3y: 88% (7/8) for *S+*, 70% (16/23) for *S-* ; p=NS *S+*: 1 death due to no access to medication (Hurricane) *S-*: 5/7 caused by rejection

Schumacher KR (33,34) 2012 Full article Prospective Case series *2002-2011*	Pediatric	14 HLA-sensitized	5± 2.8 mo after last RTX dose	cPRA measured by ELISA or FC or Luminex. with unacceptable antigens if MFI >7000 Virtual XM *PRA >10%*	IVIG 2g/kg one dose RTX 375 mg/m2 per dose (serial infusions if CD20 B-cells >10-20 cells/ml)	NR	57.14% (8/14) responders to desensitization Median cPRA changes: class I 50% → 17% class II 61% → 17% Potential donors pool increased from 10 to 85% (range 3-100%) 8 responders: 5 HTx, 2 delisted, 1 death prior to Htx 6 non-responders: 3 HTx, 3 delisted	No therapy-related events	Overall freedom from rejection: 87.5% (7/8) No responders had a treated rejection episode 1 AMR in a non-responder (presence of DSA)	21.4% (3/14) deaths 2 deaths from primary graft failure HTx pats survival: 75% (6/8)

Abbreviations: Abs Antibodies; ACR Acute cellular rejection; AHG anti-human globulin; AMR Antibody-mediated rejection (HD-AMR hemodynamically significant antibody-mediated rejection); ATG Anti-thymocyte globulin (rATG rabbit ATG; eATG equine ATG);ATZ Alemtuzumab; AZA Azathioprine; BAS Basiliximab; BTZ Bortezomib; CAV coronary artery vasculopathy; CnI calcineurin inhibitors; CDC Complement-dependent cytotoxicity; CPB cardio-pulmonary bypass; (c)PRA (calculated) Panel-reactive Antibody level; CS Corticosteroids; CsA Cyclosporine A; CYC Cyclophosphamide; DSA Donor-specific antibodies; DTT Dithiothreitol (DTE Dithioerythritol, cis epimer of DTT); ECZ Eculizumab; FC Flow-cytometry; HTx Heart transplantation; HD hemodynamic; IA Immunoadsorption; IS immunosuppressive treatment; IVIG intravenous immunoglobulin (HD-IVIG high-dose IVIG, LD-IVIG low-dose IVIG); MMF Mycophenolate Mofetil; MPA Mycophenolic acid; MTT Mean time-to-transplant; MTX Methotrexate; NF-MACE: non-fatal major adverse cardiac events; NR Not reported; NS Not significant; PE Plasma Exchange; PP Plasmapheresis; PTLD post-transplant lymphoproliferative disease; RTX Rituximab; S sensitized; TAC Tacrolimus; TG Thymoglobulin; T treated; Tx Transplantation; VAD Ventricular assist device; XM Crossmatch.
d days; mo months; pats patients; y years; w weeks.

- **ABO-incompatible Recipients**

Our main aim was not to discuss ABO-incompatible (ABOi) heart transplantation, as it refers to very specific situations, mainly involving infants with a not fully mature immune system). Transplantation from an ABO-incompatible donor is usually contraindicated because of the risk of hyperacute rejection mediated by preformed antibodies in the recipients to blood-group antigens of the donor, leading to rapid thrombosis in the vasculature of the graft. As newborn infants has an immature complement system, do not yet produce isohemagglutinins and has low serum anti-A and/or anti-B antibody titers until the age of 12 to 14 months, this contraindication may not apply to them. We indeed found only one case series reporting about adults undergoing ABOi heart transplantation. We however describe here some landmark studies that illustrate the specific situation of "natural" sensitization against donor blood group antigens.

West et al. (35) published one of the pioneer studies. They identified 10 ABOi heart transplant recipients and 10 ABO-compatible heart transplant recipients and analyzed their outcomes. The only desensitization procedure was plasma exchange (PE) done on cardiopulmonary bypass. No hyperacute rejection was reported, no significant increase of acute cellular rejection was observed in the ABOi cohort and no other supplementary morbidity was attributable to ABO-incompatibility. The overall survival was 80% with follow-up ranging from 11 months to 4.6 years. Furthermore, with the possibility to transplant across ABO-incompatibility barrier, the mortality rate among infants on the waiting list declined from 58% to 7%. The safety, feasibility and excellent short-term outcome led to a United Network for Organ Sharing (UNOS) policy change in 2006, allowing the listing of infants across the blood group barrier for heart transplantation in the United States (36).

Dipchand et al. (37) reported the only prospective study, comparing the outcome between 35 pediatric patients who received ABOi heart transplantation versus 45 pediatric patients who received ABO-compatible heart transplantation. They used the experience and the procedure of West et al. (35). The immunosuppression protocols used were strictly the same, except for the recipients of ABOi hearts who had donor-specific isohemagglutinins (DSI) titers $\geq$1:8. These patients received an additional treatment of plasma exchange which reduced the DSI titer to $\leq$1:2. There was no significant difference regarding the rejection rate or long-term patient survival with both groups having a 74% survival rate at seven years.

Henderson et al. reviewed the Pediatric Heart Transplant Study (PHTS) group data which included 34 transplant centers in the USA and in Canada. The study analyzed the outcome of over five hundred pediatric heart transplantations among which 85 were ABOi heart transplantation. They found no significant difference regarding neither the rejection rate nor the patient survival between the two groups. The ABOi recipients seemed even to experience less infectious events. The relative short follow-up of one year was chosen to include as many patients as possible. The study however did not specify the induction and maintenance immunosuppressive protocols used in each subgroup.

Irving et al. (38) analyzed the outcome of 30 pediatric ABOi heart transplant recipients by separating the cohort into two subgroups according to their donor-specific isohemagglutinins (DSI) titers. The different tools used to minimize the incidence of post-transplant rejection consisted of PE on bypass, immunoadsorption and rituximab. Although the study showed a worse mortality rate in the high-DSI titers group, none of the three deaths were directly related to the ABOi nature of the transplantation. Post-

transplant cardiac function in all surviving patients was good. Moreover, some patients (44%, 4/9) seemed to have developed B-cell tolerance with an absence or very low levels of isohemagglutinins. Also in this study, 2 patients had donor-specific anti-HLA antibodies (DSA) at the same time and received an additional treatment of IVIG, rituximab, mycophenolate mofetil (MMF), bortezomib and immunoadsorption.

Roche et al. (39) reported about the experience of two pediatric cardiac transplantation centers in the UK (one of the two centers being the same than the one analyzed by Irving et al, see above). Using PE on bypass, DSI was reduced in 11/14 patients who presented DSI before transplantation. Over the total of 21 heart transplant patients, only one case of biopsy-proven acute cellular rejection was recorded. In some patients (2/11) significant titers of DSI were repeatedly detected but no antibody-mediated rejection occurred, despite persistent expression of corresponding antigens on the graft's vascular endothelium. The follow-up duration in this study ranged from 9 to 19 months, with a survival rate of 100%.

Urschel et al. (40) made a retrospective survey by the pediatric transplant patients of 6 centers in 4 countries. They counted 58 ABOi heart transplantations in 57 patients. The patients underwent PE of 2- to 3-fold their blood volume perioperatively if the DSI was >1:8. They reported excellent survival rate, with 100% at one year, 96% at five years and 69% at 10 years, ranging above those reported in the ISHLT registry for this age group, despite the high-risk profile of the cohort. 4 graft losses were identified, but none of them were attributed to the presence of blood group antibodies, just as for the 2 cases that need retransplantation.

Such observations suggest a state of immune hyporesponsiveness or even acquired immune "accommodation" or tolerance towards donor blood group antigens, a state favored by the immature immune system of infant recipients. Although this phenomenon has also been described in ABOi kidney transplantation, it may play a bigger role in the success of pediatric ABOi heart transplantation. The exact mechanisms of accommodation need to be studied, but the presence of DSI and C4d staining on graft biopsies, without overt acute antibody-mediated rejection or allograft vasculopathy suggest that B-cell tolerance is probably not the sole mechanism of graft acceptance. (41)

Tydén et al. published the only paper which analyzed ABOi heart transplantation in the adult population. In their small serie, they performed 2 ABOi and 4 ABO-compatible heart transplantations, and treated them with preoperative plasmapheresis, rituximab and IVIG. The outcomes were excellent, with both groups of patients having normal cardiac function after transplantation. Acute cellular rejection occurred in both groups, but all the episodes occurred within the first 6 months postoperatively. An interesting finding was that post-operative biopsy specimens showed positive C4d staining in both groups of patients, but with no histological signs of rejection and clinical stable patients.

- **HLA-sensitized ABO-compatible Recipients**

We identified 23 records, relating to 20 different studies. There were 3 prospective studies with 1 cohort study and 2 case series. 11 retrospective cohort studies were found and 6 case series completed our research findings. As compared to the literature concerning ABOi heart transplantation described here above, we mainly inventoried

studies which were describing adult populations (9 studies). 6 studies reported about pediatric population, and 5 studies did not specify the age range of the patients.

Two groups of investigators produced about a third of the literature we found (9 records). The first group at Columbia University in New York published a total of 4 articles between 1998 and 2003 about heart transplantation in HLA-sensitized patients.

Itescu et al. (11) investigated in 1998 the effects of the presence of anti-HLA antibodies in sensitized individuals awaiting heart transplantation and distinguished the outcomes between patients presenting antibodies against MHC class I molecules and antibodies against MHC class II molecules. IgG antibodies against MHC class II molecules in recipient serum at the time of transplantation was found to be a major risk factor for high-grade cellular rejections, while anti-MHC class I IgG antibodies seemed to increase the waiting time to transplantation. They did not report about the use of desensitization protocols prior to transplantation

Collaborators from the same study group (12) published the only prospective cohort study found in our search. They compared the immunological outcomes of sensitized patients after implantation of a Left Ventricular Assist Device (L-VAD) and their responses to different immunomodulatory treatments (IVIG with cyclophosphamide versus plasmapheresis with cyclophosphamide). The sensitized cohort which underwent a treatment with IVIG combined with cyclophosphamide had an excellent outcome, with a mean time-to-transplantation similar to non-sensitized patients. Treatment with plasmapheresis and cyclophosphamide essentially lead to the same mean reduction of alloreactivity (PRA levels) but required longer treatments. Comparison of the adverse effects between treatments based on IVIG or plasmapheresis showed that not only IVIG was faster in achieving optimal conditions for transplantation, but had also a more favorable adverse events profile, with much less systemic infections and no systemic anaphylactic reactions. IVIG did cause acute renal failure, but only in patients who received a high-dose treatment. These results suggested that IVIG (at the dose of 2 g/kg) has a better safety profile than plasmapheresis under these described conditions. A more detailed analysis of the anti-HLA antibodies showed that only the presence of anti-HLA class I antibodies significantly increased the waiting time to cardiac transplantation, as compared to presence of anti-HLA class II antibodies.

Using these encouraging results using IVIG, John et al. (14) administered the same treatment to an enlarged cohort, using 1 to 3 monthly courses cyclophosphamide at the dose of 0.5-1.0g/m2, and IVIG at the dose of 2g/kg. The treated patients had a transplantation rate of 54% within 2 months, which is more than 20% higher than the transplantation rate of the untreated patients for the same period. Infections were the main adverse effects (22% systemic fungal infections, 12% CMV diseases), together with immune complex disease (evidenced by fever, arthralgia and maculopapular rashes), found in 4/26 treated patients (15%) and reversible acute renal insufficiency (defined by >50% increase in serum creatinine levels) in 15% of the patients. During the follow-up period which ranged from 6 to 38 months, no malignancies were observed. The rejection episodes happened significantly less frequently in the HLA-sensitized treated group, compared with the HLA-sensitized untreated group and were similar to the non-sensitized patient group. However, even if the rejection rates were significantly lower in the treated patients, the survival at one year was similar between all the groups (sensitized and treated, sensitized untreated, non-sensitized).

The same collaborators (13) reported about two cohorts of patients who became sensitized after the insertion of a LVAD. One of the cohorts beneficiated from a preoperative cyclic treatment with cyclophosphamide and IVIG, completed with additional

cyclophosphamide in the first months after transplantation. The outcomes were clearly in favor of the treatment group, as all patients were successfully transplanted across a negative crossmatch, while 14% of the patients from the sensitized untreated group died while awaiting transplantation. The mean time to transplantation of the treated sensitized patients was not significantly different from non-sensitized patients. No significantly higher occurrences of adverse effects like infectious complications or malignancies were observed. On the contrary, the patients who received cyclophosphamide presented less CMV diseases and systemic fungal infections. This study demonstrated that cyclophosphamide together with IVIG pre-transplantation was effective and safe for decreasing recipients' alloreactivity, shortening transplant waiting time and reducing allograft rejection.

The other main contributor to our literature is a group at the Cedars-Sinai Heart Institute in Los Angeles with 5 publications including 3 abstracts. Patel et el. (15) evaluated in a highly sensitized patient group the efficiency of a desensitization protocol using IVIG and rituximab, followed by additional bortezomib if the patient was refractory to the initial treatment. They observed a mean cPRA decrease of 40%, with 89% of the patients presenting significant reduction. This study group (17) then published in 2011 a case-series involving the management of 6 highly-sensitized patients (cPRA>50%) refractory to an initial desensitization treatment with rituximab and IVIG. After administrating up to five times bortezomib at a dosis of 1.5mg/m2 over 2 weeks, each time preceded by plasmapheresis, mean cPRA dropped 27% (from 62% to 35%) and 4 patients (66.7%) could be transplanted with a negative FCXM and 75% patient survival with a follow-up ranging from 6 to 17 months post-transplantation. Overall there were two deaths, both attributed to infection and sepsis. Two aspects of the study remain to be pointed out. First, there was notably no treated rejection episode during the follow-up period. Secondly, the investigators observed a phenomenon of antibody rebound, as previously described (42). This condition implies that sensitized transplant recipients need to undergo regular anti-HLA/DSA monitoring. Subsequently, they extended their initial study to a prospective case-series encompassing 30 highly-sensitized patients. Using the same immunosuppressive management (bortezomib and plasmapheresis), they obtained a mean decrease in class I and class II PRA of 76.9% and 55.6%, respectively. Over 70% of the patients were successfully transplanted with an acceptable freedom from rejection rate (73.9%), a moderate infection rate (33% at one year) and an excellent outcome (100% survival rate at one year).

More recently, the same team (19) carried out a pilot study using eculizumab, a C5 complement inhibitor, on 9 highly pre-sensitized patients (mean PRA before transplantation of 92%). Eight patients were successfully transplanted without a prospective crossmatch. One death occurred, due to perioperative purulent mediastinitis. Even with an average positive retrospective T- and B-cell flow-cytometry crossmatch, the overall outcome at 12 months was good, with 75% rate of freedom from rejection, 100% patient survival with good cardiac graft function and no record of any treated infections.

Also from the same team at Cedars-Sinai, Kobashigawa et al. (16) treated 21 pre-sensitized patients with a combination using plasmapheresis, IVIG and rituximab, and successfully transplanted the entire cohort with negative prospective CDC crossmatches. The outcome was compared between a sensitized untreated cohort and a non-sensitized cohort of patients awaiting heart transplantation. While the treated patients had an increased incidence of antibody-mediated rejection at one year, there was no significant difference in cardiac allograft vasculopathy or in overall survival at five years. 6/21

recipients had a positive retrospective FCXM, but these patients had similar outcomes in comparison with negative crossmatch patients.

We found only a minority of reported studies which use only one treatment procedure for desensitization. The pilot study using only eculizumab beside the maintenance regimen (which usually consisted of calcineurin inhibitor-based triple immunosuppressive regimen) was already described here above. We found two other studies which used a monotherapy-based desensitization protocol. Dowling and his team (20) described the clinical evolution of 4 previously non-sensitized patients who developed high PRA after a LVAD insertion. Using solely IVIG, the group observed a significant drop in the PRA shortly after initiating treatment, with a mean percent decrease in PRA at 6 months of 97 $\pm$ 2%. Holt et al. (21) studied the outcome of 13 previously sensitized patients who underwent heart transplantation with retrospective positive crossmatches. All the pre-sensitized patients of the cohort had PE or plasmapheresis on bypass, and the recipients of a crossmatch positive graft had additional plasmapheresis and cyclophosphamide postoperatively and longer T-cell depleting therapies. The incidence of infection and rejection episodes was significantly higher in the crossmatch positive cohort in the first six months but not afterward. With a survival rate of 73% at 3 years post-transplant, their protocol allowed heart transplantation across a positive crossmatch which resulted in a reasonable short-term patient survival.

We found 5 records from three study groups describing the combination of plasmapheresis or PE with IVIG. Robinson et al (22) reported 4 cases of heart transplantation in highly pre-sensitized patients. Using a procedure involving plasmapheresis and IVIG right before transplantation, they did not observe any adverse events, including no reaction during IVIG infusion or infections. At the time of publication (average post-transplant follow-up of 37 weeks), all the recipients were alive and well. Pisani et al. (23) also used the association of plasmapheresis and IVIG immediately prior to heart transplantation in 16 pre-sensitized patients. They observed a mean PRA reduction of about 20%. Despite more frequent positive crossmatches, pulmonary hypertension and requirement for mechanical circulatory support, there was no significant difference in mortality between sensitized and non-sensitized patients at a mean follow-up of 22 months after transplantation. A team in Philadelphia (24,25) compared the outcomes of sensitized heart transplant recipients after receiving one or multiple cycles of IVIG and plasmapheresis with a cohort of non-sensitized heart transplant recipients. 89% of the treated patients had a significant decline in alloantibodies levels. About one fifth of the patients experienced an antibody-mediated rejection episode during the average follow-up of 21 months. The survival in the treated patients' cohort was 89%, a significantly better rate compared to the non-sensitized control group. One of the patients was reported to have increased PRA at 57 months of follow-up, with presence of DSA, but no loss of function of the graft. This patient merits further thorough immunological investigations.

Daly et al. (26) described in 2013 their experience concerning 12 cardiac transplantation performed in highly sensitized patients despite a positive CDC crossmatch. The recipients underwent plasmapheresis or PE on cardiopulmonary bypass and had post-operatively additional plasmapheresis cycles and multiples IVIG courses. The positive crossmatch heart transplant recipients had more infectious events than the recipients of hearts with negative crossmatches, which was expected as these patients underwent a deeper immunosuppressive treatment. While the positive crossmatch cohort

had significantly more severe (hemodynamically significant) AMR episodes, the total rate of rejection occurrence was similar between both groups. The allograft survival was 58.3% in the highly sensitized group, with follow-up ranging from one year and a half to fifteen years post-transplantation.

Other regimens have also been described. Jacobs et al. (27) enrolled 8 pre-sensitized pediatric patients and compared their outcomes with a group of 52 non-sensitized untreated patients. The pre-sensitized patients were treated pre-operatively with a combination of IVIG, cyclophosphamide, MMF and plasmapheresis. Half of them received a positive retrospective crossmatch transplant. The pre-sensitized cohort presented a higher rate of rejection episodes that did not reach statistical significance (1 per patient vs. 0.66 per patient, in pre-sensitized and non-sensitized, respectively) but a significantly lower overall survival (50% vs. 85%, p=0.04). Among the 8 pre-sensitized patients, 4 had a positive retrospective crossmatch, and this subgroup contributed mostly to the higher death rate observed in the pre-sensitized group, with only 1 death (25%) recorded in the negative crossmatch group.

From the same research group, Asante-Korang et al. (28) described the outcome of 70 pediatric heart transplant recipients. Using a regimen combining plasmapheresis or PE, IVIG, cyclophosphamide and rituximab depending on the degree of sensitization, they transplanted 14 highly sensitized (PRA>10%) patients with 4 recipients having positive retrospective flow-cytometry crossmatches. The recipients did not significantly suffer from a higher infection rate in comparison to the 56 control patients (PRA≤10%). While highly sensitized patients presented acute rejection episodes much earlier after transplantation, overall rates of acute rejection as well as cardiac allograft vasculopathy were similar between both groups. Survival was also comparable between the two groups with excellent rates of 93% for the highly sensitized group and 80% for the low sensitized group.

A team in Dallas, Texas assessed the efficacy of an immunomodulatory treatment in reducing antibody production in a cohort composed of highly sensitized patients with LVAD (mean peak PRA = 79%) (29). With a treatment combining cyclophosphamide, IVIG, methotrexate, MMF and IA, they obtained a PRA reduction of 65% in the treated group versus 13% in the sensitized untreated group. With these results, this group showed that even with higher maximum PRAs and a longer waiting time (110 days for the untreated group versus 290 days for the treated group), highly sensitized patients carrying a LVAD can still be successfully bridged to transplantation.

One study group in Toronto (30) which had studied the feasibility of ABOi pediatric heart transplantation reviewed their experience in heart transplantation of HLA-sensitized pediatric recipients. Using IVIG, MMF, PE, rituximab and additional plasmapheresis in positive crossmatch patients, they obtained relatively good short-term outcome, with a survival of 89% at 3 months, and an infection rate which was not higher in comparison with other cardiac surgery patients. The medium-term outcome was also encouraging, with no AMR developed after 6 months.

Lick and collaborators (31,32) reviewed the use of alemtuzumab as induction treatment for highly sensitized heart transplant recipients (PRA > 70%). After experiencing 3 successful transplantations in patients with positive retrospective crossmatches, they extended their study. Using plasmapheresis on bypass and alemtuzumab as induction treatment just after the surgery, they transplanted 8 new patients. While only one recipient had a negative retrospective crossmatch, the outcome

was overall good with only one highly pre-sensitized patient who died at 22 months postoperatively after being deprived from access to medication.

Schumacher et al. (33,34) compared 14 pediatric patients with PRA > 10%. Patients were given one single dose of IVIG (2g/kg) and repeated infusions of rituximab (375mg/m2/infusion) until the CD20 count was less than 10-20 cells per milliliter. Mean cPRA was significantly decreased, with the anti-HLA antibodies with higher starting MFI demonstrating the most dramatic decrease. 8 patients had significant cPRA decrease and were considered as “responders”. These patients saw their potential donors pool increase from a mean value of 10% to 85%. Interestingly, 6 of them required more than one infusion of rituximab to reduce their PRA. 5 treatment-responders and 3 non-responders underwent heart transplantation and were well at the time of the publication, which corresponded to an average follow-up of 5 months after the last rituximab infusion. Only one patient who was a non-responder experienced a rejection episode. 3 deaths were reported: 1 responder patient who died prior to heart transplantation and 2 patients who presented a primary graft failure. The overall reported survival rate and transplanted patient survival rate were 78.6% and 75%, respectively.

Discussion

- **ABO-incompatible Recipients**

Concerning ABOi heart transplantation, most of the literature reports and experiences are in the pediatric population. While there are no standardized and worldwide accepted protocols, most studies point toward some consensus: ABOi heart transplantation seems to be feasible and relatively safe, especially if the recipients is very young, and if preoperative DSI titers are low. All the studies that we found used a process to remove the blood group specific antibodies perioperatively (PE, plasmapheresis or IA), usually on cardiopulmonary bypass, in addition to classical induction drugs (antithymocyte globulin or basiliximab) and standardized triple immunosuppressive regimen with a calcineurin inhibitor (tacrolimus or cyclosporine), an antimetabolite drug (azathioprine or mycophenolate mofetil) and quickly tapered corticosteroids. In some cases, other substances were used either to boost the immunosuppressive effect especially when the preoperative DSI titers were high (rituximab, IVIG), or to spare the kidney in case of acute renal failure (sirolimus, everolimus).

There are multiple mechanisms that could explain such good outcomes, and most of them are tightly linked to the fact that these patients are very young. Indeed, as the immune system is immature at birth, the pediatric subjects, and in particular infants (age < 12 months), still have defective alloimmune responses at the time of transplantation. Their antibody production is not fully functional and they have not yet developed antibodies to blood group antigens, which have been described to appear only at about six to eight months of age, after gut colonization (43,44). Thus, this very young population has an immunologic immaturity which might explain the success of these procedures with relatively low incidences of acute rejection despite blood group barriers. Other mechanisms which could explain the good results are the induction of immune tolerance and/or immune accommodation. While these phenomena have also been described in adults, it is reasonable to think that they might happen more often in

subjects that still have to develop their immune system in the presence of foreign antigens. In our search, immune tolerance was observed by two groups (38,45), as in some ABOi heart transplant recipients DSI disappeared. Immune accommodation is the explanation suggested by Roche et al. (39) to explain the absence of antibody-mediated rejection despite continuous presence of DSI at significant titers. These mechanisms were also suggested to occur in ABOi renal transplantation. The upper age limit at which there is a clear risk of hyperacute humoral rejection in ABOi heart transplantation remains controversial, as is the limit of DSI titers. The United Network for Organ Sharing (UNOS) guidelines has set a titer of >1:4 and recommends an age of less than two years except in specific circumstances (36).

Concerning the pediatric population, the reported studies showed very positive outcome with good to excellent patient survival that are often comparable to recipients of ABO-compatible heart grafts, with very rare hyperacute rejection episodes and few to none severe infections or morbidity. Having the option of ABOi heart transplantation seems to bring even superior results, as it lessens the waiting time on the transplantation list, diminishes the mortality on list, and hence the overall mortality. However, because of the relatively few total number of infants undergoing heart transplantation, there may be a statistical bias in the analysis of outcome. Longer follow-ups and larger cohorts are necessary to make definitive conclusions. Concerning ABOi heart transplantation in adult recipients, we only found one case series. Therefore, we cannot make any conclusions. However, it appears that adults with relatively low DSI titers can undergo ABOi heart transplantation with good short-term results.

- **HLA-sensitized ABO-compatible Recipients**

HLA-sensitized recipients were mostly studies in adult population. The desensitization regimens were varied, with the use by order of decreasing frequency of: IVIG, plasmapheresis or PE, cyclophosphamide, rituximab, bortezomib, alemtuzumab and eculizumab. Cyclophosphamide was mostly used in earlier studies, while the use of rituximab emerged later. Apart from the treatment protocols, the studies were very heterogeneous in the assays used to measure anti-HLA antibodies and define sensitization. 7 studies used CDC assay, 3 used flow-cytometry detection, 1 used ELISA methods, 7 used the Luminex® technology and 2 did not mention the techniques that were used. Five groups reported a switch toward a more accurate method of detection in the middle of the study time. Not surprisingly, the more recent the study was, the newer detection technology was used. Another variable was the definition of sensitization. While most studies (13/20 studies) used PRA or cPRA cut-off >10%, other studies set cut-off up to >70%. Finally, only 4 studies required a negative prospective crossmatch in order to proceed to transplantation, while 11 either did not require a prospective crossmatch or underwent transplantation even with a positive crossmatch. Four other studies did not give details on their requirements prior to transplantation.

In HLA-sensitized recipients, monotherapy-based desensitization protocols were a rare choice, with only 2 studies. In most studies, combinations of extracorporeal purification and immunomodulatory drugs were used. IVIG was the most used treatment (15/20 studies), followed by plasmapheresis/PE (12/20 studies). Cyclophosphamide was also very prevalent (7/20 studies), mainly in the older studies that we have included. Rituximab was used in 5 more recent studies. Results were overall good in all the studies that we found, but selection and publication bias are factors to be considered to appreciate our findings

Overview of the different drugs: the facts and the debate

Because of the variety of the desensitization regimens used for HLA-sensitized patients, we thought appropriate to discuss the different substances.

Cyclophosphamide

Cyclophosphamide belongs to the alkylating agent family which interferes with DNA replication, thus inhibiting cell division and proliferation as well as preventing the synthesis of proteins such as alloantibodies. It affects both B-cell and T-helper-cell functions, i.e. inhibition of antigen uptake and presentation, alloantibody production and, to a lesser extent, cellular immunity. Long-term use of its oral form has been limited by significant complications, such as bone marrow toxicity, hemorrhagic cystitis or development of malignancies. The intravenous administration has been associated with similar therapeutic efficacy but with much less complications.

This drug has been used in 8 of our reported studies. The dosing ranged from 0.5mg/kg to 1mg/kg. 3 of them did not report about infectious or other adverse events. 2 studies found an infection rate which was not significantly different from patients who did not receive cyclophosphamide. In one study reporting about its use in sensitized LVAD recipients, cyclophosphamide-treated patients had even lesser systemic fungal infections and CMV disease (46). With the development of newer drugs with lesser adverse effects and toxicity, cyclophosphamide is nowadays seldom used either as desensitization agent or in standard maintenance immunosuppressive regimen.

Plasmapheresis, plasma exchange, immunoadsorption

Plasmapheresis, plasma exchange and immunoadsorption are methods to remove proteins from the blood. They differ in the specificity of the removed molecules and in the price. They are associated with minor reactions such as skin rash, itching, tachycardia, headache, nausea, and paresthesia; but can also lead to major adverse events such as anaphylaxis with hypotension and airway edema. Another main undesirable effect associated especially with the lesser specific techniques, is the depletion of the coagulation factors, leading to bleeding complications.

Data in renal transplantation (47) suggest that plasmapheresis results in reduction in anti-HLA antibodies which lasts only few days, which implies that its use would be beneficial only if the transplantation occurs within days after the last treatment. For these reasons, blood epuration methods are more often used in living donor kidney transplantation, as the timing of the transplantation is predictable. It is also used in ABOi living donor kidney transplantation, most of the time associated with rituximab (48–50). In our review, all the studies reporting about ABOi heart transplantation used plasmapheresis, PE or apheresis, usually one session at the time of transplantation while on cardiopulmonary bypass, often as sole treatment and with acceptable short- and medium-term results. The reduction of donor-specific isohemagglutinins at the time of transplantation could prevent hyperacute rejection, while the medium- to long-term outcome seem to be explained by the development of either tolerance mostly in infants (51,52) and/or selective accommodation in older patients (53,54). In the HLA highly-sensitized patients, these extracorporeal purification methods were sometimes performed at the time of transplantation on bypass, but more often weeks before transplantation, with one or multiple cycles. As their use was always combined with other treatments in all but one study, we could not conclude on their efficacy.

Intravenous immunoglobulin (IVIG)

The exact mechanisms of immune modulation by IVIG are currently still not completely understood. They are known to have powerful immunomodulatory effects on auto-immune and inflammatory diseases, acting through modulation of Fc receptors, interference within the complement system and cytokines network, provision of anti-idiotypic antibodies, and effects on the function and differentiation of T cells (55). Regarding allosensitization, many potential mechanisms of action have been suggested to explain the efficacy of IVIG (56), including: induction of anti-idiotypic circuits modifying alloantibody levels, modulation of cytokine activity, interaction with antigen presenting cells through the Fc receptor, inhibition of innate immunity through disruption of complement activity, interference with the proliferation and activation of B cells.

There are compelling data showing good results of IVIG application in kidney transplantation, which suggest that this therapy can reduce allosensitization and acute rejection episodes, therefore resulting in better long-term outcome (57). In our literature review, 15 studies mentioned IVIG as part of their desensitization regimen, and only one had it as sole treatment. The dosing varied from 500mg to 3g/kg, and an empirical dose of 20g was given in some studies (22,23,25). IVIG was combined with various treatments such as cyclophosphamide (7 reports), plasmapheresis or plasma exchange (9 reports), rituximab (4 reports) or bortezomib (1 report). As such, while IVIG could be safely and successfully associated with other immunomodulatory treatments, the available data did not allow any conclusions concerning the use of IVIG alone.

In our two case reports, we describe the use of a desensitization regimen involving rituximab, plasma exchange and monthly IVIG (2g/kg). In both cases, PRA values fell after repeated IVIG infusions. While plasmapheresis in patient 2 did decrease his sensitization status, the effects were only transient before a quick rebound back to the initial titer. The other immunosuppressive treatments did not bring notable changes to the anti-HLA antibodies titers. Repeated IVIG infusions may have been the key to the long-term desensitization effect in both of our patients.

Plasmapheresis and IVIG: a frequent combination

While plasmapheresis and IVIG were rarely used as single treatments in the studies that we reviewed, their association was the most frequent regimen used in heart transplantation, with respectively one report concerning ABOi transplantation and 9 HLA-sensitized recipients. In the past, investigators had combined plasmapheresis with cyclophosphamide or antilymphocyte globulin to prevent B-cell activation and resynthesis of anti-HLA antibodies (58). IVIG represent a less toxic alternative to modulate B cells and have other immunomodulatory effects, as discussed above. Two studies used a triple desensitization regimen by adding cyclophosphamide to prevent rebound in B-cell immunoglobulin synthesis (25,27). The association plasmapheresis-IVIG was also reported in renal patients (59).

Rituximab

Rituximab is a chimeric anti-CD20 monoclonal antibody. It induces B-cell apoptosis through complement-dependent lysis, and depletes B cells not only in peripheral blood but also in the lymph nodes and to some extent in transplanted organs (60). However it does not affect plasma cells as they do not express the target antigen. It has been approved for the treatment of B-cell lymphomas, various auto-immune diseases and was reported to be effective in the treatment of antibody-mediated transplant rejection. Its use comes also with various adverse effects, such as a high association with infectious diseases and infection-related deaths. It can also allow reactivation of JC polyomavirus,

resulting in progressive multifocal leukoencephalopathy, as it has been described in patients with systemic lupus erythematosus (SLE). Some other specific complications are late onset neutropenia, thrombocytopenia, cytokine release syndrome or cardiovascular complications.

While it is not a licensed use, the literature harbors many studies recalling its application in renal transplantation and reviews summarizing the experience in this field (60). It is widely pre-operatively used in ABOi renal transplantation with outcomes that compare favorably to ABO-compatible procedures, although no strong evidence exist (61). It is also peri- and post-operatively used in HLA-sensitized recipients, with stronger evidence of its benefits, although assessment of its direct effect is difficult as it is often combined with other immunomodulatory procedures/drugs. The usual dosing is multiple cycles of 375mg/m2 but some reports suggest that the dosing could be reduced in the setting of transplantation while retaining all the efficacy (61). In our literature review, 5 reports mentioned the use of rituximab as desensitization agent for HLA-sensitized patients. In all studies, it was administered in combination with IVIG or plasmapheresis, if not both. The outcomes were overall encouraging, with a decrease of the sensitization status (cPRA) and hence access to a bigger donor pool. One explanation of the synergistic superior effects of this association in comparison to IVIG alone is that, while IVIG has many immunomodulatory properties, it does not affect the plasma cells. Rituximab, while not having a direct effect on fully differentiated plasma cells, induces the apoptosis of B cells that can indirectly reduce the antibody production (34). This association was investigated in sensitized renal transplant recipients by Vo et al. (62) who compared the outcome of IVIG with placebo versus IVIG with rituximab in an initially blind placebo-controlled trial. The trial was quickly discontinued and "unblinded" as the rituximab arm showed significant better results and less adverse events.

Bortezomib

Bortezomib is an inhibitor of the proteasome. It is approved for the treatment of refractory multiple myeloma, and has been described as rescue strategy for the treatment of refractory humoral rejection. It exerts its effects on the 26S proteasome by inhibiting the degradation of misfolded proteins, leading to plasma cell apoptosis and inhibition of antibody production. In contrast, IVIG, rituximab and antithymocyte globulin all have no direct effect on the antibody production by plasma cells. Jawdeh et al (63) reviewed its efficacy as a desensitization agent in kidney transplantation and concluded that bortezomib could provide sustained reduction in anti-HLA antibodies, allowing for increased transplantation rate. The main dreaded adverse event is, like for all immunosuppressive drugs, severe infections. Experience from its use for the treatment of multiple myeloma recounts thrombocytopenia, neutropenia, anemia and peripheral neuropathy as other possible undesirable effects (64).

We found only one group which published data about its application to heart transplantation (15,17,18). Associated to rituximab and IVIG, it was found that even the initially refractory patients saw significant reduction in PRA. In a prospective study (18) examining its effect if associated with plasmapheresis, 73% of a highly sensitized population group (cPRA>50%) were transplanted with a 100% survival rate at one year. Because of the limited data, we could not determine the relative contribution of each components of this desensitization regimen. The use of bortezomib has been reported in lung (65) and kidney transplantation (66,67). Associated to plasmapheresis, rituximab and IVIG, this proteasome inhibitor demonstrated a benefit in a group of 18 broadly sensitized (cPRA≥80%) lung transplant candidates with 50% undergoing transplantation with negative virtual crossmatch. In renal transplantation, it was combined with rituximab

and plasmapheresis (66) or IVIG (67), and resulted in more transplantation conversion. However, available data are still limited with no consistent results.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody which targets CD52, a molecule densely present on mature T and B lymphocytes as well as natural killer cells. It leads to cells death though antibody-mediated cellular cytotoxicity (ADCC) and complement activation pathways. It is used in the treatment of various hematologic malignancies, and has been described as a tolerogenic induction agent in solid-organ transplantation. Its use was significantly associated with an increased risk of opportunistic infections, in particular CMV replication/disease. It induces profound and sustained lymphopenia (several months), which is the reason why the introduction of antimetabolite drugs (mycophenolate mofetil, azathioprine or sirolimus) is often delayed post-transplantation.

Alemtuzumab was used after transplantation by Lick and collaborators (31,32). After undergoing plasmapheresis on cardiopulmonary bypass, it was administered as induction agent together with a maintenance immunosuppressive regimen containing a calcineurin inhibitor and quickly tapered corticosteroids. With almost no infectious occurrences and excellent short- and mid-term survival, alemtuzumab showed promising results to successfully bring highly sensitized patients to heart transplantation. In the literature, a group from Pittsburgh, USA (68) administered this drug to cardiac transplant recipients who were not necessarily highly sensitized and found a similar 12-months survival rate but with a better freedom from rejection, despite lower calcineurin inhibitor levels and no use of steroids.

Eculizumab

Eculizumab is a monoclonal antibody targeting the terminal complement component C5. It can hence inhibit the complement cascade that is activated through binding of DSA to the antigen on the surface of donor cells. It has been approved for the treatment of paroxysmal nocturnal hemoglobinuria as well as hemolytic uremic syndrome (69).

We found one abstract reporting the use of eculizumab for highly sensitized heart transplant patients. Infused perioperatively and postoperatively as single desensitization agent, together with antithymocyte globulin induction and standard triple immunosuppressive maintenance regimen, eculizumab facilitated heart transplantation despite sensitization with acceptable outcomes at 12 months. Stegall et al. (70) treated 26 highly sensitized recipients of living donor renal transplants with eculizumab and found a significant decrease in AMR (7.7% vs. 41.2%, p=0.0031) compared to a historical control group. Moreover, the treatment was well tolerated with no increase in the infections rate. However, more data are needed as some reports are less favorable (71) and the duration of such therapy has not yet been established. Finally, the current cost of eculizumab may limit extensive studies and a wider use in transplantation.

Other substances

Some novel substances are currently under investigation by various research groups.

Belimumab, a fully human recombinant monoclonal antibody to BAFF, is currently approved for SLE. A phase II clinical trial of desensitization in patients awaiting kidney transplantation has been conducted, but was terminated early for a lack of efficacy (NCT01025193). Another phase II trial analyzing its efficacy in preventing kidney allograft rejection also did not show promising results (NCT01536379).

IgG endopeptidase is an enzyme that cleaves IgG, which may be useful in lowering antibody levels in sensitized patients prior to receiving solid organ transplantation. Phase II trials are taking place (NCT02224820).

Tocilizumab is a humanized monoclonal antibody which antagonizes the Interleukin-6 receptor and is FDA-approved for polyarticular juvenile idiopathic arthritis. A recent phase I/II trial of tocilizumab treatment for 10 sensitized renal patients refractory to standard desensitization protocols resulted in 5 transplantation that were CDC crossmatch negative (72).

Conclusion and perspectives

Sensitization against HLA antigens is a growing problem in the field of both adult and pediatric cardiac transplantation, because of the improved surgical outcomes and the development of ventricular assist devices which increase the transplant candidates but also the immunization risks. Morbidity and mortality due to rejection still hinder the outcome of heart transplantation. With the development of immunological assays, we can better understand and appreciate the state of immunization of the patients on the waiting list. The emergence of newer specific drugs allows nowadays an array of possible treatments.

There are still many questions about the best approaches: timing of desensitization, drugs used and dosing. We observe the increasing use of newer biologics (bortezomib, eculizumab, alemtuzumab) for desensitization. While preliminary results have shown a relative safe adverse events profile, we still lack data about their efficacy. These new treatments may offer a good alternative for patients that are highly sensitized and/or refractory to a first line of desensitization treatment. From our experience, even if it initially did not show drastic changes, repeated monthly IVIG eventually achieved desensitization of 2 highly sensitized heart transplant recipients.

A key limitation to our findings is the quality of the studies that we found. Randomized controlled prospective trials bring the best evidence but cannot be conducted when patients can be facing a life-death situation. Double-blind trials are also difficult to conduct for the same reason. Studies on a larger scale would bring more information and more meanings to the conclusions. Another problem is the lack of uniformity between studies (inclusion criteria, outcomes) making comparison difficult or impossible. Biases are also to be pointed out. Despite our will to systematically review the literature, selection bias has to be considered as well as publication bias.

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