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Article type : Original Report

TITLE PAGE

Title: Seroresponses and safety of 13-valent pneumococcal conjugate vaccination in kidney transplant recipients

Running headline: Pneumococcal vaccination in kidney transplant recipients

Word count: 3538

Number of tables: 3

Number of figures: 2

Funding: None

Keywords: Pneumococcal antigen, Serotype-specific antibody response, Luminex technology, Kidney transplantation.

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ABSTRACT

Background

Conjugated pneumococcal vaccine is recommended for kidney transplant recipients however their immunogenicity and potential to trigger allograft rejection though generation of de-novo anti-human leucocyte antigen antibodies has not been well studied.

Methods

Clinically stable kidney transplant recipients participated in a prospective cohort study and received a single dose of 13-valent conjugate pneumococcal vaccine. Anti-pneumococcal IgG was measured for the 13 vaccine serotypes pre and post vaccination and functional anti-pneumococcal IgG for 4 serotypes post-vaccination. Anti-human leucocyte antigen antibodies antibodies were measured before and after vaccination. Kidney transplant recipients were followed clinically for 12 months for episodes of allograft rejection or invasive pneumococcal disease.

Results

Forty-five kidney transplant recipients participated. Median days between pre-and post-vaccination serology was 27 (range 21-59). Post-vaccination, there was a median 1.1 to 1.7-fold increase in anti-pneumococcal IgG antibody concentrations for all 13 serotypes. Kidney transplant recipients displayed a functional antibody titre ≥1:8 for a median of 3 of the 4 serotypes. Post vaccination, there were no de novo anti-human leucocyte antigen antibodies, no episodes of biopsy proven rejection or invasive pneumococcal disease.

Conclusion

A single dose of 13-valent conjugate pneumococcal vaccine elicits increased titres and breadth of functional anti-pneumococcal antibodies in kidney transplant recipients without stimulating rejection or donor specific antibodies.

MANUSCRIPT

INTRODUCTION

Pneumococcal vaccination is widely recommended for solid organ transplant recipients to prevent community acquired pneumonia and invasive pneumococcal disease.[1,2] The incidence of invasive pneumococcal disease is estimated to be up to 41 times higher in transplant recipients compared with the general population,[3,4] with rates in kidney transplant recipients (KTRs) of 104 per 100,000 transplanted patients per year.[5] There is also an increased risk of mortality from pneumococcal pneumonia and invasive pneumococcal disease compared with the general population.[6] Case fatality rates in transplant recipients range from 10% in young adults to 21% in those over 65 years.[6]

The main virulence factors of *Streptococcus pneumoniae* are the capsular polysaccharides, which can inhibit phagocytosis and antibodies against the polysaccharides protect against invasive infection.[7] Antibodies against pneumococcal capsular polysaccharides are generated through colonisation or disease[8] however these antibodies can decline, either quantitatively or functionally over time. Pneumococcal vaccination focuses on inducing or boosting serotype specific antibody concentration with the additional advantage of conjugate vaccination of T-cell recruitment potentially resulting in improved functional antibody.[8]

Two types of pneumococcal vaccines are currently licensed and available for routine use: the pneumococcal polysaccharide vaccines (PPV) and pneumococcal conjugate vaccines (PCV). PPV consists of purified pneumococcal polysaccharides that act as T-cell independent type two antigens. These antigens induce a restricted IgG response and poor generation of memory B cells.[9] In contrast, PCV was developed to enhance immunogenicity by covalent conjugation to carrier proteins. Peptides from the carrier proteins interact with T cells via Major Histocompatibility Complex (MHC) Class 2 receptors on antigen presenting cells, recruiting T cell responses against the conjugated polysaccharide antigens. T helper cells can promote B cell differentiation into antibody producing plasma calls or memory B cells.[10,11] The generation of this immunologic memory may be crucial in solid organ transplant recipients, in whom immunity to pneumococcus wanes quickly after polysaccharide vaccination. Lindemann *et al* demonstrated a 3-fold decrease in antibody titers two years following PPV23 vaccination in kidney transplant recipients.[12]

In immunocompetent patients, evidence for the effectiveness of pneumococcal polysaccharide vaccine in reducing IPD is poor.[13,14] A Cochrane review of 23-valent pneumococcal polysaccharide vaccine demonstrated an efficacy of 74% in protecting against vaccine serotype IPD. There was no effect in protection against all cause pneumonia and mortality.[15] In contrast, pneumococcal conjugate vaccines have been shown to prevent vaccine type bacteremic and non-bacteremic pneumonia, and invasive pneumococcal

disease.[16] Current international guidelines[1] recommend both PPV and PCV for kidney transplant recipients (KTRs) however immunogenicity and safety data is scarce. Laboratory outcomes following pneumococcal vaccination can be tested via serotype-specific IgG concentrations or functional antibody concentration, measured by opsonophagocytic assay (OPA).[17-20] Serotype specific antibody concentration identifies and quantifies the presence of pneumococcal antibody while OPA provides information as to whether these antibodies are capable of opsonizing and killing pneumococci. OPA are particularly important to assess in immunocompromised patients or those with pre-existing immunity as there may be discordance between antibody concentration and opsonic concentrations.[21]

A key safety issues to consider when recommending vaccination to solid organ transplant recipients (SOTR) is the risk that vaccination may trigger allograft rejection. It is hypothesized that vaccination could stimulate graft rejection through stimulation of alloreactive T and B cells.[22] This is particularly relevant to adjuvanted PCVs that are specifically bioengineered to increase immune activation. There have not been any studies specifically examining the development of de-novo HLA antibodies nor graft rejection in SOTR vaccinated with PCVs.

The aims of this study were to examine serological and functional responses to the 13-valet conjugated pneumococcal vaccine (PCV13) among KTRs and determine if PCV13 vaccination is associated with the development of de novo donor specific antibodies (DSA) or allograft rejection. The hypothesis of this study was that PCV13 was immunogenic and safe in KTRs.

MATERIALS AND METHODS

Setting

A prospective cohort study, assessing seroresponses of KTRs was performed at Monash Health, a tertiary referral center in Victoria, Australia. Monash Health is a 1500-bed academic health service that performs approximately 90 kidney transplants per year and has 850 kidney transplant recipients who receive ongoing follow-up care. Investigators attempted to contact all KTRs by post, to offer vaccination with the PCV13 vaccination and an invitation to participate in the study. Due to the financial cost of laboratory testing for this study, the first 58 KTRs consenting patients were then enrolled in the study at a subsequent transplant clinic visit. The study was approved by the human research ethics committee of Monash Health (13085A) and written informed consent was obtained from all participants.

Inclusion and exclusion criteria

KTRs were eligible to participate if they were aged ≥18 years, were at least 3 months post-transplant and had not received pneumococcal conjugate vaccine previously. KTRs with a

known allergy to pneumococcal vaccine, recently augmented immunosuppression to treat rejection, or infectious illness just prior to or at the time of the study were excluded.

Data collection

Patient demographics, comorbidities, cause of end-stage kidney disease, and medication use including current and previous immunosuppressive drug regimen were assessed at vaccination. Kidney transplant function was assessed by the glomerular filtration rate estimated (eGFR) using the CKD-Epi formula. KTRs were specifically asked if they had previously received pneumococcal polysaccharide vaccine and results were recorded as yes, no or unknown. Additional documentation to corroborate receipt of vaccination was sought in the medical record.

Vaccination procedures

Participants attended the hospital's outpatient vaccination clinic where they received a single 0.5ml intramuscular dose of PCV13 (Prevnar-13™/Prevernar-13™, Pfizer).

The vaccine contained polysaccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F individually conjugated to a nontoxic diphtheria toxin cross-reactive material CRM_{197} protein. The vaccine contained 2.2 µg of each polysaccharide (except for serotype 6B (4.4 µg), along with 5.0 mM succinate buffer, 0.85% sodium chloride, 0.02% polysorbate 80, and 0.125 mg of aluminum as aluminum phosphate adjuvant per 0.5-ml dose.[16] Blood samples was collected immediately prior to vaccination and at 1 month post-vaccination to assess seroresponses and immediately prior to vaccination and at 3-6 months post-vaccination for DSA assessment. Patients were asked if they developed any adverse reactions such as pain and swelling, fever or rash following vaccine administration.

Blood collection

Blood samples were tested for anti-PCV13 antibodies. KTRs underwent venepuncture just prior to vaccination, one month post-vaccination for anti-PCV antibodies and then 3 months later for anti-HLA antibodies.

Anti-pneumococcal antibody assays - IgG to PCV13

IgG antibody to capsular polysaccharides from all 13 vaccine *Streptococcus pneumoniae* serotypes were measured by standard Enzyme-linked immunosorbent assay (ELISA) assay after serum samples were absorbed to neutralise antibody to cell wall polysaccharides. Serotype-specific anti-pneumococcal IgG was measured for the serotypes in PCV13 using a

previously published modified ELISA method[17] and the international reference serum, 007sp (FDA/CBER).[17] Results are reported in µg/ml of serotype-specific IgG. Pre- and post-vaccination samples were tested in the same run.

Anti-pneumococcal antibody assays -Opsonophagoytic assay to PCV13

Functional serotype-specific anti-pneumococcal IgG was measured post-vaccination for four serotypes (1,4,9v and 23F) in PCV13 using a multiplexed opsonophagocytic assay.[18] The serotypes for OPA assay were selected based on the most immunogenic serotype from the ELISA results for each multiplexed OPA. The antibiotic resistance of the bacteria used in each multiplexed assay were as follows : Optochin resistant (serotypes 4,18c,7f and 3), spectinomycin resistant (serotypes 6b, 19f and 1), streptomycin resistant (serotypes 5, 9v and 14) and trimethoprim resistant (serotype 6a,19a and 23f). Serial dilutions of heat inactivated infant sera (IgG remains intact) were incubated with cultured HL-60 phagocytic cells (American Type Culture Collection, Manassas, VA, US), rabbit complement (Pel-Freez, Arkansas, USA) and a mix of cultured antibiotic resistant streptococcus pneumoniae. After 45 minutes, the serial dilutions were plated to selective THYE agar plates. At 24 hours, the number of colonies per dilution was measured using a ProtoCol 3 colony counter (SynopticsLtd, UK). A control serum sample, a complement control (no serum) and a bacterial control (no complement) were included in each assay. Results were recorded as an opsonic titre, which is the reciprocal of the last serum dilution with at least 50% killing when compared to the average growth in complement control wells. An OPA ≥8 was accepted as a positive response.[18]

Anti-HLA antibodies

Sera from a subset of patients (n=15) were assessed for de novo anti-HLA antibodies. Cost precluded testing all patients and hence to reduce variability the subgroup was selected by excluding patients with eGFR<30 mL/min/1.73m^2 and age >65 which are known associates of reduced vaccine responses.[23] Five control KTRs, who did not receive vaccination, had sera collected at the same time points. Anti-HLA antibody assays were performed by the Victorian Transplantation and Immunogenetics Service (Parkville, Australia) using Luminex class I and II single antigen beads (One Lambda, Canoga Park, CA, USA).

Outcome Definitions

Seroprotection for PCV13 was defined as a geometric mean titre of ≥1.0µg/ml.[24] Baseline seroprotection for PCV13 was defined as a geometric mean titre of ≥1.0µg/ml before vaccination. Seroconversion for PCV13 was defined as a geometric mean titre of ≥ 1.0µg/ml plus a two-fold rise in titre [24]

Clinical outcomes

KTRs were reviewed in outpatient clinics every three months for 12 months post-vaccination. Investigators were notified when KTRs were admitted to hospital and the reasons for admission were determined. Additionally, at the end of the follow-up period a chart review was conducted for all participants to ensure no clinical events had been missed. Severe proven pneumococcal infection was defined as an infection requiring admission whereby patient had clinical symptoms plus *Streptococcus pneumoniae* isolated from the clinical site of infection. All rejection episodes were biopsy proven. Allograft biopsies were performed on the basis of a sustained rise in serum creatinine or for surveillance at 12 months post-transplantation, where relevant.

Statistical analysis

Median and interquartile ranges were used to describe continuous variables when the data was not normally distributed. Number and percentages were used to describe categorical variables. IgG antibodies to each polysaccharide antigen were quantified pre-vaccination and post-vaccination and geometric mean titres were calculated. Change in antibody concentrations pre-vaccination and post-vaccination were determined by Wilcoxon signed-rank testing. Statistical significance was set at a p-value of 0.05. Analyses were conducted using Stata Version 15 (College Station, Texas, USA) and GraphPad Prism Version 7 (GraphPad Software, La Jolla, California, USA).

RESULTS

Characteristics of participants

Fifty-eight (7%) of a possible 850 KTRs consented to this study and received PCV13 vaccination. Of the 58 subject who completed pre-vaccination testing, 45 completed both pre and post-vaccination serological and OPA testing. The median time between pre-vaccination and post-vaccination serological testing was 27 (Range 21-59) days. The baseline demographics of the 45 subjects who completed all testing are presented in Table 1. The median age was 56.1 (47.0-63.9) and 60% were male. The median time from transplant was 2.24 years (1.1-5.7). A combination of tacrolimus plus mycophenolate plus prednisolone was the most common immunosuppressive regimen in 81.0%. All participants received basiliximab as induction therapy. The participants in this study were similar in age and other demographic details to the cohort of kidney transplant recipients at our institution who were not vaccinated as part of the study.[25] No subjects had previously received a pneumococcal

conjugate vaccine, however 27 (77%) had previously been vaccinated with PPV23 (Merck & Co., Inc.).

Seroresponses to PCV13

Data on the seroresponses to each serotypes is presented in table 2 and figures 1 and 2. Overall the number of serotypes for which patients had seroprotection significantly increased from a median of seven (IQR 3.0-10.0) pre-vaccination to nine (IQR 4.0-11.0) post-vaccination ($p<0.001$). The median fold increase in anti-pneumococcal IgG antibody concentrations ranged from 1.1 to 1.7- fold across all 13 serotypes (Figure 1). For KTRs with and without baseline seroprotection, the median fold increase in anti-pneumococcal IgG antibody concentrations ranged from 1.0 to 2.0-fold and 1.1 to 3.4-fold, respectively. Seroconversion differed by serotype with the greatest responses to 19A and 18C. The serotypes with the least response were 3 and 14. Figure 2 describes the percentage of subjects achieving seroconversion post-PCV13 vaccination for each serotype. Overall, 12 (27%) subjects seroconverted to zero serotypes, 26 (58%) seroconverted from 1 to 8 serotypes and 7 (16%) seroconverted to ≥9 serotypes.

Four weeks post-vaccination, KTRs displayed an OPA titre ≥1:8 for 3 of the 4 serotypes tested (Table 2). For KTRs without baseline seroprotection, there was an OPA titer of ≥1:8 for 2 of the 4 serotypes and for KTRs with baseline seroprotection, there was an OPA titer of ≥1:8 for 4 of the 4 serotypes.

Adverse events

Of the fifteen patients (35.7%) who underwent anti-HLA antibody testing, 12 (80.0%) had pre-existing anti-HLA antibodies in pre-vaccination sera and in five (33.3%) patients these included DSA (Table 3). After vaccination, no new DSA were detected in any patient (0 of 15, 95%CI 0-22%) and there was no significant change in DSA mean fluorescence intensity for those with pre-existing DSA. Five patients had one to three additional anti-HLA Abs detected post-vaccination without a common HLA antigen target. In 3 patients with pre-existing non-DSA anti-HLA antibodies 1 or more antibodies were no longer present on post-vaccination sera (Table 3). No previously unsensitised patient developed any *de novo* anti-HLA antibodies. A similar pattern was observed in the non-vaccinated control transplant patients with one of five developing a new non-DSA antibody.

There were no cases of biopsy proven rejection at 12 months post-vaccination.

Pneumococcal vaccination was well tolerated by 44 patients (97.7%). One patient developed

arthralgia of hands, shoulders, knees, ankles and feet 24 hours following vaccination. This patient did not require hospital admission and symptoms resolved spontaneously after 48 hours. At 12 months after PCV13 vaccination, no vaccine recipients had developed documented, microbiologically proven pneumococcal infections.

DISCUSSION

This is the first study to our knowledge that examines seroresponse rates of PCV 13 in a solid organ transplant population. Here we demonstrate that a single dose of PCV13 elicits an increase in antibody concentrations for all pneumococcal serotypes and an increase in the number of serotypes with protective antibody titres. OPAs on selected serotypes suggested that the antibodies were functional for three of the four serotypes. The vaccine was well tolerated and did not result in graft rejection or the development of donor specific antibodies in the subset tested. Given the increased risk of pneumococcal infection and its attendant high case fatality rate in KTRs, this study supports guideline recommendations for the use of PCV13 in this immunocompromized group.[1,2,26]

There have been limited studies examining anti-HLA antibodies and allograft rejection following pneumococcal vaccination and ours is the only study, to our knowledge, that has specifically examined the quantification of anti-HLA antibodies following PCV13 vaccination in solid organ transplant recipients. Lindemann *et al* demonstrated that there was no increased anti-HLA antibody production following vaccination with a pneumococcal polysaccharide vaccine.[27] Pneumococcal polysaccharide vaccine is a T cell independent vaccine in contrast to PCV, which induces T cell responses and is specifically designed to stimulate more robust immune activation. Theoretically, the risk of de novo antibody generation would be higher with adjuvated PCV than PPV due to non-specific effects of the adjuvant leading to alloimmune stimulation. Increased anti-HLA antibody production and allograft rejection has been reported in renal transplant recipients vaccinated with adjuvanted influenza vaccine[28],however several other studies did not corroborate this concern. [29,30] Studies examining allograft rejection following PCV7 vaccination were in keeping with our study with no increase in rejection.[27,31,32]The studies examining PCV7 vaccination however, did not assess anti-HLA antibody production. [27,31,32] Our findings that DSA are not induced with adjuvanted pneumococcal vaccination provides reassurance but further studies with larger patient numbers are required for verification.

All subjects demonstrated a serological response to PCV13, however overall the immunogenicity was poor. Only 16% achieved seroconversion to greater than nine serotypes. There have been few studies of PCVs in solid organ transplant recipients and a

lack of standardization of definitions between studies make them difficult to compare.[31-35] Kumar *et al* performed a randomized controlled trial whereby PPV23 was compared with PCV7 followed by PPV23 in KTRs. There were 30 KTRs in each arm. 73% KTRs who received PCV7 followed by PPV23 had an antibody response to at least one serotype and 37%–53% had OPA responses to individual serotypes at 8 weeks. Notably, there was no difference in seroresponses between the arms.[5] Three years following vaccination, geometric mean titers declined significantly for 6 of 7 serotypes.[36] A similar study performed in liver transplant recipients found that 81% of those who received PCV7 had antibody and OPA responses to at least one serotype at 8 weeks.[32] Tobrudic *et al* performed a randomized controlled trial whereby PCV7 was compared to PCV7 followed one year later by PPV23 vaccine. There were 40 KTRs in each arm. 77% of KTRs who received PCV7 vs 93% KTRs who received PCV7 followed by PPV23 had antibody and OPA response to at least one serotype at 8 weeks. These rates of seroconversion appear higher than those in this current study however it is important to note that the serological cut off of 1.0 μg/ml [24] used in this study is substantially higher than that used in other studies, which makes the immunogenicity of the vaccine in this cohort appear inferior. International recommendations for the serological cut offs for clinical correlates of protection are based on a limited data.[37-40] In particular, for pneumococcal conjugate vaccines, the anti-capsular polysaccharide antibody concentration and OPAs that correlate with clinical protection are uncertain.[41,42] For PCV7, an anti-capsular polysaccharide antibody concentration of 0.35μg/ml aggregated across all seven serotypes is recommended as the correlate of clinical protection.[43] However, more recent data suggests this cut off should be higher.[42] Andrews *et al* measured serotype specific antibody concentration in infants after two doses of either PCV7 or PCV13 and linked these to a registry of invasive pneumococcal disease. The aggregate correlate of protection against invasive pneumococcal disease for PCV7 and PCV13 was 0.59 μg/ml and 0.98, respectively.[42] To further complicate the situation, the amount of antibody required to prevent clinical diseases differs according to serotype, as well as site of clinical pneumococcal infection.[41,42,44,45] A recent study found that OPA and IgG antibody assays do not correlate well using current values for protective immunity against the pneumococcus in immunosuppressed transplant recipients.[46]

This study showed that both the antibody and OPA responses four weeks post PCV-13 vaccination were substantially lower than healthy controls of similar ages.[16,26,47] Bryant *et al* examined serological responses to PCV13 among healthy controls aged 60-64 and found GMT responses were more than twice and OPA responses 15-500 times that of our patients. Importantly, these patients were given a primary pneumococcal vaccine course compared with our cohort in which three quarters had previous exposure to PPV. In our

study, a single dose of PCV13 was administered because of the number of patients with PPV vaccination within the last five years and because it is not recommended to re-dose due to potential hyporesponsiveness.[32] The data regarding the effect of prior vaccination is conflicting. Blumberg *et al* demonstrated that when heart transplant recipients received PPV vaccination, those previously vaccinated with PPV had increased seroresponse compared to heart transplant recipients undergoing primary pneumococcal vaccination course.[48] Differing PCV dosing regimens, prior vaccination and the timing between PPV23 make comparison between studies challenging.[32-34] To further complicate matters, for a number of patients in our study we found limited records of previous pneumococcal vaccination. This represents a limitation of our study as we do not have accurate information about length of time between previous PPV23 and the current PCV13, as there may have been recall bias.

This is a single center, non-randomized study. The small sample size may not be representative of the total population and is not large enough to provide definitive evidence regarding de novo antibodies to HLA following PCV13 vaccination. The results of this study may not apply to pediatric patients or recipients of other types of solid organs as these groups were not included. Infections such as pneumonia that were managed as an outpatient were not captured.

The low numbers meant that it was not possible to perform multivariable analysis to identify risk factors associated with poor seroresponses. Immunocompromize has been associated with poor response to pneumococcal vaccination[15] but other specific host factors that decrease seroconversion are not well defined. Unlike influenza vaccination whereby the use of mycophenolate mofetil reduces vaccine efficacy[23], Kumar *et al* found no association between type of immunosuppression and PCV7 vaccine efficacy in liver or kidney transplant recipients.[5,32] Gattinger *et al* could not find an association between PCV vaccine response and age, sex, time from transplantation or immunosuppression.[34] The absence of a concurrent control group makes it difficult to interpret the results.

In summary, this study demonstrated that KTRs mount significant seroresponses to PCV13 but they appear blunted compared to reports in healthy controls. As the participants in this study only received a single dose of PCV13, it is possible the seroresponses could be improved with a booster dose or prime boosting with PPV23, as recommended by guidelines. Future research in larger transplant cohorts could analyze factors related to vaccine efficacy and the optimal dosing strategies.[1,2] The vaccine is safe and did not trigger the development of anti-HLA antibodies or allograft rejection. Our data supports guideline recommendations for PCV vaccination of KTRs. Further research could examine the efficacy and safety of PCV in larger populations of kidney transplant recipients through multicenter studies or using registry data.

DISCLOSURE

CD received an unrestricted educational grant from Pfizer Inc. No other authors declare any disclosures.

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Table 1. Demographics

Characteristic		n (%) or median (IQR)
Age (years)		56.1 (47.0-63.9)
Sex	Male	27 (60)
Ethnicity	Caucasian	32 (71)
	Asian	7 (16)
	Other	6 (13)
Cause of ESKF	Diabetes	11 (24)
	IgA	6 (13)
	Glomerulonephritis	4 (9)
	PCKD	9 (20)
	Reflux	3 (7)
	Hypertension	2 (5)
	Vascultitis	4 (9)
	Other	6 (13)
No. of previous grafts	0	42 (93)
	≥1	3 (7)
Transplant duration (years)		2.2 (1.1 – 5.7)
Medications	Tacrolimus	38 (84)
	Mycophenolate	38 (84)

	Prednisolone	42 (93)
	mTOR inhibitor	3 (7)
Tacrolimus level (µg/l)		4.9 (4.0 – 5.5)
Mycophenolate dose (mg/day)		1080 (1000 - 1500)
eGFR (ml/min/1.73m^2)		48.9 (41.0 – 68.4)
Rejection episode*		1 (2)
Prior PPV23 vaccination		33 (73)

PCV13 = 13-valent pneumococcal conjugate vaccine IQR = interquartile range PPV23 = 23 valent polysaccharide pneumococcal vaccine KTRs = kidney transplant recipients ESRF = end-stage kidney failure, PCKD = polycystic kidney disease mTOR inhibitor = Mammalian Target of rapamycin inhibitor * Rejection episode requiring corticosteroid therapy and occurring 12 to 3 months prior to study enrolment

Table 2. Seroresponses to the 13-valent pneumococcal conjugate vaccination

Serotype	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
Geometric mean titre µg/ml, median (IQR) n=45													
Pre-vaccination	0.75 (0.52 - 1.1)	0.45 (0.33 - 0.61)	0.37 (0.25 - 0.69)	0.78 (0.58 - 1.00)	1.20 (0.94 - 2.04)	1.26 (0.89 - 1.70)	0.75 (0.52 - 1.10)	0.69 (0.48 - 1.00)	3.93 (2.74 - 5.60)	0.94 (0.62 - 1.42)	1.87 (1.37 - 2.55)	2.48 (1.78 - 3.45)	0.99 (0.63 - 1.25)
Post-vaccination	1.49 (1.00 -2.01)	0.80 (0.44 - 0.83)	1.00 (0.67 - 1.49)	1.57 (1.06 - 2.33)	2.09 (1.47 - 2.91)	2.06 (1.46 - 2.91)	1.99 (1.33 - 2.99)	1.38 (0.91 - 2.09)	5.74 (3.90 - 8.44)	2.50 (1.61 - 3.80)	3.58 (2.44 - 5.24)	3.77 (2.67 - 5.33)	1.87 (1.12 - 2.90)
Opsonophagocytic assay µg/ml, median (IQR) n=45													
	8.00 (4.00-152.5)		4.00 (4.00-46.00)					102.00 (4.00-882.0)					11.00 (4.00-138.00)

Table 3. Development of anti-HLA antibodies in PCV13 vaccinated kidney transplant recipients n=15

Patient number	Number of anti-HLA antibodies pre-vaccination	Number of anti-HLA antibodies post-vaccination	De novo non-DSA anti-HLA antibodies
1	4	4	
2	4	6	DP28 and DP20
3	2	1	
4	2	2	
5	8	8	
6	10	10	B47 (Cw15 lost)
7	6	5	(DP5 lost)
8	5	5	
9	3	3	
10	5	8	B15, DP5 and DP11
11	5	5	
12	41	16	B76 (26 others lost)
13-15	0	0	0
Total	95	73	

HLA = Human Leukocyte antigen

FIGURE LEGEND

Figure 1. Pneumococcal antibody concentrations in 45 kidneys transplant recipients pre- and four weeks post-vaccination with 13-valent conjugate pneumococcal vaccine. Median and interquartile range are given separately for 13 serotypes of capsular polysaccharides. All 13 pre and post pairs $p>0.001$.

Figure 2. Percentage of kidney transplant recipients achieving seroconversion post 13-valent pneumococcal conjugate vaccine according to serotype.
Seroconversion was defined as a ≥two-fold rise in pre-vaccination titre and a serotype specific antibody concentration of ≥1.0 µg/ml.

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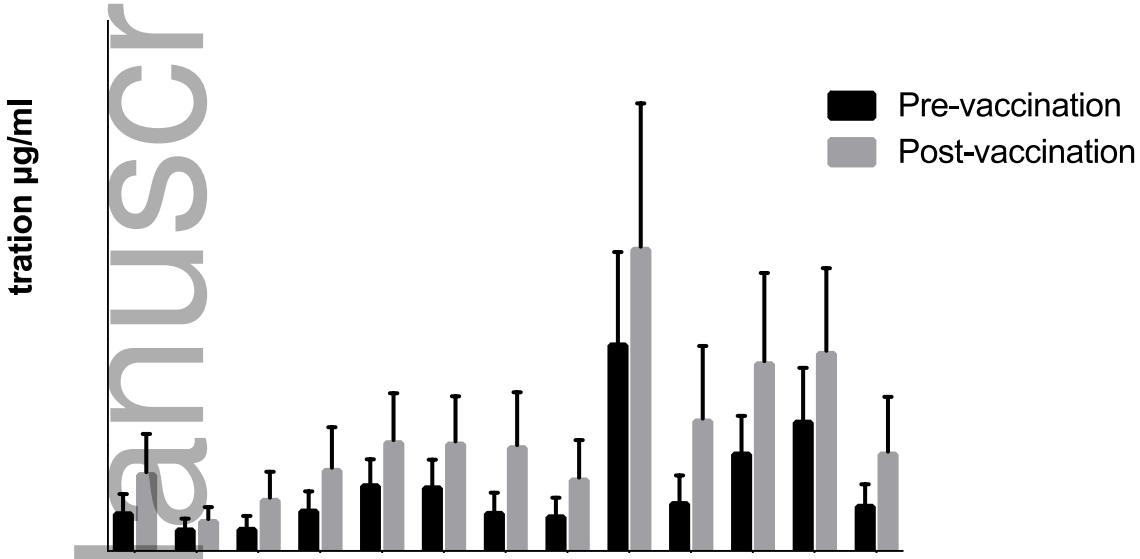

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