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Immunogenicity and safety of double dosage of pneumococcal vaccines in adult kidney transplant recipients and waiting list patients: A non-blinded, randomized clinical trial

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ABSTRACT

Background: Pneumococcal prime-boost vaccination is recommended for solid organ transplant recipients, but is not thoroughly tested in this population. Furthermore, a pneumococcal vaccine dose effect has never been investigated, though observed in healthy adults. To assess whether a double dose of 13-valent pneumococcal conjugate vaccine (PCV13) and of 23-valent pneumococcal polysaccharide vaccine (PPV23) increases the immunogenicity of prime-boost vaccination in kidney transplant recipients (KTRs) and patients on the kidney transplant waiting list (WLPs), a phase 3, randomized, non-blinded trial was conducted.

Methods: KTRs and WLPs were in parallel groups assigned either normal or double dosage of both vaccines 12 weeks apart. A 'protective response' was an average geometric mean concentration $\geq$ 1 mg/L based on 12 vaccine shared serotype-specific IgG antibodies. Furthermore, number of antibodies with $\geq$ 2-fold rises and individual serotype-specific antibody concentrations were evaluated. Follow-up was 48 weeks.

Results: Seventy-four KTRs and 65 WLPs were enrolled. In WLPs, double dosage resulted in a significantly higher proportion of participants with a 'protective response' (66.7%), 5 weeks after PPV23, compared to normal dosage (35.5%), p = 0.015. KTRs exhibited no dose effect. After PPV23, all four groups had increased their number of serotypes with $\geq$ 2-fold rises ($p \leq 0.05$ for both WLPs groups; $p \leq 0.01$ for both KTRs groups). Vaccines were safe, well tolerated and still immunogenic at week 48.

Conclusions: Data suggests that double dosage of pneumococcal vaccines used according to the prime-boost strategy might be recommendable for WLPs. Furthermore, our data supports PPV23's additive effect to PCV13 in KTRs and WLPs. (EudraCT: **2016–004123-23**)

1. Introduction

Kidney transplant recipients (KTRs) are reported to have 4–9 times greater the risk of invasive pneumococcal disease (IPD) than the background population [1,2]. Hence, immunization is important. Pneumococcal vaccineś effectiveness in preventing IPD has been established in the general population [3,4]. However, it remains unproven in solid organ transplant (SOT) recipients. Antibody levels and opsonophagocytic tests are used to estimate efficacy. Pneumococcal vaccines were introduced in 1977 with the 14-valent pneumococcal polysaccharide vaccine (PPV14) and replaced in 1982 with the 23-valent pneumococcal polysaccharide vaccine (PPV23). They are immunogenic from the age of 2 years. In 2007, the 7-valent protein-conjugate polysaccharide vaccine (PCV7) was introduced for routine use in children. This resulted

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in a decrease of IPD in all age groups; a combination of direct vaccine effect and herd protection [5]. In 2010, the 13-valent pneumococcal conjugate vaccine (PCV13) was introduced. The conjugate vaccines are also immunogenic in adults [6], and they are used for immunization in several populations with severely increased risk of IPD. In KTRs and patients on the kidney transplant waiting list (WLPs), the current recommendation is PCV13 followed by PPV23 with 8 weeks interval [7]. This prime-boost approach is based on results from populations with sickle cell anaemia, Hodgkin lymphoma, and HIV [8–10]. The theory is, that PCV13 generates a T cell–dependent response producing memory B cells, which may be boosted by PPV23 to produce larger amounts of antibody than PCV13 or PPV23 alone [11]. KTRs are reported to exhibit a lower and less robust response to PPV14, compared to healthy controls [12]. The main reason is the lifelong immunosuppressive treatment that inhibits lymphocytes and general immune functions [13]. Dosage of pneumococcal vaccines have been found to correlate with immunogenicity in immunocompetent adults [14–16]. In adult KTRs and WLPs, the prime-boost approach is not well tested, and varying vaccine dosage has never been tested. Hence, a randomized trial was initiated, to investigate whether prime-boost vaccination with double dosage of PCV13 and PPV23 enhances the serologic response.

2. Materials and methods

2.1. Trial design

This is a multi-centre, phase 3, parallel-group, randomized, non-blinded clinical trial in adult WLPs and KTRs. The trial was conducted at 3 university hospitals in Denmark (Rigshospitalet, Copenhagen University Hospital, Copenhagen; Zealand University Hospital, Roskilde; Odense University Hospital, Odense) with enrolment from Aug. 2017 through Feb. 2019. Participants were randomized to receive either normal dosage (ND) vaccination (PCV13 (0.5 ml) followed by PPV23 (0.5 ml)) or double dosage (DD) vaccination (PCV13 (1.0 ml) followed by PPV23 (1.0 ml)). Vaccines were given with 12 weeks interval. This interval was chosen to coincide with KTRs′ visits to the outpatient clinics. The trial was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice. Signed informed consent was obtained. The trial was approved by the Danish Data Protection Agency (18/20444), the Regional Committees on Health Research Ethics for Southern Denmark (S-20170018), and the Danish Medicines Agency (2017053413) and registered in European Clinical Trial Database (EudraCT **2016–004123-23**).

2.2. Participants

Participants were recruited from a previous survey on vaccine uptake [17] and enrolled by the principal investigator. Eligible participants were KTRs and WLPs, ≥18 years of age. KTRs had received their transplant within the last 18 months. Exclusion criteria were acute transplant rejection at enrolment, pregnancy, and previous PCV13 vaccination. Prior kidney transplantation was not an exclusion criteria for WLPs, if the person was dependent on dialysis. Baseline demographic data, comorbidities, kidney failure data and transplant data were collected. Randomization was 1:1, using random block sizes, stratified for patient groups (KTR/WLP). Randomization and data collection were performed by principal investigator and study nurses using REDCap electronic data capture tools [18] hosted by Odense Patient data Explorative Network [19].

Blood samples were drawn at baseline (PCV13 injection), 12 weeks after baseline (pre-PPV23 injection), 17 weeks and 48 weeks after baseline. Blood samples were stored at − 80 °C until analysis.

2.3. Objectives

The primary endpoint was number of participants that reached a ′protective response′ five weeks after PPV23 (week 17). A 'protective response' was obtained, if the participant had an average pneumococcal antibody geometric mean concentration (GMC) ≥ 1 mg/L. This was calculated as a mean over the 12 pneumococcal serotype-specific IgG antibodies. In Denmark this is reported as protective immunity in adults following PPV23.

Secondary endpoints included; ′protective response′ at week 12 and 48; number of ≥ 2-fold increases in serotype-specific antibodies at week 12, 17 and 48; increases in the 12 individual antibodies following PPV23. Furthermore, pairwise correlations at week 17 between average pneumococcal antibody GMC level, number of antibodies with ≥ 2-fold increases, age, time elapsed since transplantation (KTRs) and dosage of immunosuppressants (KTRs). Additionally, assessment of whether a positive CMV IgG at baseline affects immunogenicity (latent CMV, defined as sero-positivity for anti-CMV antibodies, has been associated with poor PPV23 response [20]. Lastly, safety was assessed.

2.4. Vaccines

PCV13 (0.5 ml) contains polysaccharides of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F individually conjugated to nontoxic diphtheria toxoid CRM197. The vaccine is formulated to contain 4.4 μg of 6B and 2.2 μg of each of the other 12 saccharides, in 5.0 mM succinate buffer, pH 5.8, 0.85% sodium chloride, 0.02% polysorbate 80 and contains 0.125 mg aluminum as aluminum phosphate. PPV23 consists of purified capsular polysaccharide and contains phenol as a preservative. PPSV23 (0.5 ml) vaccine contains serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9 N, 9 V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F. The vaccine is composed of 25 mg of each polysaccharide in phenol, sodium chloride and water. Vaccines were administered intramuscularly.

2.5. Laboratory methods

Pneumococcal serotype-specific IgG antibodies were determined for 12 serotypes (1, 3, 4, 5, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F) included in both PCV13 and PPV23 using an in-house Luminex method based on the procedure previously described [21]. This method permits the simultaneous measurement of all 12 analytes in a single sample. Each sample was analysed in duplicate and repeated, if the coefficient of variation between duplicates was > 20%. Antibody concentrations were expressed as mg/L and results above the range of the standard curve were assigned a value of 50 mg/L.

Baseline cytomegalovirus IgG antibody (CMV IgG) was analysed using LIAISON® based on an indirect chemiluminescent immunoassay and intended for the quantitative determination of IgG antibodies to CMV in human serum and plasma. Results were expressed as positive or negative.

2.6. Safety assessment

Local reactions (pain, erythema, and enema) at site of injection, and systemic reactions (fever, headache, arthralgia, and myalgia) were reported as adverse events (AE). In addition, all serious AE up to week 17 were recorded.

2.7. Statistical analysis and sample size

Logarithm transformed serotype-specific IgG antibody concentrations were used for statistical calculations and, when presented, GMCs with 95% confidence intervals (95% CIs) were used. The average pneumococcal antibody GMC was calculated as follows. A mean for the 12 logarithm transformed antibodies was calculated and then exponentially transformed back. In pairwise correlations tests the result was evaluated as a continuous variable, but as a binary variable in term of 'protective response' obtained (average pneumococcal antibody GMC $\geq$ 1 mg/L). For remaining continuous variables, median and interquartile range (IQR) were reported. For dichotomous and categorical variables, number and percentage were listed relative to patients in the group. Normality was assessed with Shapiro-Wilḱs test. Non-paired continuous variables were analysed with Student 's *t*-test if normally distributed and Wilcoxon rank-sum test if not. Paired data were analysed with Wilcoxon signed-rank test or Student's *t*-test. Spearman's correlation was used for pairwise correlations between continuous variables. Dichotomous and categorical variables were analysed using Chi-squared, McNemaŕs or Fisher's exact test as appropriate. A two-sided p-value $\leq$ 0.05 was considered statistically significant. Statistical analysis were performed using STATA 17 (Stata Corp, College Station, TX).

Based on a similar study [22], we estimated that a sample size of 39 patients in each arm would be required to detect an improvement in primary outcome of 30% (ND 50%) with 80% power and a two-sided alpha level of 5%.

3. Results

3.1. Study population

Of 236 patients screened, 142 accepted, and 65 WLPs (ND = 32; DD = 33) and 74 KTRs (ND = 39; DD = 35) were randomized and received PCV13. After PCV13, 1 participant died and was only included in baseline analysis. After blood sampling week 12, another 3 participants were excluded without receiving PPV23 (1 died and 2 withdrew consent). Additionally, two more withdrew consent after PPV23 (Fig. 1). Demographic characteristics are depicted in Table 1. Median age was 52 years (IQR: 41–61) and 69.0% (96/139) were males. Six WLP-ND and 12 WLP-DD were treated with immunosuppressants (p = 0.113). A few significant differences were present between KTR-ND and KTR-DD in comorbidity and ethology of kidney failure (Table 1). Median weeks between vaccines were 12 (IQR: 11.3–13.0), from baseline to week 17, 17.7 weeks (IQR: 16.6–19.1), and from baseline to week 48, 48.6 weeks (IQR: 47–52.4).

3.2. 'Protective response' (average pneumococcal antibody GMC $\geq$ 1 mg/L).

Proportion of participants with a 'protective response' in WLP-ND were statistical comparable with WLP-DD at baseline (WLP-ND, 2 (6.3%); WLP-DD, 1 (3%); p = 0.613) and week 12 (WLP-ND, 12 (37.5%); WLP-DD, 18 (54.6%); p = 0.168). Five weeks after PPV23, WLP-DD had a significantly higher proportion of participants with a 'protective response' than WLP-ND (20 (66.7%) vs. 11 (35.5%); p = 0.015). At week 48 the difference were no longer significant (WLP-ND, 9 (29%); WLP-DD, 12 (38.7%); p = 0.421). In KTRs, there was no significant differences between the dosage groups at any visit. Baseline (KTR-ND, 0 (0%); KTR-DD, 2 (5.7%); p = 0.220), week 12 (KTR-ND, 7 (18.4%); KTR-DD, 7 (20%); p = 0.864), week 17 (KTR-ND, 13 (35.1%); KTR-DD, 9 (25.7%); p = 0.386) and week 48 (KTR-ND, 9 (25.7%); KTR-DD, 5 (14.7%); p = 0.371). As the only group, KTR-ND demonstrated a significant increase in proportion of participants with a 'protective response' at week 17, compared to week 12. Fig. 2 illustrates the proportion of participants with a 'protective response' at each study visits.

3.3. Number of serotype-specific IgG antibodies with a $\geq$ 2-fold increase from baseline

Fold increase in concentration was determined by dividing the post-vaccination concentration with the baseline concentration (at no point did the 50 mg/L cut-off result in a missed $\geq$ 2-fold increase).

Median number of antibodies with a $\geq$ 2-fold increase with IQR are displayed in Fig. 3. There were no significant differences between WLP-ND and WLP-DD, or between KTR-ND and KTR-DD at any visit. A significant increase was observed in all groups from week 12 to week 17 following PPV23. Median fold rises in serotype-specific antibody concentrations at week 17 ranged from 1.4 to 3.0 in KTRs and 2.9 to 8.7 in WLPs, and 13% of KTRs and 4% of WLPs had zero $\geq$ 2-fold antibody increases. Participants with a $\geq$ 2-fold increase in each of the 12 serotype-specific IgG antibodies at week 17 are presented in Supplement Fig. 1 to illustrate eventual differences in the 12 serotypeś vaccine response.

3.4. Changes in 12 pneumococcal serotype-specific IgG antibody concentrations

Each serotype-specific IgG antibody GMC increased after each of the two vaccines with a few exceptions, following PPV23 (WLP-ND, serotype 6B; KTR-ND, serotype 19A) (The results for each serotype are presented in Fig. 4 + Supplement Table 1). Significant increases were observed following PPV23 in KTR-ND (serotype 3, 4, 5 and 18C), KTR-DD (serotype 1, 3, 14 and 18C) and in WLP-DD (serotype 1 and 14). At week 48, all 12 serotype-specific IgG antibody GMCs were still significantly higher than baseline values, though they had declined. There were no significant differences between dosage groups in neither KTRs nor WLPs at any time point. Participants achieving an antibody concentration $\geq$ 1 mg/L in each of the 12 serotypes at week 17 are presented in Supplement Fig. 2 to illustrate eventual differences in the 12 serotypeś vaccine response.

3.5. Correlations week 17

In WLPs, average pneumococcal antibody GMC correlated positively with number of antibodies with $\geq$ 2-fold increase (rho = 0.762; p $\leq$ 0.001). Neither correlated with age.

In KTRs, average pneumococcal antibody GMC correlated negative with age (rho = -0.261; p = 0.027) and positively with number of antibodies with a $\geq$ 2-fold increase (rho = 0.555; p $\leq$ 0.001). Furthermore, time since transplantation correlated negative with daily dosage of prednisolone (rho = -0.677; p $\leq$ 0.001), tacrolimus (rho = -0.340; p = 0.004), and mycophenolate mofetil (rho = -0.642; p $\leq$ 0.001). Time since transplantation or dosage of immunosuppressants did not correlate with number of $\geq$ 2-fold antibody increases or average pneumococcal antibody GMC.

At week 17, a positive CMV IgG response at baseline did not seem to have an impact on average pneumococcal antibody GMC level or number of antibodies with a $\geq$ 2-fold increase in neither WLPs nor KTRs. In WLPs, treatment with any immunosuppressant at baseline (n = 17) resulted in a lower number of antibodies with a $\geq$ 2-fold increase week 17, compared to those without (p = 0.006), and average pneumococcal antibody GMC was lower, but not statistically different (p = 0.099).

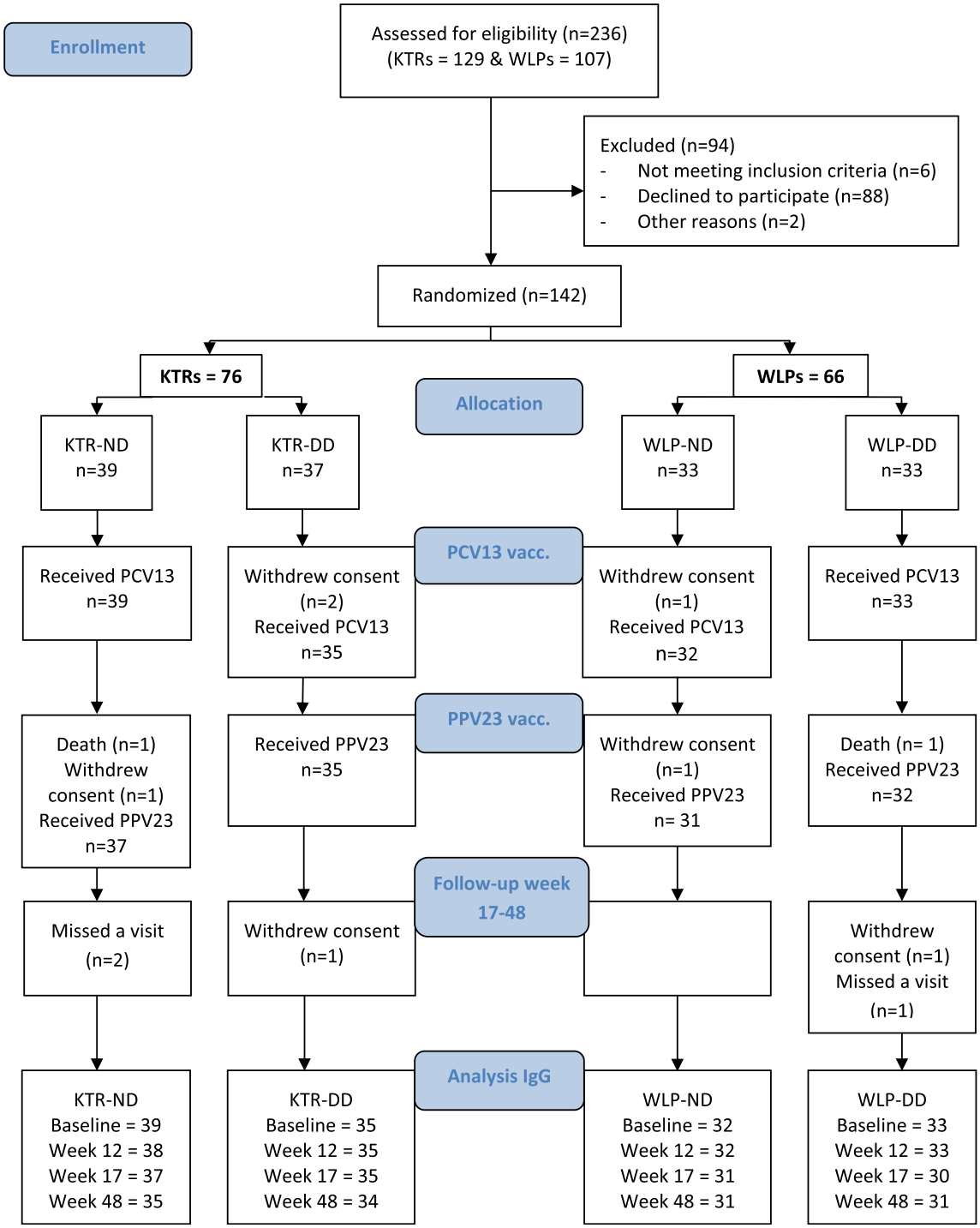

Fig. 1. CONSORT flow diagram showing eligible patients throughout the study. Abbreviations: WLPs, kidney transplant waiting list patients; KTRs, kidney transplant recipients; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage; KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; PCV13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide.

3.6. Safety data

During the study period, 55 serious AEs were reported. None were considered to be caused neither by PCV13 nor by PPV23. Overall, 82 local and 104 systemic AEs were reported. Of local reactions, 48 were treatment related and equally distributed between PCV13 and PPV23. These were primarily pain at injection-site. Fifteen of the systemic AEs were treatment related and predominantly related to PPV23. These were mainly fever and generalized muscle pain. There were no differences between the four groups in number of reactions reported or number of participants reporting. One case of IPD was observed in a WLP with fatal outcome after PCV13. The serotype was 10A.

Table 1
Baseline characteristics, comorbidities, immunosuppressive medication, dialysis treatment and transplantation data.

	WLPs			KTRs		
	WLP-ND (n = 32)	WLP-DD (n = 33)	p-value	KTR-ND (n = 39)	KTR-DD (n = 35)	p-value
Age, median years (IQR)	55 (44–60)	51 (40–62)	0.679	50 (41–62)	51 (41–60)	0.944
Males, n (%)	17 (53.1)	22 (66.7)	0.265	28 (71.8)	29 (82.9)	0.259
Prior PPV23, n (%)	1 (3.1)	0	1.000	1 (2.6)	1 (2.9)	1.000
Comorbidities, n (%)						
• Chronic heart disease incl. hypertension	21 (65.6)	22 (66.7)	0.929	22 (56.4)	28 (80.0)	0.030
• Neurological disease	9 (28.1)	7 (21.2)	0.518	5 (12.8)	10 (28.6)	0.147
• Rheumatic disease	7 (21.9)	6 (18.2)	0.710	9 (23.1)	4 (11.4)	0.231
• Diabetes	5 (15.6)	5 (15.2)	1.000	7 (17.9)	11 (31.4)	0.177
• Malignancy (prior or current)	4 (12.5)	1 (3.0)	0.197	5 (12.8)	2 (5.7)	0.435
• Other[£]	7 (21.9)	7 (21.2)	0.948	14 (35.9)	6 (14.1)	0.070
Positive CMV IgG, n (%)	20 (62.5)	21 (63.6)	0.924	25 (65.8)	22 (62.9)	0.794
Etiology of kidney failure, n (%)						
• Adult polycystic kidney disease	6 (18.8)	6 (18.2)	0.953	6 (15.4)	4 (11.4)	0.740
• Anomalies or infections	8 (25.0)	3 (9.1)	0.108	9 (23.1)	2 (5.7)	0.050
• Diabetes	5 (15.6)	5 (15.2)	1.000	1 (2.6)	8 (22.9)	0.011
• Hypertension	4 (12.5)	4 (12.1)	1.000	3 (7.7)	7 (20.0)	0.176
• IgA nephropathy	2 (6.3)	3 (9.1)	1.000	7 (18.0)	2 (5.7)	0.158
• Unknown/Other[#]	7 (21.9)	12 (36.4)	0.199	13 (33.3)	12 (34.3)	0.931
Immunosuppressive medication, n (%)						
• Calcineurin inhibitor	2 (6.3)	2 (6.1)	1.000	38 (97.4)	35 (100)	1.000
• Mycophenolic acid	2 (6.3)	6 (18.2)	0.258	37 (94.9)	35 (100)	0.495
• Steroids	5 (15.6)	7 (21.2)	0.751	14 (35.9)	11 (31.4)	0.685
• Other[§]	2 (6.3)	2 (6.1)	1.000	2 (5.1)	0	0.495
Dosages at baseline; median (IQR)						
• Tacrolimus mg/day,	NA	NA		4 (3–5)	4 (3–6)	0.332
• Mycoph. mofetil mg/day	NA	NA		1500 (1500–2000)	1500 (1500–2000)	0.780
• Prednisolone mg/day	NA	NA		6.25 (5–10)	7.5 (5–10)	0.589
Time since transplant; median days (IQR)	NA	NA		142 (83–271)	132 (71–337)	0.840
Donor status KTRs, n (%)						0.186
• Living donor	NA	NA		12 (30.1)	16 (45.7)	
• Deceased donor	NA	NA		27 (69.2)	19 (54.3)	
Dialysis, n (%)			0.670			
• Haemodialysis	26 (81.3)	25 (75.8)		NA	NA	
• Peritoneal dialysis	2 (6.3)	5 (15.2)		NA	NA	
Pre-trial transplantation in WLPs, n (%)	10 (31.3)	10 (30.3)	1.000	NA	NA	
Transplantation during study, n (%)			0.274			
• Between PCV13 and PPV23	4 (12.5)	2 (6.1)		NA	NA	
• Between PPV23 and week 17	4 (12.5	1 (3.0)		NA	NA	
• Between week 17 and 48	3 (9.4)	6 (18.2)		NA	NA	

In Abbreviations: WLPs, kidney transplant waiting list patients; KTRs, kidney transplant recipients; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage; KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; n, number of participants; PPV23, 23-valent pneumococcal polysaccharide; IQR, interquartile ratio; NA, not applicable.
[£] Other co-morbidities consists of non-malignant diseases (pulmonary, gynecological, abdominal or chronic infections).
[#] Other consists of vasculitis, amyloidosis, renal cancer, Alports disease, haemolytic uremic syndrome or unknown glomerulonephritis.
[§] Other medications consists of Azathioprine, Everolimus, Quinine or Secukinumab.

4. Discussion

In this randomized clinical trial, we demonstrated that prime-boost vaccination with DD PCV13 and PPV23, in WLPs, elicited a significantly higher proportion of participants with a 'protective response' five weeks after PPV23, compared to ND. In KTR-DD, no dose effect was observed. The vaccines were safe and well tolerated.

Three studies have tested DD of pneumococcal conjugate vaccines in formerly PPV23 vaccinated healthy adults. One study establish that DD PCV13 produced superior opsonophagocytic responses in 7/12 serotypes, compared to ND [16]. Another study showed statistically higher GMCs and opsonophagocytic responses in 2/7 serotypes after DD PCV7, compared to ND [14], whereas the third study displayed no significant difference between ND and DD PCV7, although a dose effect was observed [23]. Our study is not directly comparable to these, as we did not collect blood samples 1 month after PCV13. Hence, we can only conclude on the effect of the overall vaccine schedule. As both KTR-ND and KTR-DD performed poorly, maintenance immunosuppressants may be reducing PCV13́s immunogenicity. Corticosteroids, mycophenolic acids and calcineurin inhibitors have a widespread attenuating effect on the immune system, mostly on T-cells [13]. PCV13 is dependent on antigen-specific T-cells to (co–)stimulate memory B-cell and proliferation of antibody-secreting plasma cells [11], and evokes a reduced humeral response in KTRs compared to healthy adults [24]. A former study established that half dose PPV14 resulted in a slightly reduced vaccine response, compared with standard dose in health adults [15]. Accordingly, WLP-DD increased its proportion of participants with a 'protective response' after PPV23. In prior studies, immunogenicity of PPV14 in KTRs has been assessed only slightly reduced, compared to controls [25–27], maybe because PPV14 is T-cell independent. However, no dosage effect was observed after PPV23 in the present KTR cohort and the proportion of KTRs that achieve a 'protective response' after PPV23 appears low, compared to WLPs. Part of the explanation for this may be that our KTR cohort is heavily immunosuppressed, as they are relatively newly transplanted, or maintenance immunosuppressants are different, compared to earlier studies.

The superior achieved 'protective response' in WLP-DD at week 17 seem to be a combination of both pneumococcal vaccines. Kidney failure patients may benefit from this vaccine strategy, just as

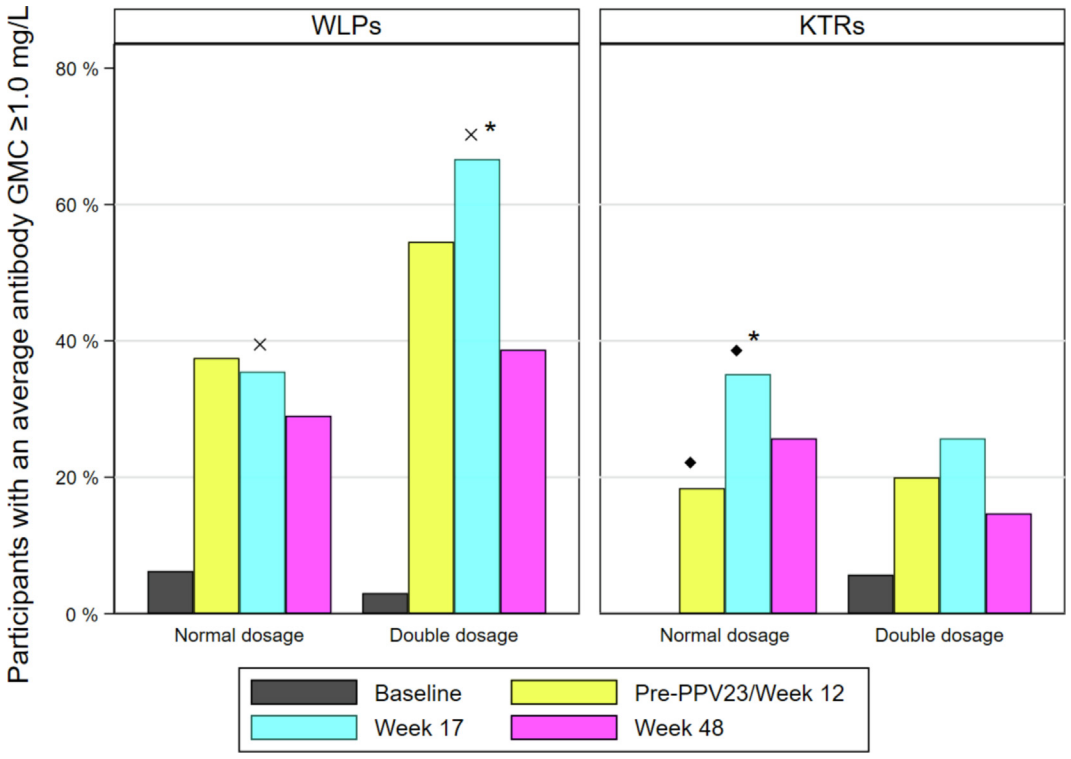

Fig. 2. Proportion of participants with a 'protective response' (average pneumococcal antibody GMC ≥ 1 mg/L) at baseline, week 12 (pre-PPV23), week 17 and week 48. Any significant differences between normal dosage and double dosage groups and from week 12 to week 17 within groups are shown. Abbreviations: GMC, geometric mean concentration; WLPs, kidney transplant waiting list patients; KTRs, kidney transplant recipients; PPV23, 23-valent pneumococcal polysaccharide vaccine. P-values for significantly higher results: *≤0.05.

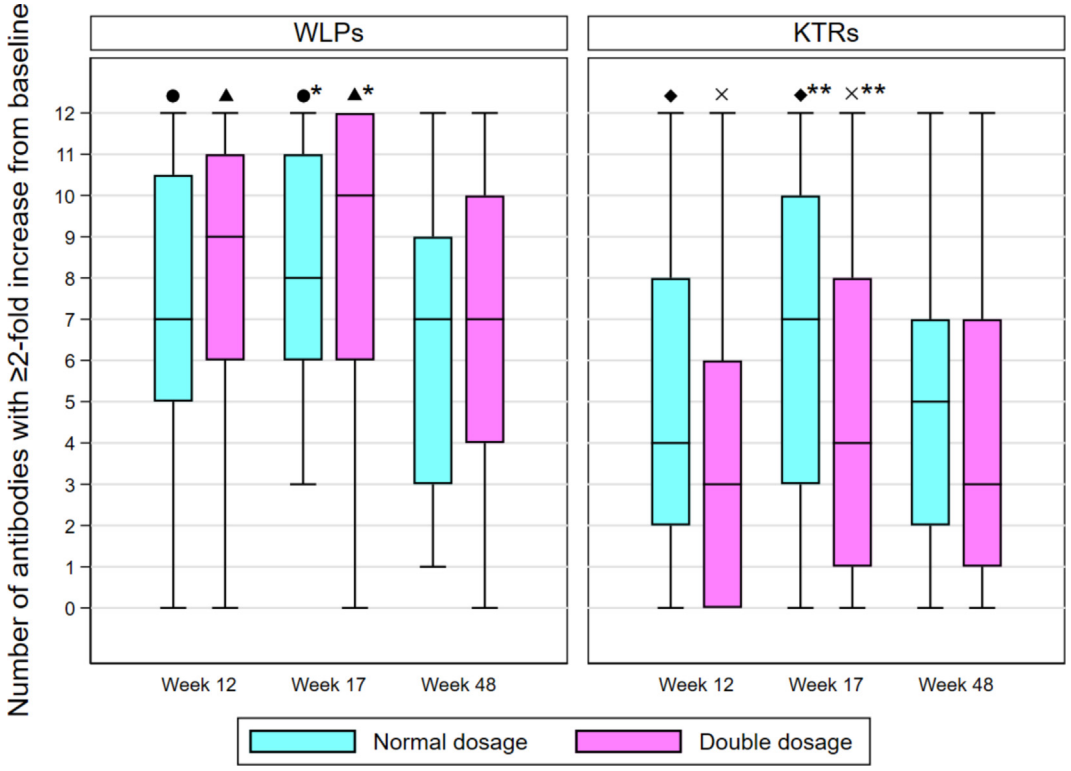

Fig. 3. Median number of ≥ 2-fold antibody increases with interquartile ranges at week 12 (pre-PPV23), week 17 and week 48. Whiskers marks minimum and maximum values. Significant differences from week 12 to week 17 within each group are shown. There were no significant differences between normal dosage and double dosage groups. Abbreviations: WLPs, kidney transplant waiting list patients; KTRs, kidney transplant recipients; PPV23, 23-valent pneumococcal polysaccharide vaccine. P-values for significantly higher results: *≤0.05, **≤0.01.

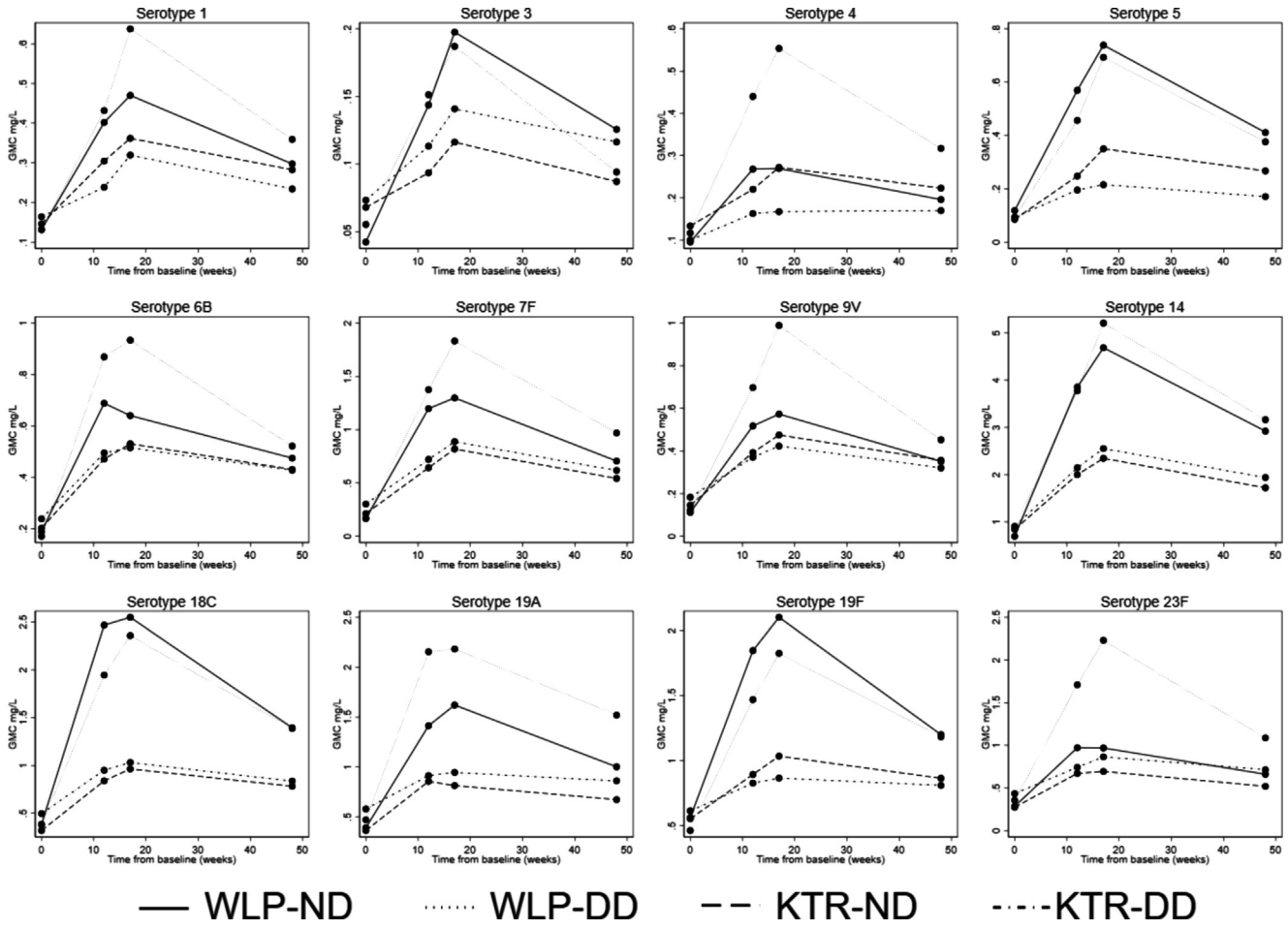

Fig. 4. Geometric mean concentrations for 12 pneumococcal serotype-specific IgG antibodies in mg/L at baseline, week 12 (pre-PPV23), week 17 and week 48. Note that the scale of the y-axis is different for all serotypes. Abbreviations: GMC, Geometric mean concentration; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage; KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; PPV23, 23-valent pneumococcal polysaccharide.

double doses of the hepatitis B vaccine is used [28]. However, we cannot elucidate how DD vaccination may affect the response to revaccination and eventual hyporesponsiveness. Hyporesponsiveness following PPV23 is described [6,29], but appears to be a phenomenon that primarily occurs if the previous PPV23 is a short time ago [30,31].

When evaluating the prime-boost strategy in KTRs, we observed significant increases in 4/12 antibody concentrations and in number of antibodies with $\geq$ 2-fold increases following PPV23 in both groups. In addition, KTR-ND significantly increased number of participants achieving a ′protective response′. A prior study displayed no additive effect from prime-boost vaccination, in adult KTRs with 12 months between vaccines [22]. The same has been observed in paediatric KTRs [32], and in paediatric and adult SOT recipients [33,34]. A small study in lung transplant recipients (n = 23), showed significant increases in 2/12 serotypes after PPV23 boosting [35]. Both WLP groups exhibited significant rises in number of antibodies with $\geq$ 2-fold increases, and WLP-DD in 2/12 antibody concentrations. We consider our results supportive of PPV23́s additive effect to PCV13 in both KTRs and WLPs.

Seven months after PPV23 (week 48), we observed that the concentrations of all 12 antibodies had dropped, mostly in WLPs. This concurs with previous studies showing rapid drops in antibody concentrations post-immunization especially in patients on hemodialysis [36,37]. However, antibody concentrations for all serotypes were still significantly above baseline in all groups, thus confirming some long-term vaccine induced immune protection. Corresponding decreases in participants with a ′protective response′ and number of $\geq$ 2-fold antibody were seen. WLP-DD performed slightly better than WLP-ND on the measured parameters, though not statistical different.

Different thresholds of protection following pneumococcal vaccination have been proposed. The World Health Organization has defined a PCV13 post-vaccine level of serotype-specific IgG antibodies $\geq$ 0.35 mg/L to be an adequate protective response in infants and children [38], but there is no consensus on protective levels for adult SOT recipients or adults in general. When PPV23 is used in the diagnosis of primary immune deficiencies, a post-vaccine antibody level $\geq$ 1.3 mg/L in 70% of tested serotypes is defined as protective [39]. In the present study, an average pneumococcal antibody GMC $\geq$ 1.0 mg/L based on 12 serotype-specific IgG antibodies was selected in line with national practice following PPV23 in adults. As the protective level of serotype-specific IgG antibodies is not clearly defined for KTRs or dialysis patients, an evaluation of antibody functionality would have been relevant. An opsonophagocytosis assay could provide a more in-depth picture of vaccine protection in our participants, as immunosuppression may affect antibody functionality. Opsonophagocytosis is the primary method by which antibodies promote the clearance of S. pneumonia. Even though the protective threshold for an opsonophagocytosis assay is not completely established in immunosuppressed adults [40], it could provide clinical relevant

information. Overall, both vaccines were well tolerated with generally mild reactions and no safety differences between different dosages. Former studies reported that DD of PCV13 and PCV7 were associated with higher frequencies of local reactions than ND, but reactions tended to be mild to moderate and self-limited [16,41].

The strength of this study is that it followed a prospective, randomized, controlled design to evaluate the endpoint. Furthermore, results are applicable when planning pneumococcal vaccination in connection with kidney transplantation, as we chose to enrol only patients with kidney failure, who were on the transplant waiting list, and KTRs who had received a transplant within the last 18 months. However, there are some limitations. The study was underpowered to analysis of primary endpoint, as the number of eligible participants was limited. We were not able to include the desired number of participants. More than expected declined to join, and due to the schedule of the study we had to stop inclusion. Additionally, it is an open-label trial. However, the vaccines were provided and blood samples were drawn mainly by staff not involved in data analyses or interpretation, and blood samples were analysed by blinded staff. Furthermore, we did not include a control group, or a group who only received e.g. DD PPV23. This could have added further important results, whether prime-boost vaccination is comparable to PPV23 vaccination. Furthermore, we did not collect blood samples after both vaccines with the same time interval. Hence, an eventual drop in antibody concentrations shortly after PCV13 may not have been taken into account. Due to small numbers, multivariate analysis was not performed to identify risk factors associated with poor vaccine response, only pairwise comparisons. Lastly, we used serotype-specific IgG antibodies as a surrogate marker for vaccine efficacy.

5. Conclusion

Double dosage of pneumococcal vaccines used according to the prime-boost strategy might be recommendable for WLPs. Furthermore, our data supports PPV23́s additive effect to PCV13 in newly transplanted KTRs as well as in WLPs.

6. Authorship contribution statement

LL, ISJ, CB, SSS, and LB are responsible for the conception and design. LL collected data, performed data analysis and wrote the article. CSJ is responsible for pneumococcal serology analysis. ISJ and LL are responsible for project administration. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.05.040.

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