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ORIGINAL ARTICLE

Desensitization for renal transplantation: depletion of donor-specific anti-HLA antibodies, preservation of memory antibodies, and clinical risks

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Summary

Desensitization protocols reduce donor-specific anti-HLA antibodies (DSA) and enable renal transplantation in patients with a positive complement-dependent cytotoxic cross-match (CDC-CXM). The effect of this treatment on protective antibody and immunoglobulin levels is unknown. Thirteen patients with end-stage renal disease, DSA and positive CDC-CXM underwent desensitization. Sera collected pre- and post-transplantation were analysed for anti-tetanus and anti-pneumococcal antibodies, total immunoglobulin (Ig) levels and IgG subclasses and were compared to healthy controls and contemporaneous renal transplant recipients treated with standard immunosuppression alone. Ten patients developed negative CDC-CXM and enzyme-linked immunosorbent assay (ELISA) and underwent successful transplantation. Eight recipients achieved good graft function without antibody-mediated or late rejection, BK virus or cytomegalovirus infection. One patient had primary non-function due to recurrent oxalosis, and one patient with immediate graft function died from septicaemia. Seven recipients required post-operative transfusion and three developed septicaemia. DSA remained negative by ELISA at 12 months, but were detectable by Luminex®. Anti-tetanus and anti-pneumococcal antibodies, total Ig and IgG subclasses were below the normal range but comparable to levels in renal transplant recipients who had not undergone desensitization. Desensitization protocols effectively reduce DSA and allow successful transplantation. Post-operative bleeding and short-term infectious risk is increased. Protective antibody and serum immunoglobulin levels are relatively preserved.

Introduction

For patients with end-stage kidney disease (ESKD) renal transplantation is the preferred form of renal replacement therapy, with improved mortality and quality of life compared to dialysis. Allo-antibodies against human leukocyte antigen (HLA) are formed by exposure from previous blood transfusions, tissue allografts, pregnancy [1], or rarely cross-reactivity following infection [2,3] or its treatment [4]. Patients with an elevated panel reactive antibody (PRA) titre are disadvantaged because of difficulty finding an immunologically compatible graft. A positive complement-dependent cytotoxic T-cell cross match (CDC-CXM) remains an absolute contraindication

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to transplantation [5]. A positive B cell crossmatch (BXM) caused by DSA is a risk factor for acute rejection and premature allograft loss [6–8].

In Australia, 12% of ESKD patients waiting for a deceased donor renal transplant have PRA>50% [9]. Although waiting times for unsensitized recipients have not changed over the last 10 years, they have increased significantly for sensitized patients. In the US in 2002, the waiting time for listed ESKD patients with PRA > 10% was nearly twice that for unsensitized patients [10]. In 2006, 14.9% of listed patients had PRA > 40%. Prolonged waiting time on dialysis brings the attendant complication of higher mortality rates post transplantation [11]. Sensitized patients have a higher risk of rejection and overall poorer graft survival post-transplantation [9].

The advent of desensitization techniques [plasmapheresis, intravenous immunoglobulin (IVIg), anti-CD20 monoclonal antibody: rituximab] facilitates transplantation by reducing or eliminating donor-specific antibodies (DSA), and producing a negative CDC-CXM [12–14]. Rituximab effectively depletes circulating B cells [15], and both plasmapheresis and IVIg reduce DSA titres. The use of more sensitive solid phase assays, such as enzyme-linked immunosorbent assays (ELISA) and Luminex®, allow more accurate monitoring of the anti-HLA repertoire [7,16]. However, significant immunosuppression is not without risk, particularly infection, in an already susceptible population. There are very little data regarding infectious complications and protective antibody levels in patients who have undergone such potent immunosuppression [17]. We sought to determine the concomitant effect of desensitization treatment on long-term DSA levels and allograft outcome, memory antibody and immunoglobulin (Ig) levels and overall infection risk.

Methods

Highly sensitized patients and desensitization protocol

Thirteen sequential sensitized ESKD patients from a single transplant centre were identified and ten (10) were transplanted between November 2005 and July 2009. All had live donors with a positive CDC-CXM and DSA. Elective desensitization was undertaken: a single injection of Rituximab (Roche, Basel, Switzerland) at 375 mg/kg was administered 14 days prior to transplantation, followed by five alternate day sessions of plasmapheresis (complete blood volume exchange with 4% albumin and fresh frozen plasma) and intravenous immunoglobulin (Sandoglobulin, Novartis, Basel), 0.1 g/kg for 4 doses then 2 g/kg for the final dose. Oral mycophenolate mofetil (Cellcept, Roche) 1 g twice daily was commenced 12 days prior to transplantation. Recipients were successfully desensitized and transplanted when CDC-CXM and ELISA became negative. All patients received induction immunosuppression (basiliximab, Simulect®, Novartis, Basel, Switzerland) followed by standard immunosuppression (prednisolone, mycophenolate mofetil, tacrolimus), three additional sessions of plasmapheresis post-transplantation, CMV prophylaxis (valganciclovir for 3 months), and *Pneumocystis carinii* prophylaxis (trimethoprim-sulfamethoxazole or inhaled pentamidine for 6 months). Patients were screened 3-monthly for BKV and CMV according to a standardized protocol. Infection data were compared with contemporaneous renal transplants ($n = 226$) performed at the same centre during the same time period, receiving identical antibiotic prophylaxis and viral monitoring. Data were analysed using Intercooled STATA (v11.0; STATA Corporation, College Station, TX, USA).

Donor-specific antibody monitoring

Recipient serum taken prior to desensitization and before and after each plasma exchange session was assessed for DSA by CDC-CXM, ELISA (Quik-ID class I and II, GTI Diagnostics, WI) and either Lifecodes class I and class II ID or single antigen beads (GenProbe, Stamford, CT, USA) as described previously [7]. American Society of Histocompatibility and Immunogenetics (ASHI) accredited CDC incubation assays were performed on separated T- and B-cells, isolated by CD2 and CD19 Dynabeads® (Invitrogen, Carlsbad, CA, USA) respectively. Longer incubation times were used to increase class I assay sensitivity: 40 min incubation following combination of lymphocytes and serum, and again after addition of complement, compared to 30 and 35 min respectively for class II cross-matching. AHG enhancement was not used and ≥10% cell death was considered positive. Auto-cross-matches were performed to detect autoantibodies and all positive CDC-CXM were performed with DTT-treated sera. The ELISA employed microwells coated with HLA Class I and Class II glycoproteins and was analysed using standard techniques (Bio-tek ELx800 series microplate reader, Progen Scientific Ltd, London, UK). Luminex® beads were incubated with patient serum in a 96-well Millipore filter plate, in conjunction with the manufacturer's reagents. The mixture was incubated for 30 min in the dark, at room temperature. A phycoerythrin (PE)-conjugated anti-human IgG was then added to each well and incubated. All assays included manufacturer's positive and negative controls. Data acquisition was performed using Lifematch Fluoroanalyzer® (GenProbe), imported into the Lifematch QuickType™ Analysis software (GenProbe) and analysed.

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Measurement of peripheral blood B cells

Whole blood was collected from desensitized patients pre- and post-rituximab administration. Circulating B cells were detected with Simultest® antibodies (anti-CD3 clone SK7 and anti-CD19 clone 4G7, BD Biosciences, NJ) using flow cytometry (FACScan; Becton Dickinson, San Jose, CA, USA).

Antibody assessment

Serum samples were collected from successfully desensitized (negative CDC-CXM and ELISA) recipients pre-transplantation and 1, 3 and 6 months post-transplantation. Samples were analysed for anti-tetanus antibodies (tetanus toxoid antibody by ELISA LP510 123, SouthPath, Bedford Park, Australia, analysed by Benchmark Plus ELISA reader, Bio-Rad, Hercules, CA, USA), anti-pneumococcal IgG antibody (Pneumococcal capsular polysaccharide assay kit MK 012, The Binding Site, Birmingham, UK; analysed by Triturus EIA Analyser, Grifols, Barcelona, Spain) and immunoglobulin subclasses (BN™Combi kit, LK 001 TB, The Binding Site, analysed on Beckman Coulter Image 800 immunochemistry system, Beckman Coulter, CA, USA). Anti-tetanus and anti-pneumococcal antibodies were chosen because all recipients were initially immune. The same testing was repeated on contemporaneous renal transplant recipients ($n = 15$) who had not undergone desensitization but received comparable immunosuppression (induction with basiliximab and immunosuppression with prednisolone, tacrolimus and mycophenolate mofetil).

Results

Effect of desensitization on anti-HLA antibodies

Thirteen consecutive highly sensitized patients (eight female, five male) with 14 previous renal allografts and over 100 cumulative years on dialysis underwent desensitization (Tables 1 and 2). Six patients with a prior transplant had undergone transplant nephrectomy to remove the antigen-bearing kidney, and five (out of these six) were successfully desensitized. Four patients had not had a prior transplant and DSA were presumably secondary to pregnancy and/or transfusion (all were women with spouses as living donor). Ten patients (77%) became CDC-CXM negative. ELISA for DSA also became negative allowing transplantation, although DSA (both class I and II) were still detectable by Luminex® in six patients, albeit at reduced levels (Tables 1 and 2). Measurements (both ELISA and Luminex®) continued on a 3-monthly basis: ELISA remained negative at 12 months (data not shown), and DSA remained detectable by Luminex® (single antigen bead) in three successfully transplanted recipients (Tables 1 and 2). Three patients remained CDC-CXM positive with detectable DSA and were not transplanted (Tables 1 and 2); further desensitization treatment was deferred due to the development of cutaneous nocardiosis in one patient, and failure to reduce DSA in the remaining.

Clinical course [18]

Eight recipients (80%) achieved good allograft function (baseline serum creatinine <160 μmol/l, Tables 1 and 2). One patient developed primary non-function due to recurrent oxalosis and another patient with immediate graft function died from sepsis. Seven patients (70%) received significant transfusions (≥5 units) post-operatively despite normal pre-operative coagulation studies (international normalized ratio, activated partial thromboplastin time and platelet count); one patient required additional activated factor VII. One patient refused transfusion on religious grounds (Hb 46 g/l). There were three episodes of acute cellular rejection (<2 months post-transplant), but no humoral rejection and no late rejection. Patients received protocol biopsies within the first 2 weeks ($n = 9$) and at 6 months post-transplantation ($n = 8$); one was C4d positive at 6 months but did not meet the criteria for chronic antibody-mediated rejection and did not have DSA by Luminex®.

In the desensitized cohort ($n = 10$), one patient developed cutaneous varicella zoster virus (VZV) infection and three patients (30%) developed septicaemia within 1 month post-transplant resulting in one death (day 12). No desensitized patients developed BKV or CMV. During the same observation period, 226 additional patients not requiring desensitization were transplanted at the same centre. Post-transplant, there were 13 admissions for urinary tract infection, 21 admissions for respiratory tract infection (two fungal), 21 admissions for infections at other sites (including one case of cryptococcal meningitis) and three cases of septicaemia (1.3%). Twenty-two patients (9.6%) had BKV (in blood and urine), 3 (1.3%) had JCV in blood and urine, and 48 (21%) were diagnosed with CMV requiring treatment.

Peripheral blood B cells

The B cell population was assessed pre- and post-rituximab using flow cytometry, and demonstrated a sustained absence in the peripheral circulation up to 6 months following transplantation (data not shown).

Protective memory antibody status and immunoglobulin levels

Anti-tetanus and anti-pneumococcal antibody levels in desensitized recipients pre- and post-transplantation

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Table 1. Demographic, anti-HLA antibody profile and outcome data for patients undergoing desensitization for kidney transplantation.

Patient/ntnt gender	Age at transplant (years)	Time on dialysis (years)	Previous transplants/ nephrectomy (no.)	PRA peak (%), DSA	Luminex MFI pre-desensitization	Luminex MFI pre-transplant	Luminex MFI at last f/u	Time to last f/u (years)	Creatinine at last f/u (μmol/l)
1 F	36.2	13.6	2/Y (2)	99%	17000	0	0	4.18	127
				A2	17000	0	0		
				A68					
2 M	48.7	4.8	2/Y (1)	60%	5654	0	0	3.75	90
				DR7					
3 M	53.3	25.8	2/Y (1)	99%	18000	0	0	3.6	115
				A24					
4 M	52.6	29.3	1/N	99%	14220	11000	11181	No transplant	–
				A24	7181	167	11742		
				DR4	2532	176	2849		
				DR14	6307	–	7950		
				DQ8					
5 F	27	7.9 months	1/N	99%	9000	4500	0	2.53	154
				A31	14086	0	0		
				B27					
6 M	49	16.5	1/N	72%	4749	0	291	2.0	93
				A*0201	5308	3415	12536		
				DQ7					
7 F	53.4	2.5	0/–	0%	10085	0	0	1.21	112
				DRB1*0408					
8 F	61.8	1.5	0/–	52%	2443	1038	0	–	Primary
				B7					non-function
9 M	42.4	6	2/Y (1)	99%	2862	0	0	1.33	142
				B7	17109	6154	3694		
				DQB*05051/					
				DQA0302					
10 F	67.6	7.7 months	0/–	80%	7582	–	–	No transplant	–
				A2	4735	12141	20277		
				DR4					
11 M	54.7	4.37	2/Y (1)	99%	6221	4518	5762	No transplant	–
				A23	3162	1692	2766		
				B35	6375	4584	8000		
				B44	16948	13409	15192		
				DR7	15657	13231	8002		
				DR11	18539	16140	15590		
				DRw53	17088	12021	–		
				DQ7					
12 F	29.7	3.41	1/Y (1)	99%	2577	709	961	7.4 months	157
				A1					
13 F	62.9	1.17	0/–	99%	7130	840	0	12 days	120
				A24	6885	0	0		
				A68	7870	1178	0		
				B7					

exceeded the minimum antibody level required for immunity throughout the observation period. IgG subclasses were reduced by up to 50% compared with the healthy population (normal) reference range. However, all antibody and IgG levels were within the reference range established by our standard, non-desensitized renal transplant recipient group (Fig. 1a–g). There was no consistent trend in the levels of antibody or immunoglobulin following desensitization.

Discussion

An increasing number of patients on the deceased donor kidney transplant waiting list are highly sensitized [19].

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Table 2. HLA typing of successfully desensitized recipients and respective donors.

Patient	HLA-A	HLA-B	HLA-DR	Donor
1 Recipient Donor	30,3	18,6	3,4	Sister-in-law
	2,68	44,–	11,4	
2 Recipient Donor	1,32	7,44	1,15	Brother
	1,32	7,64	7,15	
3 Recipient Donor	3,26	35,3	1,13	Daughter
	3,24	35,6	1,13	
5 Recipient Donor	2,2	39,7	1,4	Mother
	2,31	27,7	1,4	
6 Recipient Donor	30,31	51,55	1,14	Son
	2,29	44,–	4,15	
7 Recipient Donor	25,28	35,18	13,15	Husband
	1,11	35,57	1,4	
8 Recipient Donor	02,02	15,57	0301,0401	Husband
	2,–	7,62	0404,1104	
9 Recipient Donor	1,2	51,57	7,11	Wife
	23,26	7,37	1,7	
12 Recipient Donor	2,–	51,60	8,13	Mother
	1,2	51,50	8,13	
13 Recipient Donor	2,–	44,50	7,15	Husband
	24,28	7,71	15,15	

Ten patients (out of 13 – 77%) underwent successful desensitization for renal transplantation, resulting in the reduction of donor-specific anti-HLA class I and/or class II antibodies detected by Luminex®. These patients were CDC-CXM and ELISA negative prior to transplantation. Three patients were unable to be successfully desensitized and not transplanted.

Different approaches have attempted to decrease anti-HLA antibody levels and improve successful transplantation. Plasma exchange, rituximab and immunoglobulin effectively remove DSA, but increase total immunosuppression. Infection remains a major cause of morbidity and mortality post-transplantation [18], and neither post-transplantation antibody status nor the potential infective risk of desensitization has been extensively reported. The major findings from this analysis of patients treated with the combination of rituximab, plasma exchange and IVIG are the high rate of bleeding, infectious complications, the persistence of donor-specific antibody detectable by Luminex® in the presence of good allograft function, and relative preservation of non-HLA memory antibody levels.

The majority of our cohort (77%) were successfully desensitized and subsequently transplanted, with good clinical outcomes in 80% of patients. There were significant post-operative transfusion requirements, with 70% of patients receiving at least five units of blood, despite normal clotting studies. These complications have not been reported in other case series of desensitized patients, but have led to a change in our coagulation profile monitoring with measurement of fibrinogen and factor VII levels, and administration of activated Factor VII after five units of blood and evidence of continued bleeding. Infectious complications were confined to the early (<1 month) post-transplant period. Three patients (30%) developed septicaemia, leading to one death, a higher rate than the non-desensitized cohort (prevalence 1.3%, no deaths). Data regarding the risk of infection and infection-related death following the use of rituximab in solid organ transplantation are increasingly reported [17,20,21]. The risk of infection was potentially increased due to a combination of rituximab and plasmapheresis, despite Ig replacement. There was a lack of serious, late infections observed in the follow-up period (up to 4.18 years); in particular, neither BKV nor CMV infections occurred.

This is the first study to report antibody and immunoglobulin (Ig) levels following desensitization. Anti-tetanus and anti-pneumococcal antibodies were used as markers of immunologic memory. Whilst levels exceeded that required for immunity they were relatively lower in the non-desensitized cohort and did not fall post-transplant. The higher level in the desensitized patients may be a reflection of IVIg replacement in the early phase and endogenous production in the medium-term. ELISA is not a functional measurement of antibody biological activity, although effector mechanisms presumably remain intact, regardless of Ig origin (replacement or self-synthesis). IgG subclasses play an important role in immunity to extracellular bacteria by opsonization, enhancing phagocytosis, neutralizing bacterial toxins and activating complement. Rituximab has not been shown to significantly affect serum Ig levels when treating haematological malignancy [22]. However, the effect of desensitization on Ig is not documented, and current reference ranges are applicable only to immunocompetent adults. Serum Ig levels in our desensitized cohort are comparable with the new range established by our reference group. Despite effective reduction of DSA, there appears to be no significant negative effect on long-lived antibody levels. Bone marrow-resident plasma cells (CD20-CD52-CD138+) and memory B-cells (CD20+CD52+CD138-) are primarily responsible for protective antibody production. Rituximab preferentially affects naïve and memory B-cells, but not plasma cells; rodent studies have demonstrated plasma cell maintenance and serological memory [23]. Sensitivity of CD20+ B-cells to elimination is also dependent upon the microenvironment [24]; splenic B-cells, particularly within the marginal zone, appear to be more resistant to anti-CD20 therapy. This phenomenon also explains relative preservation of anti-pneumococcal antibodies that are maintained despite profound reductions in circulating B-cells [25].

Many protocols for anti-HLA-desensitization exist [26–28], with varying success rates. Plasmapheresis alone may effectively remove the antibodies [29] and prevent

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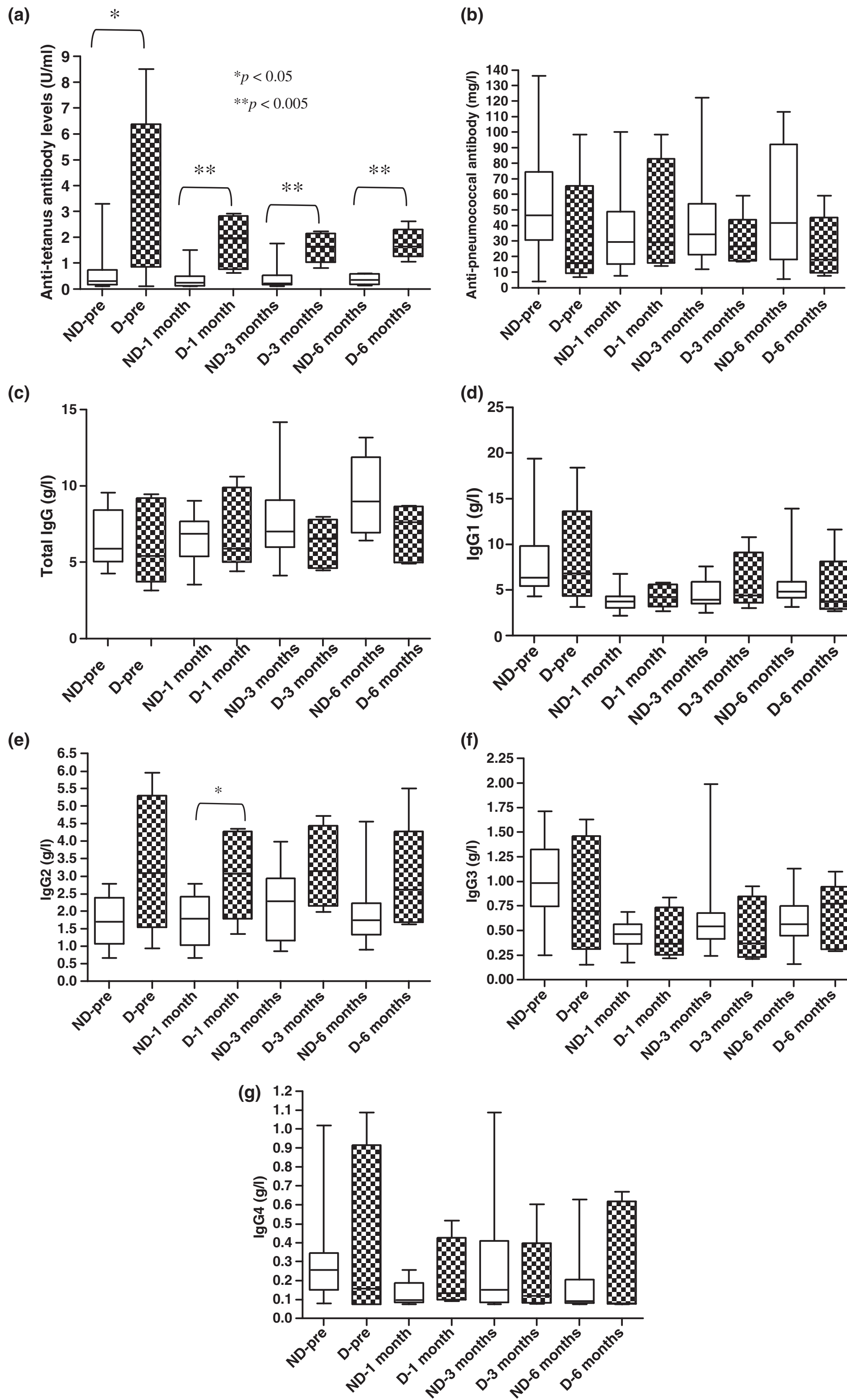

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Figure 1 Anti-tetanus antibody (a), anti-pneumococcal antibody (b), total IgG (c) and IgG subclass (d–g) levels in patients who were desensitized (D), compared to patients not desensitized (ND) prior to kidney transplantation. Measurements were taken pre-transplantation (and prior to desensitization), as well as 1, 3 and 6 months post transplantation. Antibody and immunoglobulin levels were reduced by up to 50% when compared with the reference range for immunocompetent individuals. These levels, however, were within the range provided by our new 'reference group' (non-desensitized renal transplant recipients on triple immunosuppression). Data are expressed as median (IQR) and error bars at 10th to 90th percentiles.

development of hyperacute rejection [30,31]. However, these studies have demonstrated high rates of rejection and graft loss [30], and the problem of rapid antibody re-emergence remains [32]. IVIG, which numerous immunomodulatory properties, has been successfully used to decrease allosensitization and improve transplantation rates in highly sensitized patients [26–28]. IVIG alone has been demonstrated to be less effective compared to combination desensitization treatments [33], although this may be related to overall DSA titre. Rituximab employed as a single agent has safely, but modestly, reduced anti-HLA antibodies pre-transplantation [34]. The combination of IVIG, plasmapheresis and rituximab (with and without splenectomy) has provided effective reduction of DSA in other studies [33,35], including deceased donor transplantation [36]. The rate of conversion to a negative CDC-CXM (>75%) in this study was similar to other patient cohorts [13,33,35], as were our results (80% graft survival, 90% patient survival, no antibody-mediated rejection). The use of more sensitive solid phase assays, such as Luminex®, show reduced but persistent antibody levels, in keeping with previous studies [37]. Importantly, only one transplanted patient developed C4d staining on protocol biopsy. The absence of a measurable deleterious effect of low-level DSA may be due to the development of graft accommodation [38], or persistent non-complement fixing DSA (IgG subclass 2 or 4). The subsequent effect of B-cell re-emergence and ongoing alloantibody formation on graft function is not yet known but might contribute to long-term allograft damage and longer-term follow-up is required to confirm this.

This study also raises the issue of long-term DSA monitoring. Luminex® is the most sensitive technique to detect DSA, and CDC the least sensitive assay. Luminex® detects all HLA-antibodies, including non-complement fixing IgG antibodies that may lack clinical relevance; over-reliance on this technique may reduce the chance of transplantation. Desensitized patients are clearly at greater risk of antibody-mediated rejection and allograft loss due to chronic damage [39,40]. Monitoring for an anamnestic immune response is prudent, although this may vary depending on the class and epitope of DSA [41], and is most effective when performed using single antigen beads that can track subtle changes in DSA. There is also evidence that DSA may rebound in response to inflammation [42], arguing for regular post-transplant monitoring particularly following infection or surgery. However, there are few studies looking at regular post-transplant antibody monitoring it is not known how often DSA is deleterious, at what level, and how long it may take to cause graft dysfunction.

We conclude from this study that desensitization decreases (but does not eliminate DSA), and facilitates successful transplantation in previously incompatible recipients. This study highlights the increased risk of serious post-operative bleeding and infection. Desensitization does not significantly decrease protective antibody or Ig levels when compared to standard transplant recipients. Further follow-up is required to establish the long-term effect of these protocols on the incidence of infection and malignancy.

Authorship

NMR: contributed to study design, data collection and analysis, writing and revision of the manuscript; HSE: contributed to collection and analysis of patient samples; RY: contributed to collection and analysis of patient data; SK: contributed to collection and analysis of patient samples; ET: contributed data; GDB: contributed to collection and analysis of patient samples, in addition to revision of the manuscript; NRB: contributed to study design; DG: contributed data; GRR: contributed to study design; PTC: contributed to study design.

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