Immunization of immunosuppressed patients

Knowledge, practices and serological response

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Immunization of immunosuppressed patients

Knowledge, practices and serological response

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Papers included in thesis

Paper I

The coverage of influenza and pneumococcal vaccination among kidney transplant recipients and waiting list patients: A cross-sectional survey in Denmark. Lykke Larsen, Claus Bistrup, Søren Schwartz Sørensen, Lene Boesby, Mai Thanh Thuy Nguyen and Isik Somuncu Johansen. *Transpl Infect Dis*. 2021 Jun; 23(3):e13537. DOI: 10.1111/tid.13537

Paper II

Immunogenicity and safety of double dosage of pneumococcal vaccines in adult kidney transplant recipients and waiting list patients: A non-blinded, randomized clinical trial. Lykke Larsen, Claus Bistrup, Søren Schwartz Sørensen, Lene Boesby, Charlotte Sværke Jørgensen and Isik Somuncu Johansen, *Vaccine*: Epub 2022 May 27. DOI: 10.1016/j.vaccine.2022.05.040.

Paper III

Durability of antibody response after primary pneumococcal double dose prime-boost vaccination in adult kidney transplant recipients and candidates: 18 months follow-up in a non-blinded, randomised clinical trial. Lykke Larsen, Claus Bistrup, Søren Schwartz Sørensen, Lene Boesby, Charlotte Sværke Jørgensen, Christian Nielsen and Isik Somuncu Johansen

(Submitted)

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Abbreviations

IPD, invasive pneumococcal disease

KTR, kidney transplant recipient

WLP, kidney transplant waiting list patient

IgG, immunoglobulin G

PCV13, 13-valent pneumococcal conjugate vaccine

PPV23, 23-valent pneumococcal polysaccharide vaccine

DD, double dosage

ND, normal dosage

GMC, geometric mean concentration

KTX, kidney transplantation

SOT, solid organ transplant

PY, person-years

CKD, chronic kidney disease

ESRD, end stage renal disease

PCV7, 7-valent pneumococcal conjugate vaccine

PPV14, 14-valent pneumococcal polysaccharide vaccine

CMV IgG, cytomegalovirus IgG antibody;

AE, adverse event

IQR, interquartile ranges

OR, odds ratio

95% CI, 95% confidence interval

N, number of participants

NA, not applicable

English Summary

Background

Given the high incidences of influenza and invasive pneumococcal disease (IPD) in kidney transplant recipients (KTRs), immunization against influenza and pneumococci is recommended in KTRs and kidney transplant waiting list patients (WLPs). However, prior studies have displayed low vaccine uptake and lack of immunization guidance in these patient populations. Currently, there are two commercially available pneumococcal vaccines on the market. A purified polysaccharide vaccine and a conjugate vaccine. The first vaccine induces a restricted immunoglobulin G response without recruiting T lymphocytes or generating memory B lymphocytes. The conjugate vaccine induces a T lymphocyte response, thereby promoting B lymphocyte differentiation into both memory B lymphocytes and antibody-secreting plasma cells. Pneumococcal vaccination for KTRs is recommended as prime-boost vaccination consisting of the conjugate vaccine followed by the purified polysaccharide vaccine after minimum 8 weeks. An approach adapted from other patient populations, where it has been viewed superior to the purified polysaccharide vaccine alone. Furthermore, previous studies have demonstrated a possible dose dependent vaccine response for both vaccines in healthy adults. Increased dosage of pneumococcal vaccines have never been assessed in KTRs or WLPs, and the prime-boost approach is not thoroughly tested either.

Aim of thesis

The aim of the thesis is, in KTRs and WLPs, to 1) asses the degree of vaccination with influenza and pneumococcal vaccines which are recommended according to national and international guidelines 2) assess eventual predictors influencing vaccine acceptance and uptake 3) evaluate 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.

Methods and studies

This thesis is based on a cross-sectional study and a randomized clinical trial.

Study 1. Uptake and predictors for influenza and pneumococcal vaccines in Danish KTRs and WLPs. For this cross-sectional study, a questionnaire was designed and distributed to eligible WLPs and

KTRs, during their scheduled visits to the different hospitals involved. Self-reported data on demographics, vaccine uptake and received vaccine guidance were analysed. Predictors for vaccine uptake and proportion of participants who were vaccinated were described.
Study 2. A multi-centre, phase 3, parallel-group, randomized, non-blinded clinical trial conducted to investigate whether pneumococcal prime-boost vaccination with double dosage (DD) of PCV13 and PPV23 resulted in increased immunogenicity in WLPs and KTRs, compared to normal dosage (ND). Participants were assigned in parallel groups to receive either ND or DD of both vaccines 12 weeks apart. Follow-up was 96 weeks. KTRs had to have received their transplant within the last 1½ year. Primary endpoint was difference in proportion of participants in the two dosage arms achieving a 'protective response' defined as an average antibody geometric mean concentration (GMC) ≥1 mg/L calculated as a mean across the twelve pneumococcal serotype-specific IgG antibodies measured five weeks after PPV23. Furthermore, the average pneumococcal antibody GMC level and serotype-specific IgG antibodies with ≥2-fold increases were assessed.

Results

Study 1. Totally, 220 patients participated in the survey (KTRs 54.1% and WLPs 45.9%). During the latest flu season, 92 (41.8%) participants had been vaccinated against influenza with no significant difference between the WLPs and KTRs. Significantly more WLPs had been influenza vaccinated prior to the latest season (p = 0.007). The 120 participants who were not vaccinated against influenza in the latest season stated the following three main reasons for not being vaccinated 1) I perceive my health as good, with no need for immunization (38.3%) 2) I was not informed that the vaccine was recommended (27.5%) and 3) I am afraid of side effects (17.5%).
Pneumococcal vaccine uptake ever was 9 participants (4.1%) evenly divided between KTRs and WLPs. Only 10 participants had ever been offered a pneumococcal vaccine. In multivariable analysis, any prior influenza vaccination was a positive predictor for influenza vaccination during the latest season (OR 5.79, CI95 2.44-13.76); (p<0.001), and recommendation provided by a non-physician was a negative predictor (OR 0.34, CI95 0.13-0.92); (p=0.03).
Study 2. In total, 236 patients were screened for eligibility and 139 enrolled (WLP-ND = 32, WLP-DD = 33 and KTR-ND = 39, KTR-DD = 35). Five weeks after PPV23, WLP-DD had a significantly higher proportion of participants with a 'protective response', compared to WLP-ND (20 (66.7%) vs

11 (35.5%); p=0.015). At week 12, 48 and 96, the groups were comparable. There were no significant differences between KTR-ND and KTR-DD, at any visit. Number of antibodies with ≥2-fold increases were significantly higher after PPV23 in all groups, and so were average pneumococcal antibody concentrations in 3 out of 4 groups. The average pneumococcal antibody concentration declined over time in WLPs and KTRs. However, at week 96, in all four treatment groups, the average pneumococcal antibody concentration was still significantly above baseline level, and stable, compared to week 48.

Conclusion

Both influenza and pneumococcal vaccine uptake are sub-optimal in Danish WLPs and KTRs, and lack of immunization guidance seem to be the main cause. Counselling should be made mandatory as it appears that, if the patients first have been influenza vaccinated, they will continue to do so prospectively. Furthermore, a doctor should provide the information.

Based on the randomized trial, we found that DD of both pneumococcal vaccines used according to the prime-boost vaccine strategy may be recommendable in WLPs. Moreover, PPV23 increases immunogenicity in both KTRs and WLPs, compared to that obtained by PCV13 alone.

Danish Summary – Dansk Resumé

Baggrund

I betragtning af den øgede risiko for et alvorligt forløb af influenza og for invasiv pneumokoksygdom hos nyretransplanterede, de anbefales både influenza og pneumokokvaccination. Det samme gælder for ventelistepatienter til nyretransplantation. Imidlertid har tidligere undersøgelser vist lav vaccinedækning og mangel på immuniseringsvejledning i disse patientpopulationer. I øjeblikket er to kommercielt tilgængelige pneumokokvacciner på det danske markedet. En ren polysaccharidvaccine og en konjugeret vaccine. Den første vaccine inducerer et begrænset immunoglobulin G (IgG) respons uden at rekruttere T-lymfocytter eller generere hukommelses-B-lymfocytter. Den konjugerede vaccine inducerer et T-lymfocyt respons og fremmer derved B-lymfocytdifferentiering til hukommelses-B-celler og antistof producerende plasmaceller. Pneumokokvaccination til nyretransplanterede anbefales som prime-boost vaccination bestående af den konjugerede vaccine efterfulgt af den rene polysaccharidvaccine efter minimum 8 uger. En vaccinestrategi adapteret fra andre patientpopulationer, hvor den betragtes som overlegen i forhold til en enkelt pneumokokvaccine. Tidligere undersøgelser vist en mulig dosisafhængig vaccinerespons for begge vacciner hos raske voksne; dette er aldrig blevet vurderet hos nyretransplanterede eller ventelistepatienter, og prime-boost pneumokokvaccination er heller ikke grundigt testet.

Formålet med afhandlingen

Formålet med denne afhandling er at vurdere følgende blandt nyretransplanterede og ventelistepatienter til nyretransplantation. 1) Vaccinedækningen mod influenza og pneumokokker i henhold til nationale retningslinjer. 2) Eventuelle prædiktorer, der påvirker vaccinedækningen og årsager til vaccine afslag/accept. 3) Om dobbelt dosis af både den konjugerede 13-valent pneumokokvaccine (PCV13) og den 23-valent pneumokokvaccine (PPV23) øger vaccinationsresponset efter prime-boost vaccination.

Metoder og studier

Denne afhandling er baseret på en tværsnitsundersøgelse og et randomiseret lægemiddelforsøg.

Studie 1: Vaccinationsdækning og prædiktorer for influenza- og pneumokokvaccination hos danske nyretransplanterede og ventelistepatienter. Et spørgeskema blev designet og distribueret til begge patientgrupper, i forbindelse med planlagte fremmøder på de forskellige involverede hospitaler. Selvrapporteret data om demografi, vaccinationsdækning og modtaget vaccinationsvejledning blev analyseret. Vaccinationsdækningen og prædiktorer for denne blev beskrevet.
Studie 2. Et multicenter, fase 3, parallelgruppe, randomiseret, ikke-blindet klinisk forsøg udført for at undersøge, om pneumokok prime-boost vaccination med dobbelt dosis af PCV13 og PPV23 resulterede i øget immunogenicitet hos ventelistepatienter og nyretransplanterede, sammenlignet med standard dosis. Deltagerne blev randomiseret og modtog enten ND eller DD af begge vacciner med 12 uger imellem. Opfølgningen var 96 uger. De nyretransplanterede skulle have modtaget deres graft inden for det sidste 1½ år. Primært endepunkt var forskel i andelen af deltagere i de to doseringsarme, der opnåede et 'beskyttende respons', defineret som en gennemsnitlig antistof geometrisk middelkoncentration (GMC) ≥1 mg/L beregnet på tværs af de tolv pneumokok serotype-specifikke IgG antistoffer, målt fem uger efter PPV23. Endvidere blev det gennemsnitlige pneumokokantistof GMC niveau og antal af serotype-specifikke IgG antistoffer med ≥2 gange stigninger vurderet.

Resultater

Studie 1. I alt deltog 220 personer i studiet (54.1% var nyretransplanterede og 45.9% var venteliste-patienter). I løbet af den seneste influenzasæson var 92 (41,8%) deltagere blevet vaccineret mod influenza uden nogen signifikant forskel mellem ventelistepatienterne og de nyretransplanterede. Signifikant flere ventelistepatienter var blevet influenzavaccineret forud for den seneste sæson (p = 0.007). De 120 deltagere, der ikke var vaccineret mod influenza i den seneste sæson, oplyste følgende tre hovedårsager til ikke at blive vaccineret. 1) Opfatter mit helbred som godt, uden behov for vaccination (38,3%), 2) jeg blev ikke informeret om, at vaccine er anbefalet (27.5%) og 3) jeg er bange for bivirkninger (17.5%). Pneumokokvaccinedækningen nogensinde var 9 deltagere (4,1%), ligeligt fordelt mellem de nyretransplanterede og ventelistepatienterne. Kun 10 deltagere var nogensinde blevet tilbudt en pneumokokvaccine. Med multivariabel analyse var enhver tidligere influenzavaccination en positiv prædiktor for

influenzavaccination i den seneste sæson (OR 5.79, CI95 2.44-13.76); (p<0.001), og anbefaling fra en ikke-læge var en negativ prædiktor (OR 0.34, CI95 0.13-0.92); (p=0.03).
Studie 2. I alt blev 236 patienter screenet for egnethed og 139 blev inkluderet (ventelistepatient-ND = 32, ventelistepatient-DD = 33 og nyretransplanteret-ND =39, nyretransplanteret-DD =35). Fem uger efter PPV23 havde ventelistepatient-DD en signifikant højere andel af deltagere med et 'beskyttende respons' sammenlignet med ventelistepatient-ND (20 (66,7%) vs 11 (35,5%); p=0.015). Ved uge 12, 48 og 96 var der var grupperne sammenlignelige. Der var ingen signifikante forskelle mellem nyretransplanteret-ND og nyretransplanteret-DD ved noget besøg. Antallet af antistoffer med ≥2 gange stigninger var signifikant højere efter PPV23 i alle grupper, og det samme var niveauet af den gennemsnitlige pneumokokantistofkoncentration hos 3 ud af 4 grupper. Den gennemsnitlige pneumokokantistofkoncentration faldt over tid hos begge patientpopulationer. Hos alle fire grupper i uge 96, var den gennemsnitlige pneumokokantistofkoncentration stadig signifikant højere end baseline-niveauet, og stabil sammenlignet med uge 48.

Konklusion

Både influenza- og pneumokokvaccinedækningen var suboptimal hos danske ventelistepatienter og nyretransplanterede, og manglende vaccinationsvejledning synes at være hovedårsagen. Vaccinationsrådgivning bør gøres obligatorisk, da det ser ud til, at hvis patienterne først er blevet influenzavaccineret, vil de fortsætte med dette fremadrettet. Desuden bør en læge deltage i rådgivningen. Baseret på det randomiserede forsøg fandt vi, at DD af begge pneumokokvacciner anvendt i henhold til prime-boost vaccinestrategien kan anbefales til ventelistepatienter. Desuden øger PPV23 vaccinationsresponset, hos både nyretransplanterede og ventelistepatienter, sammenlignet med den opnået efter 12 uger efter PCV13.

Introduction

Kidney transplantation

Solid organ transplantations have advanced through the years, and is for many people with end organ damage a life-saving treatment. In Denmark, the ability to offer this treatment changed in 1990 with the enactment of a new law. With this law "brain death" was now equivalent to "heart death" as a death criterion. The same year, the first heart transplantation was performed in Denmark. The first kidney transplantation (KTX) had however been performed already in 1964, but the number of procedures increased after 1990. Kidney transplant recipients (KTRs) constitute the largest part of solid organ transplant (SOT) recipients, and throughout the last ten years approximately 240 KTXs have been performed yearly in Denmark. Around two thirds of KTRs now receive a kidney from a deceased donor and the rest from living donors (1). KTRs with a living donor experience higher graft and patient survival, compared to those with a graft from a deceased donor (2).

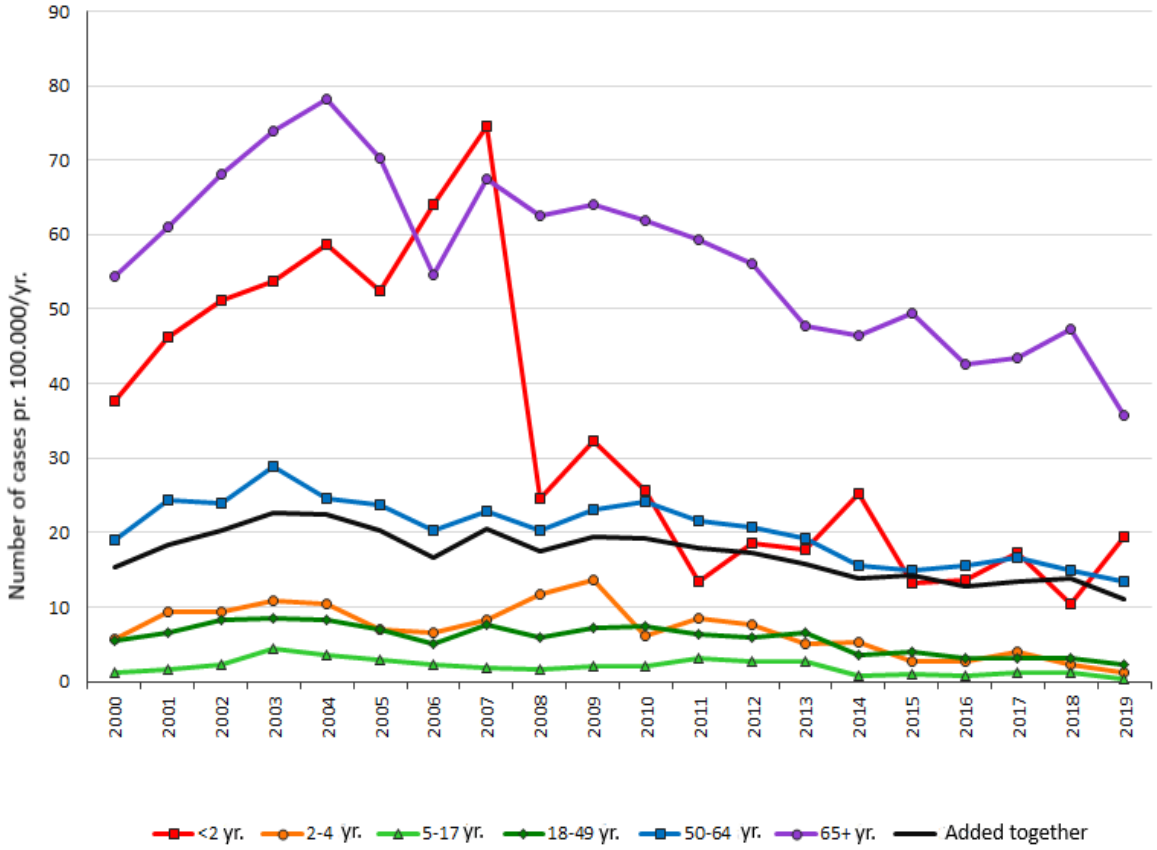
KTX, now routinely done, has increased graft survival due to improved pre- and post-transplant immunosuppressive medications, antibody screening, HLA typing, donor organ protocols, and organ preservation (3). Furthermore, pre-emptive KTX, performed before dialysis start-up, is associated with improved post-transplant outcomes, compared to KTX performed after dialysis treatment has been started. KTRs also have improved survival and quality of life, compared to patients on dialysis (2).

Lifelong immunosuppressive treatment is required in KTRs to suppress the recipient's immune response to the allograft. New drugs have been added through the years. The maintenance triple-drug regimen that presently is most used consists of the calcineurin inhibitor tacrolimus (inhibit interleukin-2 and T lymphocyte activation), the anti-proliferative drug mycophenolic acid (inhibit T-lymphocyte and B-lymphocyte proliferation) and corticosteroids (inhibit interleukin synthesis, inhibit expression of cytokine genes and induce cell death) (4). Post-transplant maintenance immunosuppressive drug therapy dosages are often reduced over time and for many KTRs lowered to a two drug therapy. Though treatment and patient follow-up have been refined over the years several issues still exists for KTRs. One being their increased risk of infections. Both by new pathogens or activation of latent, opportunistic infections.

Pneumococci

Streptococcus pneumoniae is a bacteria known for causing community-acquired pneumonia, otitis media, and sinusitis in addition to invasive pneumococcal disease (IPD) such as meningitis, osteomyelitis and sepsis. The bacteria colonizes the nasopharynx and can be transmitted to other people through droplets. There are approximately 98 serotypes divided into 21 serogroups containing 2–8 serotypes with similar capsules. The capsule protects the pneumococcus from host cell phagocytosis and is thus the key factor in virulence. Some serotypes are more invasive, compared to others (5), and around 20 serotypes are responsible for approximately 90% of IPDs. Also host factors contribute to IPD pathogenicity. In general, the highest incidences of IPD are seen among small children and the elderly (6, 7). Figure 1 shows the incidence of IPD for different age groups and the overall incidence from 2000 to 2019 in Denmark (8).

Figure 1: Age-specific and overall incidence of laboratory-confirmed cases of invasive pneumococcal disease in Denmark between 2000 and 2019. Source: Statens Serum Institut.



Pneumococcal disease in KTRs and patients with chronic kidney disease

People with primary immunodeficiency, other immunocompromising conditions, or who are treated with immunosuppressive therapy have an increased incidence of pneumococcal infections, compared to the background population (8-12). This includes KTRs and people with chronic kidney disease (CKD). In a recent Danish study, KTRs were estimated to have a four times greater risk of IPD, compared to age matched background population (incidence rates 48 vs 10-14/100000/PY) (13), while another study found it to be nine times greater (incidence rates 104 vs 11.5/100000/PY) (14). When evaluating all microbiology verified pneumococcal infections, a study from the 1970s found KTRs to have 6-28 times higher risk than the general population (incidence rates 28 vs 1-5/1000/PY) (15). KTRs hospitalized with pneumococcal pneumonia exhibit a higher cumulative incidences of graft failure, than those without pneumococcal pneumonia (16). Overall, comorbidities and older age increase mortality related to pneumococcal diseases (17, 18). Although KTRs have an increased risk of pneumococcal infections, heart, lung and liver transplant recipients may experience higher incidences (14, 19, 20).

People with CKD, are reported to have 8 times higher the risk of IPD, than the general population (incidence rate 88.6 vs 10.6/100000/PY) (21). A study including only adults with CKD, assessed the risk of IPD to be 22 times greater, compared to that in healthy adults (incidence rate 41.44 vs 1.91/100000/PY) (22). In dialysis patients the risk of IPD has been reported to be 18 times (incidence rate 89 vs 4.8/100000/PY) (8) and 22 times higher (incidence rate 340 vs 15.1/100000/PY), respectively, than in the background population (6). Generally, patients with CKD seem to experience an increasing risk of bloodstream infections as their kidney function decline. However, for *S. pneumoniae* the risk seems unchanged (23).

Vaccine effectiveness: Pneumococcal vaccines

Studies have found, PCV13 and PPV23 to be effective for the prevention of IPD and pneumococcal pneumonia (especially vaccine-serotype pneumococcal pneumonia), in the general population and the elderly, although vaccine effectiveness is lower in the elderly, (24-29). However, the estimated vaccine efficacy varies somewhat between studies. Moreover, the vaccines provides no protection against all-cause pneumonia. Yet, protective vaccine effectiveness has not been established in

immunocompromised populations. A review estimated that use of pneumococcal vaccine especially combined with influenza vaccination in patients treated with dialysis is associated with lower risks of all-cause mortality (30).

Influenza disease in KTRs and patients with CKD

Influenza in humans is mainly caused by 2 types of influenza virus: A and B. Outbreaks of influenza occur almost every winter in varying degrees of severity. The virus belong to the family of Orthomyxoviridae and causes acute, usually self-limited, febrile illness. On the surface of influenza A and B viruses are the proteins hemagglutinin (H) and neuraminidase (N). These help facilitate viral binding and release of the virus. Influenza A is classified based on the hemagglutinin and neuraminidase diversity, the 2 main circulating subtypes being H1N1 and H3N2. The 2 circulating lineages of influenza B are Victoria and Yamagata.

In SOT recipients including KTRs, influenza is associated with significant morbidity (with high hospitalization and intensive care treatment rates) and increased mortality (31-37). It is furthermore estimated that influenza can result in impaired allograft outcomes (38). In patients with end-stage renal disease (ESRD), influenza is also associated with increased morbidity (39, 40) and mortality (41). This is consistent with immunocompromised patients in general (42, 43). Overall, an influenza infection also increases the short-term risk of myocardial infarction (44). It is not clear whether or not KTRs and patients with CKD have an increased risk of contracting Influenza, compared to the background population. The risk of laboratory-confirmed influenza was in a study fivefold higher among KTRs, compared to the general population (37). However, it is not certain this is due to a true increased incidence, or if there is a different test strategy and admissions policy in KTRs, compared to the general population. A 10 year study reported the influenza incidence in KTRs to be 4.3/1000/PY (33). However, during the 2009 worldwide influenza pandemic the H1N1 incidence was estimated to be 22/1000/PY (31), and in another study from 2009-2014 the incidence of influenza A was 26.5/1000 PY (36).

Vaccine effectiveness: Influenza vaccines

In healthy adults, inactivated influenza vaccines can reduce the proportion of those who contract influenza (45, 46), though their impact is modest. However, if the circulating virus and the vaccine strain are well matched, effectiveness increases. In children, effectiveness is assessed higher (47). However, influenza vaccination has been associated with a reduced risk of critical and life-threatening influenza illness in the general population (48-50) and in SOT recipients (51, 52) including KTRs (53). It has not been established that influenza vaccination reduces the risk of contracting influenza, in SOT recipients or patients with ESRD. In these populations, influenza vaccination has been found to be associated with a reduction in overall mortality (30, 54).

Immunization in KTRs and WLPs

To protect against serious illness or from becoming infected at all, immunization against vaccine preventable infections is recommended in KTRs and WLPs. Some vaccines are generally recommended, such as influenza, tetanus or pneumococcal vaccines. Other vaccines should be provided depending on the KTR's residence area or travel pattern, e.g. tick-borne encephalitis or rabies (55, 56). Live attenuated vaccines are contradicted in KTRs due to immunosuppression, whereas inactivated vaccines can be used. So, if live attenuated vaccines are to be provided, it should be done prior to transplantation.

Immunization against Influenza

Vaccines

Two types of influenza vaccines are available currently; inactivated influenza vaccines and live attenuated influenza vaccines. Both types are produced to protect against 3 or 4 different seasonal influenza viruses. Currently, these are influenza A (H3N2), pandemic A (H1N1) and 1 (or 2) influenza B lineage viruses. Inactivated influenza vaccines exist in different variants, high-dose, adjuvanted or cell-based instead of egg-based.

In SOT recipients, the use of double-dose or booster-dose of the inactivated influenza vaccines seem to enhance immunogenicity (57, 58)

Recommendation

Influenza vaccination is recommended annually in WLPs and KTRs (55, 59, 60) and based on national guidelines for certain populations and availability, a high-dose influenza vaccine may be used. Vaccination as early as 1 month post-transplant has been deemed safe and immunogenic (61).

Immunization against Pneumococci

Vaccines

Three pneumococcal vaccines are currently in use.

Pneumococcal conjugate vaccines

Synflorix, GSK (10-valente) - children aged 6 weeks to 5 years. Not available in Denmark.

Each 0.5mL dose contains:

- 1 µg of pneumococcal polysaccharide serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3 µg of serotype 4, conjugated to a total of 9–16 µg of non-typeable Haemophilus influenzae (NTHi) protein D, 3 µg of serotype 18C conjugated to 5–10 µg of tetanus toxoid, and 3 µg of serotype 19F conjugated to 3–6 µg of diphtheria toxoid, adsorbed onto 0.5 mg of aluminium phosphate
- 4.3 mg of sodium chloride and water for injection. This vaccine contains no preservative.

Prevenar 13, Pfizer (13-valente) - children from 2 months of age and adults. Available in Denmark.

Each 0.5 mL dose contains:

- 2.2 µg of pneumococcal purified capsular polysaccharides for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F, and 4.4 µg of serotype 6B, conjugated to non-toxic diphtheria CRM197 protein and adsorbed onto aluminium phosphate (0.565 mg)
- Succinic acid, polysorbate 80, aluminium phosphate, phosphate, and sodium chloride in water for injection.

Plain polysaccharide pneumococcal vaccine

Pneumovax 23, MSD (23-valente) – children from 2 years of age and adults. Available in Denmark.

Each 0.5 mL dose contains:

- 25 µg of each capsular polysaccharide antigen (serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F)
- Sodium chloride, water for injection, and phenol (0.25 percent) added as a preservative.

Furthermore, Merck's 15-valent pneumococcal conjugate vaccine and Pfizer's 20-valent pneumococcal conjugate vaccine are coming on market.

Recommendation

It is recommended, that adult WLPs and KTRs receive PCV13 followed by PPV23 at least 8 weeks later. A second PPV23 should be repeated 5-6 years after the first dose (55, 60, 62). The expectation is that this approach provides a better vaccination response than a single vaccine.

KTRs and patients with ESRD have been reported to have a less robust pneumococcal vaccine response, compared to healthy controls (63).

Vaccine immunology

The conjugate vaccines were developed, because pure polysaccharide vaccines does not elicit an adequate response in infants and young children. In conjugate vaccines, the polysaccharide is attached (conjugated) to something else. In many conjugate vaccines to the diphtheria or tetanus toxoid protein. These proteins are very easily recognized by the immune system and helps generate a stronger immune response to the polysaccharide as they are recognised by the helper T lymphocytes. Naive B lymphocytes encounter the protein conjugated polysaccharide antigen that initiates B lymphocyte activation. Peptides on the surface of antigen-specific B lymphocytes binds T helper lymphocytes, which causes the B lymphocyte to differentiate into immunoglobulin-

secreting plasma cells or memory B lymphocytes. The B memory lymphocytes remember the antigen, so that if the body encounters it later, antibodies can be produced which bind to the antigen/bacteria, thereby facilitating phagocytosis. Hence, an immunological memory.

When using the pure polysaccharide vaccine, antigens encounter naive B lymphocytes that differentiate into immunoglobulin-secreting plasma cells. However, as polysaccharides alone cannot elicit a T lymphocyte reaction, no memory B lymphocytes are produced (64).

Pneumococcal prime-boost vaccination

The theory behind this vaccination strategy, is that immunization with a conjugate vaccine produces memory B lymphocytes that can be re-stimulated with a polysaccharide vaccine. Thereby producing larger amounts of antibody than the pure polysaccharide vaccine alone (65). The pneumococcal prime-boost vaccination strategy was originally adapted from studies with Haemophilus influenzae b vaccines (66). Afterwards studies on pneumococcal vaccines were conducted. One study found, that patients with Hodgkin's disease who received the 7-valent pneumococcal conjugate vaccine (PCV7) followed by the PPV23 after 1 year developed significantly higher antibody concentrations in 3/6 serotypes than those who received PPV23 alone (67). In patients with sickle cell disease, aged 2 years or older, those vaccinated with 2xPCV7+PPV23 with eight weeks interval had a significantly higher antibody concentration in 2/7 serotypes and significantly higher opsonophagocytic antibody titers in 5/7 serotypes measured 3-6 weeks after the last vaccine, compared to patients receiving a single PPV23, (68, 69). In adults living with HIV, with a CD4 lymphocyte cell counts between 200-500 cells/µL, those vaccinated with PCV7+PPV23 had significantly more participants achieving a twofold increase in sero-specific IgG antibody concentrations and a concentration >1µg/ml in 5-7 serotypes, than the patients receiving PPV23 alone (70).

The pneumococcal prime-boost vaccination strategy has never been tested in dialysis patients overall or in WLPs. In KTRs, the studies are scarce.

Pneumococcal vaccines - antigen doses

Different antigen doses have been tested to assess impact on immunogenicity. Three studies, testing DD of pneumococcal conjugate vaccines in formerly PPV23 vaccinated healthy adults, have been conducted. Jackson et al tested PCV7 in previously PPV23 vaccinated heathy adults and showed that a larger antigen dose enhanced immunogenicity. The 1.0 ml PVC7 group achieved statistically higher antibody concentrations and opsonophagocytic response in 2/7 serotypes, compared to the standard 0.5 ml PCV7 group (71), whereas Lode et al. did not find any significant differences, when they basically performed the same study, though a dose response was observed (72). Jackson et al. also tested 1.0 ml and 0.5 ml PCV13 doses in previously PPV23 vaccinated heathy adults and found the 1.0 ml dose to have a superior opsonophagocytic response in 7/12 serotypes, compared to the 0.5 ml dose (73).

A small study evaluating the pneumococcal prime-boost vaccination strategy in patients with rheumatoid arthritis and treated with biological disease-modifying antirheumatic drugs using 1.0 ml PCV13 found no significant dose effect (74).

Carlson et al. conducted a study testing three different 14-valent pneumococcal polysaccharide vaccine (PPV14) antigen doses (12.5 µg, 25 µg and 50 µg). Standard dose PPV14 contains 14 specific capsular polysaccharide antigens, each in 50 µg amount (PPV23 contains 25 µg for comparison). The study showed that minimum 80% of the participants receiving the 50 µg dose, achieved a ≥4fold antibody increase in each of the 14 measured serotypes. In the groups vaccinated with 25 µg or 12.5 µg doses this was achieved in 9/14 serotypes (75). However, the differences between the 25 µg and 50 µg doses did not reach statistical significance.

No studies are available investigation pneumococcal vaccine doses in WLPs or KTRs.

Protective antibody levels

There is no clear consensus on protective antibody levels, following pneumococcal vaccination in immunocompromised adults or in healthy adults for that matter. The World Health Organization has defined a post-vaccine level of serotype-specific IgG antibodies ≥0.35 mg/L following conjugate pneumococcal vaccines to be a protective response in infants and children (76). However, children aged <1 year displays the lowest serotype-specific IgG concentrations, whereas,

from approximately 1 year of age, there is an overall increase in antibody levels. The antibodies keep increasing with age into adulthood with levels well above 0.35 mg/L, without providing protecting against IPD in the elderly (77). This general increase is unrelated to pneumococcal vaccination. In the diagnosis of primary immune deficiencies, a post-vaccine antibody level ≥1.3 mg/L in 70% of tested serotypes is defined as protective. Or a two-fold increase, if pre-vaccine levels are already above 1.3 mg/L (78). The magnitude of the post-PPV23 antibody response is also different for each serotype (79). A geometric mean across 12 measured serotype-specific antibodies ≥1.0 mg/L is used for adults in Denmark following PPV23.

Uptake of pneumococcal and influenza vaccines in KTRs and WLPs

The vaccines are overall safe and an easy way to reduce morbidity and mortality. However, studies from other countries have showed that ESRD patients and KTRs often appear to have low coverage of pneumococcal and influenza vaccines, even though the vaccines are recommended (80-83). Reasons for this may be found in the healthcare system and includes lack of immunization guidance or vaccination setup. Also, the patient's knowledge and attitudes toward vaccination may influence the decision to vaccinate (83). Furthermore, in the general population, socio-demographics have been found to be predictors of vaccination, such as female sex, higher level of education, and household income (84). Data are scarce regarding this area in Denmark, and no studies exist involving Danish WLPs and KTRs.

Objectives

The objectives of this thesis are among Danish WLPs and KTRs:

1. To investigate influenza and pneumococcal vaccine uptake (study 1).
2. To identify predictors and barriers for influenza and pneumococcal vaccine uptake (study 1).
3. To investigate if double dose (DD) of both PCV13 and PPV23 would elicit a greater vaccine antibody response, compared to normal dose (ND) of both PCV13 and PPV23 (study 2).
4. To assess eventual predictors associated with pneumococcal vaccine response (study 2).
5. To asses durability of the pneumococcal vaccine response over time (study 2).

Materials & methods

Both studies overall

The two studies were done as a collaboration between three nephrology departments in Denmark, where two also serve as kidney transplant centres. The sites were Copenhagen University Hospital, Rigshospitalet, Copenhagen; Zealand University Hospital, Roskilde and Odense University Hospital, Odense.

Participants were recruited by the principal investigator that covered all sites alternately or local study nurses. Eligible participants were adult KTRs and WLPs. KTRs had received their transplant within the last 18 months. WLPs were allowed to have received a kidney transplant previously, but it´s function had to be extinct.

Study data were collected and managed using REDCap electronic data capture tools (85, 86) hosted by Odense Patient data Explorative Network (87).The randomization in study 2 was also performed in REDCap.

Study 1 (Paper I)

Design

The method of this study was a cross-sectional survey. A questionnaire was used to obtain the participants' self-reported vaccine uptake against influenza and pneumococcal disease. The inclusion period was from Aug. 2017 until Feb. 2019. This period was extended during the study as a larger number of patients than expected, refused to participate. The participants were approached during outpatient and dialysis visits. Paper questionnaires were completed either by the participant alone or with help at request.

Data

The questionnaire consisted of four parts

1. Sex, age, ethnicity, educational level, and annual household income last year.
2. Known allergies, influenza vaccine uptake the latest season, influenza vaccine uptake any prior season, intention to have the influenza vaccine the next influenza season, who

informed about vaccine opportunity, attitudes towards influenza vaccination/reasons for not being vaccinated.

3. Pneumococcal vaccine recommended ever, who informed about vaccine opportunity, pneumococcal vaccine uptake ever (with reason, name and year), and attitude towards pneumococcal vaccination/reasons for not being vaccinated.
4. Information regarding vaccine trial.

Influenza vaccine uptake the latest season was defined as having received a vaccination in the latest Oct. - Feb. period. Most questions could be answered, 'don't know' or 'don't want to disclose'. If these answers were provided, or if the question were not filled in (No data), it was reported as such in results.

The questionnaire is added as *Supplement material 1.*

Study 2 (Paper II + III)

Design

The study is a multi-centre, phase 3, parallel-group, randomized, non-blinded clinical trial.

Stratified for patient groups, participating WLPs and KTRs were randomized 1:1 to be pneumococcal prime-boost vaccinated with two different vaccine dosages. Randomization was done in random block sizes.

Participants were randomised to receive one of the following

- ND treatment which was 0.5 ml PCV13 followed by 0.5 ml PPV23
- DD treatment which was 1.0 ml PCV13 followed by 1.0 ml PPV23.

The vaccines were given 12 weeks apart. Vaccines were administered intramuscularly and composed as described in section: *Introduction - Immunization against Pneumococci – Vaccines.*

Exclusion criteria

- Acute transplant rejection at enrolment
- Pregnancy
- Earlier PCV13 vaccination

Primary endpoint

The primary endpoint was the number of participants that reached a 'protective response' five weeks after the PPV23 (week 17). A 'protective response' is specified as an average pneumococcal antibody geometric mean concentration (GMC) ≥1 mg/L calculated as a mean across the 12 serotype-specific IgG antibodies. This corresponds to what is normally used in Denmark to assess protective immunity after PPV23 in adults. The calculation is described in the statistics section.

Secondary endpoints

- 'Protective response' at week 12, 48 and 96.
- Average pneumococcal antibody GMC levels at week 12, 17, 48 and 96.
- Number of serotype-specific IgG antibodies with ≥2-fold increases from baseline (range 0-12 for each participant) at week 12, 17, 48 and 96. Fold increase in concentration was determined by dividing the post-vaccination concentration by the baseline concentration.
- Changes in each of the 12 pneumococcal serotype-specific IgG antibodies.
- Pairwise correlations between average pneumococcal antibody GMC level and number of antibodies with ≥2-fold increase, age, or lymphocytes (T cells, B cells and NK cells). In KTRs, also days passed since transplantation and baseline dosage of tacrolimus (mg/day), mycophenolate mofetil (mg/day) and prednisolone (mg/day).
- Assessment of whether a positive cytomegalovirus (CMV) IgG at baseline affects immunogenicity (latent CMV, defined as sero-positivity for anti-CMV antibodies, has been associated with poor PPV23 response (88).
- For WLPs, assessment of whether immunosuppression and KTX during the study influenced the 'protective response', average antibody GMC level, and number of antibodies with ≥2-fold increases.
- Study safety.

Data

The participants' demographic data, comorbidities, medical reasons for kidney failure and kidney transplant data were assembled from patient records.

There was five visits during the study period: Baseline, week 12, week 17, week 48 and week 96.

Participants had blood samples drawn at each visit, and these were stored at −80°C until analysis. A missed visit did not exclude the participant from the next visit if both vaccines had been provided.

Safety assessment was done up to week 17, and all serious adverse events (AE) were recorded. Local reactions (pain, erythema, and oedema) at site of injection, and systemic reactions (fever, headache, arthralgia, and myalgia) were reported as AE.

Laboratory methods

Pneumococcal serotype-specific IgG antibodies were determined for 12 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) included in both PCV13 and PPV23 using an in-house Luminex method (89). Antibody concentrations were expressed as mg/L. Results above the range of the standard curve were assigned a value of 50 mg/L.

Cytomegalovirus 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.

Enumeration of T-, B- and NK-cells was performed using fresh EDTA blood stained with the BD Multitest™ 6-color TBNK reagent in BD TruCount tubes. Samples were analyzed on a BD FACSCanto™ II flow cytometer with BD FACSDiva software.

Serotype-specific IgG antibodies concentrations were determined at every visit, and CMV IgG and lymphocytes were measured at baseline.

Approval and permissions

The National Data Protection Agency (no. 16/26770) had approved the survey and it was conducted in accordance with the Helsinki Declaration.

The clinical 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). Signed informed consent was obtained for the trial, and it was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice.

Statistical analysis

Study 1 (Paper I)

In description of dichotomous and categorical variables, the number and percentage of patients were listed relative to the total number of patients, for whom the information was available, and analysis was done using the chi-squared test or Fisher's exact test as appropriate. For continuous variables, the median and interquartile rage (IQR) were reported and comparisons were done using Wilcoxon rank-sum test.

Factors associated with influenza vaccine uptake latest season were assessed with univariable logistic regression. Odds ratio (OR) with 95% confidence intervals (95% CIs) were calculated. Variables for multivariable logistic regression analysis were selected from a cut-off of p-value < 0.1. The model was assessed using goodness of fit test. A two-tailed p-value ≤ 0.05 was considered statistically significant. Statistical analysis were performed using Stata version 15 (StataCorp, College Station, TX, USA).

Study 2 (Paper II + III)

Each of the twelve serotype-specific IgG antibody concentrations were logarithmic transformed for statistical calculations. When antibody concentrations were presented, GMCs with 95% CIs were use. An average pneumococcal antibody GMC was calculated as a mean across the 12 serotype-specific IgG antibody concentrations, for each participant at each study visit. The calculation was as follows. A mean value for the 12 logarithm transformed antibodies was calculated and then exponentially transformed back. The average pneumococcal antibody GMC was evaluated as a binary variable in terms of 'protective response' obtained (average pneumococcal antibody GMC ≥ 1mg/L) and, otherwise, as a continuous variable. For remaining continuous variables, median and interquartile range (IQR) were reported. Non-paired continuous variables were analysed with Student's t-test if normally distributed and Wilcoxon rank-sum test if not. Normality was assessed with Shapiro-Wilk's test. For paired data the Student's t-test and Wilcoxon signed-rank test were used. For dichotomous and categorical variables, number and percentage were listed relative to patients in the group and were analysed using Chi-squared, McNemar's or Fisher's exact test as appropriate. Spearman's correlation was used for pairwise

correlations between continuous variables. A two-sided p-value ≤0.05 was considered statistically significant. Statistical analysis were performed using STATA 17 (Stata Corp, College Station, TX, USA).

Sample size

Based on a similar study (90), 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 achieving 50%) with 80% power and a two-sided alpha level of 5%.

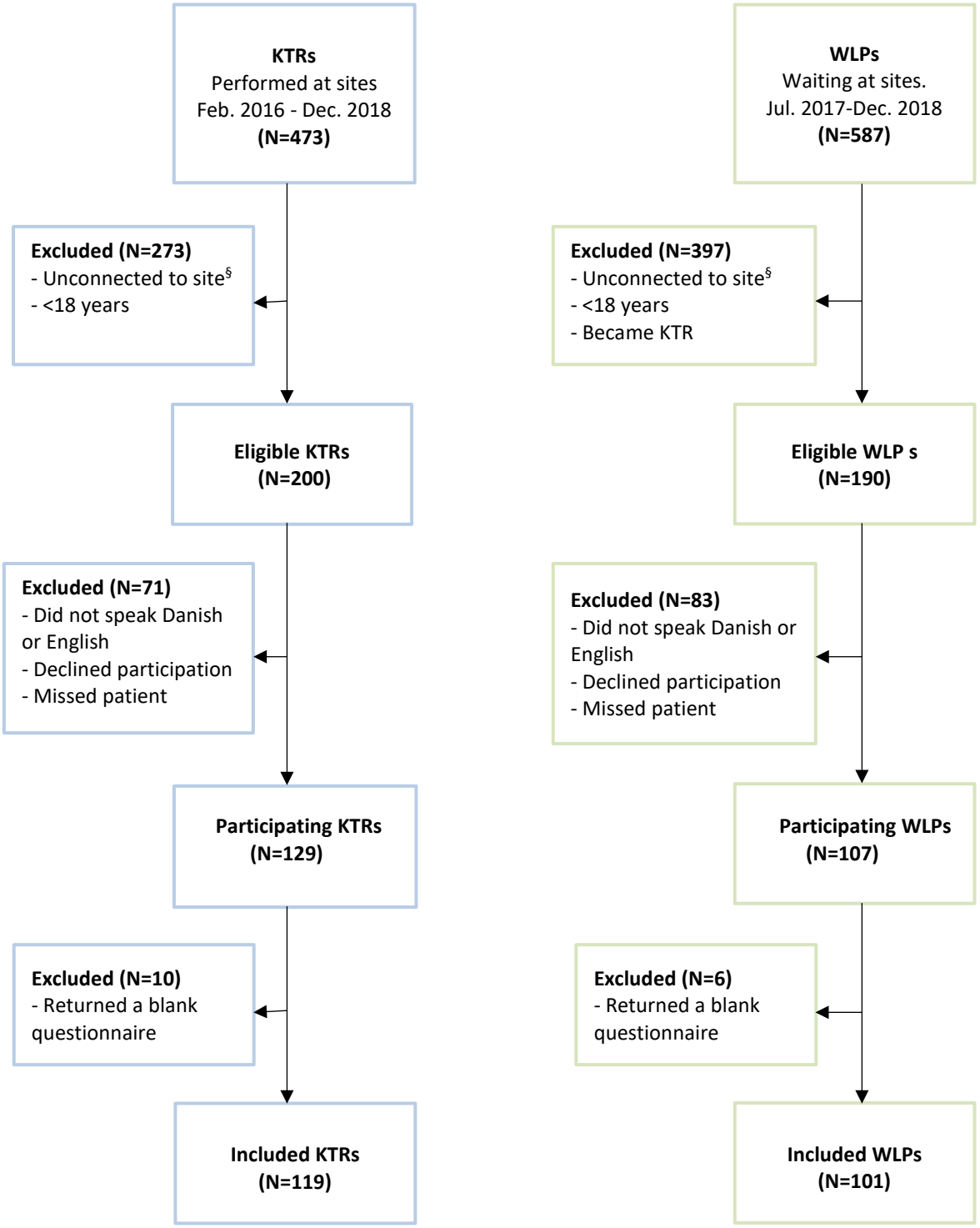
Results

Study 1 (Paper I)

Patient population

In total, 236 patients agreed to participate in the survey. Sixteen questionnaires were excluded as they were returned blank, thus leaving 220 participants in the study. Enrolment flow is depicted in Figure 2. Due to the legislation regarding anonymous research, the exact numbers for each exclusion reason were not available. Baseline characteristics are summarized in Table 1. KTRs made up 54.1% (N=119) of the population and WLPs 45.9% (N=101). Overall, median age was 52 years (IQR 42-61) and 66% (N=145) were men. There were more men in the KTR group, but the group were not statistical different from WLPs (p=0.06), and the groups were otherwise comparable.

Figure 2: Study 1 - Enrolment flow chart.



Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient.
§: KTRs and WLPs who had their follow-up at other hospitals.

Table 1: Demographics and socio-economic characteristics for patients on the kidney transplant waiting list and kidney transplant recipients.

Characteristics	Total N=220 N (%)	WLPs N=101 N (%)	KTRs N=119 N (%)	*p-value*
Age, median years (IQR)	52 (42-61)	51 (43-62)	53 (41-61)	*0.97*
Gender, male	145 (66)	60 (59.4)	85 (71.4)	*0.06*
Highest education				*0.21*
Primary school up to 10th grade	42 (19.1)	20 (19.8)	22 (18.5)	
Vocational education and training	84 (38.2)	31 (30.7)	53 (44.5)	
High school or equal to	26 (11.8)	14 (13.9)	12 (10.1)	
University or equal to	61 (27.7)	32 (31.7)	29 (24.4)	
Don't want to disclose	2 (0.9)	1 (1.0)	1 (0.8)	
No data	5 (2.3)	3 (3.0)	2 (1.7)	
Annual household income				*0.91*
<40.000€	69 (31.5)	27 (26.7)	42 (36.3)	
≥40.000€	100 (45.5)	40 (39.6)	60 (50.4)	
Don't want to disclose	8 (3.6)	4 (4.0)	4 (3.4)	
Don't know	33 (15)	21 (20.8)	12 (10.1)	
No data	10 (4.6)	9 (9.0)	1 (0.9)	

Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient.

Influenza and pneumococcal vaccination

Data for influenza and pneumococcal vaccination are summarized in Table 2. Ninety-two (41.8%) participants had received an influenza vaccine in the latest season and 117 (53.2%) in any previous season. WLPs and KTRs were statistically comparable with regards to the latest season but not for any previous season, were significantly more WLPs had been vaccinated (p = 0.007).

The information source recommending influenza vaccination was most often the general practitioner (30.9%). There was a tendency for a larger proportion of WLPs to be recommended the influenza vaccine, compared to KTRs, but not statistical significant (p=0.051) when evaluation if any recommendation had been given at all (general practitioner, nephrologist, other persons, personal information seeking) or not. Of the 39 participants who had not been informed together with the 21 participants who did not answer the question, only one individual had been vaccinated against influenza last season.

There were no differences between the groups regarding the reported reasons for not being influenza vaccinated (p=0.25). The most common reasons for not receiving the vaccine were; perception of good health with no need for vaccination (38.3%); was not informed that is was recommended (27.5%); and afraid of side effects (17.5%).

Ten participants (4.5%) with a median age of 56 years (IQR 39-64) had been offered a pneumococcal vaccine at some point, and 9 participants (4.1%) had accepted. They were equally divided between KTRs and WLPs. A general practitioner or a nephrologist had recommended the vaccine. Six participants could recollect why the pneumococcal vaccine had been recommended. Primarily it was for not being immunocompetent. One participant had requested the vaccine.

Factors associated with influenza vaccination in the latest influenza season.

In unadjusted analysis, any prior influenza vaccination was the strongest predictor for receiving an influenza vaccination in the latest season (OR 8.77 (CI95 4.52-17.02), p<0.001), compared to having never been vaccinated (Table 3). Age ≥65 years was a predictor for receiving an influenza vaccine in the latest season (OR 3.22 (CI95 1.31-7.95), p=0.01), compared to age <45 years and age group 45-64 years. Advice given by non-physicians was a negative predictor (OR 0.25 (CI95 0.10-0.62), p<0.001), compared to advice from a general practitioner. It was also statistically different, compared to advice from a nephrologist or personal information search. Remaining factors were not associated with influenza vaccine uptake. Thus age, information source and any prior influenza vaccination were then included in multivariable analysis. Influenza vaccination during any prior influenza season was still a positive predictor for influenza vaccine uptake in the latest season (OR 5.79 (CI95 2.44-13.76), p<0.001) and advice from non-physicians was still a negative predictor (OR 0.34, CI95 0.13-0.92) (p=0.03). Age was no longer significantly associated. Due to the small reported pneumococcal vaccine uptake no regression analysis was performed.

Table 2: Influenza and pneumococcal vaccination uptake for patients on the kidney transplant waiting list and kidney transplant recipients.

	All participants N=220 N (%)	**KTRs** N=119 N (%)	**WLPs** N=101 N (%)	**p-value**
Influenza vaccine uptake in the latest season				*0.293*
No	120 (54.6)	70 (58.8)	50 (49.5)	
Yes	92 (41.8)	47 (39.5)	45 (44.6)	
Don't know	4 (1.8)	0	4 (4)	
No data	4 (1.8)	2 (1.7)	2 (2)	
Influenza vaccination any prior season				*0.007*
Yes	117 (53.2)	54 (45.4)	63 (62.4)	
No	94 (42.7)	61 (51.3)	33 (32.7)	
Don't know	6 (2.7)	2 (1.7)	4 (5)	
No data	3 (1.4)	2 (1.7)	1 (1)	
Information source recommending influenza				*0.051#*
General practitioner	68 (30.9)	38 (31.9)	30 (29.7)	
Nephrologist	39 (17.7)	14 (11.8)	25 (24.8)	
Other (family, nurse, work place and media)	31 (14.1)	20 (16.8)	11 (10.9)	
Through personal information seeking	22 (10)	11 (9.2)	11 (10.9)	
Not recommended influenza vaccination	39 (17.7)	27 (22.7)	12 (11.9)	
No data	21 (9.6)	9 (7.6)	12 (11.9)	
Reasons for not vaccinating latest season (N=120)				*0.251*
Perception of good health; don't see the need for/effect of vaccination; don't want the vaccine.	46 (38.3)	23 (32.9)	23 (46)	
Was not informed that it is recommended for me	33 (27.5)	23 (32.9)	10 (20)	
Afraid of side effects	21 (17.5)	10 (14.3)	11 (22)	
Was not informed that it was free of charge	7 (5.8)	6 (8.6)	1 (2)	
Ill at the time of vaccination	5 (4.2)	3 (4.3)	2 (4)	
Don't want to disclose	2 (1.7)	2 (2.9)	0	
No data	6 (5)	3 (4.3)	3 (6)	
Ever pneumococcal vaccine offered				*0.750*
No	192 (87.3)	102 (85.7)	90 (89.1)	
Yes	10 (4.6)	5 (4.2)	5 (5)	
Don't know	15 (6.8)	10 (8.4)	5 (5)	
No data	3 (1.4)	2 (1.7)	1 (1)	
Pneumococcal vaccine accepted (N:10)				*NA*
Yes	9 (90)	4 (80)	5 (100)	
No	1 (10)	1 (20)	0	

Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient; NA, not applicable

Difference between KTRs and WLPs whether any recommendation at all was obtained.

Table 3: Factors associated with influenza vaccine uptake in the latest season for 212 participants

Factors	Vaccinated (N=92) N (%)	OR (95% CI)	p-value	aOR (95% CI)[§]	p-value
Influenza vaccine prior seasons					
No	16 (17.4)	Ref.		Ref.	
Yes	72 (78.3)	8.77 (4.52-17.02)	<0.001	5.79 (2.44-13.76)	<0.001
Age group					
<45 years	20 (62.5)	Ref.		Ref.	
45-64 years	48 (40.7)	1.25 (0.66-2.37)	0.49	0.87 (0.37-2.05)	0.75
≥65 years	22 (33.3)	3.22 (1.31-7.95)	0.01	2.28 (0.68-7.66)	0.18
Information source					
General practitioner	44 (47.8)	Ref.		Ref.	
Nephrologist	25 (27.2)	1.04 (0.44-2.46)	0.93	2.16 (0.74-6.31)	0.16
Other[£]	10 (10.9)	0.25 (0.10-0.62)	<0.001	0.34 (0.13-0.92)	0.03
Through personal info. seeking	12 (13.1)	0.60 (0.22-1.60)	0.31	1.16 (0.37-3.66)	0.80
Not informed	0	-		-	
Sex					
Male	55 (59.8)	Ref.		No data	
Female	37 (40.2)	1.57 (0.89-2.78)	0.12		
Patient groups					
WLPs	45(48.9)	Ref.		No data	
KTRs	47(51.1)	0.75 (0.43-1.29)	0.29		
Annual household income					
<40.000 EUR	25 (27.2)	Ref.		No data	
≥40.000 EUR	41 (44.6)	1.18 (0.62-2.24)	0.61		
Education level					
Primary school up to 10th grade	16 (17.4)	Ref.		No data	
Vocational educ. and training	40 (43.5)	1.50 (0.69-3.24)	0.30		
High school or equal to	7 (7.6)	0.55 (0.19-1.62)	0.28		
University or equal to	27 (29.4)	1.23 (0.55-2.76)	0.62		

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient.

£: family, nurse, work place or media

§: 148 case were included in the multivariable analysis

Study 2 (Paper II + III)

Patient population

The study population is presented in Figure 3. Of 236 WLPs and KTRs screened, 142 accepted to participate. Following randomisation, 65 WLPs (ND = 32; DD = 33) and 74 KTRs (ND = 39; DD = 35) were vaccinated with PCV13. Within 12 weeks from baseline, 1 participant died and was only included in baseline analysis. After blood sampling week 12, another 3 participants were excluded without receiving PPV23 (1 was too ill to vaccinate at week 12 (died afterwards) and 2 did not want to receive PPV23). After week 12, another two withdrew consent, two died, one dropped out. Five missed a visit without being excluded.

Baseline characteristics, comorbidities, immunosuppressive medication, dialysis treatment and transplantation data are depicted in Table 4. Median age for all participants was 52 years (IQR: 41–61) and 69% (96/139) were males. At baseline, 6 WLP-ND and 12 WLP-DD were treated with immunosuppressants (p=0.113). In comorbidity and aetiology of kidney failure, a few significant differences were present between KTR-ND and KTR-DD.

Median time from baseline to PPV23 was 12 weeks (IQR: 11.3-13.0), from baseline to week 17, 17.7 weeks (IQR: 16.6-19.1), from baseline to week 48, 48.6 weeks (IQR: 47-52.4) and from baseline to week 96, 97.1 weeks (IQR: 94.6-100.1).

Figure 3: Study 2 - Consort flow chart of study population

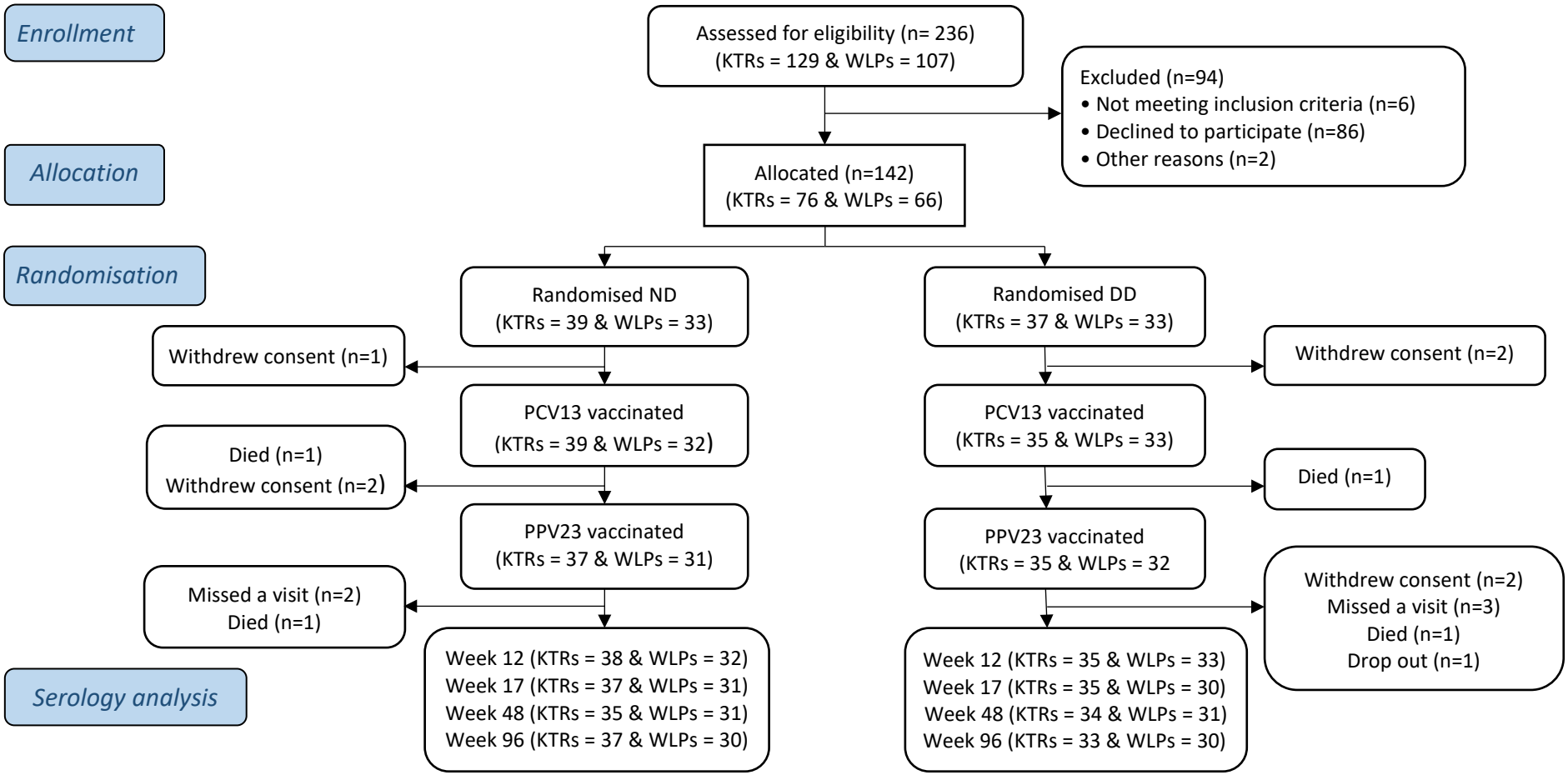


Table 4: Baseline characteristics, comorbidities, immunosuppressive drugs, dialysis treatment and transplant data.

	WLPs			KTRs		
	ND	DD	p-value	ND	DD	p-value
Participants, N	32	33		39	35	
Age, median years (IQR)	55 (44-60)	51 (40-	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 (%)						
Cardiovascular disease	21 (65.6)	22 (66.7)	0.929	22 (56.4)	28 (80)	0.030
Neurological diseases	9 (28.1)	7 (21.2)	0.518	5 (12.8)	10 (28.6)	0.147
Rheumatic diseases	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)	0.197	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
Aetiology 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)	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)	0.176
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
Immunosuppressiva, 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
Baseline dosage, median (IQR)						
Tacrolimus, mg/day	NA	NA		4 (3-5)	4 (3-6)	0.332
Mycoph. Mofetil, g/day	NA	NA		1.5 (1.5-2.0)	1.5 (1.5-2.0)	0.780
Prednisolone, mg/day	NA	NA		6.25 (5-10)	7.5 (5-10)	0.589
Time since KTX, median days	NA	NA		142 (83-271)	132 (71-337)	0.840
Donor status, 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 KTX, N (%)	10 (31.3)	10 (30.3)	1.000	NA	NA	
WLPs: KTX during study, N (%)			0.274			
Before week 12	4 (12.5)	2 (6.1)		NA	NA	
Week 12 - 17	4 (12.5	1 (3)		NA	NA	
Week 17 - 96	11 (34.4)	11 (33.3)		NA	NA	

Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient; ND, normal dosage; DD, double dosage, KTX, kidney transplantation. £: Other co-morbidities consists of non-malignant diseases (pulmonary, gynaecological, 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.

'Protective response' (an average pneumococcal antibody GMC ≥1 mg/L).

Proportion of participants in each of the four groups with a 'protective response' at each study visit are depicted in Table 5. WLP-DD responded significantly better than WLP-ND at week 17, p=0.015. At the other visits, the groups' responses were statistical comparable. KTR-ND and KTR-DD were statistical comparable at all visits. KTR-ND demonstrated a significant increase in proportion with a 'protective response' from week 12 to week 17 following PPV23 as the only group (p=0.031). The results are also presented in Supplement Figure 1a for WLPs and Supplement Figure 1b for KTRs.

Table 5: Kidney transplant waiting list patients and kidney transplant recipients in each group reaching a 'protective response' at each study visit.

Visit	**WLP-ND** N (%)	**p-value§**	**WLP-DD** N (%)	**p-value§**	**p-value€**
Baseline	2 (6.3)		1 (3)		*0.613*
Week 12	12 (37.5)		18 (54.6)		*0.168*
Week 17	11 (35.5)	*1.000*	20 (66.7)	*.250*	*0.015*
Week 48	9 (29)		12 (38.7)		*0.421*
Week 96	8 (26.7)		12 (40)		*0.273*
Visit	**KTR-ND** N (%)	**p-value§**	**KTR-DD** N (%)	**p-value§**	**p-value€**
Baseline	0		2 (5.7)		*0.220*
Week 12	7 (18.4)		7 (20)		*0.864*
Week 17	13 (35.1)	*0.031*	9 (25.7)	*.500*	*0.386*
Week 48	9 (25.7)		5 (14.7)		*0.371*
Week 96	8 (21.6)		3 (9.1)		*0.197*

Abbreviations: 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

§: p-value for increases in 'protective response' week 17, compared to week 12 within each group.

€: p-value for difference in 'protective response' between ND and DD groups at each visit.

Average pneumococcal antibody GMC as a continuous variable

Average pneumococcal antibody GMC with 95% CI for each treatment group at each study visit are depicted in Table 6. There were no significant differences in average pneumococcal antibody GMC between WLP-ND and WLP-DD or between KTR-ND and KTR-DD at any visit. Compared to week 12, WLP-ND did not achieve a significant increase in average pneumococcal antibody GMC at week 17. Yet, the three remaining groups did. All four groups continued to have an average pneumococcal antibody GMC level at week 96 that was significantly higher than at baseline.

Table 6. Pneumococcal average antibody geometric mean concentration for kidney transplant waiting list patients and kidney transplant recipients at each study visit.

Visit	WLP-ND GMC (95% CI)	p-value§	p-value#	WLP-DD GMC (95% CI)	p-value§	p-value#	p-value€
Baseline	0.20 (0.15-0.26)			0.21 (0.16-0.28)			*0.766*
Week 12	0.82 (0.54-1.25)		*≤0.001*	0.93 (0.61-1.42)		*≤0.001*	*0.657*
Week 17	0.92 (0.62-1.35)	*0.451*	*≤0.001*	1.19 (0.80-1.78)	*0.018*	*≤0.001*	*0.332*
Week 48	0.57 (0.39-0.84)		*≤0.001*	0.67 (0.45-0.98)		*≤0.001*	*0.548*
Week 96	0.56 (0.37-0.84)		*≤0.001*	0.77 (0.55-1.07)		*≤0.001*	*0.235*
Visit	**KTR-ND** GMC (95% CI)	**p-value§**	**p-value#**	**KTR-DD** GMC (95% CI)	**p-value§**	**p-value#**	**p-value€**
Baseline	0.22 (0.17-0.27)			0.26 (0.20-0.34)			*0.277*
Week 12	0.48 (0.33-0.70)		*≤0.001*	0.47 (0.34-0.66)		*≤0.001*	*0.945*
Week 17	0.57 (0.39-0.83)	*0.014*	*≤0.001*	0.54 (0.38-0.76)	*0.035*	*≤0.001*	*0.840*
Week 48	0.44 (0.30-0.64)		*≤0.001*	0.44 (0.32-0.60)		*≤0.001*	*0.976*
Week 96	0.47 (0.34-0.65)		*≤0.001*	0.41 (0.31-0.54)		*≤0.001*	*0.497*

Abbreviations: 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; GMC, geometric mean concentration; CI, confidence interval.
§: p-value for average pneumococcal antibody GMC week 17, compared to week 12 level within each group.
#: p-value for average pneumococcal antibody GMC, compared to baseline level within each group.
€: p-value for differences in between ND and DD groups at each visit.

In Supplement Figure 2a-e for WLPs and in Supplement Figure 3a-e for KTRs, log-transformed average antibody GMCs for each of the study participants are presented together with group mean, median, IQR and visibility of the border for obtaining a 'protective response'.

Number of serotype-specific IgG antibodies with a ≥2-fold increase from baseline (range 0-12)

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

Median number of antibodies with a ≥2-fold increase obtained at each visit are presented in Table 7 for each treatment group. 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 four groups from week 12 to week 17.

Table 7: Median number of serotype-specific IgG antibodies with a ≥2-fold increase from baseline at each study visit for each kidney transplant waiting list patient and kidney transplant recipient group.

Visit	WLP-ND Median(IQR)	p-value§	WLP-DD Median(IQR)	p-value§	p-value€
Week 12	7 (5-10.5)		9 (6-11)		*0.333*
Week 17	8 (6-11)	*0.037*	10 (6-12)	*0.014*	*0.214*
Week 48	7 (3-9)		7 (4-10)		*0.224*
Week 96	7 (4-11)		9 (6-10)		*0.232*
Visit	**KTR-ND** Median(IQR)	**p-value§**	**KTR-DD** Median(IQR)	**p-value§**	**p-value€**
Week 12	4 (2-8)		3 (0-6)		*0.103*
Week 17	7 (3-10)	*0.002*	4 (1-8)	*0.003*	*0.129*
Week 48	5 (2-7)		3 (1-7)		*0.153*
Week 96	5 (3-8)		3 (1-6)		*0.172*

Abbreviations: 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.
§: p-value for increases in number of antibodies with a ≥2-fold rise from week 12 to week 17 within each group.
€: p-value for difference in number of antibodies with a ≥2-fold rise between ND and DD for every visit.

The 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. Among KTRs, 13% did not obtain any ≥2-fold antibody increases at week 17. In WLPs, it was 4%.

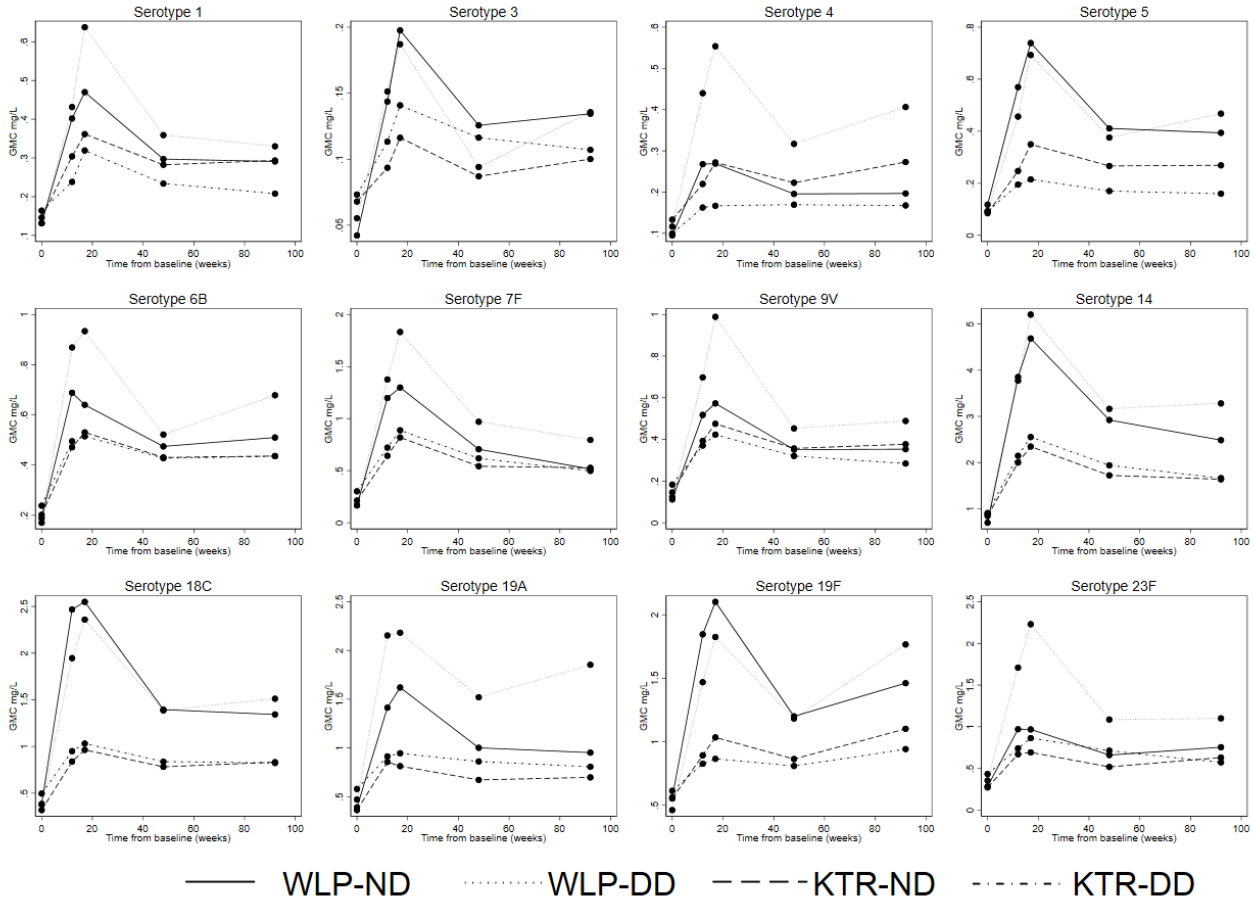
The percentage distribution of participants per obtained number of antibodies with 2-fold increases (from 0 to 12) at each study visit is shown in Supplement Figure 4a-d.

Each 12 pneumococcal serotype-specific IgG antibody concentrations

GMCs with 95% CI for the individual 12 serotype-specific IgG antibodies, with p-values for differences between and within groups, are presented in Supplement Table 1. We observed no significant differences between WLP-ND and WLP-DD, at any visit. Nor between KTR-ND and KTR-DD. Following PPV23, GMCs were significantly higher at week 17, compared to week 12 for KTR-ND in 4 serotypes (3, 4, 5 and 18C), KTR-DD in 4 serotypes (1, 3, 14 and 18C) and for WLP-DD in 2 serotypes (1 and 14). At week 17, all GMCs were significantly higher than baseline values except for serotype 19F in WLP-DD. From week 48 to week 96, GMCs stayed fairly stable (Figure 4). At week

96, antibody GMCs were significantly higher than baseline values except in 4 serotypes for WLP-DD (3, 9V, 19A and 23F).

Figure 4: Geometric mean concentrations for 12 pneumococcal serotype-specific IgG antibodies in mg/L from baseline through week 96. 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

Lymphocyte subpopulation count

A subgroup of 32 WLPs and 58 KTRs had blood samples drawn at baseline for T, B and NK lymphocyte subpopulation counts. Lymphocyte cell counts for each groups are depicted in Supplement Table 2. Compared to KTRs, WLPs had a significantly higher absolute lymphocyte cell count (median cells 1.28 x10^3/µL (IQR: 1.06-1.65) vs. 1.06 x10^3/µL (IQR: 0.77-1.58); p=0.028), CD4+ T lymphocytes (median cells 0.66 x10^3/µL (0.47-0.78) vs. 0.53 x10^3/µL (IQR: 0.27-0.72); p=0.046), and NK cells (median cells 0.18 x10^3/µL (0.12-0.26) vs. 0.12 x10^3/µL (0.09-0.17); p=0.007). For the

remaining subpopulations, there were no significant differences. We found no pairwise correlations between average antibody GMC levels at week 12 (PCV13) or at week 17 (PCV13+PPV23) and the absolute lymphocyte cell count or any subpopulation lymphocyte cell count for neither KTRs nor WLPs.

Pairwise correlations with average pneumococcal antibody GMC at week 17 in WLPs:

Average pneumococcal antibody GMC level at week 17 correlated with average pneumococcal antibody GMC at baseline (N=61, r= 0.580, p≤0.001), average pneumococcal antibody GMC at week 12 (N=61, r= 0.961, p≤0.001) and number of serotypes with 2-fold increases at week 17 (N=61, rho= 0.762, p≤0.001). No correlation with the participants' age was present. ND and DD groups were combined in this calculation.

Pairwise correlations with average pneumococcal antibody GMC at week 17 in KTRs:

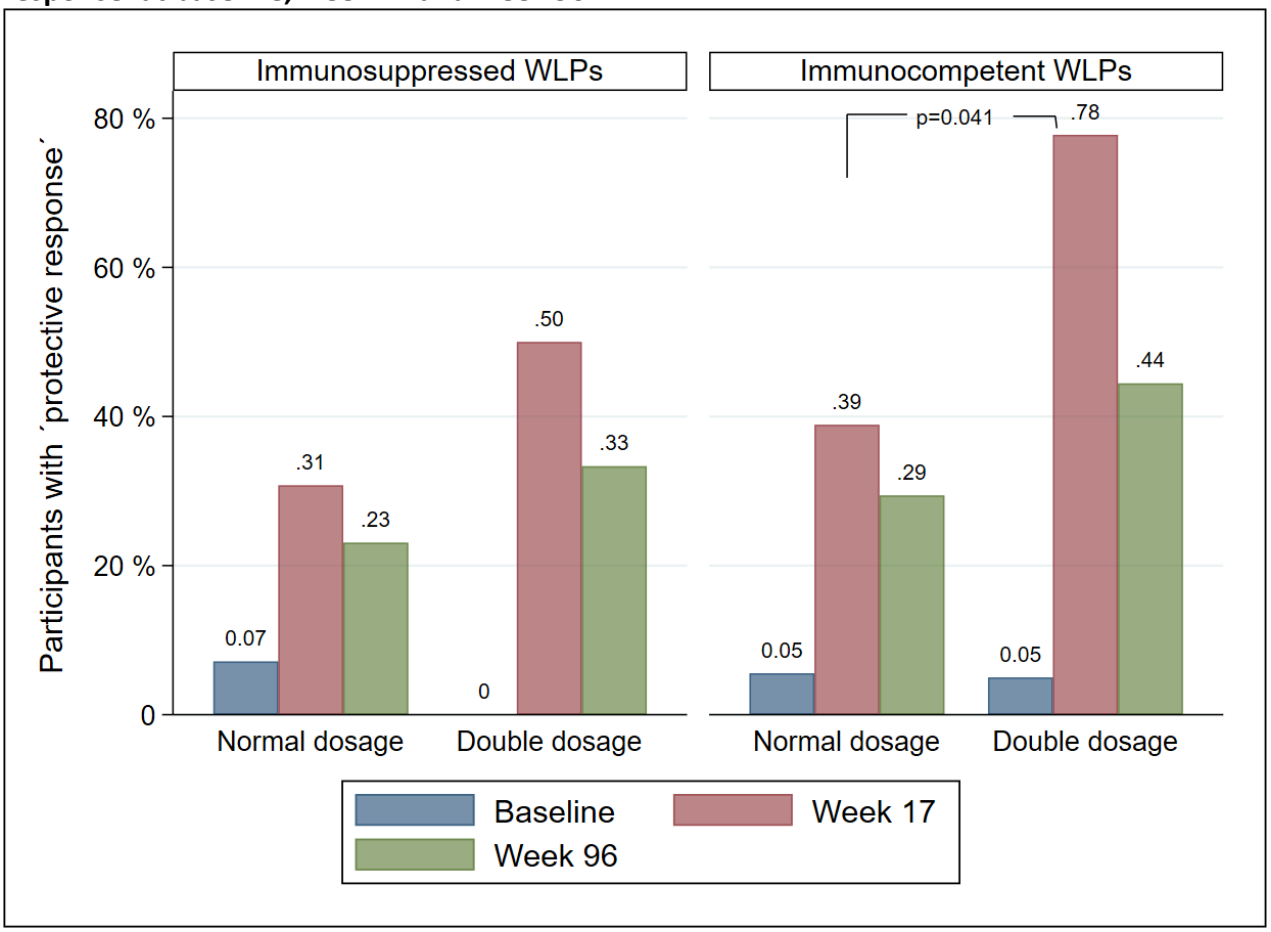
Average pneumococcal antibody GMC at week 17 correlated with average pneumococcal antibody GMC at baseline (N=72, r=0.671, p≤0.001), average pneumococcal antibody GMC at week 12 (N=72, r= 0.946, p≤0.001), number of serotypes with 2-fold increases at week 17 (N=72, r=0.555, p≤0.001) and negative with the participants' age (N=72, rho = -0.261; p=0.027). Average pneumococcal antibody GMC at week 17 did not correlate with time since transplantation at baseline, baseline tacrolimus, mycophenolate mofetil or prednisolone daily dosage. However, days since transplantation at baseline correlated negative with baseline tacrolimus (N=72, rho= -0.340, p=0.004), prednisolone (N=25, rho= -0.677, p≤0.001), and mycophenolate mofetil daily dosage (N=64, rho= -0.641, p≤0.001). ND and DD groups were combined in these calculations.

Drug-induced immunosuppression in WLPs

At week 17, twenty-five WLPs were immunosuppressed (WLP-ND = 13, WLP-DD = 12) either from received immunosuppressive medication of any kind at baseline or/and from having had a kidney transplant before week 17. Thirty-six WLPs were immunocompetent (disregarding their severe uraemia). When vaccine dosages were assessed within these 2 groups, a significant higher proportion of immunocompetent WLP-DD had a 'protective response' at week 17, compared to immunocompetent WLP-ND (p=0.041), but not at week 96. Immunosuppressed WLP-DD and

immunosuppressed WLP-ND were comparable, at both visits. Participants with a 'protective response' for immunosuppressed WLPs and immunocompetent WLPs are illustrated in Figure 5.

Figure 5: Immunosuppressed and immunocompetent WLPs (week 17) with a 'protective response' at baseline, week 17 and week 96.



Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient

Average pneumococcal antibody concentrations achieved at baseline, week 17 and 96 for immunocompetent and immunosuppressed WLPs divided into dosage groups are depicted in Table 8. There were no significant differences between ND and DD for either group at any visit. All four groups continued to have an average pneumococcal antibody concentration at week 96 that was significantly higher than at baseline.

Number of antibodies with a ≥2-fold increase from baseline, for immunocompetent and immunosuppressed WLPs divided into dosage groups are depicted in Table 9 for week 17 and 96. There were no significant differences between ND and DD for either group at any visit.

Table 8. Immunosuppressed and immunocompetent WLPs' (week 17) pneumococcal average antibody geometric mean concentrations at baseline, week 17 and week 96.

Visit	N	Immunosuppressed WLP-ND GMC (95% CI)	p-value[#]	N	Immunosuppressed WLP-DD GMC (95% CI)	p-value[#]	p-value[€]
Baseline	14	0.20 (0.13-0.34)		13	0.17 (0.11-0.28)		*0.555*
Week 17	13	0.64 (0.36-1.15)	*≤0.001*	12	0.68 (0.33-1.39)	*≤0.001*	*0.901*
Week 96	13	0.51 (0.26-0.99)	*≤0.001*	12	0.53 (0.29-0.98)	*≤0.001*	*0.910*
Visit	**N**	**Immunocompetent WLP-ND** GMC (95% CI)	**p-value[#]**	**N**	**Immunocompetent WLP-DD** GMC (95% CI)	**p-value[#]**	**p-value[€]**
Baseline	18	0.19 (0.13-0.27)		20	0.24 (0.17-0.34)		*0.351*
Week 17	18	1.18 (0.69-2.02)	*≤0.001*	18	1.74 (1.14-2.66)	*≤0.001*	*0.239*
Week 96	17	0.61 (0.34-1.07)	*≤0.001*	18	0.97 (0.66-1.43)	*≤0.001*	*0.151*

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage; GMC, geometric mean concentration; CI, confidence interval.
#: p-value for average pneumococcal antibody GMC, compared to baseline level within each group.
€: p-value for differences between ND and DD groups at each visit.

Table 9: Immunosuppressed and immunocompetent WLPs' (week 17) number of serotype-specific IgG antibodies with a ≥2-fold increase from baseline at week 17 and 96.

Visit	N	Immunosuppressed WLP-ND Median(IQR)	N	Immunosuppressed WLP-DD Median(IQR)	p-value[€]
Week 17	13	5 (4-9)	12	5.5 (6.5-9)	*0.530*
Week 96	13	7 (2-11)	12	8 (5.5-10)	*0.477*
Visit	**N**	**Immunocompetent WLP-ND** Median(IQR)	**N**	**Immunocompetent WLP-DD** Median(IQR)	**p-value[€]**
Week 17	18	9.5 (8-11)	18	11 (10-12)	*0.108*
Week 96	17	6 (5-10)	18	9 (6-10)	*0.266*

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage
€: p-value for difference in number of antibodies with a ≥2-fold increase between ND and DD for every visit.

Overall, immunocompetent WLPs had most participants with a 'protective response', compared to immunosuppressed WLPs, though the groups were statistical comparable. Compared to all KTRs, only immunocompetent WLPs did statistically better at week 17 (p=0.005) and week 96 (p=0.014). These result are presented in Supplement Figure 5.

Positive CMV IgG

Overall, 63.3% (88/139) had a positive CMV IgG response at baseline. A positive CMV IgG response was not found to have a significant impact on the proportion obtaining a 'protective response', average pneumococcal antibody GMC levels or number of antibodies with a ≥2-fold increase at week 17 in neither WLPs nor KTRs. This was also the case when vaccine dosages were taken into account.

Safety data

During the study period, 55 serious AEs were reported. None were considered treatment related. In total, 82 local and 104 systemic AEs were reported. Of local reactions, 58.5% (48/82) were treatment related and equally distributed between PCV13 and PPV23. The most frequently reported was pain at injection-site. Fifteen of the systemic AEs were treatment related and predominantly caused by PPV23. The most frequently reported were fever and generalized muscle pain. There were no differences between the dosage 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.

Discussion

Study 1 (Paper I): Vaccine uptake

The self-reported influenza vaccine uptakes on 39% for KTRs and 44% for WLPs in the latest flu season are well below the WHO goal of vaccinating 75% of key risk groups against influenza (91). However, the result agrees with the suboptimal vaccine uptake previously described by foreign studies. Here proportion of KTRs immunized against influenza was registered to be between 23% and 59% (80, 83, 92-97), and even lower (19%) in those who have received their transplant within the latest year (92). For WLPs, it has been reported that 43% to 67% are immunized against influenza in a given season (80, 82, 83, 98-100), and 24% to 77% of dialysis patients overall (81, 94, 101-103).

WLPs were more prone to have received an influenza vaccine in prior season, compared to KTRs ($p=0.007$). The explanation for this may be that the vast majority of WLPs in our cohort was waiting for a kidney from a deceased donor, whereas about 1/3 of KTRs have received their kidney from a living donor. The WLPs may therefore have had renal failure and been on dialysis for a longer time period totally, compared to KTRs. Thereby meeting the criteria for getting influenza vaccination recommended. Pre-emptive transplantation, in the KTR group, may also be a factor.

With respect to pneumococcal vaccination, only 4% of study participants reported to have been immunized against pneumococci. Other studies investigating vaccine uptake reported that 11% to 36% of KTRs (83, 95, 99), 9% to 83% of WLPs (82, 83, 98-100), and 14% to 54% of dialysis patients were immunized against pneumococci (101-104). Compared to these results, 4% is quite low. We did expected it to be lower than influenza coverage, as this was the trend in the above mentioned studies. A low immunization rate may be a result of multiple factors, such as vaccine costs for the patient, lack of structured approach to immunization, and doubt about who should perform the task. Traditionally, general practitioners have been responsible for immunization, at the patient's own request, in Denmark. Nor has immunization been a mandatory part of the pre-transplant preparation or at dialysis units in Denmark. In our cohort, 18% reported that they had not received an influenza vaccine recommendation, which must be assumed to reflect an unstructured approach to immunization in at-risk patients. Prior to the present study, there had been no national campaign for pneumococcal vaccination, but it was presented from the Danish Health Authority after study 1 was completed. It may contribute to increase vaccine adherence. Harris et al. found that over a 10

year period the pre-transplant influenza vaccine coverage in SOT candidates had increased significantly over the years (80). So perhaps there is a general growing awareness of immunization for at risk-patients. Two Danish studies, one including patients with rheumatoid arthritis and the other people living with HIV, found that 49% and 31% had received an influenza vaccine in the latest season, respectively, and 6% and 4% a pneumococcal vaccine, respectively (105, 106). Thus low vaccine uptake not only applies to our study population. These studies' results are comparable to ours and emphasize that strategies to improve immunization of chronic patient groups are highly needed in Denmark. However, improvement is possible, as previous studies have shown that increased attention and a systematic approach to immunization increases vaccination adherence (107-111). E.g. Blanchard-Rohner et al. showed that with a vaccination setup before transplantation, 96% of the candidates agreed to an influenza vaccination (82).

Study 1 (Paper I): Factors associated with vaccine uptake

In the present study, any previous influenza vaccination was a positive predictor for influenza vaccination in the latest season. This concurs with previous studies on patients with ESRD and KTRs where prior influenza or pneumococcal vaccines were positive predictors for seasonal influenza vaccination (80, 83, 94, 95, 97, 99, 103, 112, 113). Thus, if immunization has been used in the past, there is a high probability that people will continue to do so. However, it is uncertain whether it is at the request of healthcare professionals or the patient's own. This pattern is also found in other patient populations, such as the elderly and rheumatology patients (114, 115).

Receiving the influenza vaccine recommendation from, e.g., family members, nurses or the workplace was associated with not receiving the vaccine, compared to either general practitioner or nephrologist. This indicates that a recommendation from a physician has the greatest effect. This agrees with comparable studies showing that a physician's recommendation increases vaccine uptake compared to encouragement from other healthcare professionals (81, 83, 95, 97, 103). One study even found that a specialist physician's vaccine recommendation has greater impact than a general practitioner's (95).

With multi-variable adjustment, age >65 years was no longer significantly associated with increased influenza vaccine uptake. Our result may be affected by collinearity between age and any prior influenza vaccine. A previous study on SOT recipients found the same (95), whereas the majority of

studies, however, found age to be associated with influenza or pneumococcal vaccine uptake in patients with ESRD or KTRs (80, 81, 83, 92, 96, 97, 99, 112, 116). In agreement with the present study, other studies have found that education level is not association with vaccine uptake (83, 93, 94, 96-98, 103, 112, 117) though with exception (81).

The main reported reasons for not getting the influenza vaccine were; lack of information; did not deem it necessary or important; is in good health; afraid of side effects. This is consistent with other studies that also find that factors associated with decreased vaccine uptake are primarily concern over vaccine side effects (94, 95, 97, 113, 118), if the patient does not receive any recommendation or information on immunization (93, 103), if the self assed risk of a severe influenza course is low (94), or if the patient does not consider influenza to be a serious illness (113). Moreover, if vaccine effectiveness is judged to be low by patients as well as by physicians, vaccine uptake declines (93, 95).

Former studies have also showed that dialysis treatment (99, 100, 116), and longer time since KTX (92, 97) are associated with increased influenza and pneumococcal vaccine uptake. As these variable were not obtained for study 1, we were not able to evaluate them.

Study 2 (Paper II + III): Dose effect pneumococcal vaccines

The humeral response following pneumococcal prime-boost vaccination was assessed in different ways, all based on the quantitative measurements of 12 serotype-specific IgG antibodies. The primary outcome was proportion of participants that reached an average pneumococcal antibody GMC ≥1 mg/L calculated as a mean across the 12 serotype-specific IgG antibodies, where we uncovered that 2x standard dose of both PCV13 and PPV23 resulted in significantly more WLP-DD attaining a 'protective response', compared to WLP-ND, 5 weeks after immunization was completed. Hence, a significant dose effect was observed in WLPs, in accordance with previous studies which have been described in the introductory section (Pneumococcal vaccines - antigen doses) (71-73, 75). However, by week 48 - 96, the dose effect had subsided and WLP-DD and WLP-ND were statistical comparable.

The studies evaluating dose effect of pneumococcal conjugate vaccines in immunocompetent elderly measured the opsonophagocytic antibody response and levels of the individual measured serotype-specific IgG antibodies (72, 73). The present studies was not powered to detect significant

differences between the groups in average pneumococcal antibody GMC as a continuous variable, individual serotype-specific IgG antibody levels, or in number of antibodies with 2-fold increases. However, the calculations were done as secondary analyses. Though the groups were statistical comparable, all three assessment methods showed signs of WLP-DD performing slightly better than WLP-ND. Especially at week 17.

In KTRs, we found no sign of a positive dose effect, with any of the comparisons done. KTR-DD overall seemed to perform poorly, though no significant differences were uncovered between the two dosage groups. We were not able to uncover any differences, between KTR-ND and KTR-DD, in the registered baseline variables, that could explain the absence of a dose effect. Including age, as this was the only variable that correlated with average pneumococcal antibody GMC level at week 17, in KTRs. A correlation also reported in other vaccine studies (119-121). Hyporesponsiveness following a prior pneumococcal vaccine was not an influencing factor, as only two KTRs were previously vaccinated with PPV23. In a prior study, tacrolimus have been associated with lower pneumococcal antibody concentrations following PPV23, compared to cyclosporine A (122). Most KTRs in our study were treated with tacrolimus. We did however not find any correlation between daily dosage tacrolimus and vaccine response. Neither did Kumar et al. in their study (123). We can only deduce that heavy immunosuppression prior to immunization diminishes the pneumococcal prime-boost vaccine response, and a positive dose effect cannot be achieved in newly transplanted KTRs. A small study evaluating pneumococcal prime-boost vaccination using double dose PCV13 in rheumatoid arthritis patients treated with biological disease-modifying anti-rheumatic drugs found no dose effect either (74).

As all KTRs were immunosuppressed, we investigate the effect of drug induced immunosuppression in WLPs. As a proportion of WLPs received a transplant throughout the study course, a larger proportion of WLPs went from being immunocompetent at baseline to being immunosuppressed before they reached the week 17 visit. Overall, the vaccine response seemed strongest in those WLPs immunocompetent up until week 17, compared to those who were immunosuppressed. A dose effect appeared to be present in both groups, though only significantly more immunocompetent WLP-DD achieved a ‘protective response’ at week 17, compared to immunocompetent WLP-ND. However, due to very few participants in these groups, we did not expect to be able to detect any significant differences. Hence, we find our result supports DD

pneumococcal prime-boost vaccination in WLPs regardless of low level immunosuppression. Just as double the standard dose of hepatitis B vaccines are used in patients with CKD (124).

Study 2 (Paper II): Pneumococcal prime-boost vaccination

The evaluation of the pneumococcal prime-boost vaccination that was done in this study, was a comparison the vaccine-response 5 weeks after PPV23, compared to that 12 weeks after PCV13. In both KTR groups, 4/12 serotype-specific antibodies increased significantly. As did number of antibodies with ≥2-fold increases and average antibody concentration. Furthermore, significantly more KTRs in the ND group achieved a 'protective response' after PPV23. Prior studies in KTRs/SOT recipients have showed no or very marginal effect from PPV23 boosting. Tobudic et al. demonstrated no significant increases in serotype-specific antibody concentrations when adult KTRs received PPV23 a year after PCV7 (90). In paediatric SOT recipients vaccinated with 3 serial PCV7+PPV23, there were only a significant increase in 1/7 vaccine shared serotype-specific antibodies after PPV23 overall. Still, in the KTR-subgroup the difference was non-significant (125). In another study, paediatric SOT recipients displayed no additional increases in vaccine shared serotype-specific antibody concentrations after PPV23, when vaccinate with 2 serial PCV7+PPV23 (6-8 weeks between vaccines) (126). In adult heart or lung transplant recipients vaccinated with PVC7+PPV23 with 8 weeks apart, no increases in vaccine shared serotype-specific antibody concentrations were observed after PPV23 (127). However, in accordance with the present study, Hoffman et al. reported that boosting with PPV23, 8 weeks after PCV13, resulted in significant increases in 2/12 vaccine shared serotype-specific antibodies in adult lung transplant recipients (128). Both WLP groups exhibited significant rises in number of antibodies with ≥2-fold increases. WLP-DD also in 2/12 antibody concentrations and average antibody concentration.

No studies assessing pneumococcal prime-boost vaccination have been conducted in dialysis patients. Hence, no comparisons can be made.

A few former studies have also compared prime-boost vaccination with a single PPV23. Tobudic et al found the two approaches showed equal immunogenicity in adult KTRs (90). The same was observed by Kumar et al. in adult liver transplant recipient (129), and by Hoffman et al. in adult lung transplant recipients (128). Both studies with 8 weeks between vaccines.

When we evaluate our results, we must take into account, that the time interval between vaccination and antibody measurement was not the same after PCV13 (12 weeks) and PPV23 (5 weeks). Hence, an eventual drop in antibody concentrations shortly after PCV13 may not have been taken into account. This may result in an overestimation of PPV23's boosting ability in our cohort. So, we cannot strongly conclude that the PCV13+PPV23's vaccine response is superior to that of PCV13 alone. However, we do find our results supportive of PPV23's additive effect to PCV13 in both KTRs and WLPs. Also taking into account the wider serotype coverage obtained with PPV23.

Study 2 (Paper III): Durability of the antibody response

Median number of serotype-specific IgG antibodies with a ≥2-fold increase from baseline were at week 96, comparable to the week 12 results. However, the proportion of participants with a 'protective response' had declined in all groups at week 96. The average pneumococcal antibody GMC declined over time for both WLPs and KTRs, but were at week 96 still significantly above pre-immunization levels in all treatment 4 groups. The same goes for the 12 individual serotype-specific antibodies, except for WLP-DD where only 8/12 serotypes were still significantly above pre-immunization levels. These results supports a certain durability of the vaccine response and agree with previous studies in KTRs and dialysis patients, where the participants received either a pneumococcal conjugate vaccine or a plain polysaccharide pneumococcal vaccine. Kumar et al. demonstrated that three years after pneumococcal vaccination, 6/7 serotype-specific IgG antibody concentrations were still significantly higher than pre-immunization levels in KTRs (130). The same was determined one year post-immunization by Marrie et al. (131) and Oesterreich et al. (119). Vandecasteele et al. showed that one year post-immunization, dialysis patients' serotype-specific IgG antibodies were still significantly increased (132). Contrary to this, Mitra et al. demonstrated that dialysis patients one year post-immunization only had significantly increased antibody concentrations in 4/13 serotypes, compared to pre-immunization (133).

A few prior studies have found KTRs to have a better pneumococcal vaccine response, compared to dialysis patients (120, 134) . However, the majority of studies reported that dialysis patients' vaccine response is better or equivalent to that of KTRs (135-140). In the present study, immunogenicity was higher in WLPs, compared to the KTRs. This may also be influenced by the KTRs being newly transplanted, and WLPs having minor co-morbidity, compared to dialysis patients

in general. The serotype-specific IgG antibodies seem to decline more rapidly from week 17 to week 48 in WLPs, compared to KTRs, which resulted in the same for the average pneumococcal antibody GMC level. This corresponds with previous studies, were the decline in post-immunization pneumococcal antibodies is swifter in dialysis patients, compared to KTRs (120, 134, 138, 140).

Limitations

Study 1 (Paper I)

The study did not included participants from every transplant center in Denmark. Hence, the results may not be representative for all newly transplanted KTRs and WLPs in Denmark. However, two out of three centers were involved in the study. All participating KTRs had received an allograft within the last 18 months. If we had disregarded 'Date of Transplant' in inclusion, the reported vaccine uptake may have been higher, due to longer follow-up time for KTRs. Patients who declined to participate in the survey may have had a different opinion about immunization and vaccine coverage, than those who chose to participate. This could lead to sampling bias. There might have been errors in the reporting of the exposures and outcome, limiting the accuracy of data. This may be caused by participants misunderstanding the questions, unanswered questions or recall errors. Furthermore, data collection was anonymous and was not confirmed by hospital or vaccination records. Predictors for vaccine uptake may have been overlooked, as information regarding co-morbidity, medical treatment and duration of CKD were not obtained for the study.

Study 2 *(Paper II + III)*

The study was underpowered, as we were not able to include the desired number of participants. Eligible participants were screened during study 1, but more than expected did not wish to participate in study 2. Due to the study's time schedule, we had to stop inclusion. Furthermore, it was an open-label trial. Hence, especially adverse event reporting may have been influenced, by knowing which dosage the participant received. However, the primary outcome were derived from blood samples which were analysed by blinded personnel. As participants were a limited resource, we were not able to include a group that received only single or double PPV23. Due to this, we were not able to compare prime-boost vaccination with PPV23 alone which would have provided valuable information. Nor did we did not include a healthy control group. An eventual drop in antibody concentrations after PCV13, shortly after peaking, may have been overlooked, as we only drew blood samples 12 weeks after PCV13, and not after 5 weeks. This was due to two reasons; 1) our primary endpoint was the result after the full vaccine schedule and 2) it suited the scheduled attendances best at the hospitals, especially for KTRs. Serotype-specific IgG antibodies were used as surrogate markers for vaccine efficacy. It would have added further knowledge to include an

opsonophagocytic assay to judge antibody functionality. A proportion of WLPs received a kidney transplant during the study. This may have affected the results, but was inevitable. We only included KTRs who had received an allograft within the last 18 months and WLPs. Hence, data is not transferable to all KTRs or all dialysis patients.

Conclusion

The WLPs and KTRs in this study had a low influenza vaccine coverage, taking into account that both groups are recommended the vaccine annually. Furthermore, it is free of charge and relatively easily available, as both pharmacies and private physicians vaccinate. Regarding pneumococcal vaccination, the result were even lower, as under 5% of the participants were vaccinated. The main reason for not being vaccinated, with either vaccine, was a lack of vaccination counseling provided to the patient. Hence, this study demonstrates the need for a changed approach to immunization in these patient groups in Denmark, in order to comply with the national and international guidelines for immunization. As quite a few participants in the survey expressed, that they did not consider themselves as someone in need of vaccination, there also seems to be a need for patient education, regarding their disease, and the increased risk of infections that comes with it.

Based on the results from the randomized vaccine study, we conclude that the pneumococcal prime-boost vaccination with double dosage of PCV13 and PPV23 causes significantly more WLPs to reach a 'protective response', 5 weeks after immunization, assessed by the standard method used to judge obtained protective immunity after PPV23 in adults, in Denmark. In addition, we demonstrated, that the major obstacle in achieving a dose effect in our cohort seems to be immunosuppression. Primarily, in KTRs but also in WLPs. During the 1½ years follow-up, both patient populations displayed a loss of protective immunity. However, the rapid drop in serotype-specific IgG antibody concentrations decreased, and the levels stayed fairly stable week 96, compared to week 48. As a result of this, the average pneumococcal antibody GMC remains significantly above pre-immunization levels for all treatment groups. Hence, displaying a certain amount of lasting post-vaccination antibody response following pneumococcal prime-boost vaccination.

Future perspectives

In relation to clinical practice, these studies shows that, as previously mentioned, there is a need for a systematic approach to immunization of SOT candidates and recipients. Probably a cross-sectoral approach where the patient continues to receive some of the vaccines at the general practitioner following an immunization evaluation by a hospital based specialized vaccination clinic in a collaboration with a specialist at the transplantation department. Ongoing research is needed in order to further optimize vaccine responses in this vulnerable population.

This is the first study to test DD of the 13-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine in combination. As it resulted in significantly more WLPs reaching a 'protective response' post-immunization, it may be worth considering testing it in other patient populations, who may also have an impaired pneumococcal vaccine response. However, the level of immunosuppression needs to be considered. In WLPs, it would interesting to do an opsonophagocytic assay to assess the functionality of the increased antibody levels, in accordance with studies by Jackson et al. (71, 73). Additionally, a study of the impact of DD of the pneumococcal vaccines on the cellular immunity towards pneumococcal polysaccharides would be relevant (141). Furthermore, assessment of eventual enhanced hyporesponsiveness following DD PPV23 may be warranted.

Two new pneumococcal conjugate vaccines (15-valent and 20-valent) have just been approved for adults aged 18 years and older and are available for use. Consequently, pneumococcal immunization guidelines may be altered for immunocompetent and immunosuppressed people soon. The PPV23 will undoubtedly still be included, to provide the broadest serotype immunity. At least until a conjugate vaccine that covers just as many serotypes or a capsular serotype-independent pneumococcal vaccine comes on marked.

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Supplement material

Supplement Material 1

"Questionnaire survey regarding vaccination status among kidney transplant recipients and waiting list patients"

Background information

Age? _____

Sex (one cross)?
(1) Male _
(2) Female _

Status in relation to transplantation (one cross)?
(1) Awaiting kidney transplant _
(2) Kidney transplant recipient _

Which race do you describe yourself as (one cross)?
(1) Caucasian / European _
(2) Asian _
(3) African _
(6) Other, please state _____
(4) Do not know _
(5) Don't want to disclose _

What is your highest level of education (one cross)?
(1) Primary school or equivalent (up to 10 years of schooling) _
(2) Vocational education (skilled in crafts, trade, office or similar) _
(3) Upper secondary education (high school, HHX, HTX, HF or similar) _
(4) Higher education (University, vocational college, business academy or similar) _
(5) Don't want to disclose _

What was your household income before tax last year (one cross)?
(1) Below DKK 100.000 _
(2) DKK 100.000 - 299.999 _
(3) DKK 300.000 - 499.999 _
(4) DKK 500.000 - 699.999 _
(5) Above DKK 700.000 _
(6) Don't want to disclose _
(7) Don't know

Questions regarding influenza vaccination

Do you have any known allergies? (Possibly put more crosses)
(1) No _
(2) Chicken or eggs _

(3) Medicine, please specify preparation _____
(4) Other, please state _____
(5) Don't know _

Have you been vaccinated against influenza in the last period between October to February (one cross)?
(1) Yes _
(2) No _
(3) Don't know _

Have you been vaccinated against influenza other previous years apart from the latest period between October to February (one cross)?
(1) Yes _ year? _____
(2) No _
(3) Don't know _

Who has primarily informed you about the possibility of being vaccinated against influenza (one cross)?
(1) General practitioner _
(2) Nephrologist _
(4) I myself have sought information _
(3) Other person, please state _____
(5) I have not been informed of the possibilities for vaccination _

If you were not vaccinated against influenza the last period between October to February, what was the main reason (one cross)?
(1) I am allergic to chicken, eggs or other ingredients in the vaccine _
(2) I am afraid of side effects _
(3) I have previously not been informed that it was free _
(7) I have previously not been informed that it is recommended for patients like me _
(4) I was ill at the time of vaccination _
(5) Other, please state _____
(6) Don't want to disclose _

Are you considering getting influenza vaccinated next winter (one cross)?
(1) Yes _
(2) No _
(3) Don't know _

Questions regarding pneumococcal vaccination
Have you previously been offered vaccination against pneumococci (pneumonia bacterium) (one cross)?
(If the answer is no, go to the "Concluding Questions" section)
(1) Yes _
(2) No _

(3) Don’t know _

Who has primarily informed you about the possibility of being vaccinated against pneumococci (one cross)?
(1) General practitioner _
(2) Nephrologist _
(4) I myself have sought information _
(3) Other person, please state _____
(5) I have not been informed of the possibilities for vaccination _

When you were offered vaccination against pneumococci, did you choose to be vaccinated (one cross)?
(1) Yes _
(2) No _

If you chose to be vaccinated against pneumococci, we would like to ask for the following information:
(If you do not have the information, write, "Don’t know")
Year of vaccination: _____
For what reason was, the vaccination offered. _____
What was the name of the vaccine? _____

If you opted out of vaccination against pneumococci, what was the main reason (one cross)?
(1) I am allergic to ingredients in the vaccine _
(2) I am afraid of side effects _
(3) I did not think it was necessary _
(7) I did not know it is recommended for patients like me _
(4) It is too expensive _
(5) Other, please state _____
(6) Don’t want to disclose _

Concluding questions
Could you be interested in hearing more about and possibly participate in a medical trial in which kidney transplant patients and patients in need of kidney transplantation are offered vaccination against pneumococci (one cross)?

(1) Yes, please _
(2) No thanks _

If you are interested in participating in the above mentioned medical trial, we can contact you:

(1) Pr. phone (written below)
(2) Pr. E-mail.(written below)

What is your name?

_____
_____

What is your email address?

_____
_____

Please indicate which telephone number you can be contacted at:

_____
_____

Thank you for your participation.

Supplement Table 1

Geometric mean concentrations (95% confidence interval) for 12 pneumococcal serotype-specific IgG antibodies at each visit for each of the treatment groups.

		Baseline	P-value Baseline: ND Compared to DD	Week 12	P-value week 12: ND Compared to DD	Week 17	P-value week 17: ND Compared to DD	P-value week 17 Compared to week 12	P-value week 17 Compared to baseline	Week 48	P-value week 48: ND Compared to DD	Week 96	P-value Week 96: ND Compared to DD	P-value week 96 Compared to baseline
Serotype	Treatment groups	GMC (95%CI)		GMC (95%CI)		GMC (95%CI)				GMC (95%CI)		GMC (95%CI)		
1	WLP-ND	.13 (.09-.20)	.885	.40 (.21-.77)	.546	.47 (.25-.87)	.292	.814	≤.001	.30 (.16-.54)	.573	.29 (.16-.55)	.605	≤.001
	WLP-DD	.13 (.09-.19)		.43 (.25-.74)		.64 (.36-1.12)		≤.001	≤.001	.36 (.21-.62)		.33 (.20-.55)		≤.001
	KTR-ND	.15 (.10-.21)	.867	.30 (.18-.51)	.501	.36 (.22-.60)	.648	.264	≤.001	.28 (.17-.47)	.501	.29 (.18-.47)	.264	≤.001
	KTR-DD	.16 (.10-.26)		.24 (.15-.38)		.32 (.19-.55)		.012	≤.001	.23 (.14-.38)		.21 (.14-.32)		.046
3	WLP-ND	.04 (.03-.07)	.431	.14 (.08-.27)	.834	.20 (.10-.38)	.931	.248	≤.001	.13 (.07-.23)	.894	.13 (.08-.23)	.842	≤.001
	WLP-DD	.06 (.03-.09)		.15 (.09-.27)		.19 (.10-.34)		.090	≤.001	.09 (.06-.14)		.14 (.08-.22)		≤.001
	KTR-ND	.07 (.05-.10)	.749	.09 (.06-.14)	.895	.12 (.07-.18)	.915	.035	≤.001	.09 (.06-.14)	.661	.10 (.07-.15)	.698	.005
	KTR-DD	.07 (.04-.12)		.11 (.06-.21)		.14 (.07-.27)		.008	≤.001	.12 (.06-.21)		.11 (.06-.19)		.055
4	WLP-ND	.09 (.07-.13)	.537	.27 (.15-.46)	.208	.27 (.15-.47)	.103	.681	≤.001	.20 (.12-.32)	.218	.20 (.12-.31)	.026	≤.001
	WLP-DD	.12 (.08-.18)		.44 (.23-.83)		.55 (.28-1.09)		.192	≤.001	.32 (.18-.55)		.41 (.26-.64)		≤.001
	KTR-ND	.13 (.09-.19)	.277	.22 (.14-.34)	.310	.27 (.17-.43)	.074	.048	≤.001	.22 (.14-.36)	.401	.27 (.19-.40)	.055	≤.001
	KTR-DD	.10 (.07-.13)		.16 (.11-.25)		.17 (.11-.26)		.566	.010	.17 (.12-.25)		.17 (.12-.23)		.003
5	WLP-ND	.12 (.06-.24)	.743	.57 (.25-1.29)	.793	.74 (.35-1.56)	.965	.652	≤.001	.41 (.19-.88)	.938	.39 (.19-.82)	.729	≤.001
	WLP-DD	.09 (.06-.15)		.46 (.22-.97)		.69 (.33-1.44)		.797	≤.001	.38 (.18-.79)		.47 (.23-.94)		≤.001
	KTR-ND	.09 (.05-.13)	.875	.25 (.13-.46)	.749	.35 (.19-.64)	.193	.005	≤.001	.27 (.14-.52)	.368	.27 (.15-.48)	.187	≤.001
	KTR-DD	.09 (.06-.15)		.20 (.11-.34)		.22 (.12-.37)		.857	.001	.17 (.10-.30)		.16 (.09-.27)		≤.001
6B	WLP-ND	.19 (.12-.30)	.753	.69 (.31-1.52)	.656	.64 (.29-1.43)	.526	.063	≤.001	.47 (.23-.99)	.741	.51 (.26-1.01)	.487	≤.001
	WLP-DD	.17 (.11-.26)		.87 (.41-1.84)		.93 (.46-1.89)		.558	≤.001	.52 (.28-.96)		.68 (.36-1.27)		≤.001
	KTR-ND	.20 (.14-.30)	.816	.47 (.26-.85)	.956	.53 (.30-.94)	.791	.230	≤.001	.43 (.25-.75)	.719	.44 (.26-.73)	.737	≤.001
	KTR-DD	.24 (.14-.40)		.50 (.25-.98)		.52 (.27-.97)		.154	.001	.43 (.23-.79)		.44 (.24-.80)		.002
7F	WLP-ND	.17 (.10-.28)	.962	1.20 (.65-2.21)	.665	1.30 (.71-2.38)	.436	.481	≤.001	.71 (.41-1.21)	.460	.52 (.30-.90)	.303	≤.001
	WLP-DD	.17 (.10-.30)		1.38 (.71-2.66)		1.83 (.98-3.41)		.206	≤.001	.97 (.49-1.93)		.80 (.42-1.52)		≤.001
	KTR-ND	.21 (.15-.31)	.229	.64 (.36-1.14)	.834	.82 (.47-1.44)	.897	.177	≤.001	.54 (.31-.95)	.718	.53 (.32-.87)	.861	≤.001
	KTR-DD	.30 (.19-.47)		.72 (.46-1.13)		.89 (.56-1.41)		.128	≤.001	.62 (.39-.99)		.50 (.33-.75)		≤.001

Table Continued:		Baseline	P-value Baseline: ND	Week 12	P-value week 12: ND	Week 17	P-value week 17: ND	P-value week 17	P-value week 17	Week 48	P-value week 48: ND	Week 96	P-value week 96: ND	P-value week 96
Serotype	Treatment groups	GMC (95%CI)	Compared to DD	GMC (95%CI)	Compared to DD	GMC (95%CI)	Compared to DD	Compared to week 12	Compared to baseline	GMC (95%CI)	Compared to DD	GMC (95%CI)	Compared to DD	Compared to baseline
9V	WLP-ND	.11 (.07-.17)	.741	.52 (.28-.95)	.447	.57 (.33-.99)	.204	.389	≤.001	.35 (.21-.58)	.486	.35 (.22-.58)	.377	≤.001
	WLP-DD	.12 (.08-.18)		.70 (.38-1.29)		.99 (.58-1.69)		.734	≤.001	.45 (.26-.78)		.49 (.28-.85)		≤.001
	KTR-ND	.15 (.10-.22)	.451	.39 (.21-.73)	.921	.48 (.26-.88)	.685	.331	≤.001	.36 (.19-.66)	.679	.38 (.22-.63)	.441	≤.001
	KTR-DD	.18 (.12-.29)		.37 (.22-.63)		.42 (.25-.72)		.140	≤.001	.32 (.19-.55)		.28 (.17-.48)		.161
14	WLP-ND	.70 (.35-1.38)	.592	3.85 (2.10-7.06)	.969	4.69 (2.40-9.16)	.908	.290	≤.001	2.92 (1.54-	.871	2.49 (1.24-	.565	≤.001
	WLP-DD	.88 (.49-1.60)		3.78 (1.98-7.19)		5.21 (2.68-1.12)		.017	≤.001	3.16 (1.62-		3.28 (1.64-		≤.001
	KTR-ND	.84 (.51-1.40)	.849	2.00 (1.16-3.45)	.724	2.35 (1.31-4.21)	.706	.172	≤.001	1.72 (.96-3.08)	.719	1.64 (.95-2.84)	.966	.002
	KTR-DD	.90 (.52-1.58)		2.15 (1.24-3.70)		2.55 (1.45-4.51)		.019	≤.001	1.94 (1.14-		1.66 (.99-2.79)		.017
18C	WLP-ND	.39 (.25-.59)	.960	2.47 (1.45-4.20)	.875	2.55 (1.44-4.52)	.829	.456	≤.001	1.40 (.86-2.27)	.784	1.34 (.81-2.24)	.734	≤.001
	WLP-DD	.38 (.23-.62)		1.95 (1.08-3.50)		2.36 (1.23-4.54)		.734	≤.001	1.39 (.76-2.52)		1.51 (.88-2.60)		≤.001
	KTR-ND	.32 (.22-.46)	.109	.84 (.52-1.35)	.912	.96 (.57-1.62)	.933	.006	≤.001	.78 (.46-1.33)	.919	.83 (.54-1.29)	.755	≤.001
	KTR-DD	.50 (.32-.76)		.95 (.62-1.46)		1.03 (.63-1.69)		.028	≤.001	.84 (.55-1.28)		.82 (.51-1.33)		.008
19A	WLP-ND	.39 (.21-.73)	.646	1.41 (.66-3.04)	.431	1.62 (.73-3.59)	.584	.290	≤.001	1.00 (.46-2.20)	.426	.95 (.44-2.05)	.193	≤.001
	WLP-DD	.47 (.24-.91)		2.15 (1.12-4.16)		2.18 (1.04-4.60)		.271	≤.001	1.52 (.72-3.22)		1.85 (.93-3.68)		≤.001
	KTR-ND	.36 (.22-.61)	.262	.86 (.45-1.64)	.930	.81 (.41-1.62)	.879	.474	≤.001	.67 (.35-1.29)	.565	.70 (.38-1.30)	.646	≤.001
	KTR-DD	.58 (.34-.98)		.91 (.54-1.55		.95 (.52-1.72)		.987	.038	.86 (.49-1.51)		.81 (.50-1.31)		.075
19F	WLP-ND	.56 (.35-.91)	.675	1.85 (1.04-3.29)	.520	2.10 (1.18-3.74)	.784	.590	≤.001	1.20 (.69-2.08)	.816	1.46 (.86-2.48)	.501	≤.001
	WLP-DD	.46 (.31-.69)		1.47 (.79-2.73)		1.83 (.99-3.36)		.636	≤.001	1.18 (.68-2.07)		1.77 (1.07-		≤.001
	KTR-ND	.55 (.36-.85)	.824	.89 (.52-1.53)	.947	1.04 (.59-1.82)	.624	.216	≤.001	.86 (.50-1.49)	.755	1.10 (.66-1.83)	.823	≤.001
	KTR-DD	.61 (.39-.98)		.83 (.50-1.37)		.87 (.51-1.47)		.743	.185	.81 (.53-1.24)		.94 (.59-1.50)		.011
23F	WLP-ND	.28 (.19-.42)	.487	.97 (.54-1.75)	.186	.97 (.52-1.81)	.063	.992	≤.001	.66 (.39-1.12)	.197	.75 (.43-1.34)	.416	≤.001
	WLP-DD	.36 (.24-.52)		1.71 (.91-3.20)		2.23 (1.17-4.27)		.530	≤.001	1.09 (.61-1.93)		1.10 (.57-2.11)		≤.001
	KTR-ND	.27 (.19-.38)	.079	.67 (.40-1.13)	.763	.69 (.39-1.24)	.551	.093	≤.001	.52 (.33-.82)	.312	.63 (.38-1.04)	.967	≤.001
	KTR-DD	.43 (.30-.63)		.74 (.48-1.13)		.86 (.55-1.37)		.149	≤.001	.71 (.46-1.10)		.57 (.38-.87)		.166

GMC, Geometric mean concentration; CI, confidence interval; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage; KTR-ND, kidney transplant recipients – normal dosage; KTR-DD, kidney transplant recipients – double dosage; ND, normal dosage; DD, double dosage.

Supplement Table 2

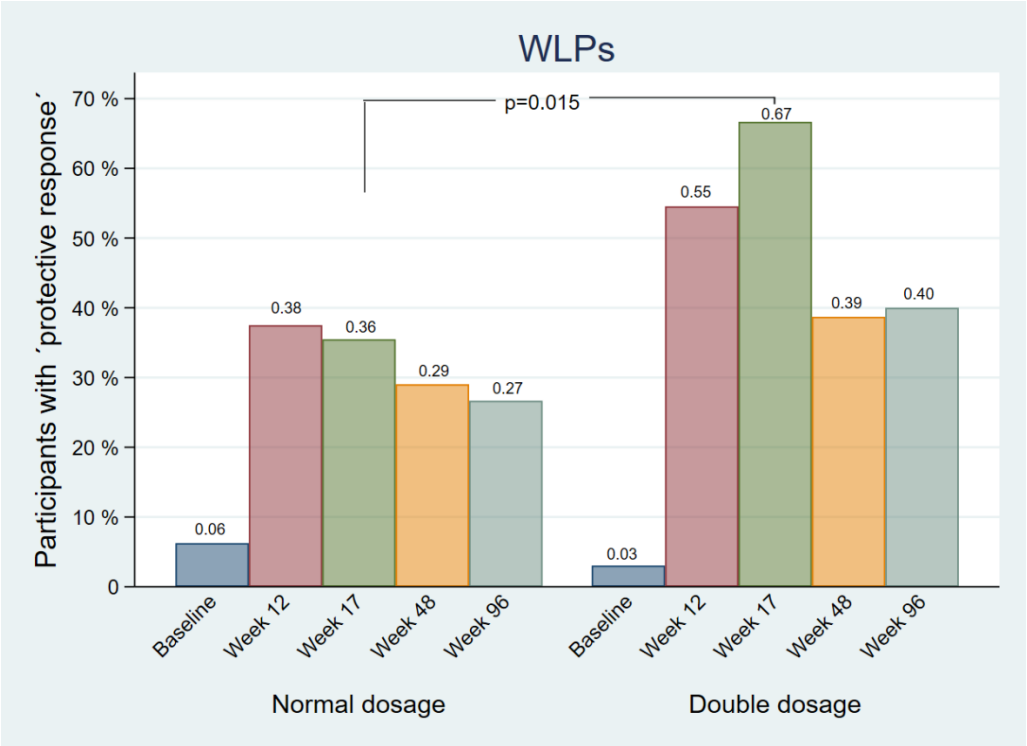
Subgroup of kidney transplant waiting list patients and kidney transplant recipients with T/B/NK cells at baseline.

	WLPs (N=32)	**KTRs (N=58)**	**p-value**
Participants with normal dosage vaccines, N (%)	16 (50)	32 (55.2)	
Age, years, *median (IQR)*	50.5 (40-58)	50.5 (41-60)	.406
Absolute lymphocyte count x 10^3/µL, *median (IQR)*	1.28 (1.06-1.65)	1.06 (.77-1.58)	.028
Total T-cells: CD3+x10^3/µL, median (IQR)	1.00 (.73-1.28)	.89 (.58-1.25)	.092
• *Helper T-cells: CD4+x10^3/µL, median (IQR)*	.66 (.47-.78)	.53 (.27-.72)	.046
• *Cytotoxic T-cells: CD8+ x10^3/µL, median (IQR)*	.31 (.19-.54)	.31 (.2-.43)	.607
B-cells: CD19+ x10^3/µL, median (IQR)	.08 (.04-.18)	.07 (.04-.13)	.357
Natural killer-cells: CD3-/CD16-56+ x 103/µL, median (IQR)	.18 (.12-.26)	.12 (.09-.17)	.007

Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient.

Supplement Figure 1a

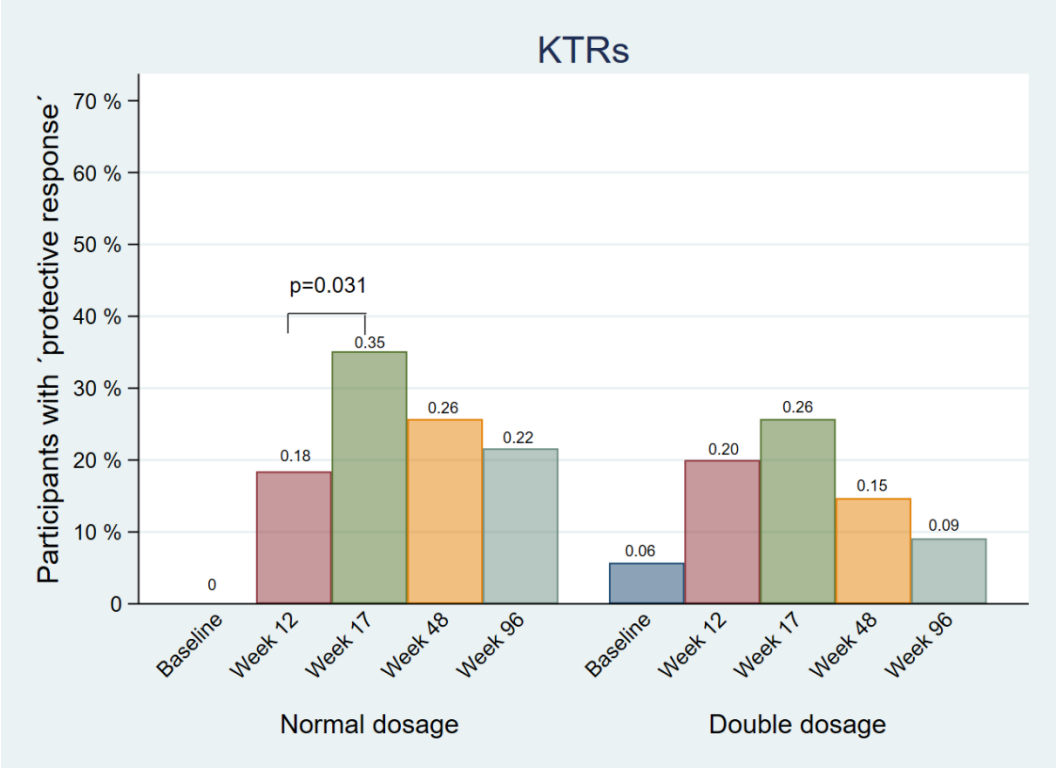
Kidney transplant waiting list patients with a 'protective response' (average antibody geometric mean concentration ≥ 1 mg/L) at each visit.



Abbreviations: WLP, kidney transplant waiting list patient

Supplement Figure 1b

Kidney transplant recipients with a 'protective response' (average antibody geometric mean concentration ≥ 1 mg/L) at each visit.



Abbreviations: KTR, kidney transplant recipient

Supplement Figure 2a-e

Kidney transplant waiting list patients: Log-transformed average pneumococcal antibody GMC value for each participant depicted together with mean, median and IQR for the group. Student t-test was used for statistical comparison of means with p-values shown for each visit. A value above the red horizontal line has reached 'protective response' (average pneumococcal antibody GMC ≥ 1 mg/L). Figure 2a-e.

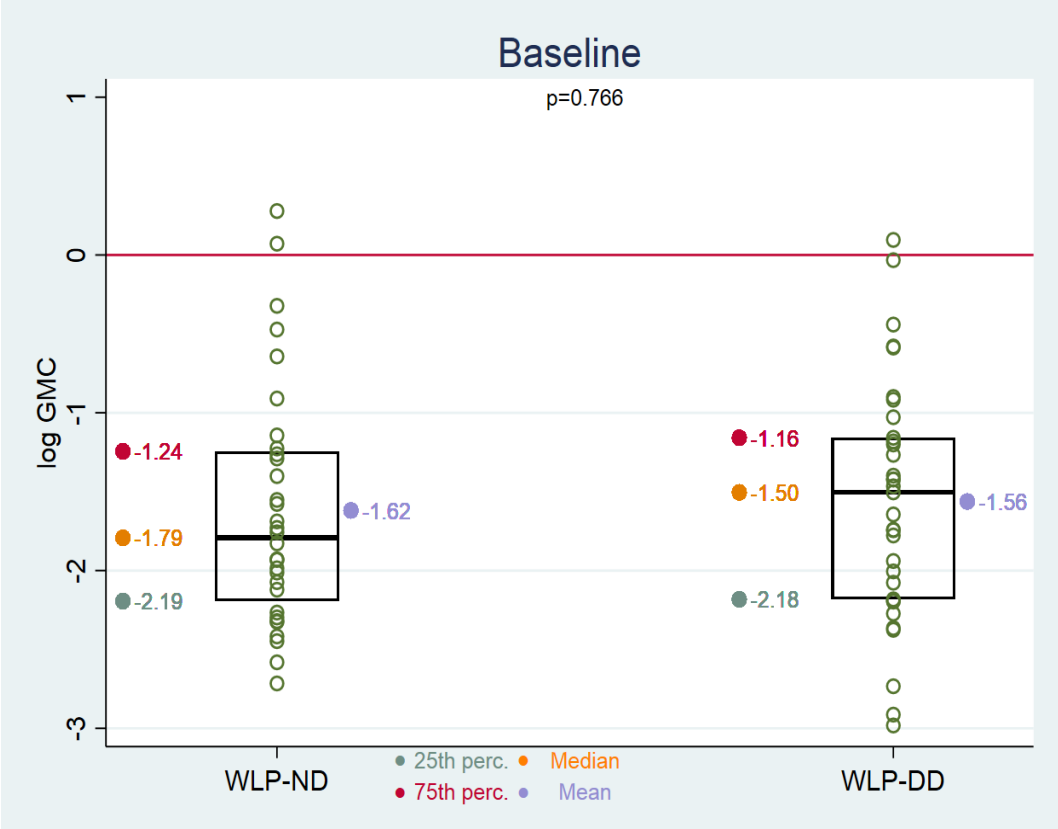


Figure 2a. Baseline

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

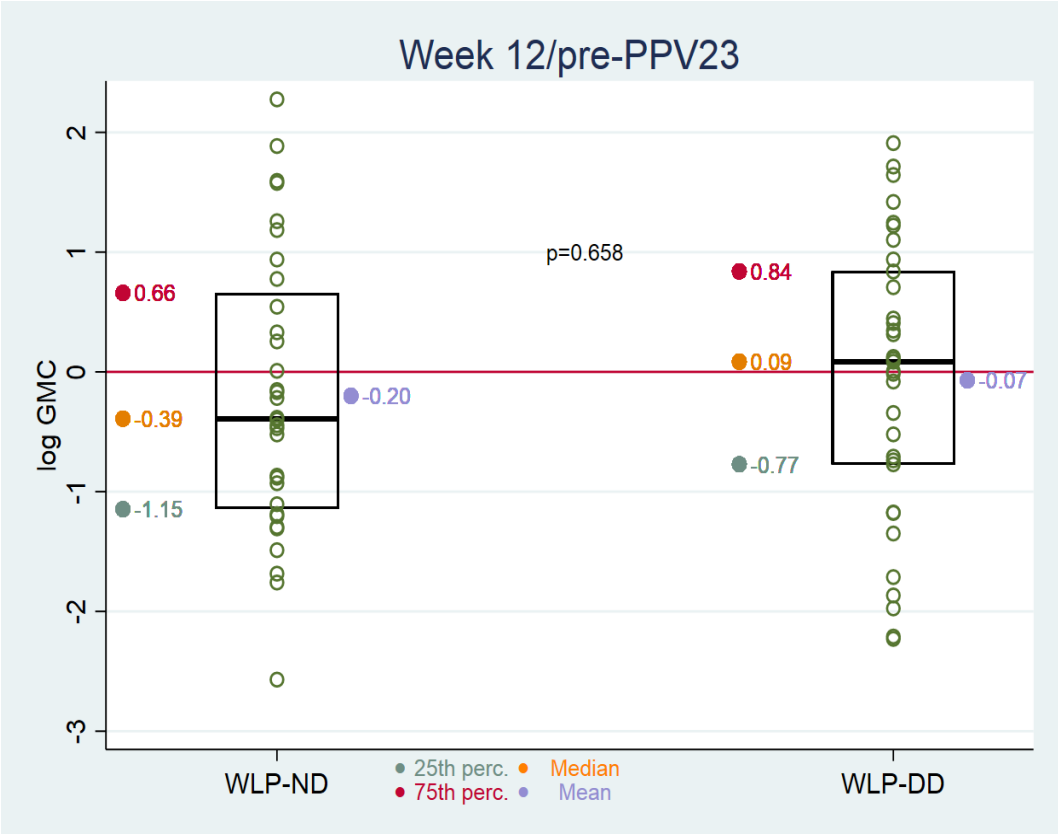


Figure 2b. Week 12

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

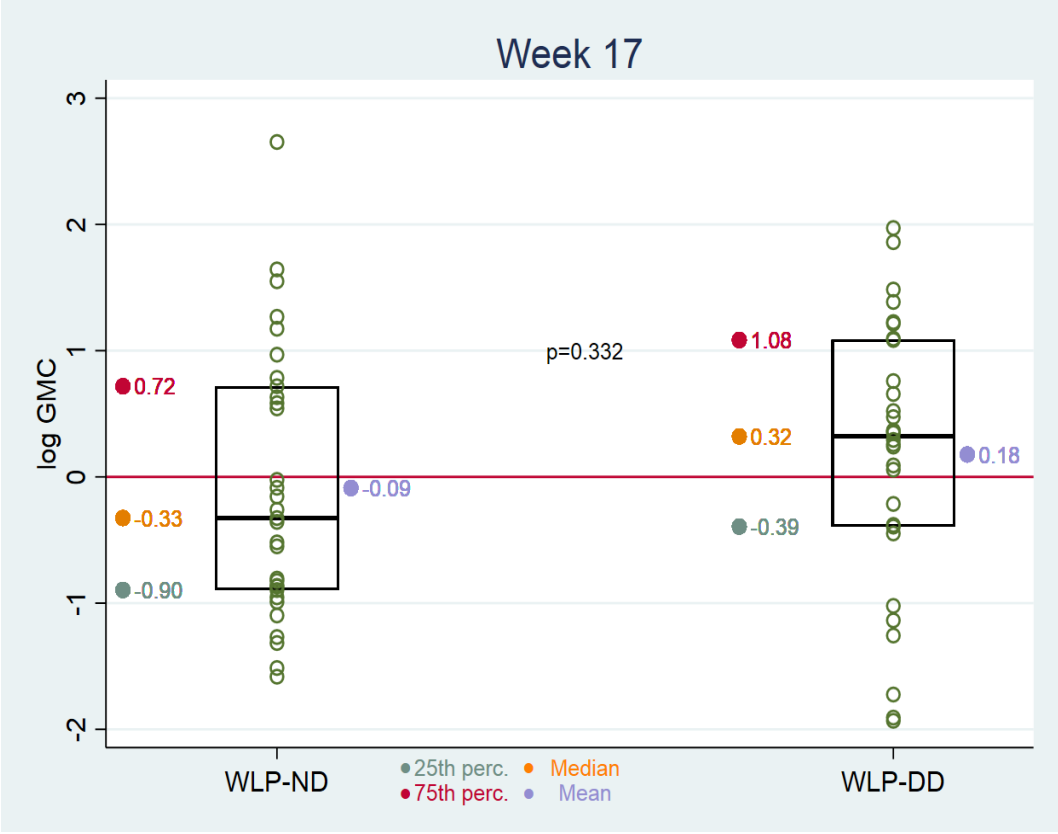


Figure 2c. Week 17

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

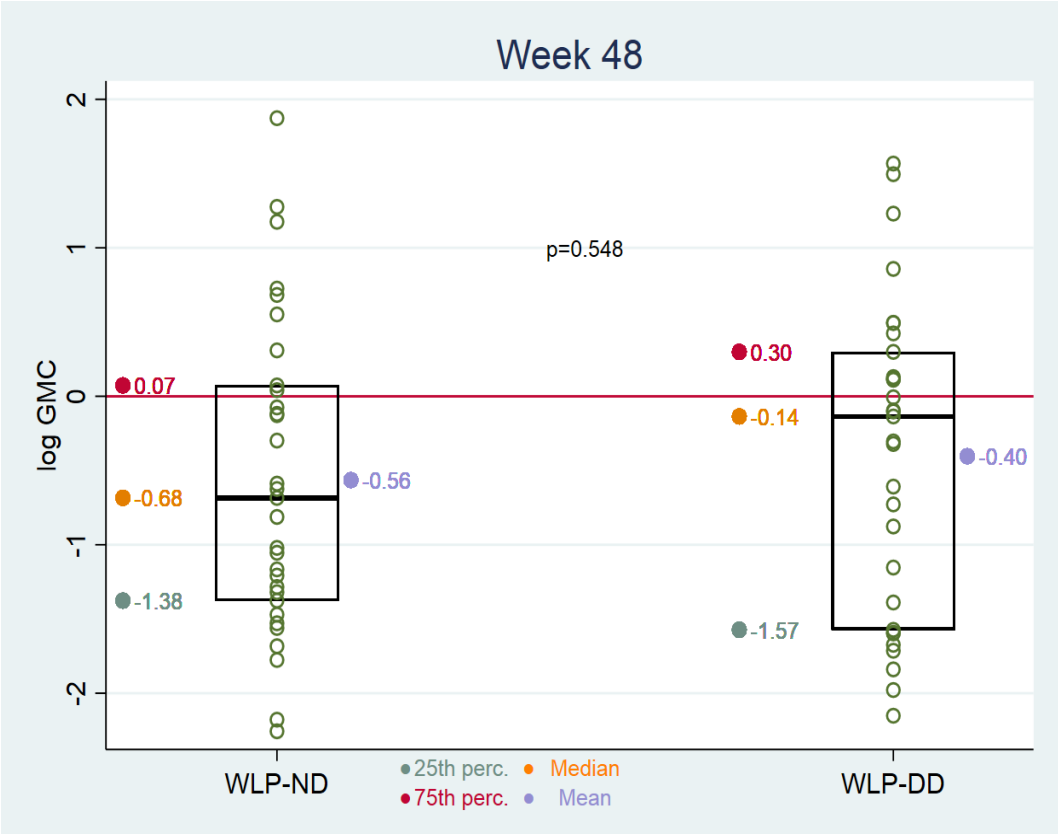


Figure 2d. Week 48

Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

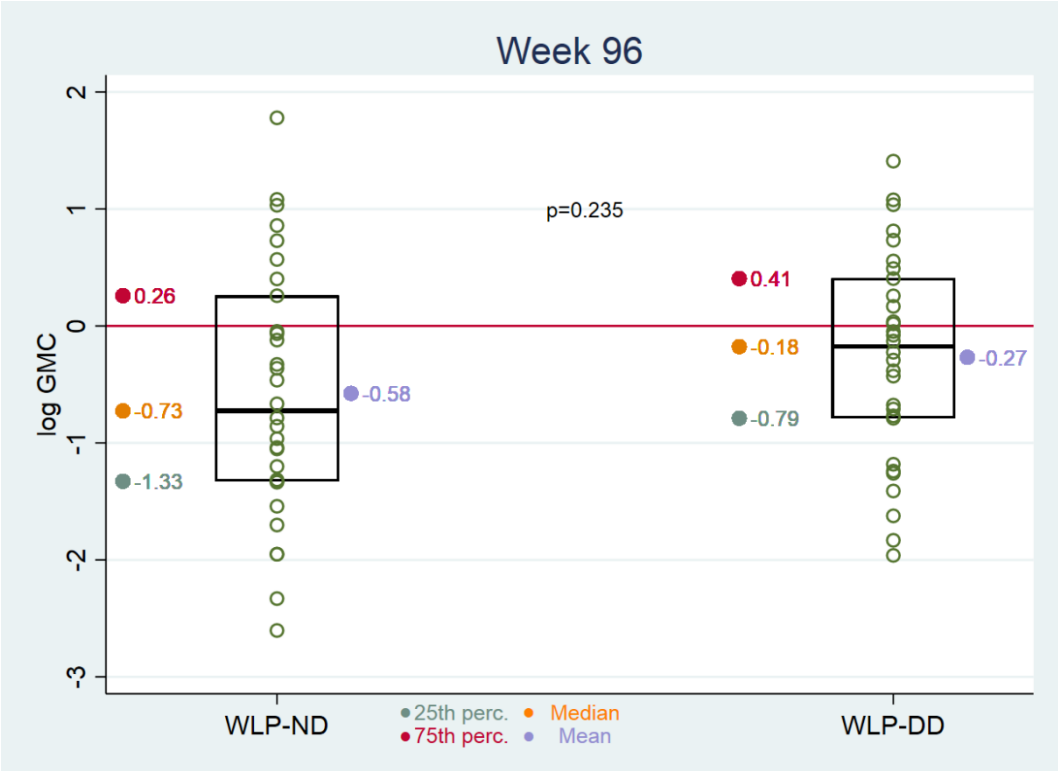


Figure 2e. Week 96
Abbreviations: WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

Supplement Figure 3a-e

Kidney transplant recipients: Log-transformed average pneumococcal antibody GMC value for each participant depicted together with mean, median and IQR for entire group. Student t-test was used for statistical comparison of means with p-values shown for each visit. A value above the red horizontal line has reached 'protective response' (average pneumococcal antibody GMC ≥ 1 mg/L). A figure for each visit. Figure 3a-e.

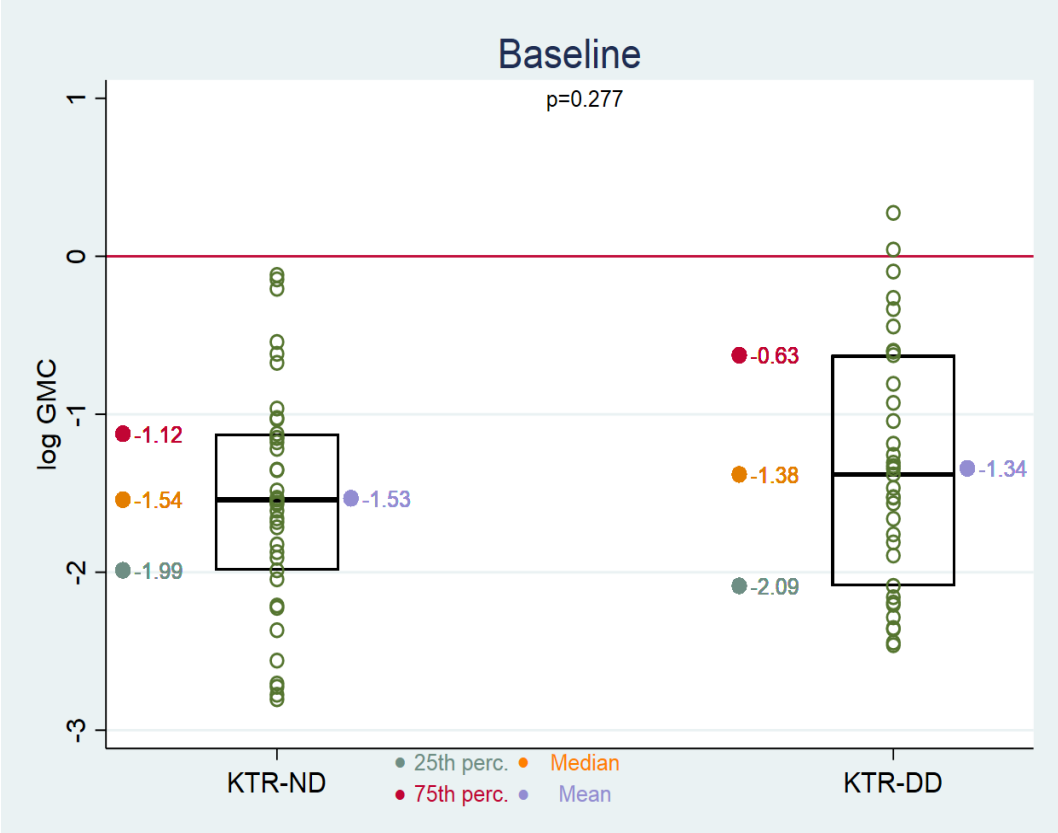


Figure 3a. Baseline
Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage.

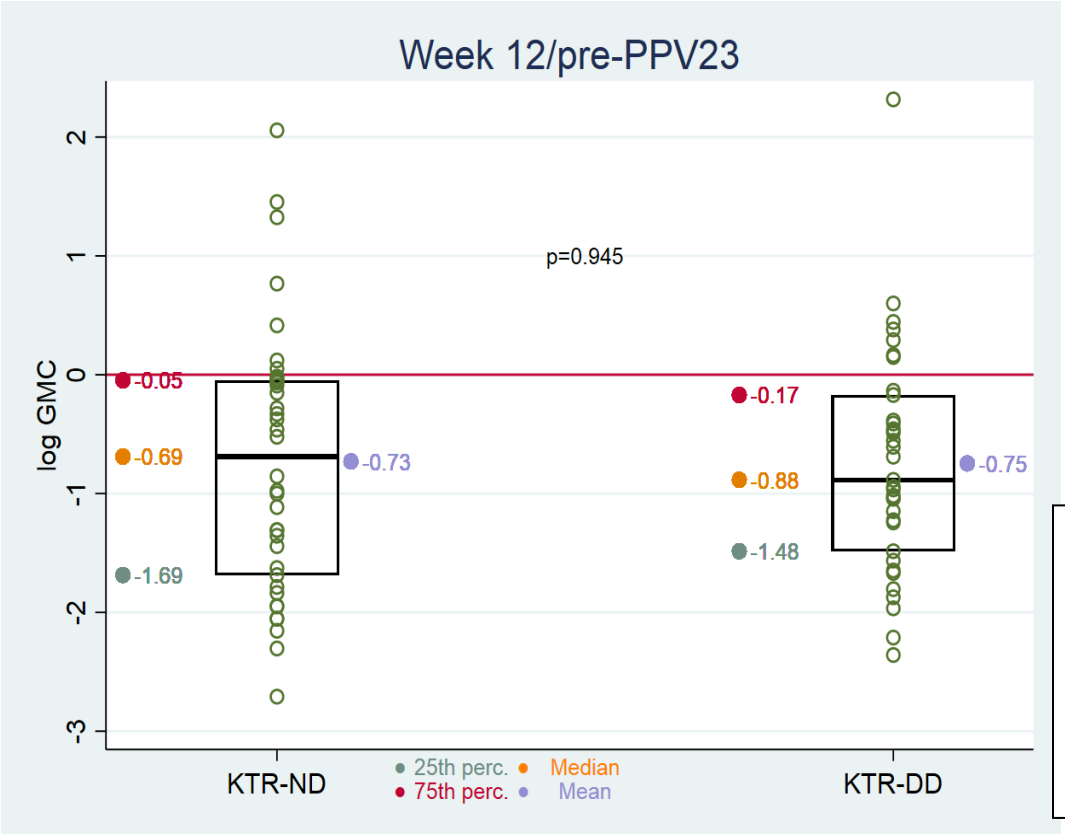


Figure 3b. Week 12 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage.

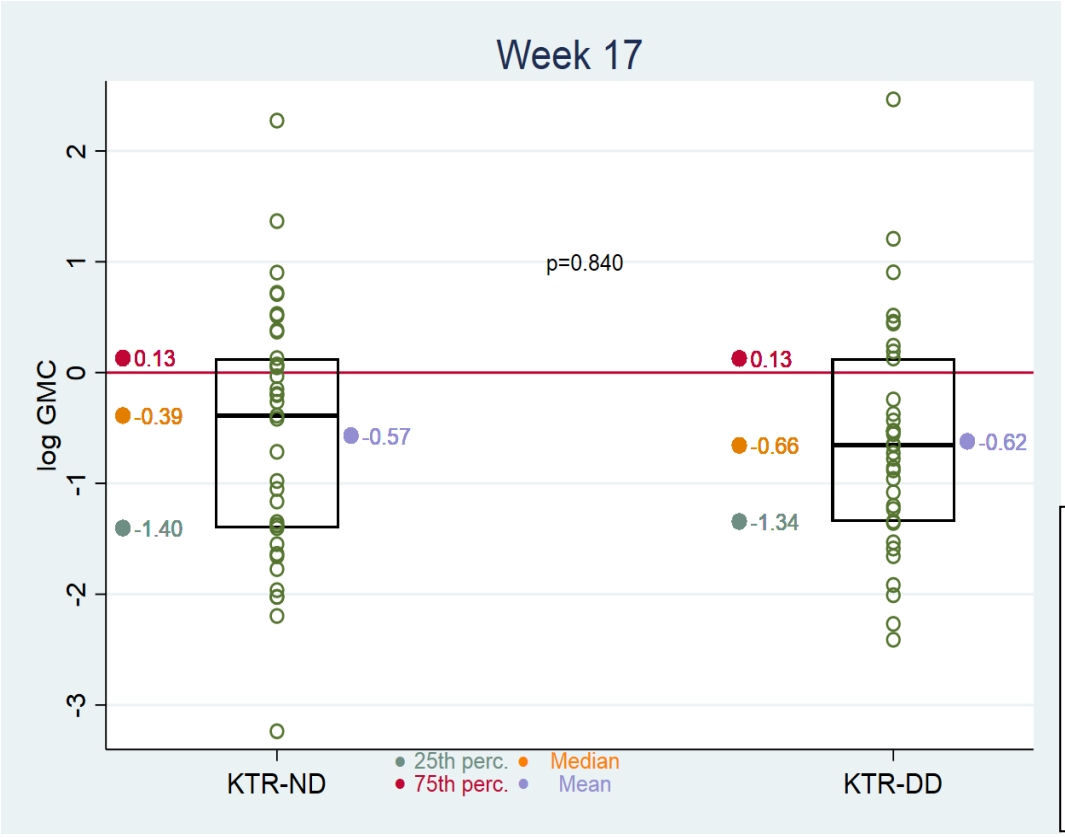


Figure 3c. Week 17 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage.

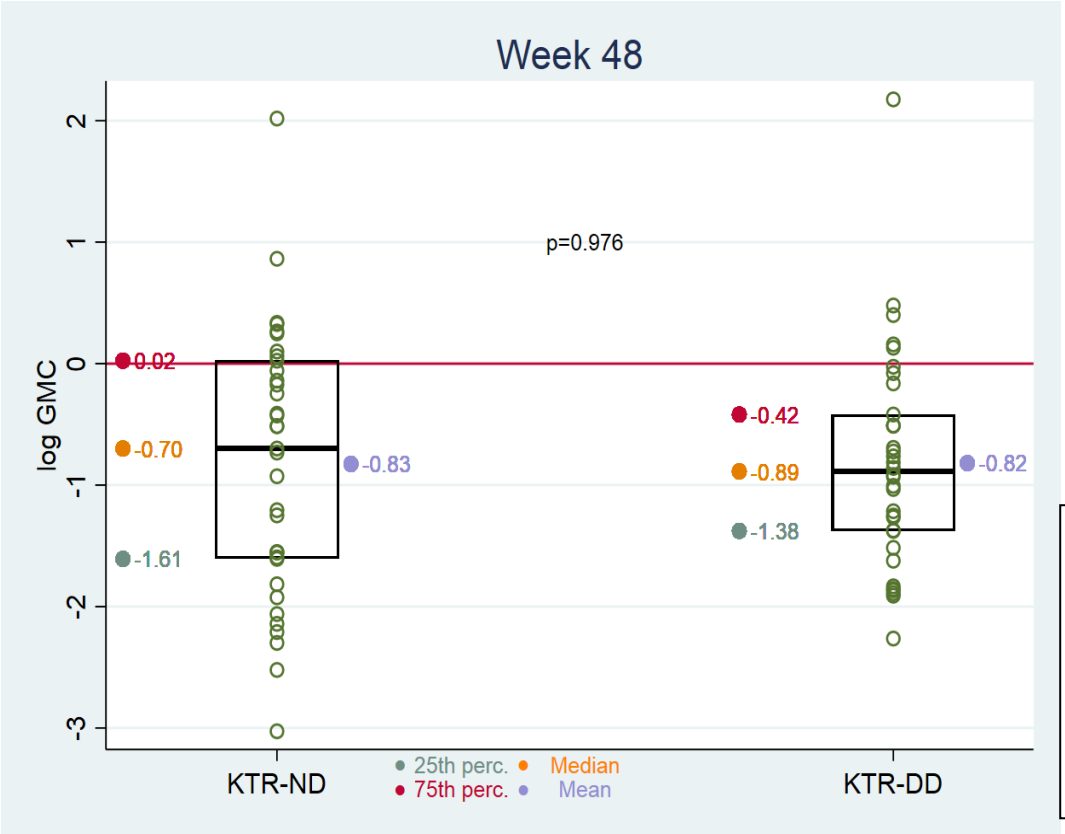


Figure 3d. Week 48
Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage.

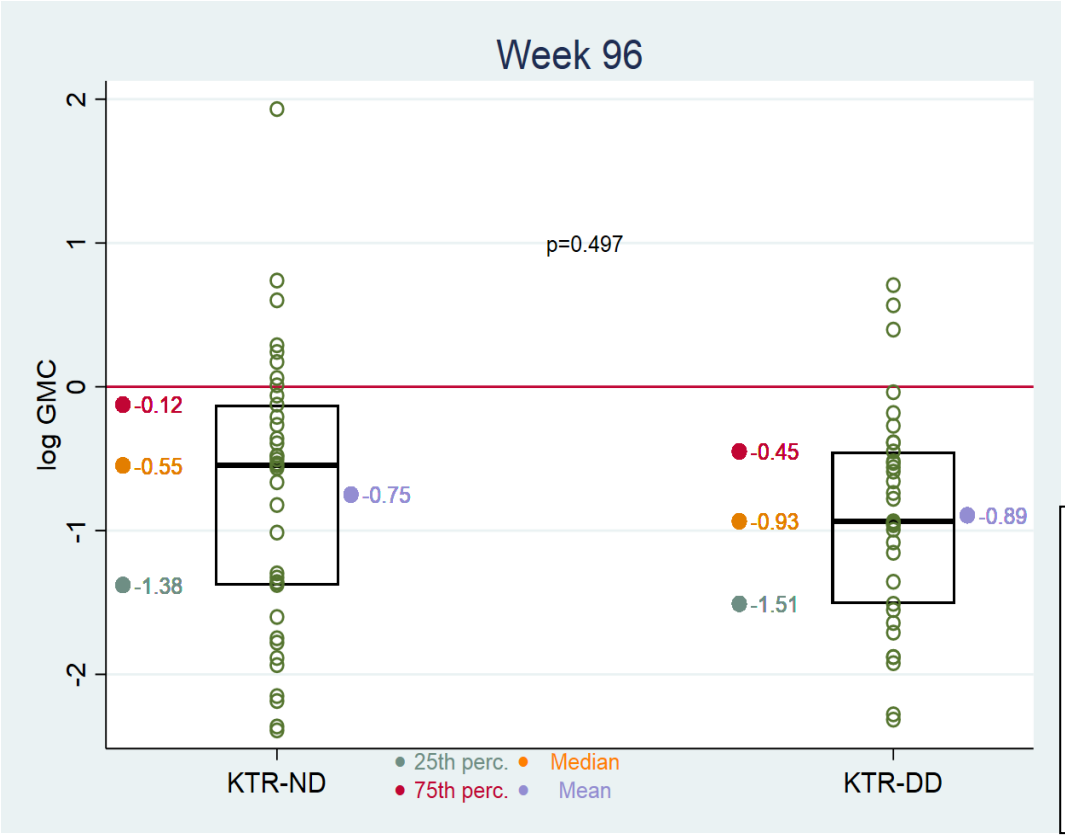


Figure 3e. Week 96
Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage.

Supplement Figure 4a-e

Participants in each of the 4 treatment groups who achieve ≥2-fold increases in 0 to 12 serotype-specific IgG antibodies at each visit. Figure 4a-d.

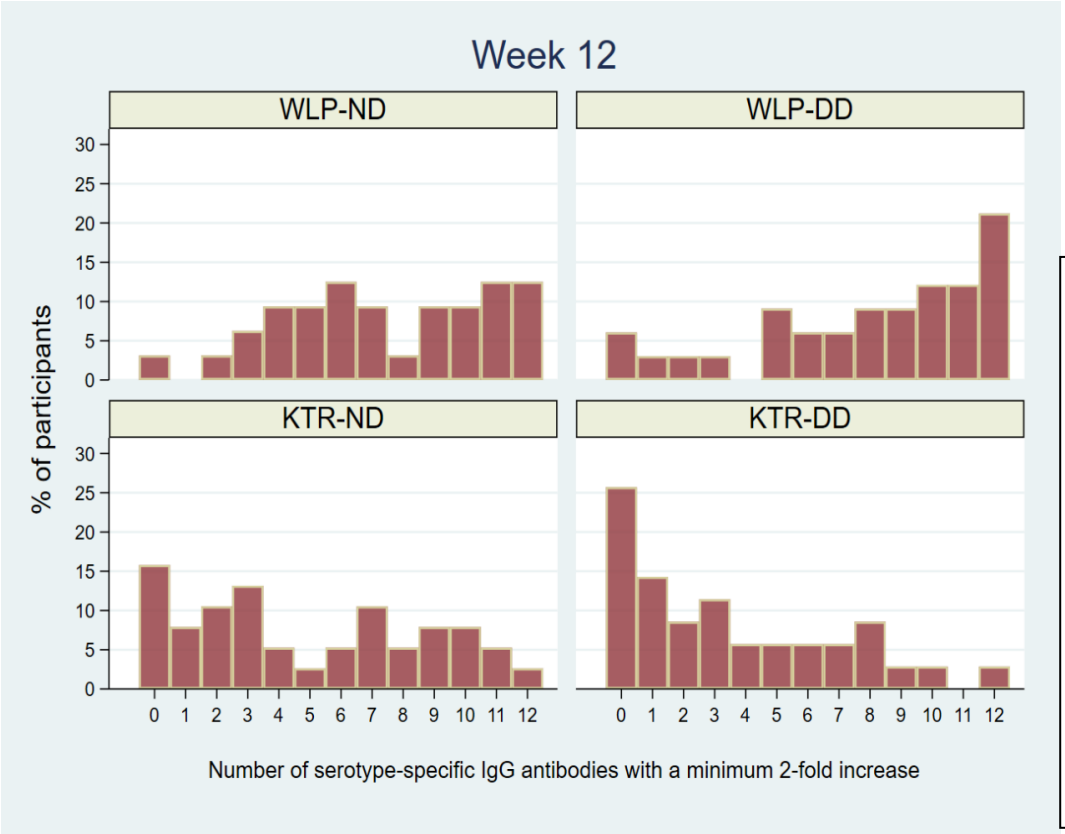


Figure 4a. Week 12 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

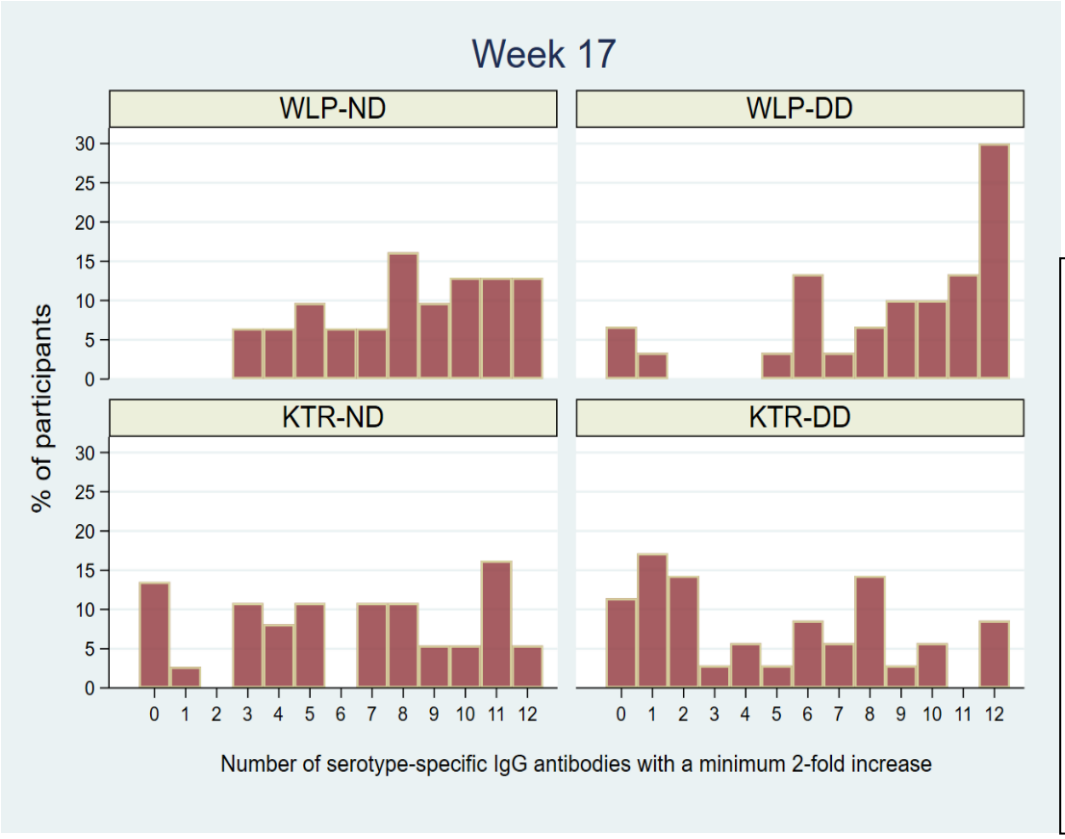


Figure 4b. Week 17 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

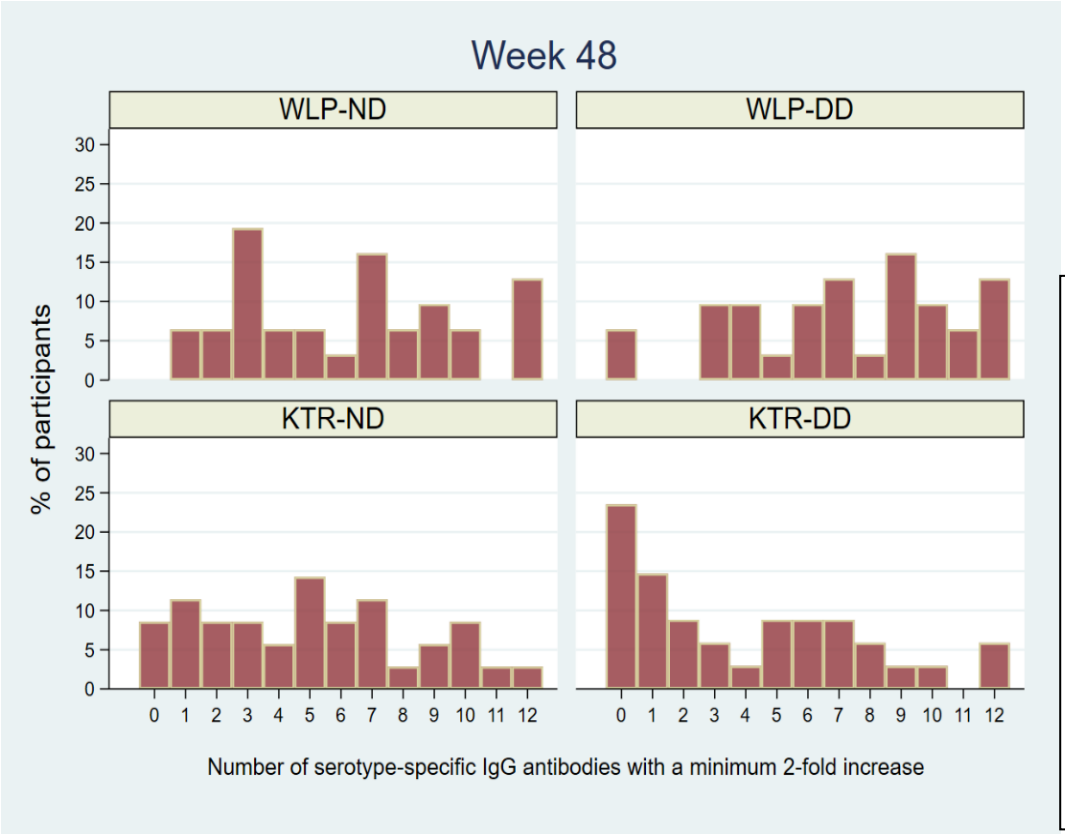


Figure 4c. Week 48 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

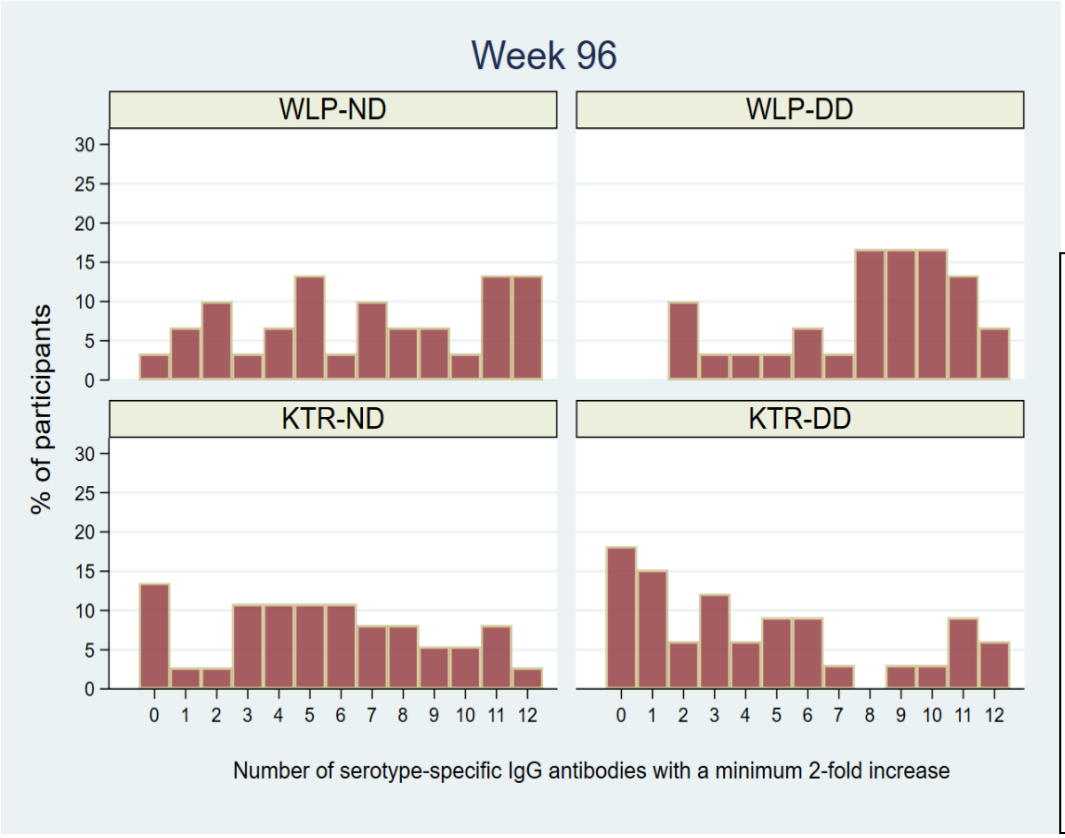
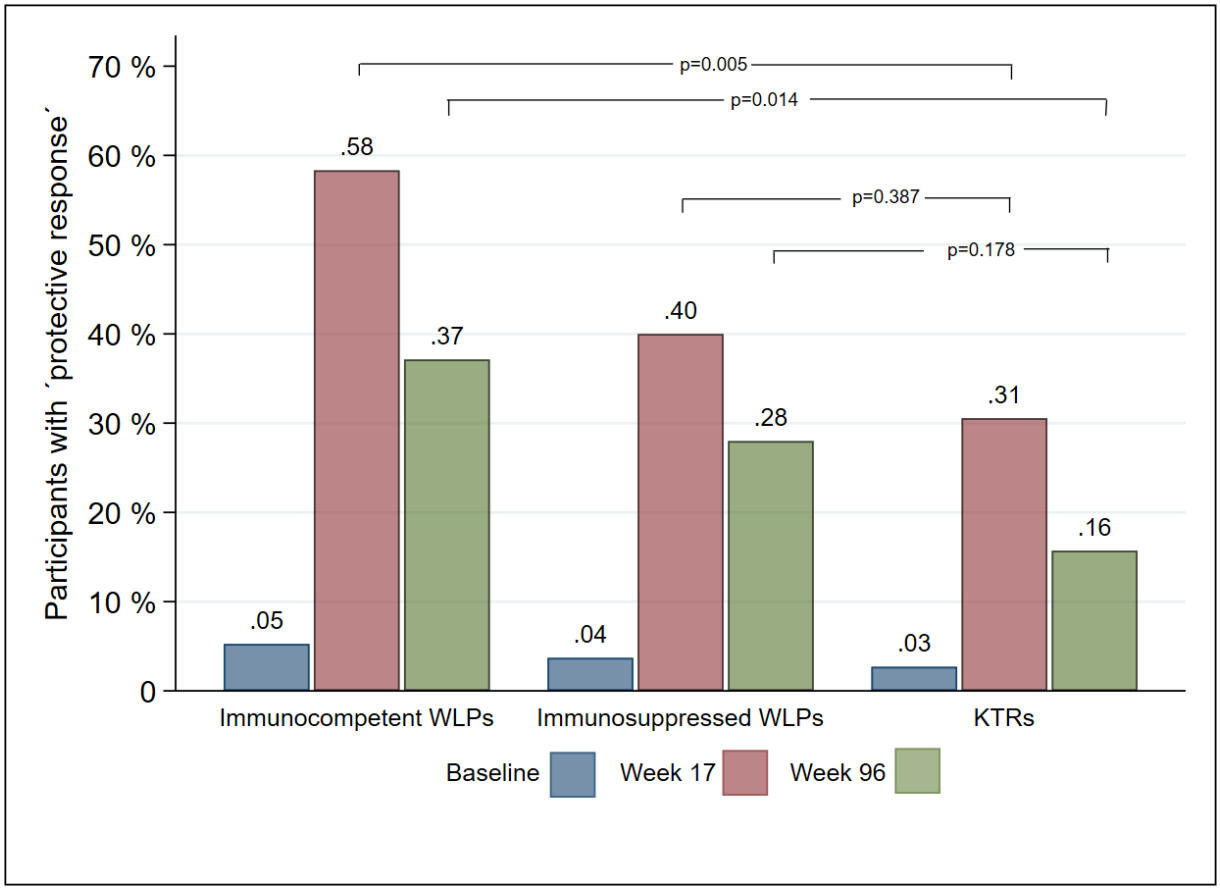


Figure 4d. Week 96 Abbreviations: KTR-ND, kidney transplant recipient – normal dosage; KTR-DD, kidney transplant recipient – double dosage; WLP-ND, kidney transplant waiting list patient – normal dosage; WLP-DD, kidney transplant waiting list patient – double dosage.

Supplement Figure 5

Immunosuppressed and immunocompetent WLPs (week 17) and KTRs with a 'protective response' at baseline, week 17 and week 96.



Abbreviations: WLP, kidney transplant waiting list patient; KTR, kidney transplant recipient.

Additional work

During my Ph.D. enrolment, I have had the privilege to work together with many talented and hardworking people. This has resulted in these publications. Even though they are independent of my thesis, they still contribute to my research education and are therefore mentioned here.

- ACTIV-3/Therapeutics for Inpatients with COVID-19 (TICO) Study Group. Efficacy and safety of two neutralising monoclonal antibody therapies, sotrovimab and BRII-196 plus BRII-198, for adults hospitalised with COVID-19 (TICO): a randomised controlled trial. *Lancet Infect Dis.* 2022 May;22(5):622-635.

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