Journal of Infection 88 (2024) 106133

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Mycophenolate mofetil hampers antibody responses to a broad range of vaccinations in kidney transplant recipients: Results from a randomized controlled study

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ARTICLE INFO

Article history:
Accepted 24 February 2024
Available online 2 March 2024

Keywords:
Vaccination
Immunocompetence
Kidney Transplantation
Tacrolimus
Mycophenolate mofetil
23-valent pneumococcal polysaccharide
Influenza
Tetanus
SARS-CoV-2

SUMMARY

Objectives: To study the effect of mycophenolate mofetil (MMF) on various vaccination responses in kidney transplant recipients.
Methods: In a randomized controlled trial (EudraCT nr.: 2014-001372-66), low immunologically risk kidney transplant recipients were randomized to TAC/MMF or TAC-monotherapy (TACmono), six months post-transplantation. One year after transplantation, in a pre-specified sub-study, recipients were vaccinated against pneumococcus, tetanus and influenza. Blood was sampled before and 21 days after vaccination. Adequate vaccination responses were defined by international criteria. A post-hoc analysis was conducted on SARS-CoV-2 vaccination responses within the same cohort.
Results: Seventy-one recipients received pneumococcal and tetanus vaccines (TAC/MMF: n = 37, TACmono: n = 34), with 29 also vaccinated against influenza. When vaccinated, recipients were 60 (54–66) years old, with median eGFR of 54 (44–67) ml/min, tacrolimus trough levels 6.1 (5.4–7.0) ug/L in both groups and TAC/MMF daily MMF dose of 1000 (500–2000) mg. Adequate vaccination responses were: pneumococcal (TAC/MMF 43%, TACmono 74%, p = 0.016), tetanus (TAC/MMF 35%, TACmono 82%, p < 0.0001) and influenza (TAC/MMF 20%, TACmono 71%, p = 0.0092). Only 7% of TAC/MMF responded adequately to all three compared to 36% of TACmono (p = 0.080). Additionally, 40% of TAC/MMF responded inadequately to all three, whereas all TACmono patients responded adequately to at least one vaccination (p = 0.041). Lower SARS-CoV-2 vaccination antibody responses correlated with lower pneumococcal antibody vaccination responses (correlation coefficient: 0.41, p = 0.040).
Conclusions: MMF on top of tacrolimus severely hampers antibody responses to a broad range of vaccinations.

Background

Kidney transplant recipients require life-long immunosuppression to protect their graft from rejection and graft loss. Currently, more than 90% of kidney transplant recipients globally use the combination of tacrolimus and mycophenolate mofetil (MMF), and 60% additionally use corticosteroids.[1] Patients using Immunosuppressive drugs are at an increased risk for malignancies and infections.[2] Infections are the third leading cause of death in solid organ transplant (SOT) recipients.[2] SOT recipients were eight times more likely to die of an infectious cause than the age-matched general population in a large transplant cohort in Australia and New Zealand.[3] Furthermore, infections cause higher morbidity and hospitalization in SOT recipients as compared to the general population.[4,5] Hospitalization rates were four-fold higher for influenza and 1.5-fold higher for pneumococcal infections in SOT recipients as compared to healthy individuals.[4,5]

Consequently, guidelines recommend various vaccinations for kidney transplant recipients, including those targeting pneumococcus, tetanus and influenza.[6] Nevertheless, kidney transplant recipients generally have lower serological vaccination responses compared to the general population, largely due to immunosuppressive drugs.[7,8] During the Covid-19 pandemic it became

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https://doi.org/10.1016/j.jinf.2024.106133

once again clear how vulnerable kidney transplant recipients are with regards to infectious burden and vaccination yield. Notably, the use of MMF was associated with reduced SARS-CoV-2 vaccination responses.[9,10] However, randomized studies addressing the effect of MMF, especially on other vaccination responses such as pneumococcus, tetanus, and influenza, remain scarce.[9,10]

We have previously performed a pilot randomized controlled study in a cohort of kidney transplant recipients using tacrolimus with or without MMF.[11] Discontinuing MMF nine months after kidney transplantation was safe in this immunological low-risk cohort, with comparable rates of rejection and graft failure and less infectious episodes.[11] Studying vaccination responses was part of this pilot study to assess the potential benefits of lowering the maintenance immunosuppressive therapy in kidney transplant recipients. This study enabled us to study responses after internationally recommended vaccinations in recipients with tacrolimus and MMF, currently the worldwide standard of immunosuppression after kidney transplantation.[1] We tested serological responses to pneumococcal, tetanus, and influenza vaccinations.

As MMF inhibits both B- and T-cell responses, we hypothesized that discontinuing MMF three month prior to vaccination would improve pneumococcal, tetanus and influenza vaccination responses in kidney transplant recipients using tacrolimus.[12]

Methods

Study design

This vaccination study was a pre-specified sub-study of a single-center randomized controlled pilot study in immunologically low-risk kidney transplant recipients.[11] This pilot study compared tacrolimus monotherapy to tacrolimus with MMF and had the following feasibility objectives: safety, consent rate to participate in an immune suppressive weaning trial and biological plausibility. The study design allowed for other outcome measures, such as the vaccination responses in the current vaccination study. Vaccination responses were part of the feasibility objective ''biological plausibility'', a surrogate marker for infectious disease and malignancy.

This trial was approved by the institutional review board of the Erasmus Medical Center and registered in the Netherlands Trial Register (EudraCT nr.: 2014-001372-66). The clinical and research activities being reported are consistent with the principles as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism' and the 'Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects'.

Patients

For this vaccination study, all kidney transplant recipients, randomized for the pilot study, who were on protocol at month 12 after transplantation, were included. Supplementary Table S1 describes the inclusion, exclusion, and randomization criteria of the pilot study. The main exclusion criterion was an immunological disease requiring additional immunosuppression (on top of tacrolimus), biopsy-proven acute rejection (BPAR) from month three onward after transplantation, or administration of lymphocyte depleting therapy.

Recipients used prednisone, tacrolimus, and MMF and prednisone was discontinued at month 5 after transplantation. Randomization took place at month 6 after transplantation and was computer-generated, in a 1:1 ratio to either TAC/MMF or TACmono (halve MMF dose at baseline (month 6) and discontinue at month 9), Supplementary Fig. S1. Target trough levels were 5–8 ug/L for tacrolimus in both arms and 1.5–3 mg/L for MMF in TAC/MMF.

Vaccination

One year (11–13 months) after transplantation, all consecutive 'on-protocol' patients received pneumococcal (0.5 ml Pneumovax23, PPV23, Merck Sharp & Dohme, New Jersey, USA) and tetanus vaccination (0.5 ml of 40 IE tetanustoxoid, Bilthoven Biologicals, Bilthoven, NL) intramuscularly from the research nurse in the outpatient clinic. If in season (October/November), recipients also received influenza vaccinations, one year (10–15 months) after transplantation (Supplementary Table S2 depicts the vaccine composition per year).

Measurements

Blood was sampled directly before and 21 days after PPV23, tetanus and influenza vaccinations. Serum was analyzed for serologic vaccination responses to pneumococcus and influenza at our center and to tetanus at the Dutch National Institute for Public Health and the Environment (RIVM).

Information about patient survival, causes of death and antibiotic use were retrieved from recipients' electronic health records (EHR). Antibiotic use was defined as the use of antibiotics for at least three consecutive days from 6 months onwards after kidney transplantation. Before vaccination, a checklist was completed about prior tetanus vaccination status.

Assays and outcomes

Serological responses to pneumococcal and tetanus vaccinations were measured by validated multiplex bead-based immunoassays, performed for routine clinical testing. Total IgG antibodies against the PPV23 vaccination serotypes 1, 3, 4, 5, 6B, 7F, 8, 9V, 11A, 14, 15B, 18C, 19A, 19F, 20, 23F were measured. The American Academy of Allergy, Asthma and Immunology (AAAAI) criteria were used to define adequate vaccination response, with post-vaccination antibody levels of ≥1.3 ug/ml with a 2-fold increase in at least 70% of tested serotypes.[13]

Tetanus vaccination response was measured using a tetanus toxoid assay. Adequate vaccination response was defined as tetanus antibody concentrations ≥1.0 IU and ≥1.5-fold increase if baseline was ≤1.0 IU or ≥2.5-fold increase if baseline was > 1.0 IU.[14]

Influenza vaccination response was measured by the hemagglutination inhibition assay (HAI).[15] An adequate vaccination response was defined as a post-vaccination antibody concentration of ≥40 IU/ml or a 4-fold increase in baseline antibody level for all 3 strains.[16]

During the follow-up of this randomized cohort, the COVID-19 pandemic unrolled. Some of our randomized recipients participated in a SARS-CoV-2 vaccination study as described by Al Fatly et al.[9] We performed a post-hoc analysis in those recipients to analyze the correlation between SARS-CoV-2, pneumococcal, and tetanus vaccination responses.

Statistical analysis

An 'on-protocol' analysis of vaccination responses was performed. Baseline characteristics were described according to distribution, with means and standard deviation for normally distributed data and medians with ranges for data with a skewed distribution. Vaccination response differences were analyzed as antibody concentrations and binary as responder vs. non-responder. For normally distributed data a students' t-test was performed and for skewed data a Mann-Whitney U test. For binary data, a Fisher's exact test was used. For the association between baseline characteristics, vaccination responses, and clinical outcomes, a Spearman's regression and a multivariate analysis was performed. Kaplan-Meier log-rank survival analysis was used to analyze patient

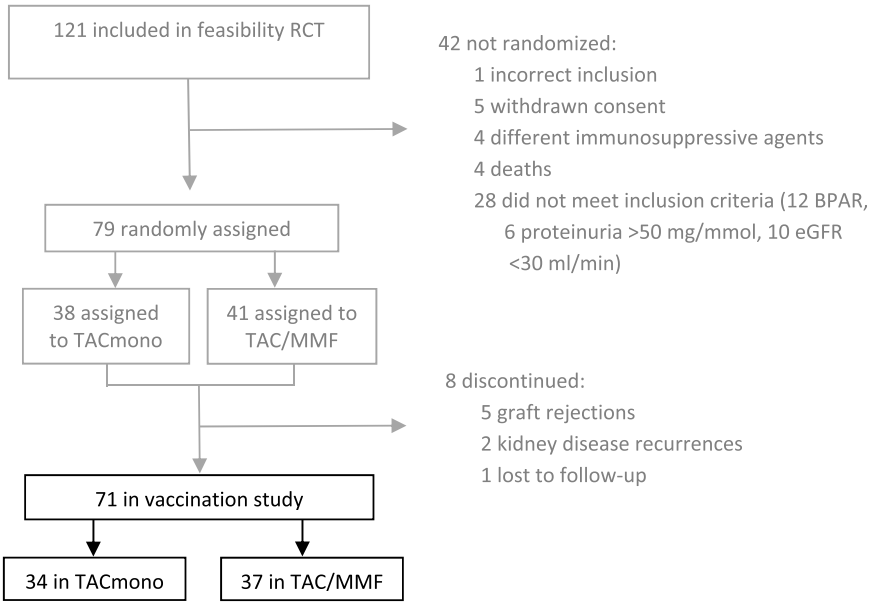

Fig. 1. Patient enrollment. BPAR, Biopsy-proved acute rejection; TACmono, Tacrolimus monotherapy; TAC/MMF, tacrolimus, and mycophenolate mofetil therapy.

survival. A sensitivity analysis was predefined on the correlation between vaccination responses and antibiotic use by excluding recipients with MMF use, as a trend towards less antibiotic use in TACmono compared to TAC/MMF was previously observed in our clinical study.[11] A post-hoc analysis was performed to compare SARS-CoV-2 vaccination responses in this same cohort to PPV23 and tetanus vaccination responses. For the association between antibody concentrations after PPV23, tetanus, and SARS-CoV-2 vaccination, a Pearson's test was performed dependent on assumptions. For the analysis IBM SPSS statistics (version 25) was used. Prism 6 (GraphPad Prism, version 5.01) and R software (version 3.6.3 GUI 1.70 El Capitan build) were used for creating figures and graphs. A p-value of < 0.05 was considered statistically significant.

Results

Between 2014 and 2018, 121 kidney transplant recipients were included in the pilot RCT.[11] After a run-in period of six months, 79 recipients were randomized to either TAC/MMF (n = 41) or to TACmono (n = 38). At the time of this vaccination study, seven recipients had discontinued the study (five rejections, two recurrences of kidney disease) and one sample was lost, resulting in 71 recipients 'on protocol' (TAC/MMF n = 37 and TACmono n = 34, Fig. 1).

Baseline characteristics

Baseline characteristics were comparable between TAC/MMF and TACmono (Table 1). Mean age was 60 (54–66) years, 51 out of 71 (72%) was male, mean eGFR was 54 (44–67) ml/min/1.73 m^2. Tacrolimus trough levels were comparable between the two groups and within target range (median (IQR): 6.2(1.3) ug/L vs. 6.4(1.8) ug/L, respectively in TAC/MMF vs. TACmono). MMF use in TAC/MMF was also within target range (trough levels median (IQR) 2.4 (2.1) mg/L; daily dose median (IQR): 1000 (500) milligrams.

Pneumococcal vaccination response

Before vaccination only four out of the 71 recipients (5.6%) already had protective pneumococcal antibody concentrations, and concentrations were similar in TAC/MMF and TACmono, with median concentrations of 1.73 (1.31–2.54) ug/ml in TAC/MMF and 1.73 (1.36–2.32) ug/ml in TACmono (95% CI −0.46 to 0.51, p = 0.85). After vaccination, serological responses were lower in TAC/MMF compared to TACmono with a median of 3.19 (1.85–5.11) ug/ml vs. 5.17 (3.76–6.70) ug/ml, respectively (95% CI 0.55 to 2.59, p = 0.0023, Fig. 2). These lower pneumococcal antibody concentrations corresponded to a lower number of recipients with an adequate vaccination response in TAC/MMF: only 16 out of 37 (43%) TAC/MMF

Table 1
Baseline characteristics of kidney transplant recipients randomized to either TACmono or TAC/MMF, vaccinated for pneumococcus and tetanus 12 months post-transplantation.

	TAC-mono (n = 34)	TAC/MMF (n = 37)
Age, median (range in years)	59 (37–71)	59 (29–80)
Sex, n male (%)	25 (74%)	26 (70%)
BMI, median (range in kg/m^2)	28 (21–36)	27 (20–35)
Current smokers, n (%)	6 (17.6%)	5 (13.5%)
Transplant type, n living donor transplant (%)	23 (68%)	21 (57%)
Primary kidney disease, n (%)		
Diabetes mellitus	10 (29.4%)	11 (29.7%)
Hypertension	7 (20.6%)	10 (27.0%)
Polycystic kidney disease	4 (11.8%)	3 (8.1%)
FSGS	1 (2.9%)	0 (0.0%)
Tumor	1 (2.9%)	0 (0.0%)
Alport's syndrome	0 (0.0%)	2 (5.4%)
Other	11 (32.4%)	11 (29.7%)
CKD-EPI eGFR, median (range in ml/min/1,73 m^2)	60 (32-105)	53 (29–84)
Proteinuria protein/creatinine ratio, median (range in mg/mmol)	18.6 (6.0-94.9)	13.9 (5.1–33.3)
TAC trough level, median in ug/l (IQR)	6.4 (1.8)	6.2 (1.3)
MMF trough level, median in mg/l (IQR)	–	2.4 (2.1)
Daily dose MMF, median in g (IQR)	–	1.0 (0.5)

TACmono, tacrolimus monotherapy; n, number; BMI, body mass index; eGFR, estimated gromerular filtration rate
; TAC, Tacrolimus; MMF, mycophenolate mofetil; IQR, interquartile range.

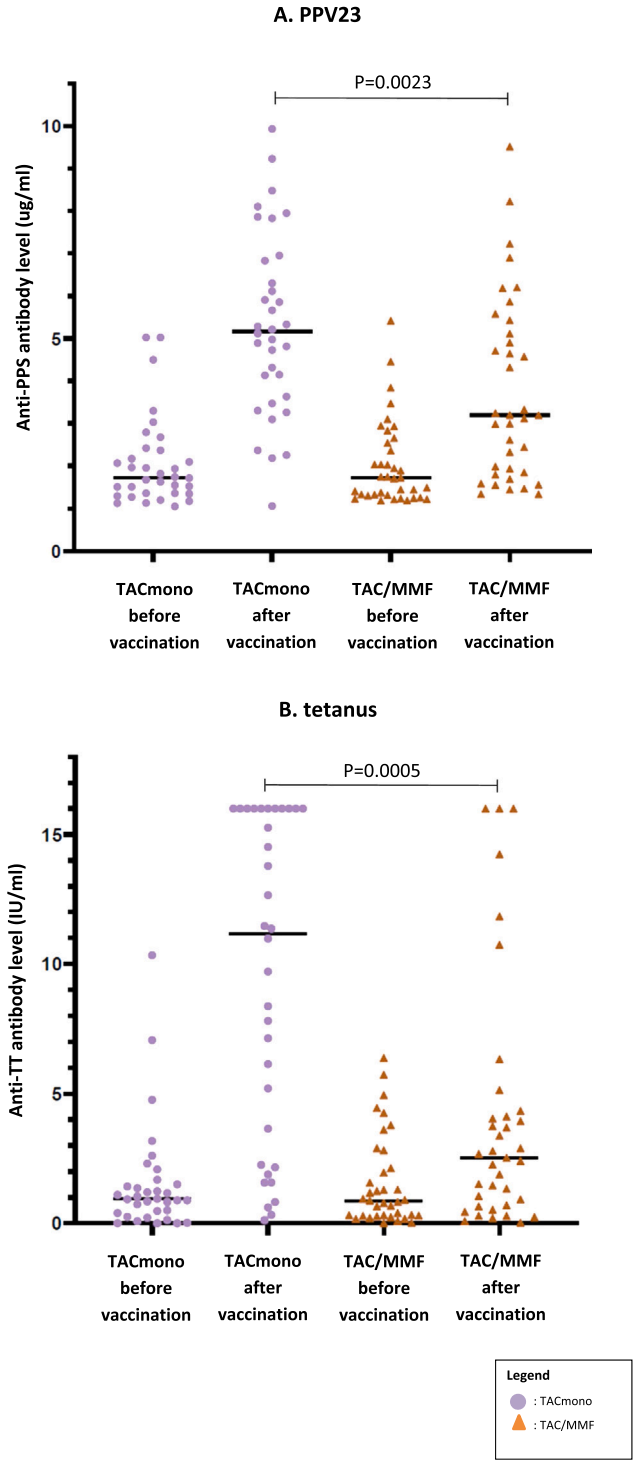

Fig. 2. Antibody levels before and after pneumococcal and tetanus vaccinations in kidney transplant recipients using tacrolimus with or without mycophenolate mofetil. Antibody levels were lower in TAC/MMF compared to TACmono after PPV23 and tetanus vaccinations: antibody levels were 3.19 (1.85–5.11) IU/ml vs 5.17 (3.76–6.70) ug/ml in TAC/MMF vs TACmono after PPV23, p = 0.0023 (A). Antibody levels were 3.98 ± 0.78 vs 9.57 ± 1.06 IU/ml in TAC/MMF vs TACmono after tetanus vaccinations, p = 0.0005 (B). PPV23: pneumovax-23 vaccination, Anti-PPS: anti-pneumococcus polysaccharide, TACmono: tacrolimus monotherapy, TAC/MMF:tacrolimus and mycophenolate mofetil therapy, Anti-TT: anti-tetanustoxoid.

recipients had an adequate serological response to PPV23 vaccinations compared to 25 out of 34 (74%) recipients in TACmono (p = 0.016, Fig. 3). Pneumococcal antibodies rose 4 times after vaccination in TAC/MMF compared to 8 times in TACmono.

Tetanus vaccination response

Based on the checklists, none of the 71 recipients had received a tetanus vaccination in the five years prior to vaccination. However, 89% already had seroprotective antibody concentrations (> 0,1 IU/ml) before vaccination, and these concentrations were comparable between TAC/MMF and TACmono (95% CI −0.96 to 0.87, p = 0.91). After vaccination serological responses were lower in TAC/MMF: mean antibody concentrations increased from 1.56 ± 0.28 IU/ml to 3.98 ± 0.78 IU/ml in TAC/MMF and from 1.54 ± 0.36 IU/ml to 9.57 ± 1.06 IU/ml in TACmono (95% CI 2.89 to 8.10, p = 0.0005, Fig. 2). This also corresponded with less recipients in TAC/MMF with an adequate vaccination response (13 out of 37 (35%) in TAC/MMF compared to 28 out of 34 (82%) in TACmono (p < 0.0001, Fig. 3). On average tetanus antibody concentrations rose 4 times after vaccination in TAC/MMF compared to 37 times in TACmono.

Influenza vaccination response

29 out of the 71 included recipients (15 TAC/MMF and 14 TACmono) received influenza vaccination with three seasonal strains according to the vaccine composition in the period 2015–2019. Influenza vaccination responses were also inferior in TAC/MMF compared to TACmono: on average, an adequate serological response was observed to just 0.4 strains in TAC/MMF vs. 1.9 strains in TACmono (95% CI 0.85 to 2.07, p = 0.0002). This translated into an adequate response to all three strains in only three out of 15 (20%) TAC/MMF recipients compared to 10 out of 14 (71%) TACmono recipients(p = 0.0092, Fig. 3).

Relation between pneumococcal, tetanus, and influenza vaccination responses

29 out of 71 recipients received all three vaccinations (PPV23, tetanus, and influenza), 15 TAC/MMF, and 14 TACmono. In these recipients an adequate serological response to at least one of the three vaccination was less often observed in TAC/MMF compared to TACmono: only 60% responded to at least one of the three vaccinations in TAC/MMF compared to 100% in TACmono (p = 0.041, Fig. 4). In TAC/MMF the number of recipients who responded adequately to all three vaccinations trended to be less: in TAC/MMF only one out of 15 recipient (7%) compared to five out of 14 (36%) in TACmono (p = 0.080).

Furthermore, higher MMF doses correlated with lower antibody responses to influenza (mean antibody response of the three strains) and tetanus, with correlation coefficients of −0.69 (p < 0.0001) for influenza, −0.49 (p = 0011) for tetanus and −0.13 (p = 0.53) for pneumococcus (Supplementary Table S3). Higher MMF trough levels also resulted in lower antibody responses with comparable correlation coefficients as for the MMF dose for influenza and tetanus vaccinations. Unlike MMF dose, sex, age, BMI, eGFR, and smoking were not related to lower antibody concentrations after any of the three vaccinations in univariate analysis.

Clinical differences in vaccination responders versus non-responders

We compared patient survival, cause of death, and antibiotic use in non-responders to both PPV23 and tetanus (non-responders, n = 18) to those who responded to at least one of these two vaccinations (responders, n = 53). The median follow-up was 6.2 years (range 4.5–8.5). Median age was 60 (59–69) in responders vs. 64 (52–67) years in non-responders, eGFR was 56 (44–67) vs. 50 (43–58) ml/min, all not significantly different. Patient survival was comparable: five out of the 18 (28%) non-responders died during follow-up compared to 15 out of 53 (28%) responders, with a mean survival of 85.3 (± 6.4) months in non-responders vs. 95.6 (± 4.2)

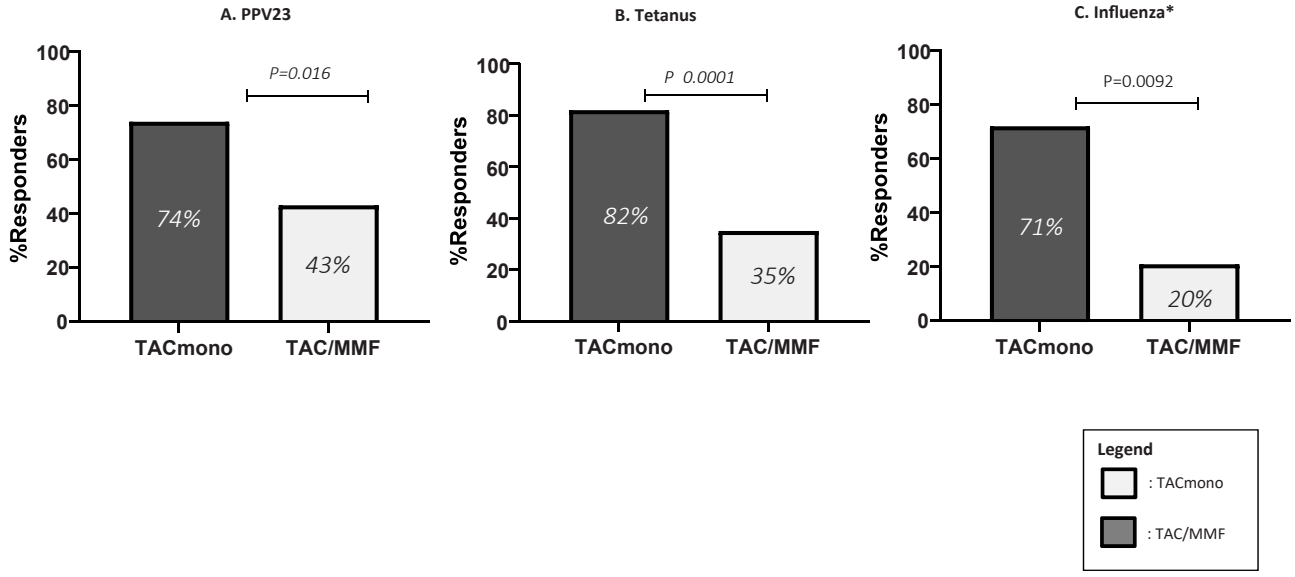

Fig. 3. Adequate serological response rate to PPV23, tetanus and influenza vaccinations in kidney transplant recipients using tacrolimus with or without mycophenolate mofetil. 43% of recipients in TAC/MMF had an adequate serological response to PPV23 vaccinations compared to 74% of the recipients in TACmono, p = 0.016 (A). This was 35% of recipients in TAC/MMF vs 82% in TACmono after tetanus vaccinations, p < 0.0001 (B), and 20% of recipients in TAC/MMF vs 71% in TACmono after influenza vaccinations, p = 0.0092 (C). * An adequate serological response to influenza was defined as an adequate response to all three influenza strains a patient is vaccinated against. PPV23: Pneumovax-23, TACmono: tacrolimus monotherapy, TAC/MMF: tacrolimus with mycophenolate mofetil therapy.

months in responders, p = 0.69. Infection-related death trended to be more frequent in non-responders: 3/5 in non-responders, compared to 2/15 in responders, p = 0.073. The other two deaths in non-responders were due to malignancies. Of the other 13 deaths in responders, five were due to malignancies, three due to cardiovascular diseases, and five due to other causes.

More non-responders had used antibiotics: 13/18 (72%) compared to 20/53 (37%) in responders, from randomization till the follow-up of median 6.2 years (range 4.5–8.5), p = 0.015. We had predefined a sensitivity analysis on antibiotic use in recipients without MMF. However, almost all non-responders were in TAC/MMF, as only three out of 53 non-responders were in TACmono, rendering such a sensitivity analysis valuable.

SARS-CoV-2 in relation to pneumococcal and tetanus vaccination responses

Of the 71 recipients who received PPV23 and tetanus vaccinations, 67 were alive with a functioning graft in 2021, of which 26 recipients participated in a SARS-CoV-2 vaccination study.[9] SARS-CoV-2 antibody concentrations were lower in TAC/MMF compared to TACmono after mRNA vaccination with a median (range) of 25.5 (1.7–135) versus 715.6 (149.7–2746.2) BAU/ml, respectively, p < 0.001. In a post-hoc analysis, recipients with higher post-vaccination SARS-CoV-2 antibodies also had higher post-PPV23 pneumococcal antibodies (correlation coefficient: 0.41, p = 0.040). This correlation was not seen between SARS-CoV-2 antibodies and tetanus antibodies (correlation coefficient: −0.15, p = 0.49, Supplementary Fig. S2).

Four out of 12 (33%) TAC/MMF recipients did not respond to any of the three vaccinations (PPV23, tetanus and SARS-CoV-2) compared to only one out of 14 (7%) in TACmono (p = 0.15, Fig. 4).

Co-administering of influenza vaccines did not affect PPV23 and tetanus serological vaccination responses

All recipients (n = 71) received PPV23 and tetanus vaccines on the same day, of whom 14 also received influenza vaccines on the same day. Co-administration of influenza did not have an effect on pneumococcal or tetanus vaccination responses, compared to the remaining 57 recipients where influenza vaccines were not co-administered (Supplemental Table S4). Co-administering of PPV23 and tetanus vaccines did not affect influenza antibody concentrations after influenza vaccination either (p = 0.61, p = 0.69, p = 0.82 for antibody level increase for each of the three influenza strains).

Discussion

In stable kidney transplant recipients using tacrolimus and mycophenolate mofetil (MMF), serological responses to PPV23, tetanus, and influenza vaccinations were severely hampered. Discontinuation of MMF three months prior to vaccination, one year after kidney transplantation, dramatically improved post-vaccination antibody concentrations and response rates.

As infectious diseases are a huge burden for SOT recipients, guidelines recommend several vaccinations.[2,6] Immunosuppressive drugs are known to weaken vaccination responses, which has gained much attention during the SARS-CoV-2 pandemic. With the high mortality rate of SARS-CoV-2 in SOT recipients and the severely impacted serological vaccination responses in patients on immunosuppressive drugs, many studies have dissected the impact of different drugs on vaccination responses. MMF hampers SARS-CoV-2 serological vaccination responses; however, randomized controlled studies are scarce, especially on the effect of MMF on immunization against other vaccinations that are recommended for SOT recipients.[9,10]

With the AAAAI criteria for pneumococcal vaccination responses, often used in immunocompromised patients, we demonstrated a higher response rate (43%) in TAC/MMF as compared to Larsen et al. (with a response rate of 35%). This difference may be due to the fact that one-third of their cohort used corticosteroids in addition to a calcineurin inhibitor and MMF.[17]

Only 6% of our cohort had seroprotective pneumococcal antibody levels before vaccination. This may differ for countries with different vaccination campaigns. In the Netherlands, the national vaccination program has only recently introduced PPV23 vaccination for people over the age of 60 years, from 2020 onwards. Only last year the recommendation for PPV23 vaccinations for kidney transplant

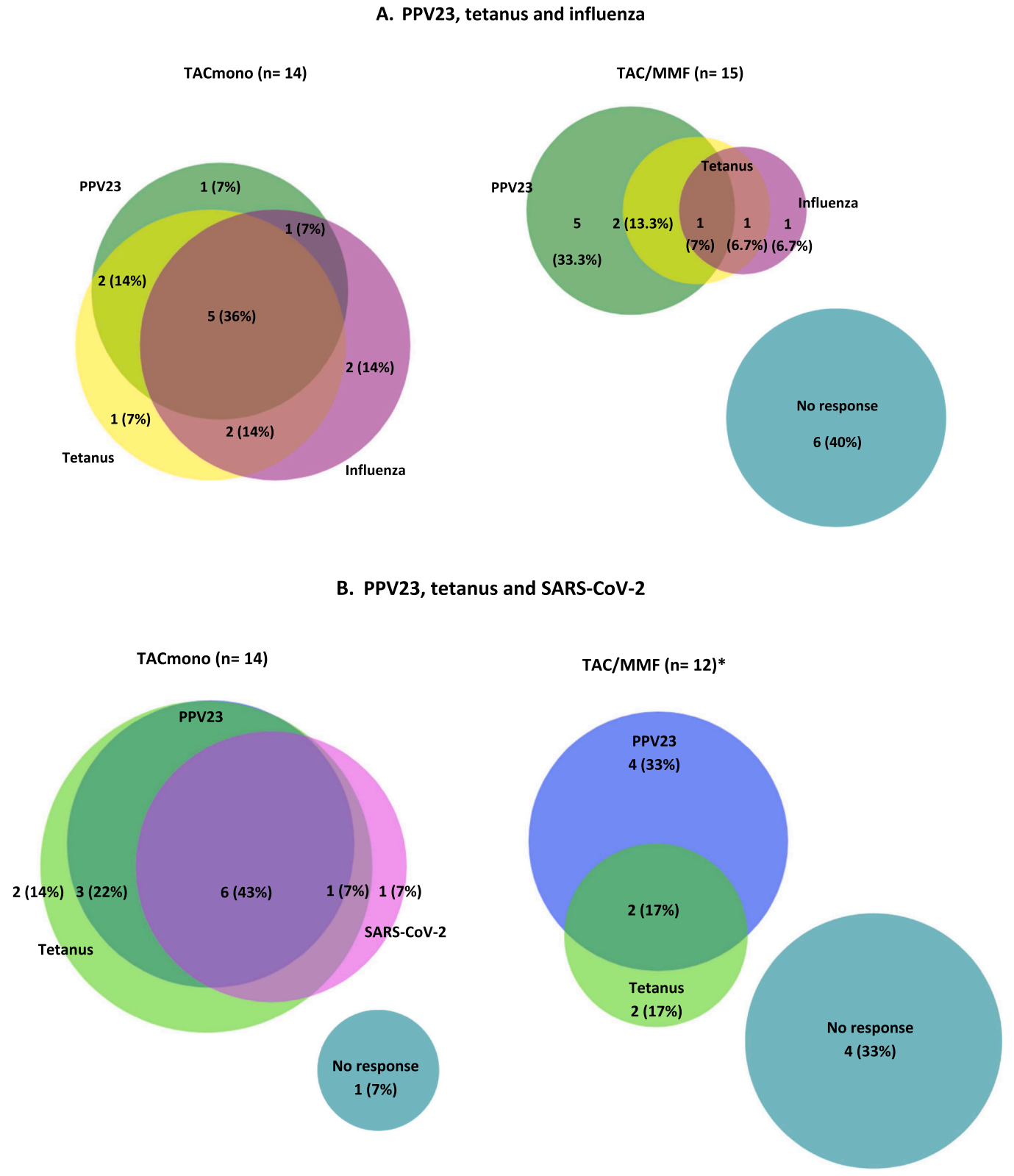

Fig. 4. Relation between different vaccination response rates in kidney transplant recipients using tacrolimus with or without mycophenolate mofetil. The number of recipients with an adequate serological response is shown in this Venn diagram. The figure depicts the 29 recipients who received PPV23, tetanus and influenza vaccinations (A), and the 26 recipients who received PPV23, tetanus and SARS-CoV-2 vaccinations out of the total 71 recipients in this study cohort (B). * In TAC/MMF none of the patients had an adequate response to SARS-CoV-2 vaccinations. TACmono: tacrolimus monotherapy, TAC/MMF: tacrolimus with mycophenolate mofetil, n: number, PPV23: pneumovax23 vaccination.

candidates irrespective of age was included in a national vaccination guideline.[18] Our findings of low seroprotection before PPV23 vaccination stress the need for such a campaign.

Only one-third of TAC/MMF recipients had adequate vaccination responses after tetanus vaccination. However, over 89% of recipients already had seroprotective antibody levels before vaccination, probably because tetanus vaccinations are part of the Dutch National Immunization Program in childhood.

Only 20% of TAC/MMF had an adequate serological vaccination response to influenza, very comparable to lung transplant recipients on MMF and cyclosporine with 20% response rate in Manuel et al.[19]

Our findings demonstrate comparable low serological vaccination response rates with tacrolimus as earlier studies have shown with cyclosporine in kidney and lung transplant recipients.[19,20] Discontinuing MMF three months before vaccination, more than doubled the number of patients with adequate responses. In TAC/MMF, 40% of recipients had inadequate responses to all three vaccinations, while all TACmono recipients responded to at least one of the three vaccinations. The inhibitory effect of MMF on serological response was only modestly dose and trough-level-dependent. These data indicate that (temporarily) reducing MMF, three months prior to PPV23, tetanus, and influenza vaccinations, may not be a good strategy to yield adequate vaccination responses.

We previously demonstrated in this cohort that discontinuing MMF, three months prior to vaccination, dramatically improved serological SARS-CoV-2 vaccination responses.[9] In other reports with a shorter interval, i.e. two weeks, between discontinuation of MMF and vaccination, no improvement in SARS-CoV-2 vaccination responses could be observed.[10]

We demonstrated that TAC/MMF results in lower antibody levels compared to TACmono after SARS-CoV-2, pneumococcus, tetanus and influenza vaccinations. This is important because higher antibody levels correlate with better protection against influenza, tetanus, and SARS-CoV-2.[16,21,22] Our findings are in line with previous studies showing that MMF impairs serological responses to various vaccinations and pathogens, including pneumococcus, influenza, and SARS-CoV-2.[7,8]

PPV23 vaccination causes immunization strictly via B-cells, while tetanus and influenza vaccinations elicit immunization via both B- and T-cells. Tetanus vaccination primarily relies on T-helper (Th) 2 rather than Th1-cells.[23,24] In our study, MMF had the most pronounced effect on tetanus responses. MMF impairs both B- and T-cells via inhibition of purine synthesis, preventing lymphocyte proliferation. It also causes apoptosis of T-cells and affects both cytotoxic and Th1 and 2-cells.[12,25] The pronounced inhibitory effect of MMF on tetanus vaccination responses could be due to impaired Th-cell activity in addition to impaired B-cell activity. For influenza, studies have demonstrated in kidney transplant recipients using calcineurin inhibitors, that MMF was associated with lower serological responses and inhibition of B- and interleukin-4 producing cells, while preserving IFN-gamma-producing T-cells.[26] Influenza vaccination responses rely heavily on antibody-dependent cellular cytotoxic protection.[27,28] This means that IFN-gamma-producing cells, responsible for cellular cytotoxic responses, might be present but not active with the lower antibody levels when MMF is used. Furthermore, lower antibody levels are associated with lower overall immunity after influenza vaccination.[16,28] We did not measure T-cell subsets in our study and we only measured immune responses in peripheral blood, while previous studies have demonstrated that MMF affects immune responses in lymph nodes as well.[29]

In our study, kidney transplant recipients on tacrolimus monotherapy had post-vaccination tetanus and pneumococcal antibody concentrations comparable to healthy adults in the literature.[30,31] Tacrolimus inhibits cytotoxic T-cell formation, T-cell proliferation, and B-cell activation by Th1-cells with less effect on Th2-cells.[32] This could explain why, despite tacrolimus, our serological responses to tetanus and pneumococcus vaccinations were mostly intact. Pneumococcal serological responses are solely B-cell dependent and tetanus is mostly B- and Th2-cell dependent.[23,24]

Unfortunately, lowering immunosuppression is not possible for many transplant recipients. The impaired responses to pneumococcal, influenza, tetanus and SARS-CoV-2 vaccinations in kidney transplant recipients using tacrolimus with MMF, emphasize the need for vaccinations before kidney transplantation and immunosuppression use. Since many vaccinations should be repeated periodically, it is important to recognize the hampered vaccination responses while patients are on immunosuppressive agents.

A limitation of our study is that we did not include healthy controls, nor did we measure T-cell responses or cytokine levels. Another limitation is that antibiotic use was recorded by interviewing the patient instead of reading the central pharmacy. Vaccines were administered on the same day, which could have dampened or synergized individuals responses.[33] In our study influenza vaccine co-administering did not alter PPV23 or tetanus vaccination responses, but we were not able to separate the effect of PPV23 and tetanus co-administration.

The strength of our study is the randomization with MMF discontinuation three months prior to vaccination while continuing tacrolimus. Our study provides real-world data on immune responses to recommended vaccinations in a representative cohort of kidney transplant recipients with an average age of 60 years and with the most commonly used immunosuppressive regimen.

This study demonstrates that MMF on top of tacrolimus severely hampered serological responses to various vaccinations in kidney transplant recipients. Together with the inferior SARS-CoV-2 serological vaccination responses and more infectious episodes in TAC/MMF compared to TACmono, the findings of this study stress the importance of identifying kidney transplant recipients who could benefit from less maintenance immunosuppression. Furthermore, we demonstrated that MMF inhibits different vaccine types, including B- and combined B- and T-cell dependent vaccines and mRNA vaccines. This makes it difficult to improve vaccination responses by changing the vaccine type. The severely hampered serological vaccination responses to pneumococcus, tetanus, and influenza warrant studies on strategies to optimize vaccination campaigns in patients with immunosuppressive agents.[9,11]

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

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

Acknowledgments

We thank Marieken Boer-Verschragen, Judith Kal- van Gestel and the other research nurses and data managers for their help in data collection. We thank the Viroscience Laboratory and the Immunology Laboratory at the Erasmus Medical Center for analyzing the vaccination responses. We disclose that we did not have any writing assistance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106133.

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