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Comparison of the immunogenicity of Dukoral® oral cholera vaccine between renal transplant recipients on either a calcineurin inhibitor or mycophenolate – A controlled trial

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ABSTRACT

Background: The evidence for recommendations regarding vaccination in solid organ transplant recipients is sparse. There is little data comparing vaccine responses between groups on different immunosuppressive drugs. This study was conducted to evaluate the antibody response to Dukoral® oral cholera vaccine in renal transplant recipients (RTR).

Methods: In a single-center non-randomized controlled clinical trial, healthy volunteers (n = 21) and renal transplant recipients (n = 30) were vaccinated with the oral whole cell/recombinant B subunit cholera vaccine Dukoral® (Valneva Inc., Vienna, Austria). The RTR were stratified according to their maintenance immunosuppressive therapy: either prednisone and a calcineurin inhibitor (cyclosporine A or tacrolimus; P/CNI group; n = 15) or prednisone and mycophenolate (P/MMF group; n = 15). All volunteers ingested Dukoral® at baseline and at day 14. Serum samples were drawn at day 0 and day 21. The primary outcome was seroconversion, defined as either a 3-fold IgA serum titer increase in anti-cholera toxin B antibodies and/or a 4-fold rise in the serum vibriocidal titer.

Results: Follow-up was complete. Seroconversion after vaccination was 57% (standard error, SE 9%) in RTR and 81% (SE 9%) in healthy controls (Relative Risk, RR 0.70; 95% CI 0.48–1.02). When stratified according to maintenance immunosuppression, the seroconversion rate was 67% (SE 12%) in the P/CNI group (RR compared with controls 0.82; 95% CI 0.55–1.25) and 47% (SE 13%) in the P/MMF group (RR compared with controls 0.58; 95% CI 0.32–1.03).

Conclusion: Adverse events were mild to moderate and transient. The response to Dukoral was weaker and the seroconversion rate was lower in renal transplant recipients than in healthy controls. In particular, those using mycophenolate had a poor response. Nevertheless, more than half of the transplant recipients seroconverted. Therefore oral vaccines should not be discarded as a potential tool for protection of solid organ transplant recipients.

This trial is registered in clinicaltrials.gov under NCT01109914.

1. Background

After kidney transplantation, immunosuppressive drugs are administered to prevent rejection, delicately balancing improved allograft survival with infectious complications [1]. In solid organ transplantation the standard of care is to administer calcineurin inhibitors (CNI) and mycophenolate. CNI inhibit the intracellular enzyme calcineurin, which plays an important role in transducing the signal from the T-cell receptor to the nucleus to allow transcription of genes encoding for cytokines including IL-2 and the expression of CD40 ligand. Therefore, CNI impair T-cell function including T-cell help to activated B-cells. Mycophenolate interferes with DNA synthesis and is cytotoxic to rapidly dividing cells, such as activated T- and B-lymphocytes. Both drugs severely inhibit the primary and secondary immune response [2]. When using these drugs, the capacity to mount a primary immune response to infection or vaccination is suppressed in a way that is hard to predict in individual circumstances, leading to a variety of reported response rates to different vaccines. There is limited data on the response rate to the majority of vaccines. Results of studies on seroconver-

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sion in solid organ transplant recipients after vaccination have been summarized by Eckerle et al. [4].

While guidelines for vaccination of solid organ transplant recipients do exist (such as stated in Kidney Disease: Improving Global Outcomes: KDIGO) [5] these only address a limited number of vaccines and additionally suffer from insufficient adherence in clinical practice, possibly due to uncertainty of transplant doctors regarding the immunogenicity of vaccines under immune suppression [6]. There is virtually no data on responses to oral vaccines [7], such as to the oral cholera vaccine [8] in solid organ transplant recipients. There is some data on the immunogenicity of the oral cholera vaccine in another immunocompromised group: HIV infected Haitian adults [9]. This study showed that 74% of HIV positive subjects seroconverted to the Inaba strain, compared with 91% of the healthy adults. Those with the lowest CD4+ count had a poorer response.

Certain groups of travelers, such as renal transplant recipients (RTR) are more vulnerable to the adverse consequences of travelers' diarrhea. Dehydration can induce kidney injury. Therefore, in some countries, Dukoral®, an oral cholera vaccine, is prescribed to immunocompromised travelers to prevent traveler's diarrhea, based on the notion that the immune response to cholera toxin B may provide protection against travelers' diarrhea caused by the heat-labile toxin of enterotoxigenic *E. Coli*, with which it shares structural and antigenic similarities [10,11]. This practice is not supported by the evidence, as is summarized in a Cochrane review [12]. Nevertheless, it raises an interesting question: To what extent is the response to oral immunization affected by immunosuppressants?

Vaccination of solid organ transplant recipients offers the possibility to study the effect of different immunosuppressive drugs on the ability to mount an immune response. To this end, we performed a non-randomized controlled clinical trial with Dukoral® oral cholera vaccine in RTR on maintenance immunosuppressive therapy with either a calcineurin inhibitor or mycophenolic acid.

2. Methods

2.1. Study design

This was a single-center non-randomized controlled clinical trial conducted between March 2010 and November 2014 at Leiden University Medical Center (LUMC) in the Netherlands. The primary objective was to evaluate the immunogenicity of 2 doses of the oral whole cell/recombinant B subunit cholera vaccine Dukoral® in RTR (n = 21), 21 days after vaccination. The RTR were stratified into two groups according to their maintenance immunosuppressive therapy, either prednisone and a calcineurin inhibitor (cyclosporine A or tacrolimus) (P/CNI group) (n = 15) or prednisone and mycophenolic acid (P/MMF group) (n = 15). Secondary objectives were to compare the immunogenicity of Dukoral® between RTR and healthy controls and to compare the immunogenicity between the P/CNI group and the P/MMF group. We originally intended to include a third group of RTR: those on prednisone and an mTOR-inhibitor (p/mTORi). However, inclusion into this category was unsuccessful due to sparsity of RTR with p/mTORi in our source population.

RTR were selected from the registry of the department of Nephrology at LUMC. All patients that met the inclusion criteria during the screening period were invited to participate by letter. Concurrently, siblings and partners of the transplant recipients were invited to participate as healthy controls. Adult RTR, with stable renal function and on a stable immunosuppressive regimen consisting of P/CNI or P/MMF were eligible. Exclusion criteria included: a history of auto-immune disease, prior cholera vaccination or infection, use of immunosuppressive medication other than a CNI or MMF, recent treatment with blood products (<3 months) and recent treatment for graft rejection (<12 months). Recent episodes of travelers' diarrhea (<6 months ago) were recorded in the CRF, but were not an exclusion criterium.

The primary immunogenicity endpoint was seroconversion among all RTR. There is no established immunological correlate of protection. The assays were performed by Crucell (Crucell Holland BV). In accordance with their specifications, seroconversion was defined as a $\geq$ 3-fold rise in serum anti-CTB IgA antibodies and/or a $\geq$ 4-fold rise in serum vibriocidal antibodies.

2.2. Vaccine and procedures

Dukoral® (The pharmaceutical company that produced the vaccine at the time of the study was Crucell Holland BV. The licence is currently held by Valneva Inc., Lyon, France), a licensed oral cholera vaccine consisting of killed whole cell monovalent *Vibrio cholerae* (serogroup O1, Inaba and Ogawa strain) combined with recombinant cholera toxin subunit B, was administered to all subjects. Subjects received the first dose upon inclusion, according to the instructions of the manufacturer (day 0), and were instructed how to store and self-administer the 2nd dose at home, 2 weeks after the first dose. Administration of the 2nd dose was verified by telephone. Subjects kept a diary of adverse events for 4 days after each dose. Subjects were invited back to the outpatient clinic for a second and final visit, 1 week after the 2nd dose (day 21), at which time the diary was collected. Blood samples were drawn upon inclusion (day 0) and at the final visit (day 21). Samples were centrifuged and serum was stored at −20 °C.

2.3. Immunogenicity assays

Anti-CTB serum IgA ELISA: rCTB peptide (Crucell) was coated (2 h at room temperature) to high binding microtiter plates (Immunon 2HB, NUNC, USA) at a concentration of 0.5 μg/mL in phosphate buffered saline (PBS). Upon coating, plates were blocked with 1% Casein blocking buffer (Novagen, Merck Millipore, Germany) to reduce the background. Heat-inactivated (30 min at 56 °C) samples, a reference sample and internal controls were diluted in blocking buffer and applied to the rCTB coated plate (room temperature). After 2 h of incubation, and washing with phosphate buffered saline containing 0,05% Tween 20, anti-human IgA HRP-labeled antibody (24 ng/mL, Jackson ImmunoResearch Europe Ltd, UK) was added for 1 h, followed by tetramethylbenzidine substrate (Sureblue, KPL Inc., USA) for detection. After 10 min a 1 M H_2SO_4 stop solution was used to stop the colorimetric reaction. The optical density (OD) was measured at 450 nm, using a microplate spectrophotometer (PowerWave 340, Bio-Tek, USA). A stored serum sample with an unequivocal reproducible response was used as the reference standard in this assay. The reference value, in relative ELISA units (EU/mL), for this sample was based on the geometric mean 50% infliction point as determined in eight subsequent assay runs. This reference curve was tested at seven 2-fold dilutions from 1/80 to 1/5120 in each assay and a four-parameter logistic (4PL) curve fit was applied. The CTB ELISA titer of individual samples was determined by correlating a single dilution of the sample in the reference curve. Titers below 0.159 were considered as 0.079 for analysis. The cut-off for seroconversion was established as a 3-fold increase of post- vs. pre-vaccination individual titers.

Vibriocidal assay: *V. cholerae* O1 Inaba El tor (strain T19479) working freezer stocks were harvested by centrifugation (2 min, 15,000 rcf) and washed twice in saline to remove the storage medium (LB medium with 15% glycerol) and diluted to a final OD600 of 0.220 ± 0.01. An assay reaction mixture was prepared by further dilution of the bacteria to 1/80 in 0.85% saline (Fluka, Sigma-

Aldrich, USA) solution, supplemented with 6.7% guinea pig complement (Calbiochem, USA). All wells of a 96-well microtiter plate (Immulon 2B, Nunc, USA) were filled with 25 μL of sample. Heat-inactivated (30 min, 56 °C) samples and internal controls were serially diluted two-fold in 0.85% saline buffer, starting from 1:5 until reaching 1:1280 dilution. An equal volume (i.e. 25 μL) of the reaction mixture was added to the serially diluted serum samples and allowed to incubate at 37 °C for 1 h and 350 rpm. After 1 h, 150 μL of fresh brain heart infusion (BHI) media (Prolab, Brazil) was added to each well, and the plates were incubated for an additional 2.5 h at 37 °C. The bacterial turbidity within each well was read at 630 nm with a microtiter-plate reader (PowerWave 340, Bio-Tek, USA) and a four-parameter logistic (4PL) curve fit was applied. The vibriocidal antibody titer was defined as the sample curve's 50% infliction point (i.e. the EC_{50}). Titers below 5 were considered as 2.5 for analysis. The cut-off for seroconversion was defined as a $\geq$ 4-fold rise in serum vibriocidal antibodies.

Both assays were performed by Crucell Holland B.V. (currently part of Janssen Pharmaceuticals) after completion of the study. Laboratory workers of Crucell were blinded to the study groups.

2.4. Statistical analyses

No formal sample size calculation was performed. Geigy scientific tables were used to estimate the confidence intervals when a total of n = 20 renal transplant patients per study arm would be included. This was judged to yield sufficient difference between the groups based on the expected outcome. Because of slow recruitment the study was terminated when 15 RTR were included in the P/CNI and P/MMF study arm.

Statistical analyses were performed using SPSS Statistics version 20.0.0.1 (IBM Corp., USA) and Excel version 2010 (Microsoft Corp., USA).

2.5. Ethics statement

All participants provided informed consent. The study was approved by the Medical Ethics Committee of Leiden University Medical Center and registered in clinicaltrials.gov under NCT01109914.

3. Results

3.1. Participants

Participant characteristics are summarized in Table 1. In total 51 subjects were enrolled and vaccinated, consisting of 30 renal transplant recipients (15 in the P/CNI arm and 15 in the P/MMF arm), and 21 healthy controls (8 patient partners and 13 non-related subjects). All subjects had a negative history for cholera infection or vaccination, and follow-up was complete. Three subjects experienced travelers' diarrhea in the 6 months before inclusion: 2 healthy controls and 1 in the P/MMF arm. None of the subjects had ever been diagnosed with cholera. The dosage of the

Table 1
Characteristics and demographics of the study arms.

Variable	HC (n = 21)	RTR (n = 30)	P/CNI (n = 15)	P/MMF (n = 15)
Male gender, n (%)	11 (52)	25 (83)	12 (80)	13 (87)
Age, median years (IQR)	49 (24)	60 (23)	56 (26)	62 (15)
Primary kidney disease, n (%)				
Glomerulonephritis	–	9 (30)	3 (20)	6 (40)
Diabetes mellitus	–	1 (3)	0	1 (7)
Hypertension/ischemic	–	4 (13)	2 (13)	2 (13)
ADPKD	–	6 (20)	2 (13)	4 (27)
Reflux nephropathy	–	3 (10)	3 (20)	0
Other	–	3 (10)	2 (13)	1 (7)
Unknown	–	4 (13)	3 (20)	1 (7)
Transplant details				
Years after transplant, mean yrs (range)	–	9.3 (1.8–17.3)	9.3 (2.0–16.6)	9.4 (1.8–17.3)
Heart beating donor, n (%)	–	13 (43)	5 (33)	8 (53)
Non-heart beating donor, n (%)	–	17 (57)	10 (67)	7 (47)
Related donor, n (%)	–	7 (23)	4 (27)	3 (20)
Unrelated donor, n (%)	–	23 (76)	11 (73)	12 (80)
Repeat transplant, n (%)	–	1 (3)	1 (7)	0
Anti-rejection therapy, n (%)	–	5 (17)	1 (7)	4 (27)
Immunosupression				
Prednisone dose, mg, mean (range)	–	7.6 (5–10)	6.7 (5–10)	8.6 (5–10)
Ciclosporin dose, mg, mean (range)	–	–	182 (150–225)	–
Ciclosporine trough level, ug/L, median (IQR)	–	–	103.5 (50.25)	–
Tacrolimus dose, mg, mean (range)	–	–	4.9 (1.5–10)	–
Tacrolimus trough level, ug/L, median (IQR)	–	–	7.8 (4.5)	–
Mycophenolate dose, mg, mean (range)	–	–	–	1929 (1440–2500)
Mycophenolate AUC, mg*h/L, mean (range)	–	–	–	64.5 (44–94)
Laboratory measurements				
Serum creatinin, μmol/L, mean (range)	–	117 (73–184)	128 (83–181)	106 (73–184)
GFR Cockroft, mL/min, mean (range)	–	–	63 (30–103)	80 (46–110)
eGFR, mL/min/1.73 m^2, mean (range)	–	–	47 (33–58)	56 (32–81)
Hemoglobin, mmol/L, mean (range)	–	–	8.2 (6.5–10.2)	8.6 (7–10)
Blood group				
A, n (%)	–	10 (33)	5 (33)	5 (33)
B, n (%)	–	6 (20)	3 (20)	3 (20)
O, n (%)	–	12 (40)	5 (33)	7 (47)
AB, n (%)	–	1 (3)	1 (7)	0

HC: healthy control, RTR: renal transplant recipient, P/CNI: prednisone/calcineurin inhibitor, P/MMF: prednison/mycophenolate, IQR: interquartile range, ADPKD: autosomal dominant polycystic kidney disease.

Table 2
Serologic response to Dukoral vaccine in RTRs.

	Healthy controls (n = 21)	RTR (n = 30)	P/CNI (n = 15)	P/MMF (n = 15)
Combined seroconversion, % (SE; n)	81 (9%, 17)	57 (9%, 17)	67 (12%, 10)	47 (13%, 7)
Risk ratio for seroconversion (95% CI)	1.0 (ref)	0.70 (0.48–1.02)	0.82 (0.55–1.25)	0.58 (0.32–1.03)
Anti-CTB IgA titer, geometric mean (95% CI)				
Baseline	0.3 (0.2–0.5)	0.2 (0.1–0.3)	0.30 (0.2–0.5)	0.2 (0.1–0.3)
Post-vaccination	4.5 (2.3–8.7)	1.0* (0.5–1.9)	2.1 (0.8–5.1)	0.4** (0.2–1.0)
Mean Fold Increase	13.4 (6.7–26.7)	4.3 (2.5–7.6)	6.9 (2.7–17.7)	2.7*** (1.6–4.7)
Vibriocidal titer, geometric mean (95% CI)				
Baseline	26.5 (9.7–72.5)	47.2 (20.0–111.6)	54.8 (15.2–197.4)	40.6 (12.4–133.4)
Post-vaccination	78.0 (26.5–229.8)	86.8 (36.5–206.3)	101.5 (26.9–383.0)	74.3 (23.5–235.0)
Mean Fold Increase	2.9 (1.8–4.7)	1.8 (1.1–3.1)	1.9 (0.9–3.7)	1.8 (0.8–3.9)

CTB: Cholera Toxin B-subunit, CI: Confidence interval (normal distribution), RTR: renal transplant recipient, P/CNI: prednisone/calcineurin inhibitor, P/MMF: prednison/mycophenolate.
* Significantly different from control (Mann-Whitney U = 166, p = 0.004).
** Significantly different from control (Mann-Whitney U = 50, p = 0.001).
*** Significantly different from control (Mann-Whitney U = 171, p = 0.006).

immunosuppressants was in the therapeutic range, as reflected by serum monitoring. Patients on MMF were slightly older and were more likely to have received past treatment for allograft rejection. The prednisone dosage was slightly higher in the MMF group, in accordance with the protocol for dosage of immunosuppressants after transplantation.

3.2. Immunogenicity (Table 2, Figs. 1 and 2)

At 21 days after vaccination, the overall seroconversion rate was 57% (SE 9%) in renal transplant recipients and 81% (SE 9%) in healthy controls (RR for seroconversion in RTR versus controls 0.70; 95% CI 0.48–1.02) (Table 2). When stratified according to maintenance immunosuppression therapy, the seroconversion rates were 67% (SE 12%) in the P/CNI group (RR for seroconversion in the P/CNI group versus controls: 0.82; 95% CI 0.55–1.25) and 47% (SE 13%) in the P/MMF group (RR for seroconversion in the P/MMF group versus controls 0.58; 95% CI 0.32–1.03).

Anti-CTB response: The geometric mean fold increase (GMFI) for anti-CTB IgA titers was 4.3 (95% CI: 2.4–7.8) for RTR and 13.4 (95% CI: 6.4–28) for healthy controls. The GMFI was significantly lower in the P/MMF group than in healthy controls (2.73 vs 13.4; p = 0.006). Vibriocidal response: Vibriocidal geometric mean titers (GMT) showed little difference between the study groups, due to high variability in baseline titers between individuals. The GMFI for vibriocidal titers was 1.8 (95% CI: 1.1–3.1) in RTR and 3.0 (95% CI: 1.8–4.8) in healthy controls. For both assays, the reverse cumulative distribution curves are provided in Fig. 1 and the individual titer plots and geometric mean titers in Fig. 2.

In total, 34 subjects seroconverted, satisfying either the anti-CTB response criterium (n = 21) or the vibriocidal response criterium (n = 3) or both (n = 10). The strength of the anti-CTB and the vibriocidal response did not correlate with each other or with patients' age, hemoglobin level, serum creatinine, immunosuppressant concentrations, cumulative prednisone dose or time since transplantation (data not shown).

3.3. Vaccine safety

There were no serious adverse events. Adverse events were mild to moderate and transient in all subjects. Healthy controls suffered more varied adverse events than RTR after the first vaccine dose (p = 0.004) but not after the second dose, adding up to a higher total amount of AEs in healthy controls. In total, 15 subjects (29%) reported any adverse event after the first vaccine dose.

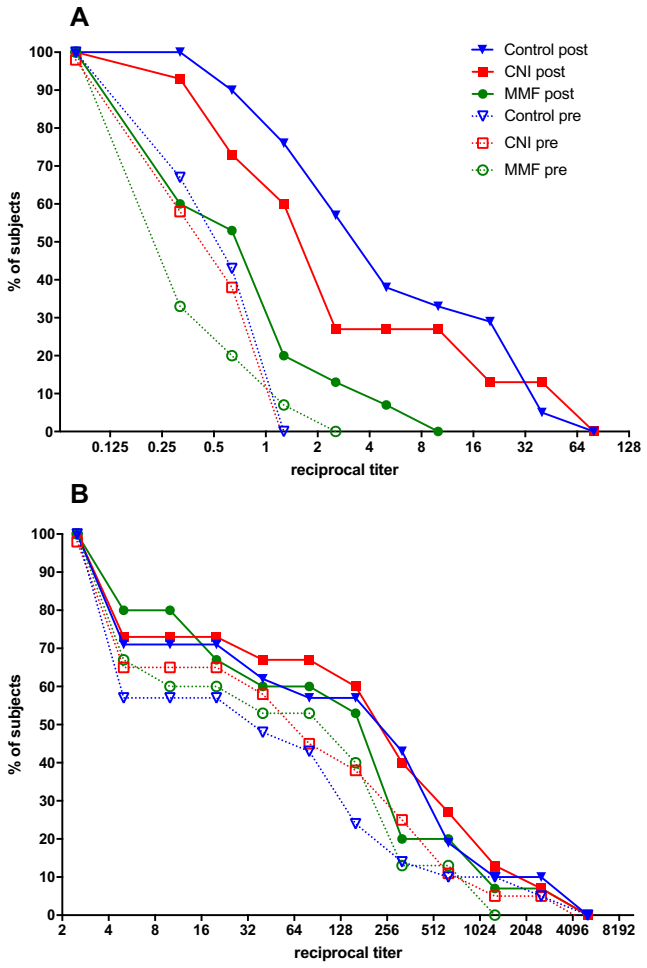

Fig. 1. Reverse cumulative distribution curves for the anti-cholera toxin B (anti-CTB) titers (A) and the vibriocidal titers (B), indicated in blue for controls, red for renal transplant recipients (RTR) on calcineurin inhibitor + prednisone (P/CNI) and green for RTR on mycophenolate and prednisone (P/MMF). Please note the ordering of anti-CTB responses where the control group has the best response, followed by the P/CNI group and lastly the P/MMF group. Also note the lack of differentiation between vibriocidal responses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In 14 subjects this was considered related to vaccination. After the second dose, 14 subjects (28%) reported any adverse event, which in 9 subjects was considered to be related to vaccination (Table 3).

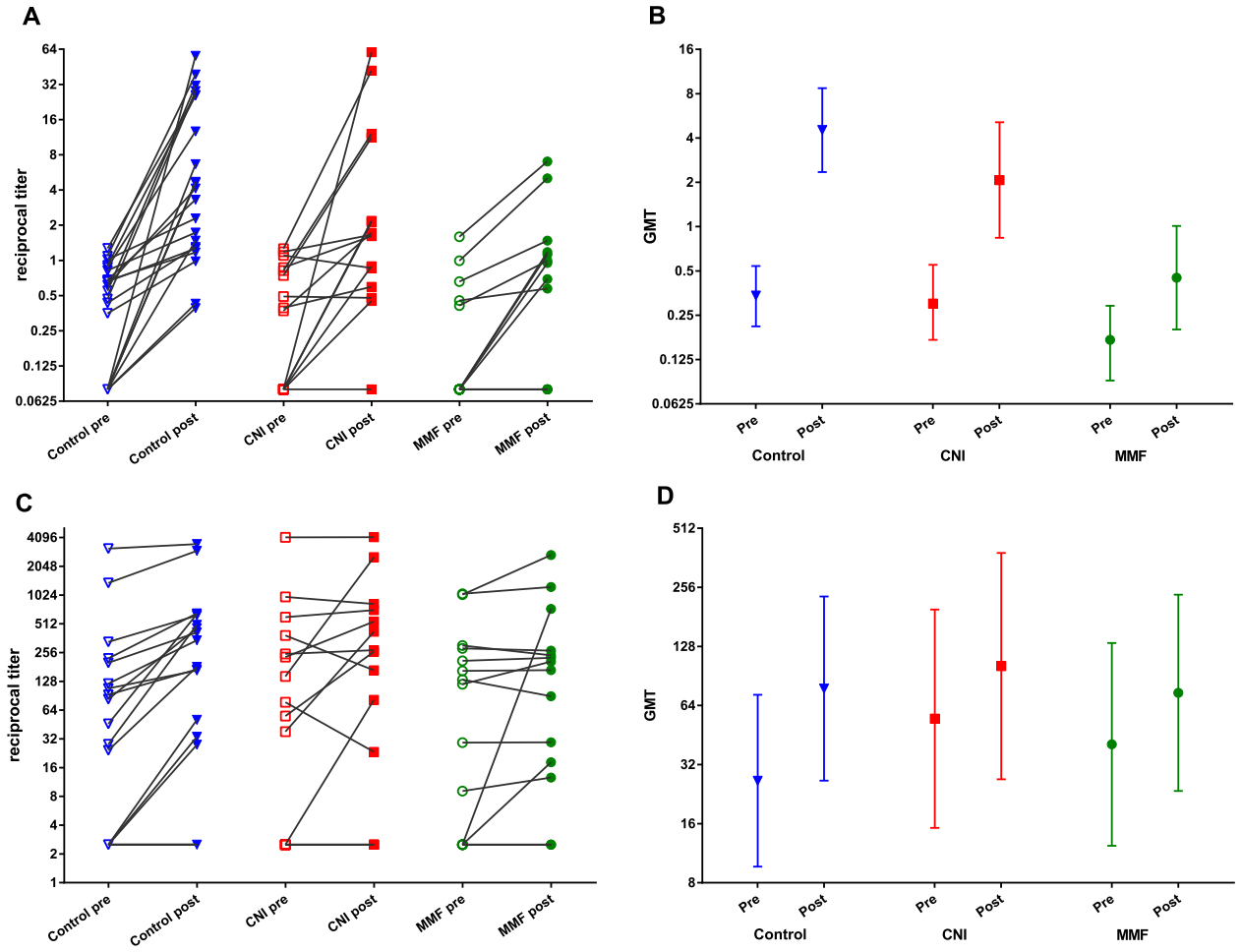

Fig. 2. Individual titer plots for anti-CTB titers (A) and for vibriocidal titers (C), and their respective geometric mean titers (B and D). Please note the differential response seen in the anti-CTB titers but not in the vibriocidal titers.

Table 3
Adverse events after vaccination.

	RTR (n = 30)		HC (n = 21)		Total (n = 51)	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
Percentage of subjects with any AE, % (n)	13 (4)	20 (6)	52* (11)	38 (8)	29 (15)	27 (14)
Frequency of various AEs, n:						
bloating	2	–	2	1	4	1
flatulence	1	1	2	2	3	3
decreased appetite	–	–	1	1	1	1
urge to defecate without production	–	–	1	–	1	–
fatigue	–	1	1	1	1	2
excess bowel noises	1	2	3	–	4	2
acid reflux	–	–	1	–	1	–
abdominal pain	1	–	1	1	2	1
nausea	1	1	–	–	1	1
myalgia	–	1	–	–	–	1
general malaise	–	2	–	–	–	2
headache	–	–	1	–	1	–
decreased visual acuity	–	–	1	–	1	–
sleeplessness	–	–	1	1	1	1
dizziness	–	1	1	–	1	1
excess micturition/fluid retention	–	–	1	–	1	–
increased menstrual discomfort	1	–	–	–	1	–

* Significant difference with RTRs after dose 1, Fisher's exact test = 0.004.

4. Discussion

This is the first trial to study the immunogenicity of oral cholera vaccine in solid organ transplant recipients. Seroconversion was 57% in renal transplant recipients and 81% in healthy controls. Vaccine responses were highest in healthy controls, followed by the P/CNI group, and lowest in the P/MMF group. Anti-toxin serum IgA responses were significantly lower in RTRs and lowest in the P/MMF group. The vibriocidal antibody response showed no significant differences between groups due to large variability in the baseline titers and responses. Our findings are in line with several other studies, which show that vaccine responses are lowest in patients on mycophenolic acid, higher in patients on calcineurin inhibitors and highest in healthy controls [3,13,14]. However for the mucosal vaccine Dukoral we had expected an even lower response in the RTR groups, based on the relatively low seroconversion to oral cholera vaccines even in healthy vaccinees [15]. The difference in seroconversion rates between RTR and HC was in line with the result of a study in HIV infected adults [9].

Significantly fewer adverse events (AEs) were reported by RTRs than by the controls, but only after the first dose. This may be due to the higher tolerance for physical discomfort developed by RTRs during the course of their previous illness. An immunological etiology seems less likely, since the difference occurred only after the first dose and within 4 days of the dose. The frequency of observed AEs were all in the range of expected side effects after oral cholera vaccination [16,17].

The results from our trial demonstrate that oral vaccines can be useful as a tool in the protection of solid organ transplant recipients and should not be dismissed beforehand.

4.1. Strengths and weaknesses

The main strength of this study is its prospective controlled design, in which only RTRs on dual therapy (prednisone and 1 other immunosuppressive agent) were included, thus enabling a clear comparison of the effect of different immunosuppressive agents on the response to vaccination. Second, there was no loss to follow-up and the trial was performed in a single transplant center which contributed to a high quality of data regarding the patient characteristics. Furthermore, the study used two assays to assess immunogenicity. Both were performed by the company that produces the vaccine in accordance with strict quality criteria. Finally, variation was minimized by limiting the number of vaccine batches to the absolute minimum achievable, since variation in cholera vaccine trials is at least partly due to the fact there is currently no *in vitro* test to evaluate and compare the potencies of different vaccine lots [18].

There are two main limitations. First, the study is relatively small and did not achieve the intended sample size. Nevertheless, the standard errors for the proportions achieving seroconversion are within acceptable limits, allowing between-group comparisons and interpretation of the results. Second, the trial lacks a third arm to assess responses in RTRs using sirolimus or everolimus, the inhibitors of the mammalian target of rapamycin (mTOR). This would have been of particular interest, as there is evidence that immunological responses remain relatively intact in subjects using mTOR inhibitors [3,19,20]. Furthermore, we did not evaluate the long-term, to assess differences in waning of titers in RTRs and controls, such as is seen following vaccination for hepatitis B [21]. Lastly, there was high variability in vibriocidal assay results. This affects the field of cholera vaccine research in general, which also suffers from a lack of a true immunological correlate of protection [15,22–24]. We did not collect data on previous travel to cholera endemic countries. In theory this may have influenced baseline titers.

4.2. Discussion of the immunogenicity measurements

In our study we used a 3-fold increase in anti-toxin antibody titer as cutoff for seroconversion instead of the more frequently used 2-fold increase. This stricter cutoff was instituted in consultation with the laboratory experts, based on their experience and because a 2-fold cutoff in an ELISA is more likely to introduce false positive results due to inter-assay variability of one dilution step.

We measured seroconversion and not seroprotection as there is no established correlate of protection against cholera. The correlation between anti-cholera serology and subsequent protection from cholera is an imperfect one [25]. The challenges of finding a correlate of protection are well illustrated by a challenge study with the antigenically similar Escherichia coli heat-labile toxin [26]. Furthermore, the endpoint in this study was seroconversion, which does not reflect the entirety of the immunological response elicited by an oral vaccine. Assays to study the mucosal immune response (fecal antibody responses, antibody secreting cell response to vaccine antigens) were not performed in this study.

The strength of the vibriocidal and anti-CTB IgA seroresponse did not correlate, probably due to the variability in the results from the vibriocidal assay. This variability is not unique to our study [17,22]. Furthermore, in contrast to larger studies on oral cholera vaccines, we did not find a correlation between patient characteristics and the immune response [13,14,27]. This is to be expected in a study such as ours, with a limited sample size.

4.3. Conclusion

In immunocompromised individuals, the response to vaccination differs, based on the type of immunosuppressant. Therefore, trials of vaccine responses in immunocompromised patients should include well defined groups. Based on this study, we conclude that the response to Dukoral was weaker and that the seroconversion rate was lower in renal transplant recipients than in healthy controls. In particular, those using mycophenolic acid had a poor response. Nevertheless, more than half of the transplant recipients seroconverted and only mild transient adverse events were observed. Therefore, oral vaccines should not be discarded as a potential tool for protection of solid organ transplant recipients.

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Disclosure

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