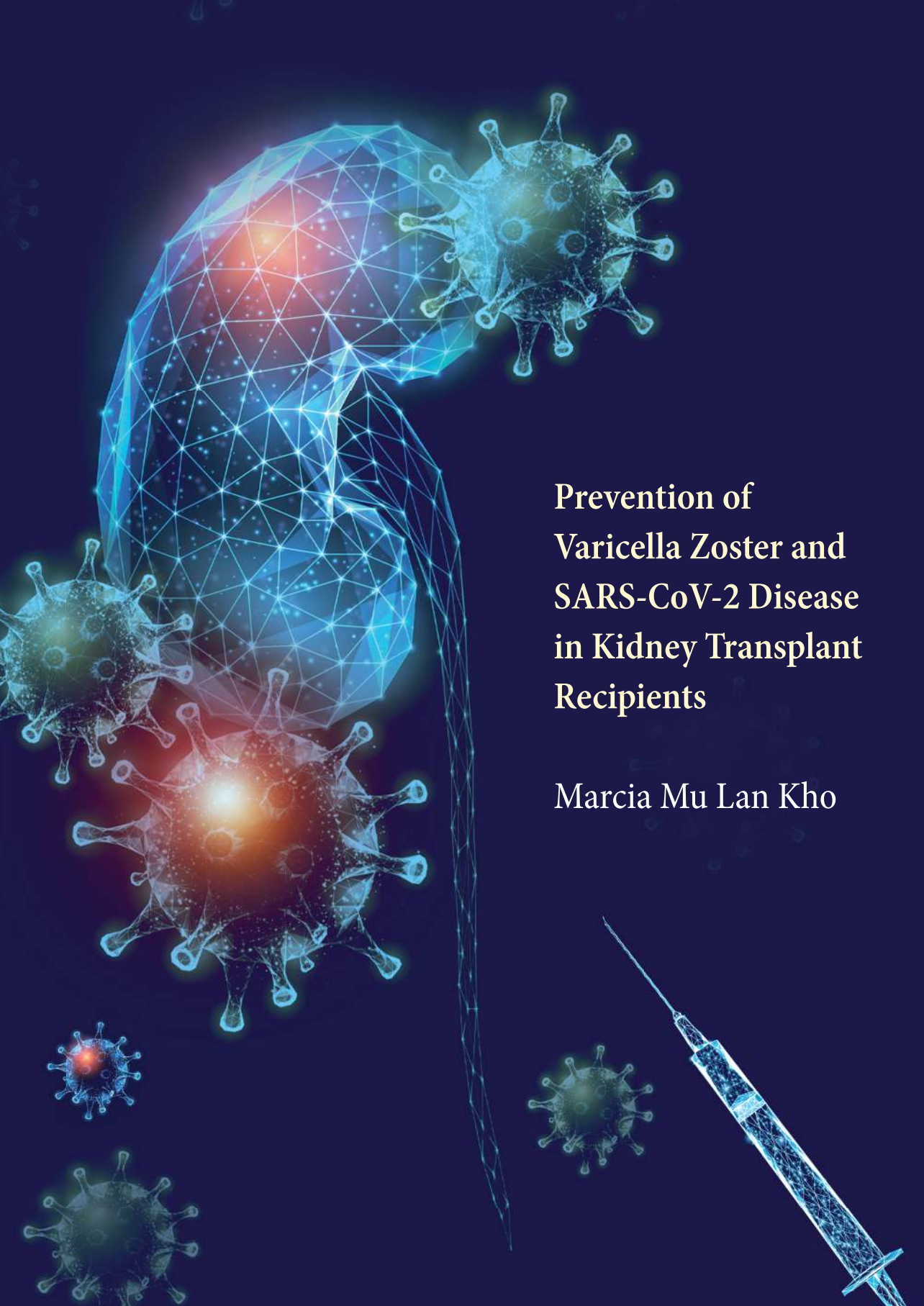

Prevention of Varicella Zoster and SARS-CoV-2 Disease in Kidney Transplant Recipients

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Preventie van varicella zoster en SARS-CoV-2 ziekte in niertransplantatie patiënten

Proefschrift

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Erasmus Universiteit Rotterdam
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rector magnificus

Prof.dr. A.L. Bredenoord

en volgens besluit van het College voor Promoties.
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Marcia Mu Lan Kho
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Erasmus University Rotterdam

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1 General introduction

GENERAL INTRODUCTION

Patients with kidney disease and patients using immunosuppressive medication have an increased risk of severe morbidity and mortality from infectious diseases. Ranking the most common causes of death, infection comes second after cardiovascular disease in patients on dialysis,[1,2] and third after cardiovascular disease and malignancy in kidney transplant recipients.[1] As cause of hospitalisation, infection rivals cardiovascular disease in both dialysis and kidney transplant patients.[3] The risk of infectious disease-related morbidity and mortality increases with the severity of chronic kidney disease (CKD).[4,5] This can be explained by accelerated ageing of the immune system, so-called immune senescence, in patients with CKD. These patients have lower thymic output and higher susceptibility for apoptosis of naïve T cells, and less diversity of T cell receptors.[6-8] Furthermore, there is impaired antigen recognition and antigen presentation by monocytes and dendritic cells.[9] On top of their immune senescence, kidney transplant recipients require chronic drug-induced suppression of their immune system to prevent transplant rejection. In general, immunosuppressive drugs aim to inhibit T and B cell activation and proliferation.[10,11]

In immunocompromised patients, viral infections are serious threats because of their contagiousness and severe disease course. Furthermore, some viruses have the ability to establish latency and to reactivate when the immune system is aged or suppressed. Vaccination induces both humoral and cell-mediated immune responses with the aim of preventing (severe) disease from viral infection or reactivation. Worldwide there are many successful vaccination programmes to prevent severe illness from infectious diseases. Since the 1960s, diseases like diphtheria, polio, measles and pertussis have practically disappeared in countries with high vaccine coverage.[12] National and international guidelines for immunocompromised patients recommend vaccination of solid organ transplant candidates and recipients, as well as their close contacts.[13-15] However, patients with chronic kidney disease, dialysis patients and kidney transplant recipients have shown impaired immune responses to vaccinations, including influenza, hepatitis B and Streptococcus pneumoniae.[16-19] This emphasizes the need for more research on vaccine immunogenicity and efficacy in these patients and strategies to improve these outcomes.

Vaccine-induced protection is often expressed in the level of antibodies that can neutralize the virus or toxin or opsonize bacteria. However, T cell responses are also required for optimal protection.[20] Whereas $CD4^+$ T cell help is important for optimal antibody responses, $CD8^+$ T cells are crucial for clearance of obligate intracellular pathogens like viruses.[21,22] Furthermore, memory B and T cells are necessary to mount an immune response upon encounter of the pathogen in the future (Figure 1).[20] It is plausible to think that T cell dysfunction is a major impairing factor in the immune response of CKD patients and kidney transplant recipients.

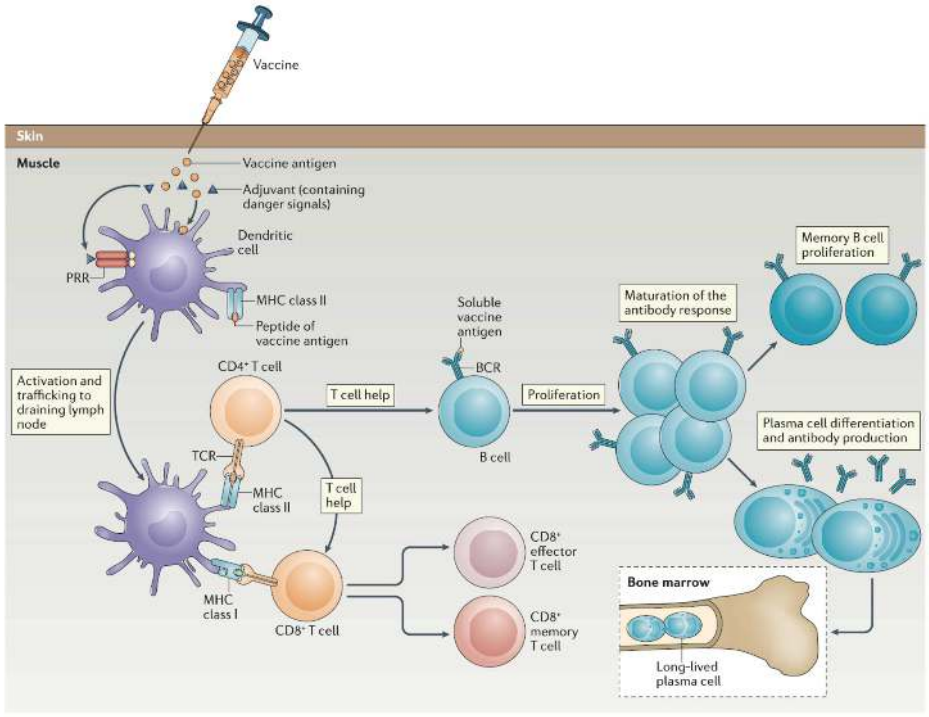

Figure 1. The generation of an immune response to a vaccine. From: Pollard AJ and Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021 Feb;21(2):83-100. Reproduced with permission from Springer Nature
PRR: pattern recognition receptors. MHC: major histocompatibility complex. TCR: T cell receptor. BCR: B cell receptor.

The varicella zoster virus is sometimes underestimated, but able to cause life-threatening disease in these patients, either as primary infection or as herpes zoster after a period of latency. Many studies on varicella vaccine immunogenicity in these patients focus only on short-term antibody response and do not include a control population.[23] There is clearly a need to fill these knowledge gaps by investigating longer term memory B and T cell responses on top of antibody responses to primary and booster varicella vaccination.

The SARS-CoV-2 virus has, more than any other, accelerated vaccine development. However, the large clinical trials which led to the approval of these vaccines by regulating authorities did not include CKD patients or kidney transplant recipients. Hence the importance of assessing vaccine immunogenicity and safety in these patients.

Varicella zoster virus

The varicella zoster virus (VZV) or human herpes virus 3, is a DNA alpha herpes virus and member of the Herpesviridae, which specifically infect humans and some primates.[24] The VZV virion consists of a linear double-stranded DNA genome in an icosahedral nucleocapsid core (Figure 2). Surrounding the core is a tegument layer, made of viral regulatory

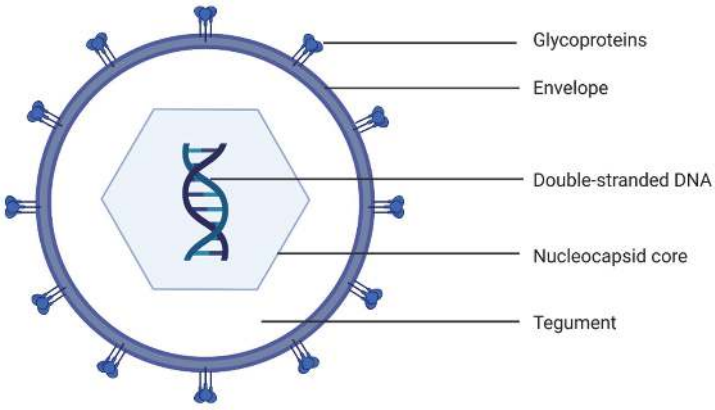

Figure 2. Varicella zoster virus structure. Adapted from "Virology", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

proteins which are needed for genome replication and activation. The outer layer is a lipid-rich envelope, containing viral glycoproteins which mediate entry of virions into human cells and induce fusion of infected and uninfected cells. VZV has specific tropism for T cells, skin and neuronal cells.[24,25]

Viral spreading occurs by droplets and aerosols from the nasopharynx and from skin lesions of infected people. VZV enters through the mucosa of the respiratory tract, proliferates in lymphoid tissues of Waldeyer's ring and infects T cells and dendritic cells. Infected T cells are modulated to disseminate the virus preferentially to the skin, but also to other organs. In the skin, innate immune responses delay virus proliferation and it takes 10 to 21 days (the incubation period) to develop the typical vesicular skin lesions. Viral transmission from the nasopharynx occurs already 1 to 2 days before skin lesions are detectable. The skin vesicles contain high amounts of virions and are an important source of transmission in the 5 to 7 days before crusts are formed. During viremia, sensory neurons in ganglia of the cranial nerves, dorsal root and autonomic ganglia are infected, and within weeks after primary infection, VZV establishes latency. From the ganglia, VZV can reactivate and upon reactivation and replication, the virus is transported by neuronal axons to the skin or other organs.[24-26]

Varicella is present worldwide, but epidemiology varies with climate. In temperate climates, it is a childhood disease and over 90% of people are infected before adolescence.[27-30] In many tropical climates, infection occurs later in life and a higher proportion of the adults is susceptible.[31,32] Primary infection with the generalized maculopapular-vesicular rash is called varicella or chickenpox. Usually, varicella is a benign and self-limiting disease, especially in otherwise healthy children. The most common complication is secondary bacterial infection of the skin. Gastro-intestinal, pulmonary and neurological complications such as

cerebellar ataxia and meningoencephalitis are rare,[30] but do occur with high morbidity and mortality in immunocompromised patients.[33,34]

VZV reactivation can occur sub-clinically or with symptoms. The clinical presentation of viral reactivation with typical skin lesions erupting unilaterally in a dermatome is called herpes zoster (HZ) or shingles. A rare form of reactivation with radicular pain without skin lesions is called zoster sine herpete. Pain resolves in most patients within 1 to 2 months. However, on average 20% of patients suffer from post-herpetic neuralgia (PHN), mostly defined as pain persisting more than 3 months after rash onset, the most frequent complication of HZ.[35] Other complications are paresis, vasculopathy causing stroke, giant cell arteritis and ocular disease.[26,36] The lifetime risk of VZV reactivation is about 30%.[37] HZ incidence rate in the general adult population ranges worldwide from 4 to 11 cases per 1000 person-years, with the highest incidence in the elderly, and appears to be increasing.[38,39]

In immunocompromised patients, especially CKD patients and solid organ transplant recipients, HZ incidence is 2- to 5-fold higher than in the general elderly population.[40-43] Immunocompromised patients are also at greater risk of PHN, cranial nerve involvement, recurrent HZ and potentially lethal disseminated varicella or herpes zoster.[33,42,44,45]

To control VZV infection and reactivation, both innate and adaptive immune responses are needed. Innate immune cells act locally in the skin and ganglions. VZV-specific antibodies prevent infection of host cells. $CD4^+$ T cells stimulate specific antibody production and assist $CD8^+$ T cells to eliminate already infected host cells and thus stop viral replication. Long-lived plasma cells, memory B and T cells are needed to suppress reactivation.[46-48] Re-exposure to the virus in the community and (subclinical) reactivation of latently residing virus maintain natural immunity against VZV. In CKD patients and kidney transplant recipients this immunity is impaired.

The first vaccine for primary varicella vaccination was developed in 1974 by Takahashi in Japan: the Oka strain live attenuated virus vaccine.[49] It has been used widely in the United States of America in routine vaccination of children. For the prevention of herpes zoster, there are currently two licensed vaccines. A live attenuated virus vaccine of the Oka strain was licensed in Europe and the USA in 2006. A recombinant subunit vaccine was licensed in 2017 in the USA and in 2018 in Europe. The three above-mentioned vaccines are effective in the general population,[50,51] but clinical trials reporting immunogenicity in CKD patients and kidney transplant recipients are limited.[52-54] Nevertheless, to prevent severe morbidity and potential mortality, both primary and booster vaccination are recommended in guidelines for solid organ transplant recipients.[13,15] Therefore, investigation of the humoral and cellular memory immune response to both types of vaccination in kidney transplant candidates is an unmet need.

SARS-CoV-2 virus

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus and a beta coronavirus. The family of corona viruses causes various diseases in animals and humans and SARS-CoV-2 probably originated in bats.[55] The coronavirus virion consists of a nucleocapsid, a membrane, an envelope and spike (S) proteins which give the virion its crown-like appearance (Figure 3). The S protein consists of 2 subunits. The S1 subunit binds the angiotensin-converting enzyme 2 (ACE2) receptor to enter host cells. The S2 subunit anchors the S protein to the virion's membrane and mediates membrane fusion when a host cell is infected.

Fusion of viral and cellular membranes creates a pore, through which the viral genome can reach the host cell cytoplasm and start replicating. Mutations in the S protein change the virus' capability to adapt its binding to human cells and to escape neutralization by the human immune system.[56] The immune response to COVID-19 involves (neutralizing) antibodies, SARS-CoV-2 specific B cells, and a balanced $CD4^+$ and $CD8^+$ T cell response.[57-60]

The coronavirus disease 2019 (COVID-19) pandemic started in December 2019 in Wuhan, China. The typical symptoms of COVID-19 are fever, dry cough, dyspnea, headache and pneumonia which may progress to respiratory failure and death.[61] Other frequent symptoms are diarrhea, vomiting, anorexia, myalgia and loss of olfactory and/or gustatory functions. Transmission occurs via droplets and aerosols from the respiratory tracts of infected people. Early in the pandemic, median incubation period was about 5 days and 98% of patients who developed symptoms did so within 8 to 16 days.[62,63] At least 30-40% of infections were asymptomatic.[64,65] Mortality rates are difficult to calculate, because asymptomatic and mild infections are often not detected and testing capacity and documentation of cause of death varied over time and regions world-wide. One systematic analysis including 53 countries, reported infection fatality rates in the pre-vaccine era of 0.002% in seven year

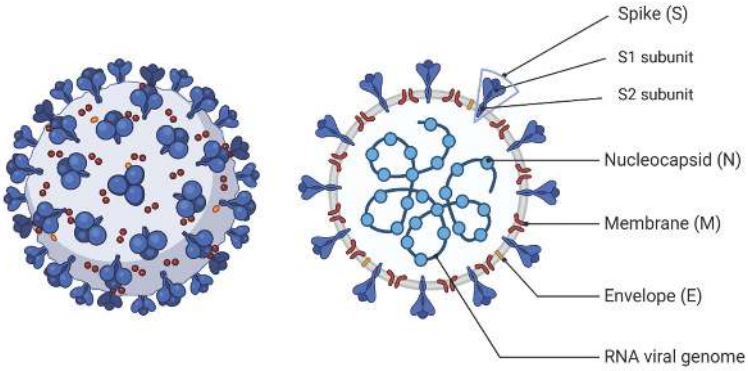

Figure 3. Human Coronavirus structure. Adapted from "Human Coronavirus Structure" by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

old children, increasing exponentially to 0.06% at age 30, 1% at age 60 and 20% at age 90, with considerable variation between regions.[66] When the SARS-CoV-2 virus evolved, people acquired immunity through infection and vaccination, symptoms, incubation time, transmission rates, and disease severity changed. When the Omicron variant was dominant, a shorter incubation time, higher transmission rates, but less disease severity were reported compared to the earlier variants.[67,68]

Patients with severely impaired kidney function (eGFR <30 ml/min/1.73m^2, CKD stages 4 and 5), on dialysis, and kidney transplant recipients were shown to be extremely vulnerable. COVID-19-associated mortality risk in these groups was reported to be 3- to 4-fold higher than in the general healthy population in the UK.[69] Furthermore, the ERA-CODA collaboration analysed data from 26 European countries showing a COVID-19 related mortality in the first month after diagnosis of 21.3% in kidney transplant patients and 25.0% in dialysis patients.[70]

Several COVID-19 vaccines were developed. In Europe three vaccine designs have been approved: mRNA vaccines, non-replicating viral vector vaccines, and protein subunit vaccines. mRNA and vector vaccines are the most frequently used. The mRNA vaccines consist of lipid nanoparticles containing mRNA molecules which encode a viral antigen. The antigen is expressed by the vaccine recipient as a protein against which immune responses are elicited. Virus vector vaccines use a weakened version of an adenovirus, that has been genetically changed so that it is not able to replicate in humans. The vector virus carries the genetic code to produce a viral protein. Protein subunit vaccines contain recombinant virus protein and an adjuvant, which elicits an immune response in the recipient.[71] The clinical trials that proved COVID-19 vaccine efficacy in the general population, included only a small number of patients with severely impaired kidney function, on dialysis or kidney transplant recipients.[72-75] Therefore, we designed a study comparing these three patient populations to a healthy control group. From our clinical trial and others, it became clear that kidney transplant recipients have a diminished response to vaccination.[76-78] So, the next logical step was to investigate approaches enhancing (booster) vaccine efficacy.

AIMS OF THE THESIS

Part 1: Varicella zoster virus

1. To study the humoral and T cell response after primary varicella vaccination in kidney transplant candidates (Chapter 2).
2. To assess the incidence and risk factors of herpes zoster in recipients of a heart, lung, liver or kidney transplant (Chapter 3).
3. To study the memory B and T cell response after booster varicella vaccination in kidney transplant candidates compared to healthy people (Chapter 4).

Part 2: SARS-CoV-2 virus

4. To study the immune response and safety of mRNA-1273 COVID-19 vaccination in patients with chronic kidney disease, on dialysis or living with a kidney transplant (Chapter 5).
5. To investigate whether alternative strategies increase the immunogenicity of COVID-19 vaccines in kidney transplant recipients who did not respond to two or three doses of an mRNA vaccine (Chapter 6).

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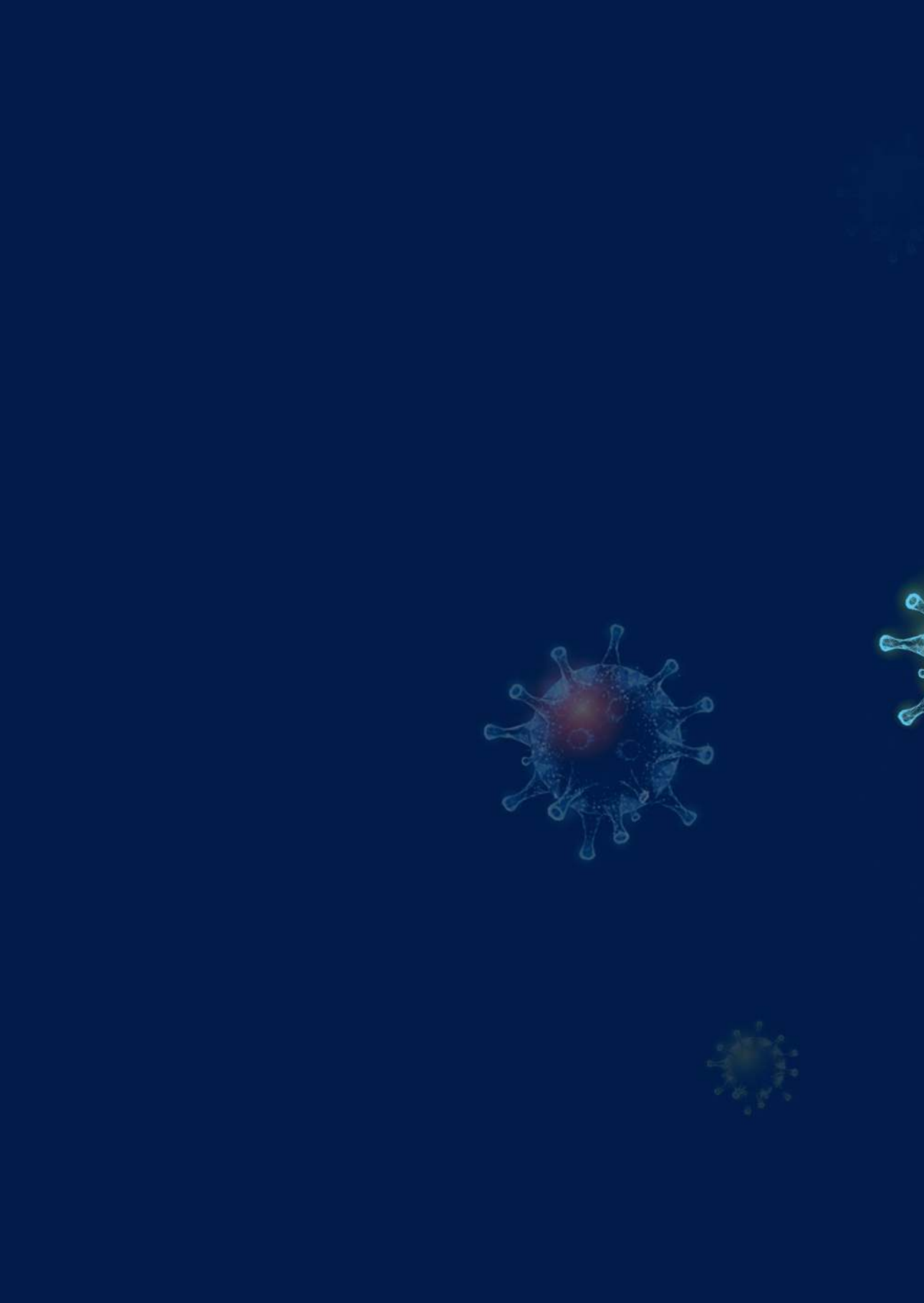

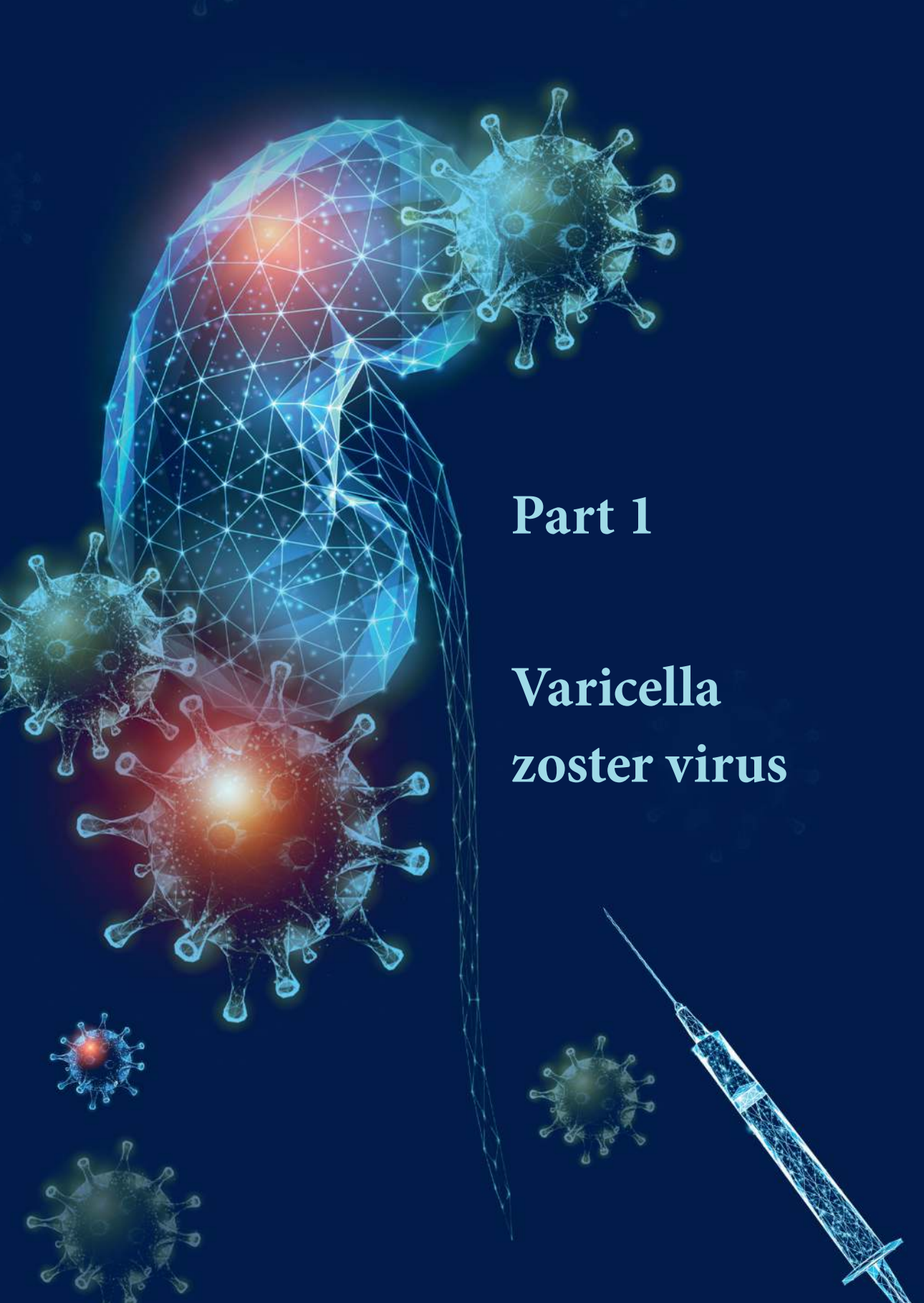

Part 1

Varicella zoster virus

2 Humoral and cellular response after varicella vaccination in VZV IgG seronegative kidney transplant candidates

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ABSTRACT

Background: In immunocompromised patients, primary infection with VZV may have a disastrous clinical course. Vaccination of VZV-seronegative patients on the waiting list for renal transplantation may prevent severe disease. However, the immunologic response of end-stage renal disease patients to peptide vaccines is far from optimal. Our question was whether end-stage renal disease patients with undetectable VZV-IgG levels were able to mount an adequate humoral and cellular response to a live attenuated varicella vaccine.

Methods: Kidney transplant candidates with undetectable VZV levels were vaccinated twice with a live attenuated varicella vaccine at an interval of 6 weeks. VZV IgG levels were analysed till 2 years after vaccination. The VZV-specific T-cell reactivity was determined prior to vaccination and after transplantation.

Results: Seventy-seven percent (40/52) of the vaccinees reached positive VZV-IgG levels after vaccination (responders). Eighty two percent (9/11) showed an increase in VZV-specific $CD4^+$ memory T-cells (both central and effector memory cells). The percentage VZV-specific $CD8^+$ memory T-cells did not increase. None of the non-responders suffered from primary VZV after transplantation. No severe vaccine-related adverse events were reported, only spontaneously resolving local skin irritation.

Conclusion: The live attenuated varicella vaccine evokes positive VZV IgG-levels and VZV-specific memory T-cells in VZV-seronegative potential kidney transplant candidates.

INTRODUCTION

The varicella zoster virus (VZV), or human herpes virus 3, causes varicella and is highly infectious. After 4 to 6 days a first, subclinical, viremia occurs, during which the virus disseminates to the viscera and sensory ganglia. Further replication occurs in reticulo-endothelial tissues and the skin is infected, resulting in the characteristic vesicles.[1,2]

In the Netherlands VZV is endemic, seroprevalence of VZV antibodies in the overall Dutch population amounts to 95%.[3] Routine vaccination against VZV is not implemented in the Dutch childhood vaccination program, because VZV is considered a relatively benign childhood-disease. In contrast, in adult immunocompromised patients primary VZV infection is rare, but is associated with high mortality and morbidity rates.[4-6] In our centre, 3.2% of adult patients on waitlist for renal transplantation was VZV seronegative. This is comparable to the percentage VZV seronegative people in the overall Dutch population. In the past, we observed three lethal primary VZV infections in adult renal transplant recipients.[7] Recently, we described one VZV IgG negative patient who received a transplant without vaccination against VZV, who developed a primo-VZV infection, involving the skin and gastrointestinal system eight months after transplantation.[8] Despite the severity of primary VZV infection in renal transplant recipients, pre-transplant vaccination of seronegative patients is not performed on a routine basis. Studies of vaccination against VZV prior to renal transplantation are mainly focussed on pediatric patients.[9-12] Little is known about VZV vaccination in adults with chronic and end-stage kidney disease.[13] Most vaccinations in patients with end stage renal disease are not very effective. Patients on dialysis show an impaired immune response compared to healthy individuals to hepatitis B[14-16] and influenza[17-19] vaccines. The same impaired response to influenza vaccines is shown in renal transplant recipients.[20,21] However, such peptide vaccines are possibly less immunogenic compared to a live attenuated virus vaccine.[22, 23]

Immunity to VZV is complex, and consists of both a humoral component by VZV-specific antibodies and a cellular component by VZV-specific effector T-cells. Both components are impaired in solid organ recipients.[24,25]

We studied a) whether a live attenuated varicella vaccine results in VZV seroconversion in VZV seronegative patients awaiting renal transplantation, b) whether VZV-specific IgG levels remain positive after transplantation, c) whether vaccination induces VZV-specific T-cell responses.

MATERIALS AND METHODS

Since May 2003, we performed a prospective study into the serologic response to a VZV vaccine, of adult VZV-seronegative patients with end stage renal failure. Included were pa-

tients in preparation for kidney transplantation, both from living as from deceased donors, with a VZV-specific IgG value below 0.9 arbitrary units (AU) by VIDAS Varicella Zoster IgG test (Bio-Merieux, Marcy l'Etoile, France).[26] Exclusion criteria for vaccination were the use of immunosuppressive drugs and pregnancy. Endpoint was VZV-seroconversion after 2 vaccinations.

Live attenuated VZV vaccine (OKA strain, Varilrix / Provarivax, ≥2700 pfu/ml, 0.5 ml; GlaxoSmithKline Beecham) was used. Vaccination was performed twice with a six weeks' interval. The vaccine was administered by subcutaneous injection in the deltoid region, with a Terumo 0.6 x 25 mm needle.

VZV-specific IgG test values were measured before vaccination, at 6 weeks (when the second vaccination was given) and at 3, 12 and 24 months after first vaccination. A value from 0.9 AU was considered positive.

Retrospectively, we studied whether a renal transplant within the first year resulted in loss of VZV-specific IgG levels at 1 year after vaccination.

Furthermore, retrospective analysis was performed to compare VZV-specific T cell reactivity before vaccination to reactivity after vaccination and transplantation. This analysis was done in 11 patients who gave informed consent to draw blood for additional tests. In their peripheral blood mononuclear cells (PBMCs) we investigated VZV-specific T-cell reactivity by measuring interferon gamma (IFN-γ) production using flow cytometry.

VZV-specific T-cell reactivity

As described before[24], mature monocyte-derived dendritic cells (moDCs) were co-cultured with VZV-infected and mock-infected human melanoma cells in a 6-wells flat bottom plate (Costar, Verviers, Belgium) at 37 °C during 24 hours. The moDCs were used as autologous antigen presenting cells (APCs) in functional T-cell assays.

In brief, autologous $CD3^+$ cells were thawed and re-suspended in medium.[24] The $CD3^+$ cells and autologous VZV-infected or mock-infected moDC were incubated for 24 hours at 37 °C of which the last 6 hours in the presence of 1 µl brefeldin A (Golgiplug, BD Pharmingen, Erembodegem, Belgium). This method mainly results in a VZV-specific memory response. Tube 1 and tube 2 contained $1x10^6$ $CD3^+$ cells and autologous moDCs infected with VZV, and tube 3 and tube 4 contained $1x10^6$ $CD3^+$ cells and $1x10^5$ autologous mock-infected moDCs. Monoclonal antibodies were used to stain the cell surface of the $CD3^+$ cells. The cells from tube 1 and tube 3 were stained with peridinin chlorophyl protein (PerCP) labelled anti-CD4 (Becton Dickinson, Erebodegem, Belgium), allophycocyanin (APC) labelled anti-CD45RO (Becton Dickinson) and phycoerythrin (PE) labelled anti-CCR7 (R&D Systems Europe LtD, Abingdon, UK). The cells from tube 2 and tube 4 were stained with PerCP-labelled anti-CD8 (Becton Dickinson), APC-labelled anti-CD45RO (Becton Dickinson) and PE-labelled anti-CCR7 (R&D Systems). Thereafter, the cells were incubated with fluorescein isothiocyanate (FITC) labelled IFN-γ (Becton Dickinson).

The net percentage VZV-reactive T-cells was determined by enumerating the number of IFN-γ producing $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cells stimulated with autologous moDCs infected with VZV.

Naïve cells are $CD45RO^-$ and memory cells are $CD45RO^+$, central memory cells are $CCR7^+CD45RO^+$, effector memory cells are $CCR7^-CD45RO^+$, and the $CCR7^-CD45RO^-$ are EMRA T-cells.[27]

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.01. The presence and absence of anti-VZV IgG antibodies were compared at different time points after vaccination by Fischer's Exact test. The number of transplant recipients with positive and negative anti-VZV IgG antibody levels at 1 year post vaccination were compared to the number of patients still on the waitlist with positive and negative levels, using Fisher's exact test.

The Wilcoxon signed rank test was used to compare the T-cell reactivity after transplantation with before vaccination.

RESULTS

A total of 52 patients (26 men, 26 women; age: median 50 years, range: 20-73) were enrolled in the study between January, 2003 and July, 2010. Patient characteristics are shown in Table 1 and clinical outcome after vaccination in Table 2.

Humoral response

At 3 months after vaccination, 37 patients had a positive (≥0.9 AU) level of VZV-specific antibodies (Figure 1). From 3 patients, the VZV IgG level was not available at 3 months after vaccination. However, these 3 patients did have positive anti-VZV IgG levels at 1 and/or 2 years after vaccination, without any signs of varicella infection after vaccination. Therefore, these patients were also considered responders. Consequently, seroconversion rate after vaccination was 77% (40 out of 52 patients).

Thirty six patients completed a follow-up time of 1 year after vaccination, 26 responders and 10 non-responders (Table 2). Twenty four of these 36 patients (67%) still had positive anti-VZV IgG levels at that time point (Figure 1), two did not.

Of the 36 patients who had a follow-up time of 1 year after vaccination, 23 received a kidney transplantation within that year. No difference was found in VZV seroconversion within the first year between transplanted and not transplanted patients (Figure 2: 16 of 23 recipients vs. 8 of 13 not transplanted patients had positive VZV-IgG at 1 year, p=0.72).

Table 1. Baseline characteristics

	Responders	Non-responders
Patients	40	12
Male / Female	20/20	6/6
Age at vaccination, years	51 (22-73)	49 (20-66)
Cause of renal failure:		
o Hypertension	7	3
o Diabetes Mellitus	5	3
o Polycystic kidney disease	2	1
o Glomerulonephritis	11	1
o Urologic	4	0
o Other	3[a]	1[b]
o Unknown	9	2
Renal Replacement Therapy: None / HD / PD[c]	14 / 19 / 7	5 / 5 / 2
Patients with nephrotic range proteinuria at vaccination	8 unknown: 4	0 unknown: 3
Place of birth		
o Europe	23	7
o Surinam/Antilles/Cabo Verde	9	4
o Asia	3	1
o Africa	3	-
o South-America	2	-

Age: median (range)

[a]: 1 tuberous sclerosis, 1 AM-amyloidosis due to Behçet's disease, 1 nephrectomy + chemotherapy for Non Hodgkin lymphoma

[b]: AL-amyloidosis

[c]: HD= hemodialysis, PD= peritoneal dialysis

Nephrotic range proteinuria: >3.5 gram/24h

Cellular response

All 11 patients who gave consent to determine VZV-reactive T cells received a kidney transplant after vaccination. VZV-reactive T cells were determined before vaccination and after transplantation (median 7.2 months after transplantation (range: 2.7-15.7)). The number of leukocytes decreased in all 11 patients from median 8.82 x 10E9/l (range: 4.47-14.70) before vaccination to 6.64 x 10E9/l (range 2.20-11.90) after transplantation (p=0.001). The transplant procedure did not affect the percentages of VZV-reactive CD4 and CD8 naive cells (data not shown). The percentage VZV-specific CD4 memory cells did significantly increase after vaccination and transplantation (p=0.04, Figure 3A). The percentage CD8 memory cells did not increase after vaccination and transplantation (Figure 3B). The increment in VZV-reactive CD4 memory cells was due to both central and effector CD4 memory cells (p=0.02 and p=0.05, respectively; Figure 4A and B). No difference was found in the percentage VZV-reactive CD8 central memory, effector memory and EMRA cells (Figure 4C, D, E).

Three patients did not reach positive VZV-IgG levels after vaccination (Figure 3A and 3B, lines A, B, C). Two of these patients had more VZV-reactive memory CD4 and CD8

Table 2. Clinical outcome after vaccination

	Responders	Non-responders
Total number patients	40	12
Kidney transplant recipients	37	10
Years post vaccination	0.7 (0 - 3.7)	0.9 (0.1 - 6.7)
Age at transplantation, years	51 (25-73)	44 (21-67)
Patients with herpes zoster	2	0
Years post vaccination	8.9 - 9.0	-
Years post transplantation	2.5 - 3.4	-
Patients died	5	5
Years post vaccination	2.6 (0.9 – 10.7)	6.0 (0.5 – 10.1)
Years post transplantation	3.2 (2.5 – 7.8)	5.9 (5.5 – 8.5)
Renal function 3 months post vaccination.	40	12
RRT: none / HD / PD	15 / 19 / 6	6 / 5 / 1
Serum creatinine (if no RRT)	364 (95-819)	296 (150-404)
Patients with nephrotic range proteinuria 3 months post vaccination	4 unknown: 6	0 unknown: 3
Kidney transplantation <1year post vaccination	18	5
Tacrolimus trough levels at 1 year post vaccination* (µg/l)	7.5 (5-13)	7 (5-9)

Years: median (range)
NT: not transplanted
RRT: renal replacement therapy. HD= hemodialysis, PD= peritoneal dialysis
Serum creatinine: median (range): µmol/l
Nephrotic range proteinuria: >3.5 gram/24h
*: trough levels of the patients with a kidney transplant at 1 year post vaccination

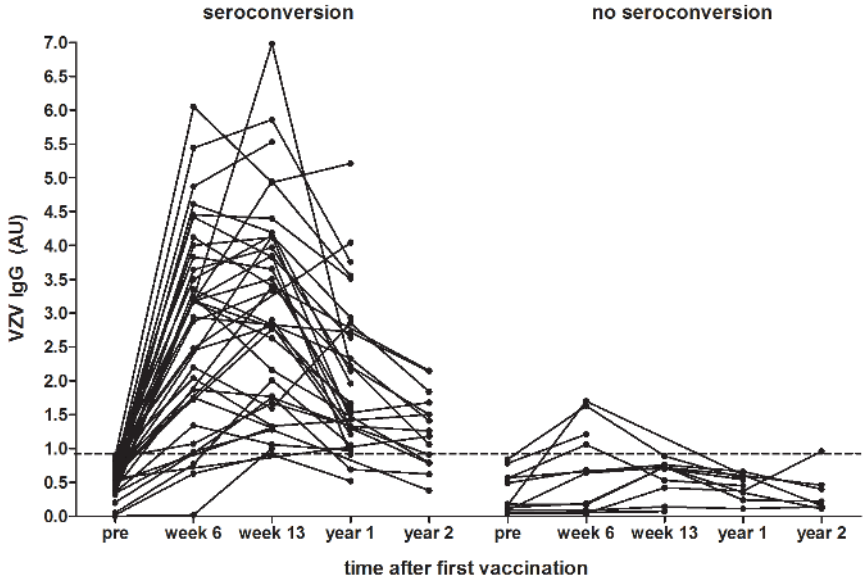
cells after transplantation than before vaccination. Kidney transplantation was performed 1 year post vaccination in patient A and 9 months post vaccination in patient B. VZV-reactive memory cells were measured 7 months post transplantation in patient A and 15 months post transplantation in patient B.

Patient C had less VZV-reactive memory CD4 and CD8 cells after transplantation than before vaccination. Her kidney transplantation was performed 2 months post vaccination and VZV-reactive memory cells were measured 11 months post transplantation. During this period her transplant function was impaired due to acute rejection and urological problems. She received a second vaccination procedure (1.3 years after first vaccination) while awaiting her second transplant. Thereafter, she reached positive VZV IgG levels, persisting after transplantation (data not shown).

Clinical outcome

Thirty seven responders and 10 non-responders received a kidney transplantation after vaccination (Table 2). Four patients died before receiving a kidney transplantation. One responder was considered not transplantable because of morbid obesity (BMI 46).

After kidney transplantation, maintenance immunosuppressive treatment consisted of tacrolimus and mycophenolate mofetil. Tacrolimus trough levels are shown in Table 2.



positive %:	0%	91%	100%	92%	73%	0%	33%	0%	0%	14%
positive patients:	0	32	37	24	11	0	4	0	0	1
total patients:	40	35	37	26	15	12	12	10	10	7

Figure 1. Anti-VZV IgG antibodies in arbitrary units (AU) and percentage patients with positive anti-VZV IgG antibodies after vaccination to prevent varicella. Significantly higher number of patients reached positive anti-VZV IgG antibodies (>0.9 AU) in all periods after vaccination compared to prior to vaccination (week 6, week 13 and year 1: $p<0.0001$, year 2: $p=0.002$)

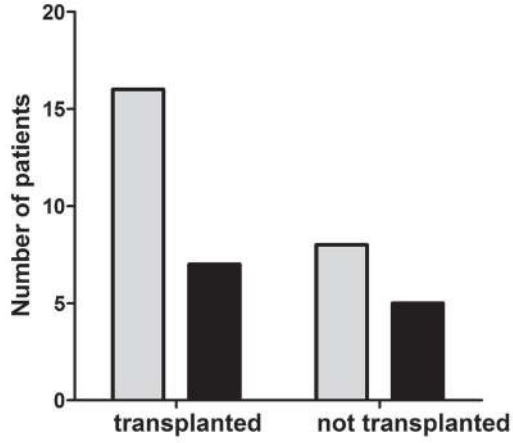


Figure 2. The number of transplanted and not transplanted recipients with positive (gray bar) and negative (black bar) anti-VZV IgG antibodies at 1 year post vaccination ($p=0.72$)

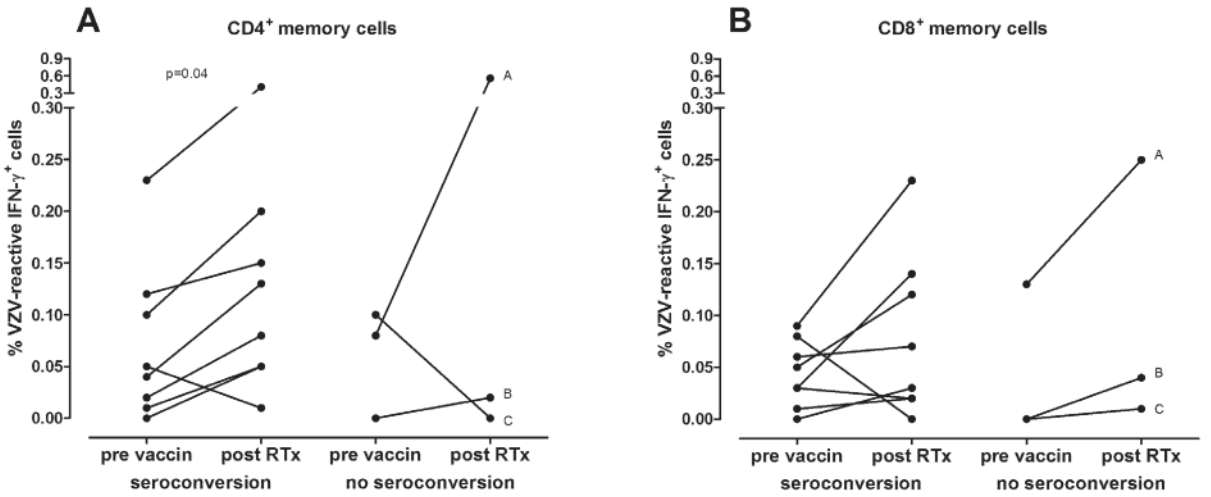


Figure 3. Percentage of VZV-reactive CD4 (A) and CD8 (B) memory cells ($CD45RO^+$) in patients with and without seroconversion to positive anti-VZV antibody levels (>0.9 AU) before vaccination and after vaccination and renal transplantation (RTx)

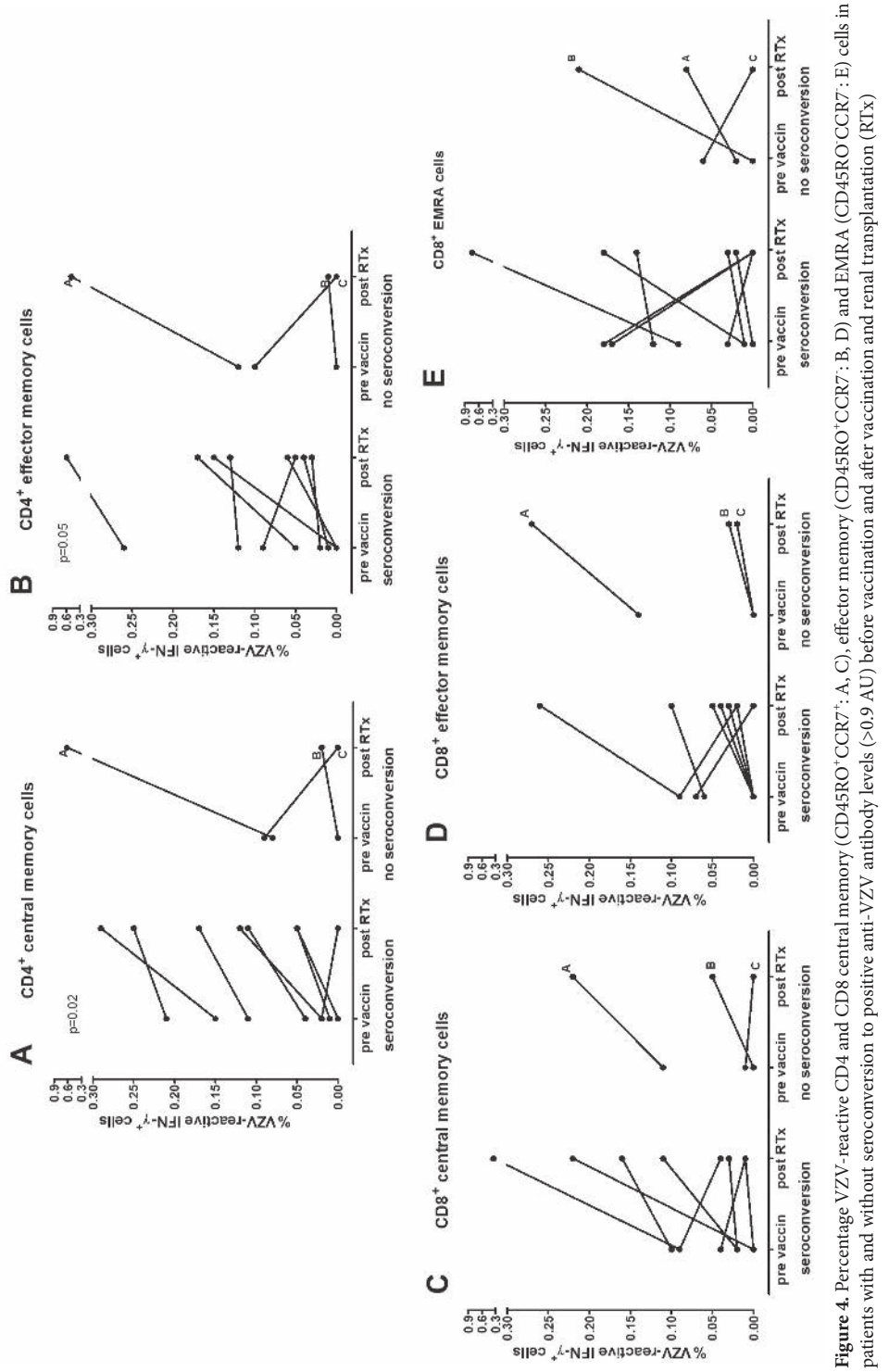


Figure 4. Percentage VZV-reactive CD4 and CD8 central memory ($CD45RO^+CCR7^+$: A, C), effector memory ($CD45RO^+CCR7^-$: B, D) and EMRA ($CD45RO^-CCR7^-$: E) cells in patients with and without seroconversion to positive anti-VZV antibody levels (>0.9 AU) before vaccination and after vaccination and renal transplantation (RTx)

Prednisolone was tapered from 20 mg daily during the first 3 months and discontinued thereafter. One patient was erroneously vaccinated while using immunosuppressive drugs because of an earlier renal transplant. He responded very well and did not suffer from varicella after vaccination.

No severe vaccine-related adverse events were reported, only spontaneously resolving pain at the injection site in one patient.

Two patients had an episode of herpes zoster. Both had shown seroconversion at 3 months after vaccination. One patient had vesicles on her head, 9 years after vaccination and 8.8 years after transplantation. She was treated with topical aciclovir and oral amitriptyline as anaesthetic. The other patient had vesicles on the left side of her chest, 3.5 years after vaccination and 2.5 years after transplantation. She received oral valaciclovir, 1000 mg thrice daily for 7 days. Both patients recovered without complications.

One patient developed a mild varicella without complications, 18 days after the first vaccination dose. She had a positive VZV-IgG level when the signs of varicella were noticed. Because we did not know whether her seroconversion was due to varicella infection or vaccination, she was considered a non-responder. She did not receive the second vaccination dose. She recovered one week later without anti-viral treatment. She did not use immunosuppressive medication (16 years after bone marrow transplantation), but was on hemodialysis since 7 months at the time of vaccination. Her leukocyte count, serum protein and albumin levels were within the normal range and she had no proteinuria.

None of the 10 non-responders who received a kidney transplant developed varicella infection.

DISCUSSION

The 77% seroconversion after varicella vaccination is higher than seroconversion rates reported after hepatitis B[14,15] and influenza[17-20] in patients with chronic renal failure. As described by Hoft in young children[28], we think it is possible that the presence of whole virus pathogen in this varicella vaccine, induces a more comprehensive immune response in patients with chronic renal failure than a peptide vaccine. Recently, a peptide vaccine was developed for prevention of herpes zoster.[29] Further research to compare its efficacy with live attenuated vaccine would be interesting.

To our knowledge, only one study was published on VZV vaccination in a small cohort of adult patients with chronic renal failure. Crespo et al.[13] found a VZV IgG antibody response percentage of 94% in 17 patients, of whom only 4 required a second dose. However, the follow-up of the study was only 4 weeks.

In the present study, determination of seroconversion was done at 13 weeks after first vaccination. Moreover, we compared patients who received a kidney transplant within 1 year after vaccination with those who did not.

We found that the percentage of patients who showed an increase in VZV-specific $CD4^+$ memory T-cells was higher (9/11 patients: 82%) than the percentage of patients with positive VZV-IgG levels at one year (24/36 patients: 67%), despite the use of immunosuppressive therapy after kidney transplantation and a significant decrease in leucocyte number. Unfortunately, from most of our patients, we did not have VZV-IgG levels of longer time prior to our study. However, CMV and/or EBV IgG levels were positive before vaccination. No history of herpes zoster or varicella was found in their medical charts. It is possible that some patients did experience a VZV infection in the past. This infection had evoked a VZV-specific memory cell response, which was boostered by the vaccination. However, their end stage renal failure could have impaired an adequate rise in VZV IgG antibodies. An example is patient A (Figures 3 and 4), who had transient detectable VZV-IgG levels in his youth, before he reached end stage renal failure. After vaccination he did not mount positive VZV-IgG levels, but showed a high response in VZV-specific CD4+ memory cells. The level of virus specific memory T-cells is possibly more representative of immunity against a virus than IgG antibodies. This could explain that even non-responders did not suffer a primary VZV infection after transplantation. Because of the small numbers of patients, the correlation between VZV-specific T-cell reactivity and vaccine response requires further investigation.

Two of the responders experienced a herpes zoster episode (9 and 3.5 years after vaccination, and respectively 8.5 and 2.5 years after transplantation). There are two possible explanations. The first is that they were not truly VZV-naïve, but had lost their VZV-specific IgG levels due to their end-stage renal disease. Then, they had a reactivation of the earlier contracted wild-type varicella virus.[30] The alternative explanation could be that the vaccine-type varicella virus established latency in these patients and was reactivated, causing herpes zoster. Herpes zoster may be caused by the vaccine-type varicella.[31] It was demonstrated in varicella vaccinated children, that half of the herpes zoster infections were due to wild-type VZV.[32] As other investigators also reported[31,33], the herpes zoster episodes after vaccination were mild and without complications. In contrast, of the VZV-seropositive (not vaccinated) recipients of a first kidney transplant in our centre, 9% (14.4 cases/1000 PY), experienced a herpes zoster episode. Twenty four percent of these patients had a complicated herpes zoster infection.[34]

In summary, prophylactic vaccination before kidney transplantation induces a T and B cell mediated response and therefore may prevent varicella after transplantation. Our study confirms that a two-dose vaccination regime with live attenuated virus vaccine is safe and effective in adult patients with chronic renal failure, and results in anti-VZV IgG seroconversion and/or VZV-specific T-cell memory in at least 77% of the vaccinees.

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3 Herpes Zoster in solid organ transplantation: incidence and risk factors

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ABSTRACT

Background: Studies on herpes zoster (HZ) incidence in solid organ transplant (SOT) recipients report widely varying numbers. We investigated HZ incidence, severity and risk factors in recipients of 4 different SOT, with a follow-up time of 6-14 years.

Methods: Records of 1033 transplant recipients after first heart (HTx: n=211), lung (LuTx: n=121), liver (LiTx: n=258) and kidney (KTx: n=443) transplantation between 2000 and 2014 were analysed for VZV-PCR, clinical signs of HZ and complications.

Results: HZ was diagnosed in 108 of 1033 patients (10.5%): 36 HTx, 17 LuTx, 15 LiTx and 40 KTx recipients. Overall HZ incidence rate after HTx (30.7 cases/1000 person-years (PY)), LuTx (38.8 cases/1000 PY), LiTx (22.7 cases/1000 PY) and KTx (14.5 cases/1000 PY) was significantly higher than in the general 50-70 year population. Multivariable analysis demonstrated age ≥50 years at transplantation (p=0.038, RR 1.536), type of organ transplant (overall p=0.002; LuTx p=0.393; RR 1.314; LiTx p=0.011, RR 0.444; KTx p=0.034, RR 0.575), CMV prophylaxis (p=0.043, RR 0.631) and type of anti-rejection therapy (overall p=0.020; methylprednisolone p=0.008, RR 0.475; r-ATG p=0.64, RR1.194) as significant risk factors. Complications occurred in 33 of 108 (31%) patients (39% of HTx, 47% of LuTx, 20% of LiTx, 20% of KTx): post-herpetic neuralgia, disseminated disease and cranial nerve involvement.

Conclusion: HZ incidence and severity in SOT recipients is most pronounced after heart and lung transplantation, in older patients and when CMV prophylaxis is lacking.

INTRODUCTION

Herpes zoster (shingles, HZ) is caused by reactivation of the varicella zoster virus (VZV). After primary infection, the virus establishes lifelong latency in dorsal root neural ganglia.[1] Virus reactivation occurs when the immune system is suppressed. Increased HZ incidence, attributed to a decline in immunity, is observed in elderly people and in patients using immunosuppressive medication.[2-10] The latter certainly applies to solid organ transplant recipients.

Both HZ incidence and HZ related complications occur more frequently and with higher severity in solid organ transplant recipients compared to the general population.[11] Severe complications are dissemination in more than three dermatomas, involvement of cranial nerves or internal organs and post-herpetic neuralgia (PHN). PHN may lead to considerable morbidity and loss of quality of life.[12] Due to use of different definitions of PHN, the reported incidence ranges from 10% to 50% of herpes zoster cases.[13] In the Netherlands, overall annual HZ incidence is 3.2 cases per 1000 person-years (PY), comparable to other West-European countries.[3] HZ incidence increases with age up to 10 cases/1000 PY^3 in people older than 80 years. In solid organ recipients however, HZ incidence has been reported to be 2-5 fold higher than in the general 80 years old population.[14-18]

Studies on HZ incidence and complications in multiple solid organ transplant (SOT) recipients report widely varying numbers. In North America and Asia HZ incidence ranges from 18/1000 PY in liver to 55/1000 PY in lung transplant recipients.[14-21] Whereas in Europe, kidney transplant studies show crude incidences of 1-8%[22,23] and overall incidences of 20/1000 PY[24] and one study of multiple SOT recipients reports an incidence of 12/1000 PY.[25] Therefore, we assessed crude and overall incidence and complications of HZ after heart, lung, liver and kidney transplantation in our centre by retrospective analysis of the medical files of adult recipients. Furthermore, we performed a detailed analysis of risk factors for developing HZ.

PATIENTS AND METHODS

Medical records of adult heart (HTx), lung (LuTx), liver (LiTx) and kidney (KTx) transplant recipients in our transplant centre between 2000 and 2014 were reviewed. Permission to extract data from hospital (pharmacy) records was granted by the local medical ethical commission: MEC-2018-1574. Because not all KTx recipients visit our outpatient clinic in case of infection, we performed an inquiry by letter and phone calls. Patients who died or lost their graft within one month after a first organ transplantation were excluded from our analysis. KTx recipients whose medical records were incomplete, mostly due to referral to another hospital, and who did not respond to our inquiries, were excluded (Figure 1).

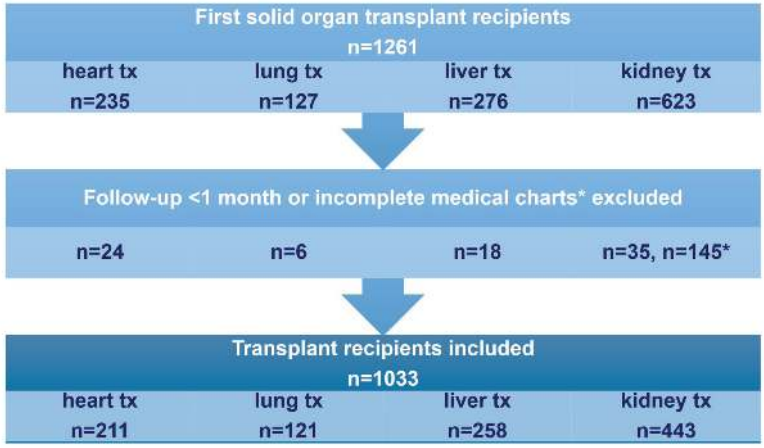

Figure 1. Adult recipients of a first solid organ transplant with a minimum follow-up of 1 month and complete medical charts were included in the analysis.
tx= transplantation

Demographic and clinical information were extracted from the medical records and included the following information: transplantation date, gender, date of birth, date of death or graft failure (if applicable), duration of follow-up, induction therapy, maintenance immunosuppressive regimen, use of methylprednisolone or rabbit Anti-Thymocyte Globulin (r-ATG) as anti-rejection therapy, cytomegalovirus (CMV) prophylaxis regimen, serum CMV-PCR results post-transplantation, patient CMV serologic status pre-transplantation, donor CMV serologic status, patient VZV serologic status (IgG positive or negative) pre-transplantation, first episode of HZ, location and number of dermatomas affected by HZ, internal organ and cranial nerve involvement, VZV-PCR results, therapy used to treat HZ and occurrence of PHN. Primary varicella zoster infections were not included in our analysis.

Localised HZ was defined as presentation of vesicles in 1 or 2 adjacent dermatomas, whereas involvement of 3 or more, or 2 not adjacent dermatomas was considered as disseminated HZ.[11] Cranial nerve involvement was scored separately. Post-herpetic neuralgia (PHN) was defined as pain in the affected dermatomas, persisting at least 3 months after onset of the skin lesions and requiring treatment with opioid analgesics, tricyclic antidepressants, gabapentin or pregabalin.[26] Infections were mostly confirmed by VZV-PCR on blood and/or blister samples, however in obvious cases HZ was diagnosed on clinical presentation only.

Statistical analysis:

Analyses were performed in SPSS, version 25, 2017. HZ incidence was expressed as percentage of the total number of patients (crude incidence) and as cases per 1000 person

years (overall incidence rate). Age at transplantation in all patients with and without HZ was compared by one-way ANOVA. Age at transplantation in patients with positive and negative VZV IgG before transplantation was also compared by one-way ANOVA. Correlation between time to HZ onset and age at transplantation was analysed with Spearman's correlation test. Univariable and multivariable Cox proportional hazards analysis using backward elimination was used to analyse the effect of multiple variables on HZ incidence. Univariable Cox proportional hazards analysis was used to analyse the effect of the type of organ transplant on complicated HZ incidence. Cases with missing values were excluded from Cox proportional hazards analyses.

RESULTS

Patient characteristics

In total, 1261 patients received a first transplant, of which 235 HTx (Tx period January 2000 - July 2014), 127 LuTx (Tx period April 2002 - March 2014), 276 LTx (Tx period January 2008 – July 2014) and 623 KTx (Tx period January 2003 - January 2009). Of the 588 KTx patients with a follow-up of more than 1 month, 145 had incomplete medical charts, due to referral to another hospital and/or no response to our inquiry, resulting in 443 patients in the KTx group and a total of 1033 patients (Figure 1). Maximum follow-up time was 14 years in HTx, 12 years in LuTx, 6 years in LiTx and 10 years in KTx recipients. Mean follow-up time was 5.5 years in HTx, 3.6 years in LuTx, 2.6 years in LiTx and 6.0 years in KTx. During follow-up 26 (12%) HTx, 21 (17%) LuTx, 29 (11%) LiTx and 14 (3%) KTx recipients died and 12 (5%) LiTx and 72 (16%) KTx recipients lost their graft.

The characteristics of the organ transplant recipients are shown in Table 1. In the LuTx group about 54% was male, whereas in the other groups the percentage of males varied between 62% and 68%. The standard immunosuppressive medication regimens at time of transplantation in each group are shown in Table 1. There are some differences in immunosuppressive medication between the transplant groups: all HTx recipients received r-ATG as induction therapy. After LiTx, patients received tacrolimus monotherapy as maintenance immunosuppressive therapy from 6 months post-transplantation. HTx and LuTx recipients received an increased dose of maintenance immunosuppression as compared to LiTx and KTx recipients (triple therapy including low dose prednisolone and higher tacrolimus target concentrations).

In general, valganciclovir was used as CMV prophylaxis. In the LuTx and KTx groups, all except CMV donor negative / recipient negative combinations received prophylaxis. In the HTx and LiTx groups only CMV donor positive / recipient negative combinations received prophylaxis. In the HTx group up to 2003, prophylactic anti-CMV immunoglobulin was given in the first 6 weeks after transplantation. Since 2003, valganciclovir was given during

Table 1. Transplant recipients' characteristics

Organ transplant	Heart	Lung	Liver	Kidney	Overall
Recipients in analysis	211	121	258	443	1033
Gender (M / F)	143 / 68	65 / 56	169 / 89	274 / 169	649 / 384
Median age at Tx (range)	51 (18-72)	54 (19-66)	53 (18-69)	51 (18-77)	52 (18-77)
Age ≥ 50 y at Tx (%)	54%	66%	58%	53%	56%
Pre-Tx VZV-IgG pos / neg / unknown	194 / 11 / 6	108 / 5 / 8	239 / 7 / 12	419 / 15 / 9	960 / 38 / 35
Induction therapy	r-ATG 211 (100%)	Basiliximab 121 (100%)	Basiliximab 258 (100%)	2006-2008: rATG in DCD: 44 (10%) Rituximab in ABO-I: 18 (4%) No induction: 381 (86%)	
Standard maintenance immunosuppression > 6 months	Tacrolimus + Mycophenolate Mofetil + Prednisolone	Tacrolimus + Mycophenolate Mofetil + Prednisolone	Tacrolimus	Tacrolimus + Mycophenolate Mofetil	
Tacrolimus target trough levels (ug/l) > 6 months	< 12 mo: 9 - 15 > 12 mo: 5 - 9	< 7 mo: 10 – 15 > 7 mo: 7 - 10	5 - 8	5 - 8	
r-ATG anti-rejection therapy (pts)	15 (7%)	0	1 (0.4%)	32 (7%)	48 (5%)
Methylprednisolone anti-rejection therapy (pts)	72 (34%)	42 (35%)	30 (12%)	102 (23%)	246 (24%)
CMV serostatus pre-transplant					
D- / R-	36 (17%)	30 (25%)	44 (17%)	96 (22%)	206 (20%)
D- / R+	71 (34%)	37 (31%)	80 (31%)	102 (23%)	290 (28%)
D+ / R-	59 (28%)	23 (19%)	45 (17%)	82 (19%)	209 (20%)
D+ / R+	45 (21%)	30 (25%)	89 (35%)	154 (35%)	318 (31%)
unknown	0	1 (1%)	0	9 (2%)	10 (1%)
CMV prophylaxis					
Yes	59[a] (28%)	91 (75%)	45 (17%)	340 (77%)	535 (52%)
No	152 (72%)	30 (25%)	213 (83%)	99 (22%)	494 (48%)
unknown	0	0	0	4 (1%)	4 (1%)

Tx= transplantation. M= male. F= female. D= donor. R= recipient. pts= patients. VZV-IgG= Varicella Zoster Virus immunoglobulin G. r-ATG= rabbit anti-thymocyte globulin. Basiliximab= InterLeukin2-receptor blocker. Rituximab= anti-CD20 antibody. DCD=donation after cardiac death. [a]: 5 patients received Immunoglobulin CMV prophylaxis.

the first 6 months after transplantation. In the LuTx group, duration of CMV prophylaxis was extended during the study period: up to 2012 valganciclovir was given during the first 3 months, since 2012 prophylaxis was extended to 6 months after transplantation. In the KTx and LiTx groups, valganciclovir was given during the first 3 months.

Most of the methylprednisolone treated rejections occurred early after transplantation, during the standard CMV prophylaxis period: 59/72 (82%) in HTx, 25/42 (60%) in LuTx, 14/30 (47%) in LiTx, 65/102 (64%) in KTx. CMV prophylaxis was not extended after methylprednisolone anti-rejection treatment.

VZV-seroprevalence

In the Netherlands, no routine VZV vaccination programme exists, neither primary vaccination for the general population nor booster vaccination for senior adults. VZV is endemic in the Dutch population, seroprevalence of VZV antibodies amounts to 95%.[27] Of our transplant candidates 3.7% (38/1033) was VZV sero-negative prior to transplantation (Table 1).

Age at transplantation was significantly higher in patients who were VZV IgG seropositive compared to seronegative before transplantation (50.4 [17.5-77.8] vs. 45.7 [19.7-75.2], p=0.024).

Two KTx patients, one LuTx patient and two HTx patients suffered from primary VZV infection, 1.2 – 8 years after transplantation. Although some had severe complications, none of them died.

Pre-transplantation vaccination of VZV seronegative patients was introduced only in the kidney transplant group (2 doses of Provarivax (Merck Sharp & Dohme B.V., Haarlem.)[28] Four of 15 VZV-seronegative kidney transplant candidates were vaccinated before transplantation with Provarivax. One of these vaccinated transplant recipients developed HZ during follow-up, at 2.7 years post-transplantation. This patient had non-complicated HZ and recovered without sequelae.

Booster vaccination of seropositive patients was not performed in any of the groups.

Incidence and severity of herpes zoster

To compare the incidence of HZ in our centre with the incidence reported by other authors, we analysed both crude and overall incidences. Only the first HZ episode after transplantation was analysed. Primary VZV infections were not included in the analysis.

The crude HZ incidence was 36/211 (17.1%) in heart, 17/121 (14.0%) in lung, 15/258 (5.8%) in liver and 40/443 (9.2%) in kidney transplant recipients (Table 2).

The overall HZ incidence is shown in Table 2 as the number of HZ cases per 1000 person years (PY), meaning the years at risk of HZ after transplantation. The HZ incidence was significantly higher after HTx (30.7 cases/1000 PY) compared to after LiTx (22.7 cases/1000 PY) and after KTx (14.5 cases/1000 PY) (Cox proportional hazards, p<0.001 in LiTx vs.

Table 2. Transplant recipients with herpes zoster

Organ transplant	Heart	Lung	Liver	Kidney	Overall
Herpes Zoster Cases / recipients	36/211 (17.1%)	17/121 (14.0%)	15/258 (5.8%)	40/443 (9.2%)	108/1033 (10.5%)
Herpes Zoster Cases / 1000 PY	30.7	38.8	22.7	14.5	22.1
Gender (M / F)	27 / 9	9 / 8	11 / 4	25 / 15	72 / 36
Median age at Tx (range)	54 (22-67)	60 (35-67)	52 (22-60)	53 (28-77)	53 (21-72)
HZ onset post-Tx Median years (range)	2.0 (0.04-10.8)	1.4 (0.08-3.8)	0.5 (0.3-4.5)	1.8 (0.04-8.9)	1.2 (0.04-10.8)
Initial HZ treatment					
Oral	32 (89%)	9 (53%)	12 (80%)	37 (92%)	90 (83%)
Intravenous	4 (11%)	7 (41%)	3 (20%)	3 (8%)	17 (16%)
No treatment	0	1 (6%)	0	0	1 (1%)
CMV-IgG pre-Tx					
D- / R-	5 (14%)	5 (29%)	3 (20%)	10 (25%)	23 (21%)
D- / R+	19 (53%)	7 (41%)	5 (33%)	7 (18%)	38 (35%)
D+ / R-	7 (19%)	3 (18%)	1 (7%)	6 (15%)	17 (16%)
D+ / R+	5 (14%)	2 (12%)	6 (40%)	16 (40%)	29 (27%)
unknown	0	0	0	1 (3%)	1 (1%)
CMV prophylaxis					
Yes	7 (19%)	12 (71%)	1 (7%)	28 (70%)	48 (44%)
No	29 (81%)	5 (8%)	14 (23%)	11 (28%)	59 (54%)
unknown	0	0	0	1 (3%)	1 (1%)
Induction therapy	r-ATG 36 (100%)	Basiliximab 17 (100%)	Basiliximab 15 (100%)	2006-2008: rATG in DCD: 2 (5%) Rituximab in ABO-I: 3 (7.5%) No induction: 35 (87.5%)	r-ATG 38 (35%) Basiliximab 32 (30%) Rituximab 3 (3%) No 35 (32%)
prior r-ATG anti-rejection therapy	3 (8%)[a]	0	0	5 (13%)[a]	8 (7%)
prior methylprednisolone anti-rejection therapy	3 (8%)	5 (29%)[b]	3 (20%)[a]	5 (13%)	16 (15%)

Tx= transplantation. VZV-IgG= Varicella Zoster Virus immunoglobulin G. PY= person years. M= male. F= female. D= donor. R= recipient. rATG= rabbit anti-thymoglobulin. [a]: 1 complicated HZ case. [b]: 3 complicated HZ cases

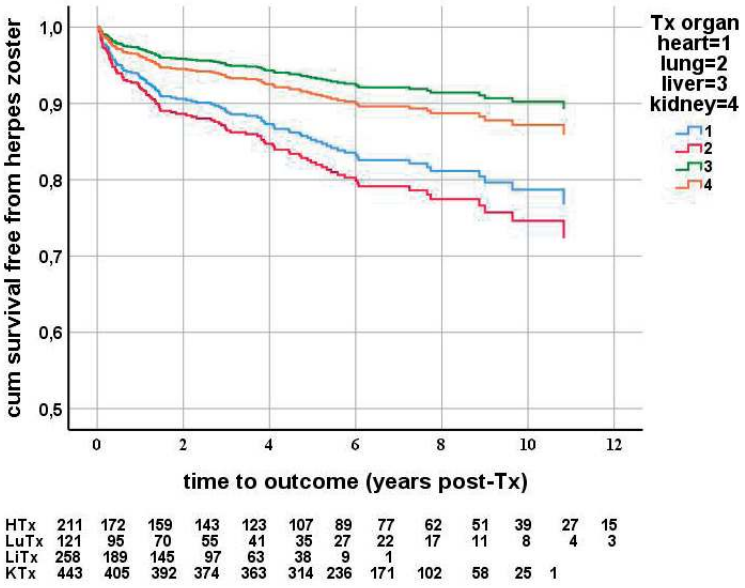

Figure 2. Herpes zoster free survival after solid organ transplantation, censored for death and graft failure. Cox proportional hazards, multivariable analysis.
First episodes of herpes zoster were counted as event. Herpes zoster incidence is significantly higher in heart recipients compared to liver (p=0.011) and kidney (p=0.034) transplant recipients. For each organ type, the number of patients at risk at each year after transplantation is described below the graph.

HTx, , p=0.003 in KTx vs. HTx). HZ incidence after LuTx (38.8 cases/1000 PY) was comparable to after HTx (Cox proportional hazards, p=0.907) (Table 2 and 4, Figure 2).

The median time to the onset of HZ was 2.0 years after HTx, 1.4 years after LuTx, 0.5 years after LiTx and 1.8 years after KTx (Table 2).

More than 80% of HZ episodes were treated with oral valacyclovir (Table 2). LuTx recipients more often received intravenous acyclovir treatment compared to recipients of other organs.

Complicated HZ incidence did not significantly differ between the 4 organ transplant groups (Cox proportional hazards, p=0.156) (Table 3). The incidences of PHN, disseminated HZ and cranial nerve involvement are shown in Table 3. One LuTx patient and two KTx recipients died due to disseminated HZ with secondary bacterial infection and encephalitis. Of the patients who had HZ after treatment for acute rejection 6 had complicated HZ. Four patients (3 LuTx, 1 LiTx) had received methylprednisolone, one HTx and one KTx patient received r-ATG (Table 2).

Table 3. Herpes zoster complications

Organ transplant	Heart	Lung	Liver	Kidney	Overall
Complicated / Total	14/36 (39%)	8 /17 (47%)	3/15 (20%)	8/40 (20%)	33/108 (31%)
RR (95% CI) p-value	Reference	2.147 (0.831-5.550) 0.115	1.166 (0.314-4.326) 0.818	0.671 (0.269-1.670) 0.391	0.156
Post Herpetic Neuralgia	7 (19%)	3 (18%)	1 (7%)	0	11 (33%)
Disseminated disease	2 (6%)	5 (29%)	2 (13%)	7 (18%)	16 (48%)
Cranial nerve involvement	5 (14%)	1 (6%)	0	1 (3%)	7 (21%)
Deceased due to complicated HZ	0	1 (6%)	0	2 (5%)	3 (9%)

Risk factors for herpes zoster

We analysed risk factors for development of HZ. Age at transplantation was not different between patients who did or did not develop HZ, neither in the entire group (51.7 [21.1-72.8] vs. 49.9 [17.5-77.8], p=0.17), nor in the HTx, LuTx, LiTx and KTx groups separately.

No correlation was found between the time to the onset of HZ after transplantation and age at transplantation (r_s = 0.009, p=0.93).

As HZ incidence significantly increases in the general population above 50 years (3), we added age categories ≥50 years and ≥60 years at transplantation as dichotomous variables. We studied the effect of the following variables on the risk of developing HZ: gender, age at transplantation, age ≥50 years at transplantation, age ≥60 years at transplantation, type of organ transplant, use of methylprednisolone or r-ATG anti-rejection therapy, induction therapy agent (no induction, basiliximab, rATG or rituximab), use of CMV prophylaxis (in all patients and in CMV seropositive recipients only), duration of CMV prophylaxis (none, valganciclovir 3 months and ≥6 months), occurrence of CMV viremia (serum CMV-PCR >1000 IU/ml) and pre-transplant VZV-IgG (positive of negative) (Tables 4 and 5).

In univariable Cox regression analysis, age ≥50 years at transplantation, type of organ transplant, use of CMV prophylaxis, duration of CMV prophylaxis, use of induction therapy, type of induction therapy and type of anti-rejection therapy significantly influenced the risk of developing HZ (Table 4).

In multivariable Cox regression analysis, successively including all above mentioned variables, age ≥50 years at transplantation, type of organ transplant, use of CMV prophylaxis and type of anti-rejection therapy were the variables significantly influencing the risk of developing HZ (Table 5). Patients ≥50 years of age had a significantly increased risk to develop HZ compared to younger patients (p=0.038, RR=1.536, CI=1.023-2.304). Compared to HTx (reference variable) the risk of HZ after LuTx was not different. The risk to develop HZ after LiTx (p=0.011, RR=0.444, CI=0.237-0.833) and after KTx (p=0.034, RR=0.575, CI=0.345-0.959) was significantly lower than after HTx (Figure 2, Table 5). Use of CMV prophylaxis significantly diminished HZ risk (p=0.043, RR=0.631, CI=0.404-0.986). The risk to develop HZ was significantly lower in patients who were treated for acute rejection with methylprednisolone, compared to those without acute rejection treatment (p=0.008, RR=0.475, CI=0.275-0.821). In the multivariable Cox regression model, no interaction was found between type of organ transplant and either age ≥50 years, use of CMV prophylaxis or type of anti-rejection therapy.

In addition, we performed a Cox regression analysis of the effect of above mentioned variables on the risk of developing HZ in all organ transplant subgroups. In the LuTx and LiTx groups, none of the variables significantly influenced the incidence of HZ. In univariable analysis in the HTx group (n=211 with 36 HZ cases) we found 2 significant risk factors for development of HZ: any anti-rejection therapy (p=0.002, RR 0.253 (0.105-0.610) and type of anti-rejection therapy (overall p=0.009; methylprednisolone p=0.003, RR 0.165

(0.050-0.542); rATG p=0.311, RR 0.541 (0.164-1.778)). In univariable analysis in CMV seropositive KTx recipients (n=256, 23 HZ cases), use of CMV prophylaxis significantly reduced the incidence of HZ (p=0.0001, RR=0.109, CI=0.032-0.371).

Table 4. Risk factors for herpes zoster, univariable analysis

Variable (reference)	Cox proportional hazards Univariable analysis	
	RR (95% CI)	**p-value**
Gender (male)	0.834 (0.559-1.245)	0.374
Age (continuous)	1.012 (0.997-1.028)	0.125
Age ≥50 years	1.672 (1.120-2.495)	0.012
Age ≥60 years	1.355 (0.889-2.066)	0.158
Organ transplant (Heart)		0.003
Lung	1.035 (0.579-1.850)	0.907
Liver	0.300 (0.159-0.565)	<0.001
Kidney	0.482 (0.297-0.783)	0.003
VZV IgG pre-transplant (negative)	2.370 (0.585-9.611)	0.227
Valganciclovir CMV prophylaxis (no)	0.629 (0.430-0.922)	0.017
CMV prophylaxis (no)		0.041
Valganciclovir 3 months	0.603 (0.404-0.900)	0.013
Valganciclovir >6 months	0.669 (0.289-1.547)	0.347
CMV prophylaxis (no)		0.086
Valganciclovir 3 months	0.603 (0.405-0.900)	0.013
Valganciclovir 6 months	0.752 (0.302-1.872)	0.540
Valganciclovir 9 months	0.430 (0.059-3.115)	0.403
Valganciclovir prophylaxis CMV R+ (no)[a]	0.550 (0.337-0.898)	0.017
CMV-PCR >1000 IU/ml	1.012 (0.627-1.634)	0.960
Induction therapy (no)	1.676 (1.114-2.522)	0.013
Induction therapy (no)		0.035
Basiliximab or Rituximab	1.537 (0.952-2.482)	0.078
rATG	1.818 (1.144-2.887)	0.011
Induction therapy (no)		0.059
Basiliximab	1.484 (0.909-2.423)	0.114
rATG	1.817 (1.144-2.886)	0.011
Rituximab	2.432 (0.746-7.928)	0.140
Anti-rejection therapy (no)		0.042
Methylprednisolone	0.531 (0.311-0.906)	0.020
r-ATG	1.301 (0.629-2.691)	0.477
r-ATG anti-rejection therapy (no)	1.481 (0.720-3.050)	0.286

VZV IgG: varicella zoster virus specific immunoglobulin G. CMV: cytomegalovirus. r-ATG: rabbit anti-thymocyte globulin. CMV R+: CMV seropositive recipient

[a]: CMV seropositive recipients without Valganciclovir prophylaxis were compared to CMV seropositive recipients with Valganciclovir prophylaxis

Table 5. Risk factors for herpes zoster, multivariable analysis

Variable (reference)	Cox proportional hazards Multivariable analysis	
	RR (95% CI)	p-value
Age (≥50 years)	1.536 (1.023-2.304)	0.038
Organ transplant (Heart)		0.002
Lung	1.314 (0.703-2.455)	0.393
Liver	0.444 (0.372-0.833)	0.011
Kidney	0.575 (0.345-0.959)	0.034
CMV prophylaxis (no)	0.631 (0.404-0.986)	0.043
Anti-rejection therapy (no)		0.020
Methylprednisolone	0.475 (0.275-0.821)	0.008
r-ATG	1.194 (0.566-2.518)	0.641

CMV: cytomegalovirus. r-ATG: rabbit anti-thymocyte globulin

DISCUSSION

Our study is one of the largest European studies that reports the incidence and severity of HZ in recipients of four solid organ transplants with a maximum follow-up time of 14 years in HTx, 12 years in LuTx, 6 years in LiTx and 10 years in KTx recipients. In addition, risk factors for the development of HZ were analysed.

The crude HZ incidence was 17.1% in heart, 14.0% in lung, 5.8% in liver and 9.2% in kidney transplant recipients. The overall HZ incidence in HTx (30.7 cases/1000 PY) and LuTx (38.8 cases/1000 PY) recipients was significantly higher compared to LiTx (22.7 cases/1000 PY) and KTx (14.5 cases/1000 PY) recipients.

Overall and crude HZ incidence rates in our KTx group are lower than the reports of KTx recipients from Canada and the USA, but higher than those from other European countries.[14-16,20,22-25] HZ incidence in our LiTx group is higher than in previous studies.[15,19]). These differences may be explained by a higher percentage of patients in North-America using T-cell depleting induction therapy and a more intense maintenance immunosuppressive regimen. In addition, in other European and Asian countries duration of CMV prophylaxis in KTx and LiTx patients is longer compared to our centre. In our HTx and LuTx groups HZ incidence is lower than in other studies[15,17,18,21], which might be due to a longer duration of CMV prophylaxis in our transplant recipients and lower tacrolimus target trough levels after 1 year post-transplantation.

HTx, LuTx and LiTx patients with infectious problems generally visit the outpatient transplant clinic. However, KTx recipients may visit their general practitioner and may go unnoticed. Therefore, KTx recipients were also contacted by letter and by telephone. This resulted in a more reliable incidence and a more complete picture of HZ complications (e.g. PHN) compared to earlier studies that lacked this approach.

In addition to crude and overall incidences of HZ, our study also focussed on severity of HZ. Post-herpetic neuralgia (PHN) and other complications of HZ are not uniformly described in the literature. In our study, PHN, dissemination and cranial nerve involvement were more often reported in HTx and LuTx compared to KTx recipients. PHN incidence, as indicated in Table 3, is lower than in other reports. This is probably due to our more strict definition of PHN: >3 months PHN plus requirement of either opioid analgesics, tricyclic antidepressants, gabapentin or pregabalin. The incidence of disseminated HZ was higher compared to other reports.[14-19,23] However, definition of dissemination was not specified in these reports.

Furthermore, we analysed potential risk factors for HZ. In multivariable analysis, type of organ transplant, age ≥50 years at transplantation, duration of CMV prophylaxis and type of anti-rejection therapy significantly influenced the risk of developing HZ (Table 5). As expected, the risk of HZ is higher in HTx and LuTx recipients, who are exposed to higher levels of immunosuppressive maintenance therapy. However, HZ incidence after KTx or LiTx is still significantly higher compared to healthy individuals (age <40 years: 2 cases/1000 PY, age 40-50 years: 1-4/1000 PY, age 50-70: 7-8/1000 PY and age >80 years: 10/1000 PY).[3] VZV and CMV are both herpes viruses, therefore CMV prophylaxis strategies might influence the incidence of HZ. Ko et al. found a lower HZ incidence per 1000 PY in KTx patients who received >3 months prophylaxis compared to patients who received pre-emptive therapy.[20] However, Fernandez-Ruiz et al. and Martin-Gandul et al. did not show a significant effect of CMV prophylaxis on HZ incidence compared to pre-emptive therapy.[23,25] In our study, patients using CMV prophylaxis did have a significantly lower risk of HZ. We did not find a significant effect of longer use of CMV prophylaxis on HZ incidence, but the number of patients receiving at least 6 months of prophylaxis (84 patients) may be too low to find significant effects. In our centre, herpes simplex prophylaxis with acyclovir is not used.

In other studies, more intensive immunosuppressive therapy was frequently reported as risk factor for HZ: anti-rejection treatment[21,24], induction therapy (mostly anti-thymocyte globulin)[16] and use of mycophenolate mofetil.[17,19] Surprisingly, we found that in patients treated for acute rejection with methylprednisolone, the risk of HZ was lower than in patients who did not experience acute rejection. Sixteen out of 246 patients with methylprednisolone treated rejection developed HZ. We should not make these assumptions due to these low numbers. However, our finding could be explained by the fact that most (overall 66%) of the methylprednisolone treated rejections occurred during the standard CMV prophylaxis period. Methylprednisolone could have diminished acute neuritis, although studies on steroid treatment of herpetic neuralgia show conflicting results.[29,30] Valganciclovir could have suppressed zoster virus reactivation in an early stage. Asymptomatic virus reactivations would then go unnoticed, but could have boosted the immune system. Another possible explanation is that patients experiencing rejection received an insufficient amount of immunosuppression and were therefore less immunocompromised compared to

patients who did not experience rejection. More potent anti-rejection therapy with r-ATG was not a significant risk factor, possibly due to the low number of patients (8 of 48 with r-ATG developed HZ). Induction therapy no longer remained significant as risk factor for HZ after multivariable analysis. In our study, type of organ transplant appeared the most powerful risk factor for HZ. The higher level of maintenance immunosuppressive medication (higher tacrolimus target trough levels) is a probable explanation. However, type of organ transplant could also reflect more than the difference in immunosuppressive burden, but a more detailed analysis of patient's frailty and co-morbidity is needed to confirm that hypothesis.

There are some limitations to our study. First, the patients included in the KTx and LiTx groups received their transplantation in different time periods compared to the HTx and LuTx groups. However, maintenance immunosuppression and CMV prophylaxis were comparable in all groups. Only induction therapy differed in the KTx group. Data on dosing of maintenance immunosuppression per patient were not analysed, due to the retrospective approach and large number of patients in this study. However, the target trough levels in each organ group are described in Table 2. Finally, we did not analyse renal function after solid organ transplantation as risk factor for HZ. Patients with end stage renal disease show premature ageing of the T-cell system[31] and a higher HZ incidence in patients with chronic renal insufficiency has been reported.[32,33]

Vaccination has been shown to be effective in the prevention of HZ in healthy elderly people[34-36] as well as in patients with chronic kidney disease.[37,38] Currently, there are two licensed HZ vaccines. One is a live attenuated vaccine[34], which cannot be given to patients using immunosuppressive medication for fear of inducing VZV infection. The other is a subunit vaccine, containing VZV glycoprotein E[35], which could be given to patients on immunosuppressive medication, because it does not contain live virus. One phase III trial of the subunit vaccine in renal transplant recipients has shown persisting humoral and cell-mediated immunity at one year after vaccination.[39] More studies are necessary to confirm that this vaccine is an effective tool to prevent HZ in organ transplant recipients.

In summary, this study shows that incidence of HZ is high after organ transplantation with severe complications. The incidence of HZ after HTx or LuTx is significantly higher than after LiTx or KTx. Use of CMV prophylaxis significantly decreases HZ incidence. A rational method to prevent HZ after organ transplantation might be to use CMV prophylaxis and in our opinion booster vaccination in seropositive transplant candidates is advisable.

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4

Boosting the VZV-specific memory B and T cell response to prevent herpes zoster after kidney transplantation

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ABSTRACT

Background: Solid organ transplant recipients are at high risk to develop (complicated) herpes zoster (HZ). Booster vaccination could prevent HZ. However, end-stage renal disease (ESRD) patients show poor immunological responses to vaccinations. We studied the effect of a live attenuated VZV booster vaccine on VZV-specific B and T cell memory responses in ESRD patients and healthy controls. NL28557.000.09, www.toetsingonline.nl

Methods: VZV-seropositive patients, aged ≥50 years, awaiting kidney transplantation, were vaccinated with Zostavax®. Gender and age-matched VZV-seropositive potential living kidney donors were included as controls. VZV-specific IgG titers were measured before, at 1, 3 and 12 months post-vaccination. VZV-specific B and T cell responses before, at 3 months and 1 year after vaccination were analysed by flow-cytometry and Elispot, respectively. Occurrence of HZ was assessed at 5 years post-vaccination.

Results: 26 patients and 27 donors were included. Median VZV-specific IgG titers were significantly higher at all time-points post-vaccination in patients (mo 1: 3104 IU/ml [1967-3825], p<0.0001; mo 3: 2659 [1615-3156], p=0.0002; mo 12: 1988 [1104-2989], p=0.01 vs. pre: 1397 [613-2248]) and in donors (mo 1: 2981 [2126-3827], p<0.0001; mo 3: 2442 [2014-3311], p<0.0001; mo 12: 1788 [1368-2460], p=0.0005 vs. pre: 1034 [901-1744]. The patients' IgG titers were comparable to the donors' at all time-points. The ratio VZV-specific B cells of total IgG producing memory B cells had increased 3 months post-vaccination in patients (0.85 [0.65-1.34] vs. pre: 0.56 [0.35-0.81], p=0.003) and donors (0.85 [0.63-1.06] vs. pre: 0.53 [0.36-0.79], p<0.0001) and remained stable thereafter in donors. One year post-vaccination, the percentage of $CD4^+$ central memory cells had increased in both patients (0.29 [0.08-0.38] vs. 0.12 [0.05-0.29], p=0.005) and donors (0.12 [0.03-0.37] vs. 0.09 [0.01-0.20], p=0.002) and $CD4^+$ effector memory cells had increased in donors (0.07 [0.02-0.14] vs. 0.04 [0.01-0.12], p=0.007). Only 1 patient experienced HZ, which was non-complicated.

Conclusion: VZV booster vaccination increases VZV-specific IgG titers and percentage VZV-specific memory T cells for at least 1 year both in ESRD patients and healthy controls. VZV-specific memory B cells significantly increased in patients up to 3 months after vaccination. Prophylactic VZV booster vaccination prior to transplantation could reduce HZ incidence and severity after transplantation.

INTRODUCTION

Herpes zoster (HZ) is a common complication after solid organ transplantation, incidence rates varying from 10 to 40 cases/1000 person years[1,2] and among patients suffering from end-stage renal disease (ERSD), with hazard ratio's from 1.4 to 3.6 compared to the general population.[3,4] The incidence of HZ in these patients is higher than the usually reported 10-12 cases/1000 person years in immunocompetent people older than 70 years.[5] In addition, the disease course in immunocompromised patients is more often accompanied by severe complications, e.g. dissemination and post-herpetic neuralgia.[5-7]

In the Netherlands, VZV is endemic. Most people are infected in childhood and VZV antibody prevalence is 95% in adults.[8] No nationwide VZV booster vaccination program exists in the Netherlands. Previously, we showed that 96.2% of solid organ transplant candidates were VZV-seropositive.[2] Prophylactic VZV vaccination in seropositive patients may boost the memory T cell and B cell repertoire and thereby reduce HZ incidence and morbidity. Unfortunately, patients with ESRD and on those on dialysis are known to build up significantly poorer antibody responses to vaccinations against influenza and hepatitis B, compared to the general population.[9,10] This is probably due to the impairing effects on the immune system of uremic toxins, malnutrition, chronic inflammation and premature thymic involution, resulting in a decreased percentage of naïve T cells and a reduction in diversity of T cell receptor repertoire in ESRD patients.[11-13] After kidney transplantation, the ability to mount an adequate response to vaccination is even more impaired by immunosuppressive medication.[14-17] Therefore, it makes sense to administer vaccinations prior to start of immunosuppressive medication, as recommended in major guidelines.[18,19] Only few other studies of VZV booster vaccination in patients awaiting solid organ transplantation have been performed[20-22], of which only one study reported both IgG and T cell responses[22] and none had healthy individuals as control group.

We investigated VZV-specific IgG titers, B and T cell memory responses to the live attenuated virus vaccine, Zostavax®, in ESRD patients awaiting kidney transplantation. Our study population was at least 50 years of age and we compared them to gender and age-matched living kidney donors.

MATERIALS AND METHODS

Participants

The present prospective study (NL28557.000.09 / MEC2009-286) was conducted between 2010 and 2015.

Patients aged ≥50 years, suffering from ESRD and awaiting renal transplantation from our outpatient clinic were enrolled. Gender- and age-matched living kidney donors

were included as healthy controls. All had positive VZV-IgG titers during assessment for transplantation or donation. All participants received one dose of Zostavax® (Sanofi Pasteur MSD NV, Brussels), 0.65 ml subcutaneously in an upper arm.

Gender, age, renal replacement therapy, serum anti-CMV IgG status (positive or negative) screening and cause of kidney failure (in patients) were collected from the hospital charts. These data are part of the standard medical screening for kidney transplant candidates and potential kidney donors.

Five years after vaccination, herpes zoster occurrence was assessed by reviewing the hospital electronic patient files and telephone calls to participants who did not have regular hospital visits in our center.

Humoral response

VZV-specific IgG antibody levels were analyzed by chemiluminescence immunoassay (Liaison® XL, DiaSorin, Saluggia, Italy) before and at 1, 3 and 12 months after vaccination in all patients and donors. The cut-off for seropositivity was set at >165 mIU/ml. This automated assay for quantitative determination of VZV-specific IgG, showed good correlation with a highly sensitive VZV-IgG time-resolved fluorescence immunoassay (TRIFIA), 67% sensitivity and 100% specificity compared to TRFIA in a British population.[23]

VZV-specific B cell reactivity

VZV-reactive B cell memory was determined before and at 3 and 12 months after transplantation. The VZV-specific B cell reactivity was determined by Elispot assay.[24] In brief, PBMC were stimulated with B cell stimulus (U-CyTech biosciences, Utrecht, the Netherlands). After an incubation period of 5 days, the cells were transferred to 96-well filter plates with PVDF membrane (Millipore, Darmstadt, Germany) coated with VZV antigen (Varicella Zoster grade 2 antigen; Microbix Biosystems Inc, Ontario, Canada) or anti-human IgG (U-CyTech biosciences) to determine the spontaneous frequency of IgG producing B-cells. After 5 hours of incubation, the cells were removed and biotinylated detection antibody (U-CyTech biosciences) was added. Thereafter, a streptavidin-HRP conjugate (U-CyTech biosciences) was added followed by addition of AEC (3-amino-9-ethyl-carbazole) substrate solution (U-CyTech biosciences). Spots were counted automatically by using a Bioreader 3000 Elispot reader (Biosys, GmbH, Karben, Germany). In all experiments at least 50 IgG producing cells per $1x10^4$ cells were determined. Data are presented as the ratio VZV-specific B-cells of the total IgG memory B-cells in PBMC.

VZV-specific T cell reactivity

VZV-reactive T-cell memory was determined before and at 3 and 12 months after transplantation. Mature moDCs were generated with a cocktail of cytokines as described before.[25] Due to the instability of cell-free VZV in cell culture, mature moDCs were infected

with VZV by co-culturing the cells with human melanoma cells (MeWo cells; American Type Culture Collection, HTB-65) infected with the vaccine Oka strain of VZV.[26,27] Mature moDCs were co-cultured with VZV-infected and mock-infected MeWo cells for 24 hours. The level of VZV infection was determined by flow cytometric analysis of the moDCs infected with VZV stained for VZV glycoprotein B (gB; Advanced Biotechnologies, Inc., Columbia, MD). Co-staining for CD86 enabled differentiation of moDCs (CD86 positive) from residual MeWo cells (CD86 negative). The level of VZV-infection was determined by subtracting the background after mock-infection from the moDCs infected with VZV. After 24 hours these moDCs were used as autologous APCs. Autologous $CD3^+$ T cells were isolated from the CD14 negative fraction and incubated with moDCs infected with VZV or mock-infected moDCs for 24 hours.[25] Briefly, tubes 1 and 2 contained $1x10^6$ T-cells and$1x10^5$ autologous moDCs infected with VZV, and tubes 3 and 4 contained T-cells and autologous mock-infected moDCs. The cells from tubes 1 and 3 were stained with peridinin chlorophylprotein (PerCP) anti-CD4, (Becton Dickinson, Erebodegem, Belgium), allophycocyanin (APC) labeled anti-CD45RO (Becton Dickinson) and phycoerythrin (PE) labeled anti-CCR7 (R&D Systems Europe Ltd, Abingdon, UK). The cells from tubes 2 and 4 were stained with PerCP-labeled anti-CD8 (Becton Dickinson), APC-labeled anti-CD45RO (BectonDickinson) and PE-labeled anti-CCR7 (R&D Systems) (Figure S1). Thereafter, the cells from all tubes were fixed and permeabilized followed by incubation with anti-human IFN-γ. The VZV-reactive T cells were determined by counting the number of IFN-γ producing $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells stimulated with autologous moDCs infected with VZV, minus the respective values obtained upon stimulation with autologous mock-infected moDCs, on the fluorescence-activated cell sorter (FACS Canto-II, Becton Dickinson). The VZV-reactive T cells are expressed as percentage of the total number of reactive T cells or (central or effector) memory cells.

Statistical analysis

Statistical analyses were performed in SPSS, version 25, 2017 and GraphPad Prism 9.1.2, 2021.

Patient and donor categorical variables were compared using Fisher's exact and Pearson chi square tests and continuous variables with Mann-Whitney test. VZV-IgG titers and VZV-specific T and B cell responses were compared between patients and donors with Mann-Whitney U test and within patient and donor groups using Wilcoxon signed rank test. Data are presented as median with interquartile range. VZV-IgG geometric mean fold rise (GMFR) was calculated for each time point after vaccination: geometric mean titer (GMT) at that time point divided by GMT pre-vaccination.

RESULTS

Participants

A total of 26 patients and 27 donors was included (Figure 1). The characteristics of the patients and donors are listed in Table 1. Gender, age and CMV serostatus were comparable between patients and donors. The follow-up of patients and donors is described in Table 2.

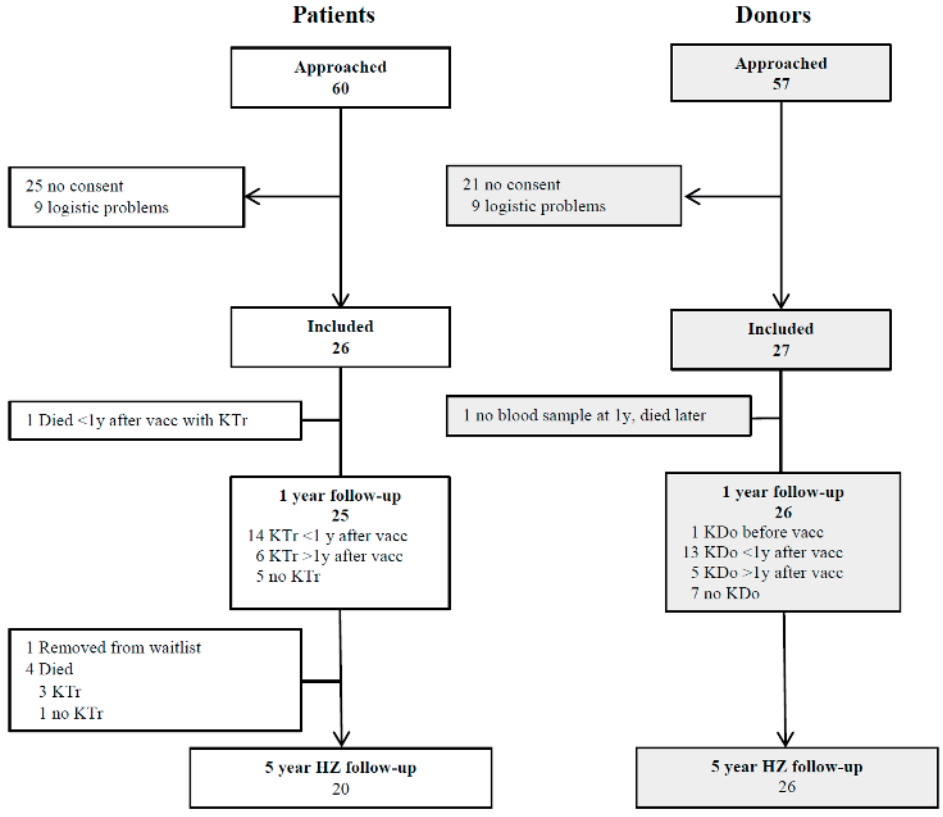

Figure 1. Participants enrolment and follow-up

KTr= kidney transplant. KDo= kidney donation. HZ= herpes zoster. Vacc= vaccination.

Eligible, potential participants were approached at the outpatient clinic and by telephone call from the investigators. They received both oral and written information. Some participants could not be included due to a tight schedule of transplantation and donation and/or temporary unavailability of the vaccine.

Table 1. Patient and donor characteristics at vaccination

	Patients	Donors	p
Number	26	27	
Gender (M/F)	14 / 12	12 / 15	0.59
Age[a] (years)	64 (50-77)	62 (52-74)	0.44
RRT			
No / HD / PD	17 / 6 / 3		
CMV-IgG			
pos / neg / unknown	19 / 7 / 0	14 / 9 / 4	0.54
Renal disease			
Hypertension	8		
Diabetes Mellitus	6		
Polycystic	5		
Glomerular disease[b]	4		
Other[c]	3		

M: male, F: female

[a]: median (range)

RRT: renal replacement therapy. HD: hemodialysis. PD: peritoneal dialysis.

CMV-IgG: anti-cytomegalovirus Immunoglobulin G

[b]: 1x glomerulonephritis eci, 2x IgA nephropathy, 1x Alport's disease

[c]: 1x acute tubular necrosis due to sepsis, 1x unknown, 1x nephrolithiasis due to cystinuria

Patients

Seventeen patients were waiting for a pre-emptive living or deceased donor kidney transplantation, whereas six patients were on hemodialysis and 3 on peritoneal dialysis (Table 1). Twenty-one patients received a kidney transplant, 15 within 1 year after vaccination and 6 more than 1 year (range 24 – 67 months) after vaccination. Five patients did not receive a kidney transplant during the study period: 3 were waiting but not on dialysis, 1 died due to heart failure and 1 was removed from the waitlist due to severe iliac artery atherosclerosis. Four patients died after transplantation (Table 2).

The characteristics of the patients who received a transplant after vaccination are described in Table 3. Five patients experienced a rejection episode within the first year (0.1 to 8 months) after transplantation, varying from 3 to 15 months after vaccination (Table 3). Their anti-HLA antibody level, expressed as panel reactive antibody (PRA) did not increase after vaccination. Two of these 5 rejections occurred in ABO-incompatible transplants.

Donors

Nineteen donors donated a kidney, 13 within 1 year after vaccination and 5 more than 1 year (range 15 – 47 months) after vaccination. One donor donated 2 months before vaccination. One potential donor died due to a malignancy almost 14 months after vaccination (Table 2). Prior to her disease, she and her recipient had been removed from the transplant program because of severe iliac artery atherosclerosis in the recipient.

Table 2. Patient and donor follow-up

	Kidney Transplant Recipients			Kidney Donors		
	Yes	**No**	**p**	**Yes**	**No**	**p**
Number (%)	21 (81)	5 (19)		19 (70)	8 (30)	
<1 year post vaccination, n (%)	15 (58)			13 (48)		
>1 year post vaccination, n (%)	6 (23)			5 (19)		
pre-vaccination, n (%)	0			1 (4)		
Age at vaccination [a]	63 (50-74)	71 (61-77)	0.41	64 (52-73)	62 (51-66)	0.41
Donor type, n (%)						
Living	14 (67)					
Deceased	7 (33)					
Time to transplantation / donation (months post vaccination)[a]	13.5 (1-67)			4.9 (-2-47)		
RRT						
No / HD / PD	13 / 5 / 3	4 / 1 / 0				
Herpes Zoster, n (%)	1 (4.8)	0		0	0	
Death[b], n (%)	4 (19)	1 (20)		0	1 (12.5)	
Time to death (months)[a]						
since vaccination	61 (6-91)	18.7			13.6	
since transplantation / donation	36 (0.3-80)	-			-	

RRT= renal replacement therapy. HD= hemodialysis. PD= peritoneal dialysis.

[a]: median + range

[b]: Four transplant recipients: 1 heart failure, 1 malignancy: lung carcinoma, 1 infection: cellulitis + sepsis, 1 unknown. One patient without transplant: heart failure. One donor: retroperitoneal sarcoma

VZV-specific IgG

25/26 patients and 26/27 donors reached the 12 month time point. VZV-specific IgG titers were significantly higher at all time-points after vaccination in patients (M1: 3104 IU/ml [1967-3825], p<0.0001; M3: 2659 [1615-3156], p=0.0002; M12: 1988 [1104-2989], p=0.01 vs. pre: 1397 [613-2248]) and in donors (M1: 2981 [2126-3827], p<0.0001; M3: 2442 [2014-3311], p<0.0001; M12: 1788 [1368-2460], p=0.0005 vs. pre: 1034 [901-1744] (Figure 2A). The patients' titers were comparable to the donors' titers at all time points: pre: p=0.64, M 1: p=0.94, M 3: p=0.79, M 12: p=0.84. GMFR was also comparable between patients and donors at all time points (Figure 2B).

The patients who did not receive a kidney transplant within 1 year post vaccination had a greater increment in IgG titers between pre-vaccination and month 12 (median 1035 [268-2063]), compared to the patients who received a transplant within the first year (450 [-13-751], p=0.033) (Figure 3).

We found no difference in VZV IgG titers at any time point between patients who were on dialysis at vaccination and patients who were not (Table S1).

Also, no difference was found between VZV IgG titers at any time point and CMV serostatus at vaccination in patients (Table S2) and donors (Table S3).

Table 3. Kidney transplant recipient characteristics

	No rejection	Rejection	p
Number	16	5	
Age (years)[a]	64 (50-74)	62 (54-70)	0.40
RRT			
No / HD / PD	11 / 4 / 1	2 / 1 / 2	
Donor type, n (%)			
Living	10 (62.5)	4 (80)	0.35
Deceased	6 (37.5)	1 (20)	
Time to KTr (months after vaccination)[a]	7 (1-67)	5 (2-12)	
Time to rejection (months)[a]			
since vaccination		5.8 (3-15)	
since transplantation		0.2 (0.2-8)	
Herpes Zoster, n (%)	0	1 (20)	
Time to Herpes Zoster (months)[a]			
since vaccination		15.6	
since transplantation		10.8	
PRA[b]			
before vaccination	8.7 (0-77) n=15	2.6 (0-13) n=5	0.30
at transplantation	0.0 (0-0) n=11	2.0 (0-8) n=4	0.27
ABO-incompatible kidney transplantation n (%)	1 (6)	2 (40)	
Induction therapy, n (%)			
Basiliximab	15 (94)	3 (60)	
Rituximab[c]	0	2 (40)	
Alemtuzumab	1 (6)	0	
Maintenance immunosuppression, n (%)			
Tac + MMF	11 (69)	1 (20)	
Tac + MMF + pred	1 (6)	2 (40)	
Other	4 (25)[d]	2 (40)[e]	

KTr= kidney transplantation. RRT= renal replacement therapy. HD= hemodialysis. PD= peritoneal dialysis. Tac= tacrolimus. MMF= mycophenolate mofetil. Pred= prednisolone

[a]: median + range

[b]: PRA= panel reactive antigen as percentage, mean + range, n= patients with available PRA

[c]: because of ABO-incompatible kidney transplantation

[d]: 2 Tac monotherapy, 1 everolimus + MMF + pred, 1 Tac + pred [e]: Tac + pred

VZV-specific B cell response

VZV-reactive B cell memory was determined pre-vaccination in 22 patients and 22 donors, at 3 months in 21 patients and 21 donors and at 12 months in 10 patients and 14 donors. Total numbers of IgG producing cells were similar between patients and donors, within the patient group and within the donor group (Figure 4A). The number of VZV-specific IgG producing memory B cells increased significantly within the first 3 months post-vaccination in both patients and donors (Figure 4B). Between patients and donors, the numbers of VZV-specific IgG producing B cells were similar. The ratio VZV-specific B cells of the total IgG producing memory B cells in PBMC had also increased significantly at month 3 in both

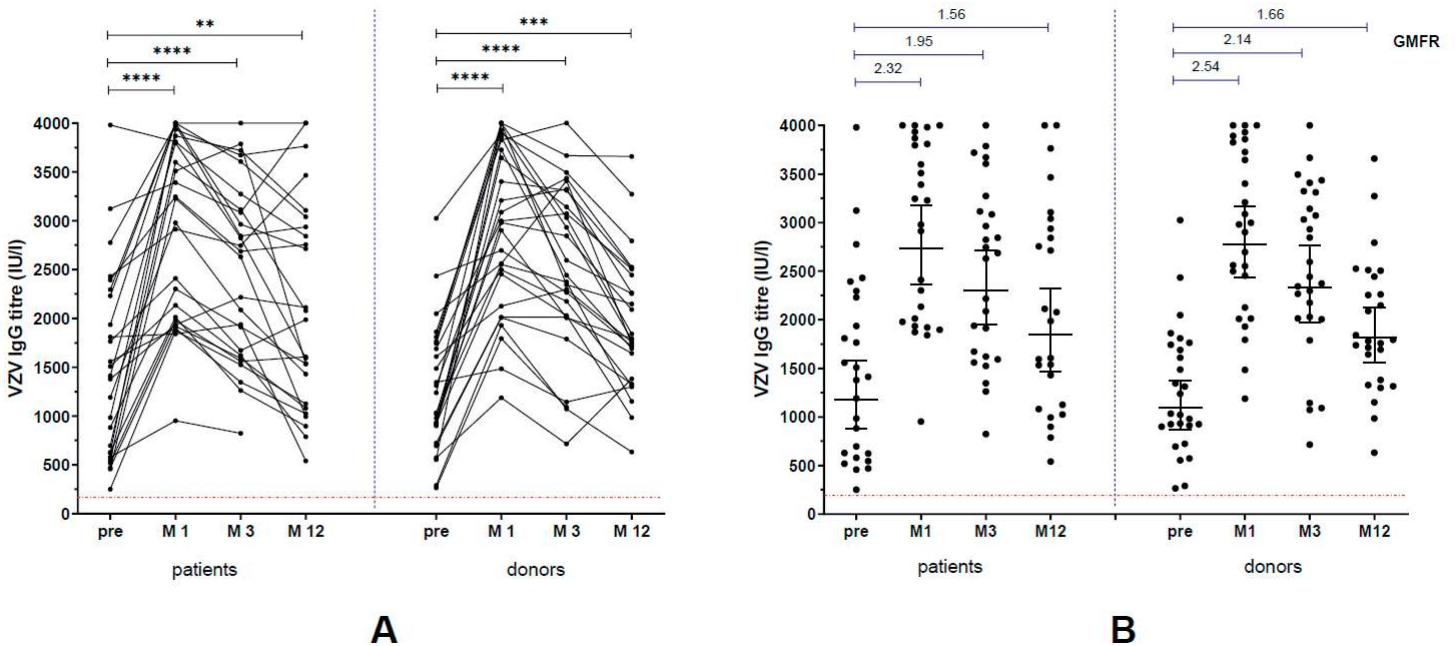

Figure 2. VZV-specific IgG response

VZV-specific IgG titres, before vaccination and at 1 month (M1), 3 months (M3) and 12 months (M12) after vaccination.

(A) **: p=0.005, ***: p=0.0002, ****: p<0.0001. Patients compared to donors: pre: p=0.67, M1: p=0.94, M3: p=0.79, M12: p=0.84

(B) Lines in scatter plots indicate Geometric Mean with 95% confidence interval. Above the scatter plots: Geometric Mean Fold Rise (GMFR) between pre and M1, M3 and M12.

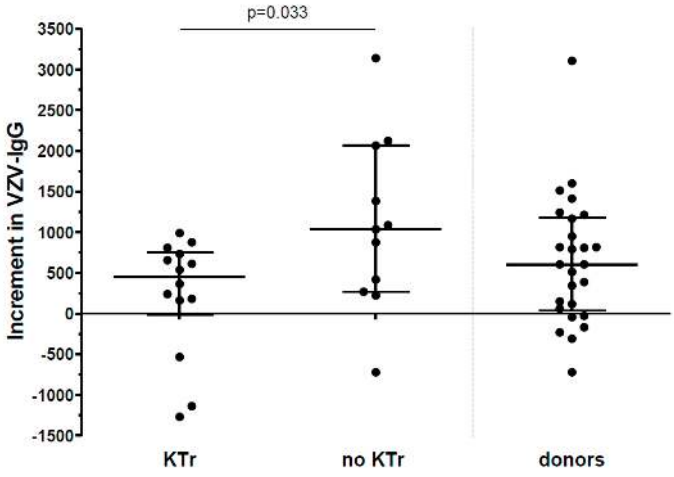

Figure 3. Increment in VZV-IgG titres between vaccination and 12 months after vaccination
Increment in VZV-IgG titres in 14 patients who received a kidney transplant (KTr) within 1 year after vaccination compared to 11 patients who were still on the waitlist (no KTr) at 1 year after vaccination and to 26 donors. Lines indicate median with interquartile range.

patients (0.85 [0.65-1.34] vs. pre: 0.56 [0.35-0.81], p=0.003) and donors (0.85 [0.63-1.06] vs. pre: 0.53 [0.36-0.79], p<0.0001) (Figure 4C). In donors this ratio remained significantly higher at month 12 compared to pre-vaccination (Figure 4C).

VZV-specific B cell data were only available in 6 patients who received a kidney transplant within 1 year after vaccination and in 4 patients who did not. Due to these low numbers of patients, statistical analysis was not performed.

We found no difference in the ratio VZV-specific of total IgG producing B cells at any time point between patients who were on dialysis (n=7) at vaccination and patients who were not (n=15) (Table S1).

No difference was found between the ratio VZV-specific of total IgG producing B cells at any time point and CMV serostatus at vaccination in patients (n=22: 5 IgG neg, 17 IgG pos) (Table S2) and donors (n=19: 8 IgG neg, 11 IgG pos) (Table S3).

VZV-specific T cell response

VZV-reactive T cell memory was determined in 18 patients and 22 donors at all time points. We compared the VZV-reactive memory cells at 3 and 12 months after vaccination with before vaccination.

The percentage of VZV-reactive $CD4^+$ memory cells (defined as $CD4^+CD45RO^+IFN\text{-}\gamma^+$) significantly increased in both donors (M3 and M12 compared to pre-vaccination) and patients (M12 compared to pre-vaccination and M12 compared to M3) (Figure 5A). No relevant difference was found in the VZV-reactive $CD8^+$ memory response (defined as $CD8^+CD45RO^+IFN\text{-}\gamma^+$) (Figure 5B).

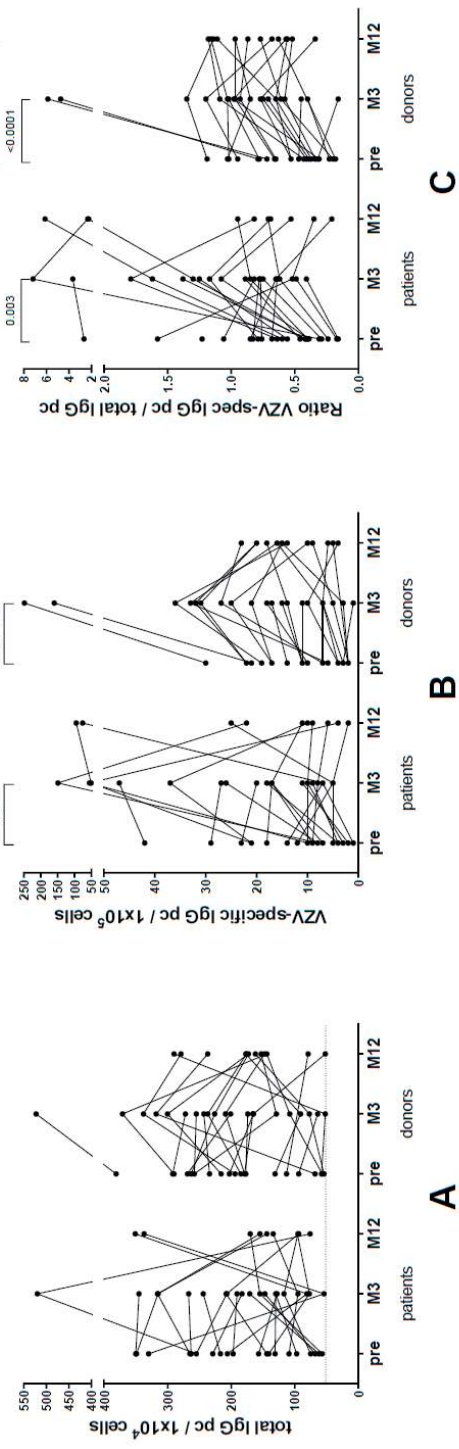

Figure 4. VZV-specific B cell response
(A) Total IgG producing B cells per 10^4 cells
(B) VZV-specific IgG producing B cells per 10^5 cells
(C) Ratio between VZV-specific IgG producing B cells and total IgG producing B cells

When we divided the VZV-reactive $CD4^+$ memory cells into $CD4^+$ central memory (CM, $CD4^+CD45RO^+CCR7^+$) and effector memory (EM, $CD4^+CD45RO^+CCR7^-$) cells, we found that in both patients (0.29 [0.08-0.38] vs. 0.12 [0.05-0.29], p=0.005) and donors (0.12 [0.03-0.37] vs. 0.09 [0.01-0.20], p=0.002) the percentage of VZV-reactive $CD4^+$ CM cells increased after vaccination and were still higher at one year after vaccination (Figure 6A). However, the VZV-reactive $CD4^+$ EM cells only increased in donors (0.07 [0.02-0.14] vs. 0.04 [0.01-0.12], p=0.007) (Figure 6B).

VZV-specific T cell memory data were available in 11 patients who received a kidney transplant within 1 year after vaccination and in 8 patients who did not. No difference was found at month 12 in percentage of VZV- reactive $CD4^+$ (total, CM and EM) nor in VZV-reactive $CD8^+$ memory cells between patients with and without a kidney transplant and between both patient groups and donors (Table S4).

We found no difference in VZV-reactive $CD4^+$ or in VZV-reactive $CD8^+$ memory cells at any time point between patients who were on dialysis at vaccination (n=6) and patients who were not (n=12) (Table S1).

No difference was found between VZV-reactive $CD4^+$ or VZV-reactive $CD8^+$ memory cells at any time point and CMV serostatus at vaccination in patients (n=18: 4 IgG neg, 14 IgG pos) (Table S2) and donors (n=18: 7 IgG neg, 11 IgG pos) (Table S3).

Adverse events after vaccination

A 68-year-old female donor developed an itching rash on face and breast (no blisters), a feeling of malaise and slightly elevated temperature one day post-vaccination. She spontaneously recovered in 4 days after vaccination. None of the other participants reported adverse events.

Herpes zoster after vaccination

One patient suffered from a mild HZ, localized just below her left breast. It occurred 16 months after vaccination, 11 months after transplantation and 9 months after anti-rejection treatment (methylprednisolone and IVIG). She was treated with oral valaciclovir for 9 days. She recovered completely within one month.

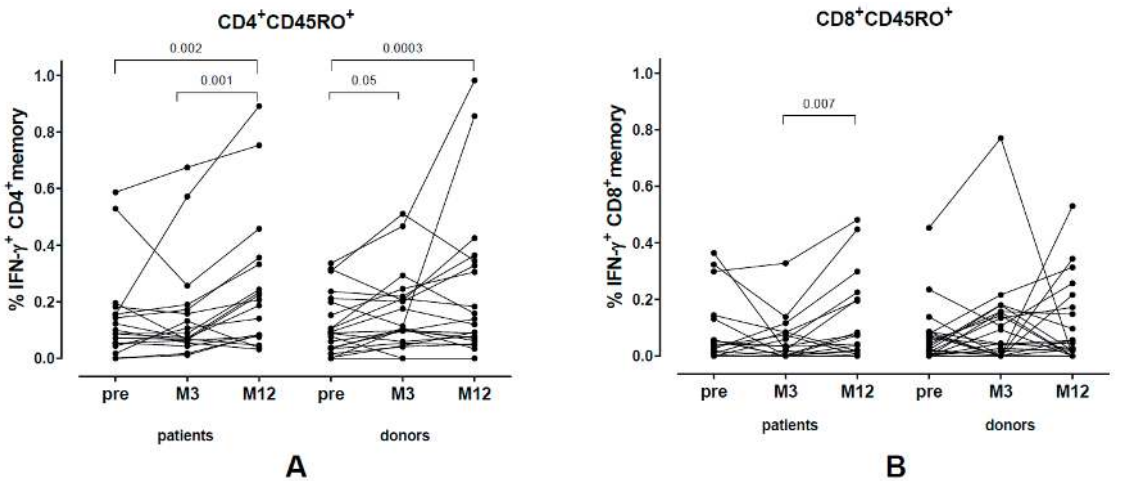

Figure 5. VZV-reactive T cell response: CD4+ and CD8+ memory
(A) Percentage VZV-specific IFN-γ producing $CD4^+$ memory cells
(B) Percentage VZV-specific IFN-γ producing $CD8^+$ memory cells

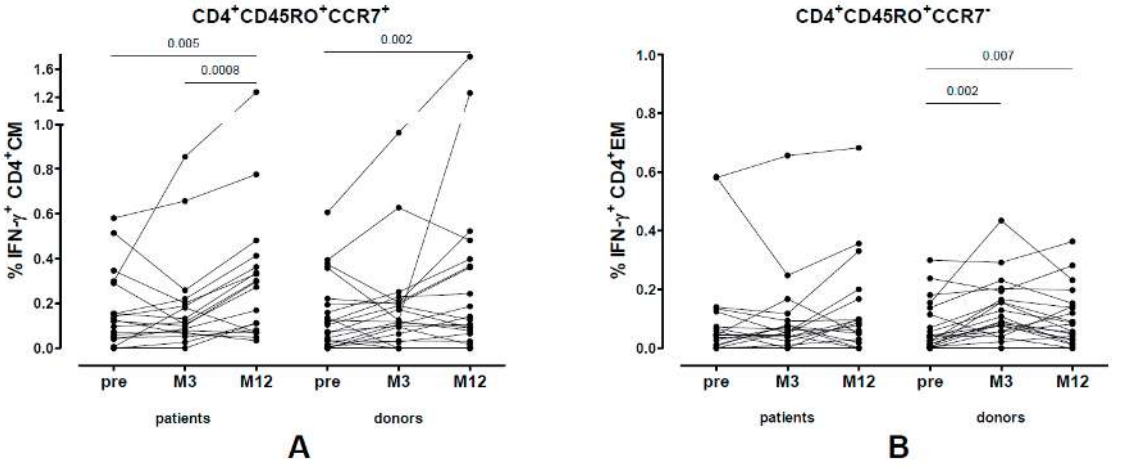

Figure 6. VZV-reactive T cell response: CD4+ central and effector memory
(A) Percentage VZV-specific IFN-γ producing $CD4^+$ central memory cells
(B) Percentage VZV-specific IFN-γ producing $CD4^+$ effector memory cells

DISCUSSION

Natural immunity against VZV is maintained during (subclinical) virus reactivation and re-exposure to the virus but declines as the immune system ages.[28] From hepatitis B vaccination in dialysis patients and SARS-CoV2 vaccination in kidney transplant patients, it is known that higher and repeated dosing (boostering) can improve antibody and cellular response against the virus.[11,29-31] As herpes zoster incidence and severity are high in solid organ transplant recipients[1,2,4], it is important to investigate whether booster vaccination can decrease HZ incidence. Vaccine efficacy can be assessed by monitoring both the humoral and cellular VZV response after booster vaccination. To effectively control VZV reactivation, cell mediated immunity is necessary and the magnitude of the cellular response correlates better with HZ severity than IgG titers.[28,32-34] Weinberg et al. reported that in elderly people, a higher cell mediated immune response (measured in VZV responder cell frequency and IFN-γ Elispot) correlated with reduced HZ morbidity, whereas VZV antibody titers did not.[32] In a nonhuman primate model with simian varicella virus infection, it was shown that depletion of $CD4^+$, and not $CD8^+$, T cells resulted in significantly higher viral loads and disseminated varicella, while the absence of B cells did not alter disease severity.[33] Given the helper functions of $CD4^+$ T cells, their absence would lead to delay and reduction of VZV-specific antibody and $CD8^+$ T cell responses.[28] $CD8^+$ T cell response to VZV has been studied less extensively than $CD4^+$ T cell responses.

To our knowledge, this is the first study comparing three parameters reflecting the immune response to VZV booster vaccination (IgG titers, B cell and T cell memory) in ESRD patients and healthy controls (kidney donors), all above 50 years of age. We found that at 3 months after Zostavax®, VZV-specific IgG titers and B cell memory had equally increased in ESRD patients compared to controls. At 1 year after vaccination, VZV-specific IgG titers remained significantly high in both groups, but the ratio VZV-specific memory B cells of the total IgG producing memory B cells had declined in patients. The percentage of VZV-reactive $CD4^+$ T cells and central memory $CD4^+$ cells were significantly increased at 1 year in both patients and controls. The percentage of VZV-reactive $CD4^+$ effector memory cells were only significantly increased in controls.

From the few studies describing immune responses to VZV booster vaccination in patients awaiting solid organ transplantation[20-22], only one study concerned ESRD patients. Miller et al. compared VZV antibody titers after Zostavax® (n=26) and after placebo vaccine (n=8). Geometric mean titer was significantly higher in the Zostavax group only at 5 weeks after vaccination, but not at 12 months.[20] Comparing the study of Miller et al. with our study, a higher percentage of patients (69%) was on dialysis at time of vaccination and only 46% received a kidney transplant thereafter, while in our study these percentages were 35% and 81% (58% within 1 year post vaccination), respectively. In the transplant recipients, Miller et al. demonstrated a more pronounced decline in antibodies, while we found comparable

levels of VZV IgG antibodies between transplant recipients and healthy donors (Figure 2A and 2B). In our study, the patients who received a kidney transplant within one year after vaccination, had lower VZV IgG titers compared to those still awaiting transplantation. Indeed, several reports demonstrated that especially mycophenolic acid impairs B cell numbers and production of IgG.[14,15] However, we found no difference in percentage VZV-specific T cells between patients who received their transplant within one year after vaccination and those who did not. Tacrolimus, the most used calcineurin inhibitor in our transplant recipients, inhibits T cell activation, including $CD4^+$ helper function, and T cell proliferation. Despite this suppressive effect, it has been shown that VZV specific $CD4^+$ and $CD8^+$ memory T cells did significantly increase after a herpes zoster episode in lung transplant patients.[24] VZV-reactive memory $CD4^+$, but not $CD8^+$ T cells also significantly increased upon *in vitro* stimulation by VZV infected dendritic cells in kidney transplant patients.[25] Recently, Wang et al. also found that in patients vaccinated prior to lung transplantation, VZV stimulated IFN-γ producing cells decreased shortly after lung transplantation, but had increased again at 6 months or longer after transplantation.[22] In patients with latent VZV infection, circulating VZV-specific $CD4^+$ memory T cells are long-lived, and these cells have skin-homing and tissue residing ability.[28] This could be one mechanism by which these cells escape the effect of immunosuppressive drugs.

We did not find any difference in immune responses to the booster vaccination between our dialysis and ESRD patients. A possible explanation could be that duration of dialysis before vaccination was limited (median 12 months, range 5-48). Tseng et al. reported that a lower HZ incidence in vaccinated ESRD patients is most prominent when vaccination was performed within 2 years of dialysis initiation.[35]

Only one patient experienced a mild and self-limiting rash after vaccination. In 5 of 21 transplant recipients an acute rejection episode was observed. Acute rejection incidence in the vaccinated transplant recipients was comparable to the general acute rejection incidence in our center in the same time period.[36] Therefore, we conclude that vaccination was safe and did not induce graft rejection. With a follow-up of 5 years, only 1 patient suffered from herpes zoster, with mild symptoms no complications and full recovery. None of the controls had herpes zoster.

However, the present study was not designed to detect significant differences in herpes zoster incidence. Furthermore, only patients who were not using immunosuppressive medication could be included, because the Zostavax vaccine contains live attenuated virus. Although there are many reports on the safety of zoster vaccines[34], caution is advised when live attenuated virus vaccination is considered in immunocompromised patients. A literature review from Price et al. reported three cases of fatal zoster vaccine infections. Of these patients, one was taking prednisone 10 mg/day, methotrexate and hydroxychloroquine and two patients had a hematologic malignancy without use of immunosuppressive medication at least 6 months before administration of the zoster vaccine.[37] Since 2018, a recombinant

subunit adjuvanted vaccine (Shingrix®) was also approved in Europe to prevent HZ. In the Netherlands, Shingrix® was only available from June 2020. As this vaccine does not contain live attenuated virus, it may possibly be given to patients using immunosuppressive drugs. Efficacy and safety have been reported in a phase 3 study with kidney transplant recipients.[38] However, regardless of the vaccine type, performing vaccination in patients *before* they receive a kidney transplant is an obvious strategy. Also SARS-CoV-2 vaccination studies have shown that vaccine immunogenicity in patients with chronic kidney disease and even on dialysis is better compared to in patients with a kidney transplant.[39,40]

In conclusion, our study showed that boosting the immune system of ESRD patients ≥50 years old with Zostavax® does significantly increase VZV-specific IgG titers and $CD4^+$ memory cells, even to comparable levels as in healthy controls of the same age. The responses persisted for at least one year after vaccination, despite the introduction of immunosuppressive medication after kidney transplantation. Given the high herpes zoster incidence after solid organ transplantation, it seems justified to perform booster vaccination in transplant candidates.

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SUPPLEMENTAL MATERIALS

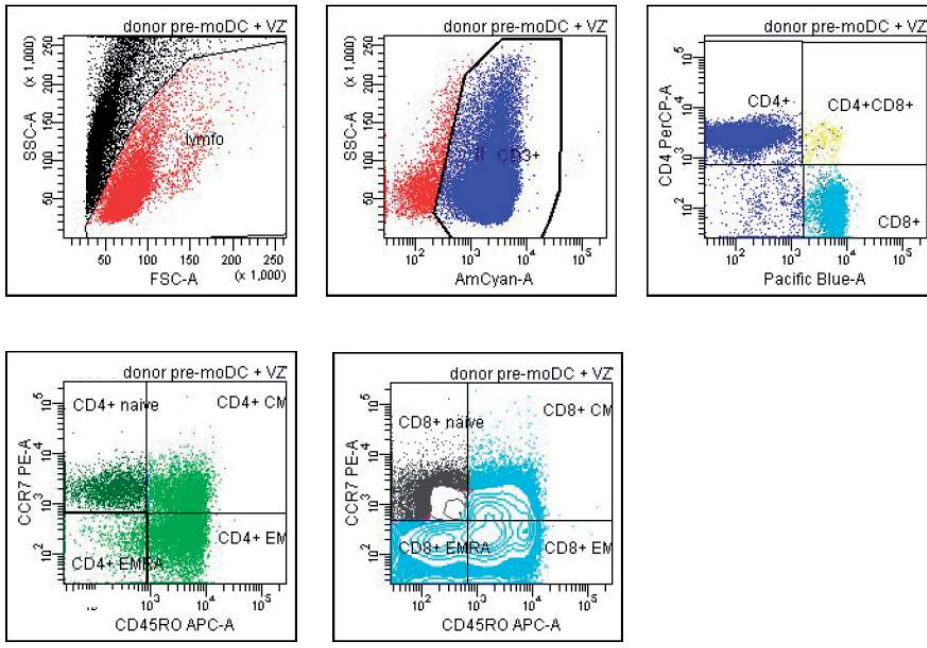


Figure S1. Representative example of the gate setting

From the lymphocyte gate, the $CD3^+$ cells were selected. Thereafter, the $CD3^+$ $CD4^+$ and $CD8^+$ cells were gated. From the $CD4^+$ and $CD8^+$ cells the naïve, central memory and effector memory cells were selected.

Table S1. VZV-specific T and B cell responses and IgG titres in patients on dialysis and not on dialysis

			CD4 TM pre	CD4 TM M3	CD4 TM M12	CD4 CM pre	CD4 CM M3	CD4 CM M12	CD4 EM pre	CD4 EM M3	CD4 EM M12
Recipients	Dialysis no	median	0,105	0,120	0,210	0,131	0,157	0,318	0,057	0,074	0,091
		25% percentile	0,046	0,066	0,083	0,043	0,081	0,089	0,016	0,041	0,050
		75% percentile	0,156	0,240	0,433	0,263	0,249	0,464	0,139	0,156	0,299
	Dialysis yes	median	0,095	0,067	0,145	0,112	0,088	0,192	0,040	0,042	0,030
		25% percentile	0,054	0,046	0,043	0,055	0,054	0,045	0,006	0,000	0,000
		75% percentile	0,186	0,106	0,229	0,304	0,138	0,309	0,063	0,068	0,097
	Dialysis no vs. yes	P-value	0,892	0,180	0,213	0,964	0,250	0,151	0,291	0,102	0,083

			CD8 TM pre	CD8 TM M3	CD8 TM M12	CD8 CM pre	CD8 CM M3	CD8 CM M12	CD8 EM pre	CD8 EM M3	CD8 EM M12
Recipients	Dialysis no	median	0,041	0,012	0,078	0,010	0,009	0,041	0,049	0,062	0,103
		25% percentile	0,002	0,000	0,017	0,000	0,000	0,000	0,014	0,000	0,007
		75% percentile	0,141	0,084	0,219	0,146	0,106	0,157	0,284	0,116	0,208
	Dialysis yes	median	0,039	0,035	0,040	0,017	0,027	0,001	0,061	0,039	0,015
		25% percentile	0,000	0,014	0,004	0,000	0,005	0,000	0,014	0,006	0,000
		75% percentile	0,114	0,138	0,177	0,139	0,166	0,279	0,063	0,075	0,088
	Dialysis no vs. yes	P-value	0,682	0,553	0,682	0,964	0,437	0,820	0,553	0,553	0,125

			B cell pre	B cell M3	B cell M12	VZV-IgG T=0 vacc	VZV-IgG T=1 mo	VZV-IgG T=3 mo	VZV-IgG T=12 mo
Recipients	Dialysis no	median	0,420	0,780	0,710	1380,0	2979,0	2630,0	1988,0
		25% percentile	0,290	0,620	0,530	626,8	2073,5	1647,5	1278,0
		75% percentile	0,790	1,300	2,260	2084,5	3839,5	3440,0	3073,0
	Dialysis yes	median	0,600	1,070	0,950	1559,0	3391,0	2844,0	1805,5
		25% percentile	0,460	0,800	0,210	563,1	1869,5	1469,5	1039,0
		75% percentile	1,230	2,133	6,130	2536,0	3791,5	3100,0	2881,0
	Dialysis no vs. yes	P-value	0,185	0,235	0,833	0,792	0,597	0,916	0,588

Table S2. VZV-specific T and B cell responses and IgG titres in CMV seronegative and CMV seropositive patients

			CD4 TM pre	CD4 TM M3	CD4 TM M12	CD4 CM pre	CD4 CM M3	CD4 CM M12	CD4 EM pre	CD4 EM M3	CD4 EM M12
Recipients	CMV IgG neg	median	0,086	0,062	0,065	0,100	0,067	0,081	0,059	0,070	0,055
		25% percentile	0,018	0,023	0,036	0,018	0,016	0,038	0,020	0,049	0,013
		75% percentile	0,142	0,160	0,270	0,147	0,183	0,338	0,120	0,089	0,087
	CMV IgG pos	median	0,106	0,098	0,216	0,131	0,124	0,300	0,046	0,053	0,091
		25% percentile	0,051	0,066	0,084	0,046	0,086	0,104	0,006	0,021	0,027
		75% percentile	0,186	0,193	0,382	0,311	0,216	0,393	0,129	0,131	0,234
	CMV neg vs. pos	P-value	0,574	0,277	0,158	0,442	0,192	0,192	0,789	0,645	0,277

			CD8 TM pre	CD8 TM M3	CD8 TM M12	CD8 CM pre	CD8 CM M3	CD8 CM M12	CD8 EM pre	CD8 EM M3	CD8 EM M12
Recipients	CMV IgG neg	median	0,054	0,046	0,149	0,109	0,139	0,343	0,062	0,058	0,070
		25% percentile	0,013	0,008	0,050	0,009	0,002	0,019	0,016	0,013	0,017
		75% percentile	0,238	0,261	0,417	0,380	0,470	0,615	0,099	0,201	0,119
	CMV IgG pos	median	0,030	0,020	0,030	0,000	0,018	0,000	0,043	0,040	0,025
		25% percentile	0,000	0,000	0,004	0,000	0,000	0,000	0,015	0,000	0,002
		75% percentile	0,134	0,084	0,196	0,048	0,038	0,146	0,151	0,104	0,207
	CMV neg vs. pos	P-value	0,721	0,574	0,158	0,158	0,382	0,061	1,000	0,798	0,798

			B cell pre	B cell M3	B cell M12	VZV-IgG T=0 vacc	VZV-IgG T=1 mo	VZV-IgG T=3 mo	VZV-IgG T=12 mo
Recipients	CMV IgG neg	median	0,600	1,250	2,340	882,5	3228,0	2630,0	2078,0
		25% percentile	0,435	0,995	0,690	470,0	1934,0	1913,0	1081,0
		75% percentile	0,925	4,410	6,130	1937,0	4000,0	3116,0	2713,0
	CMV IgG pos	median	0,460	0,775	0,710	1510,0	2979,0	2687,0	1797,0
		25% percentile	0,340	0,625	0,350	624,2	1978,0	1561,0	1100,8
		75% percentile	0,840	1,245	0,950	2393,0	3810,0	3274,0	3056,5
	CMV neg vs. pos	P-value	0,704	0,062	0,183	0,334	0,651	0,611	0,929

Table S3. VZV-specific T and B cell responses and IgG titres in CMV seronegative and CMV seropositive donors

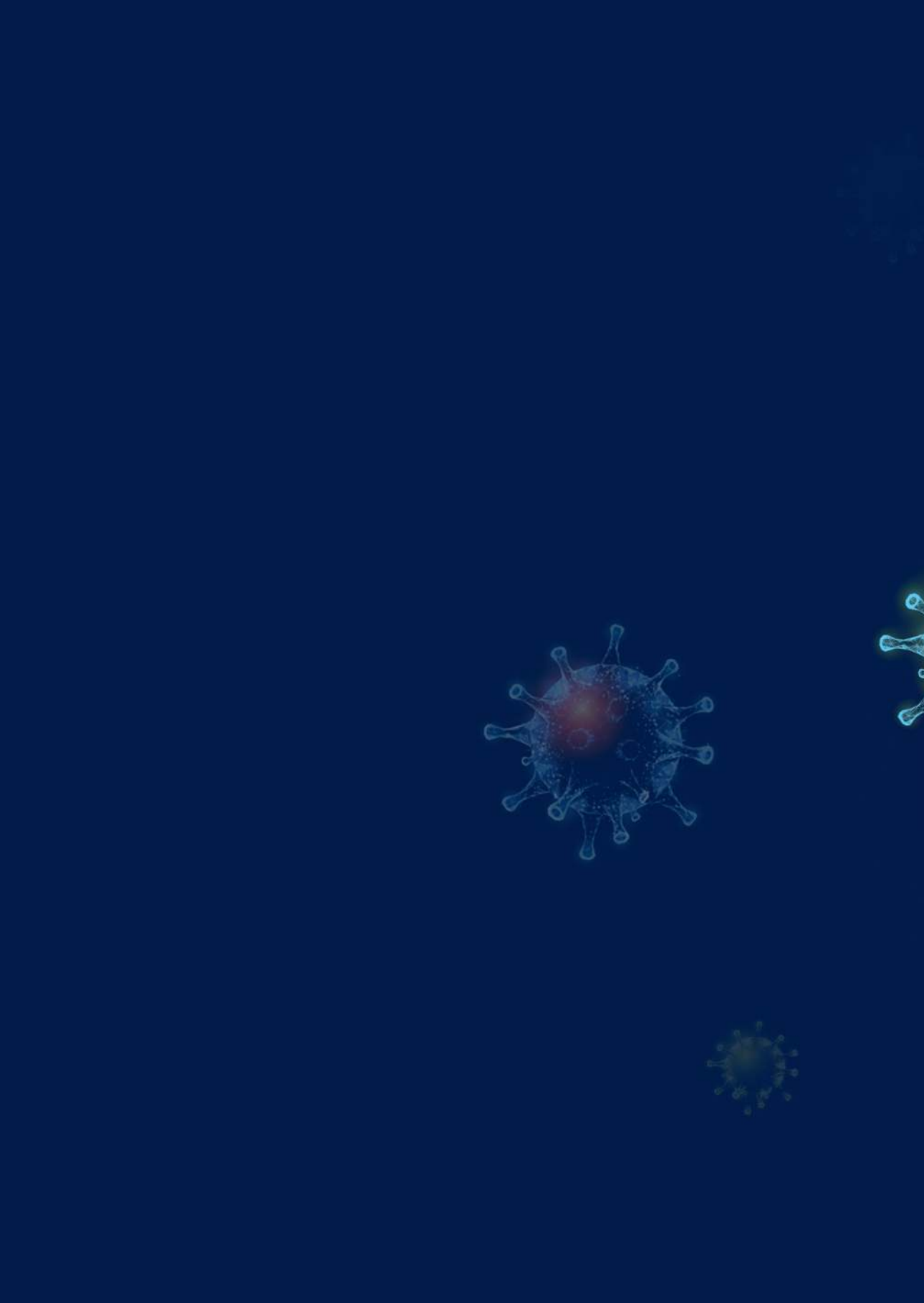
			CD4 TM pre	CD4 TM M3	CD4 TM M12	CD4 CM pre	CD4 CM M3	CD4 CM M12	CD4 EM pre	CD4 EM M3	CD4 EM M12
Donors	CMV IgG neg	median	0,034	0,054	0,048	0,035	0,033	0,031	0,035	0,108	0,082
		25% percentile	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
		75% percentile	0,153	0,219	0,305	0,159	0,198	0,360	0,138	0,231	0,153
	CMV IgG pos	median	0,106	0,176	0,159	0,105	0,173	0,142	0,044	0,088	0,120
		25% percentile	0,034	0,059	0,078	0,034	0,064	0,092	0,014	0,039	0,027
		75% percentile	0,309	0,293	0,425	0,375	0,206	0,523	0,155	0,194	0,198
	CMV neg vs. pos	P-value	0,151	0,179	0,069	0,246	0,246	0,126	0,536	0,860	0,536

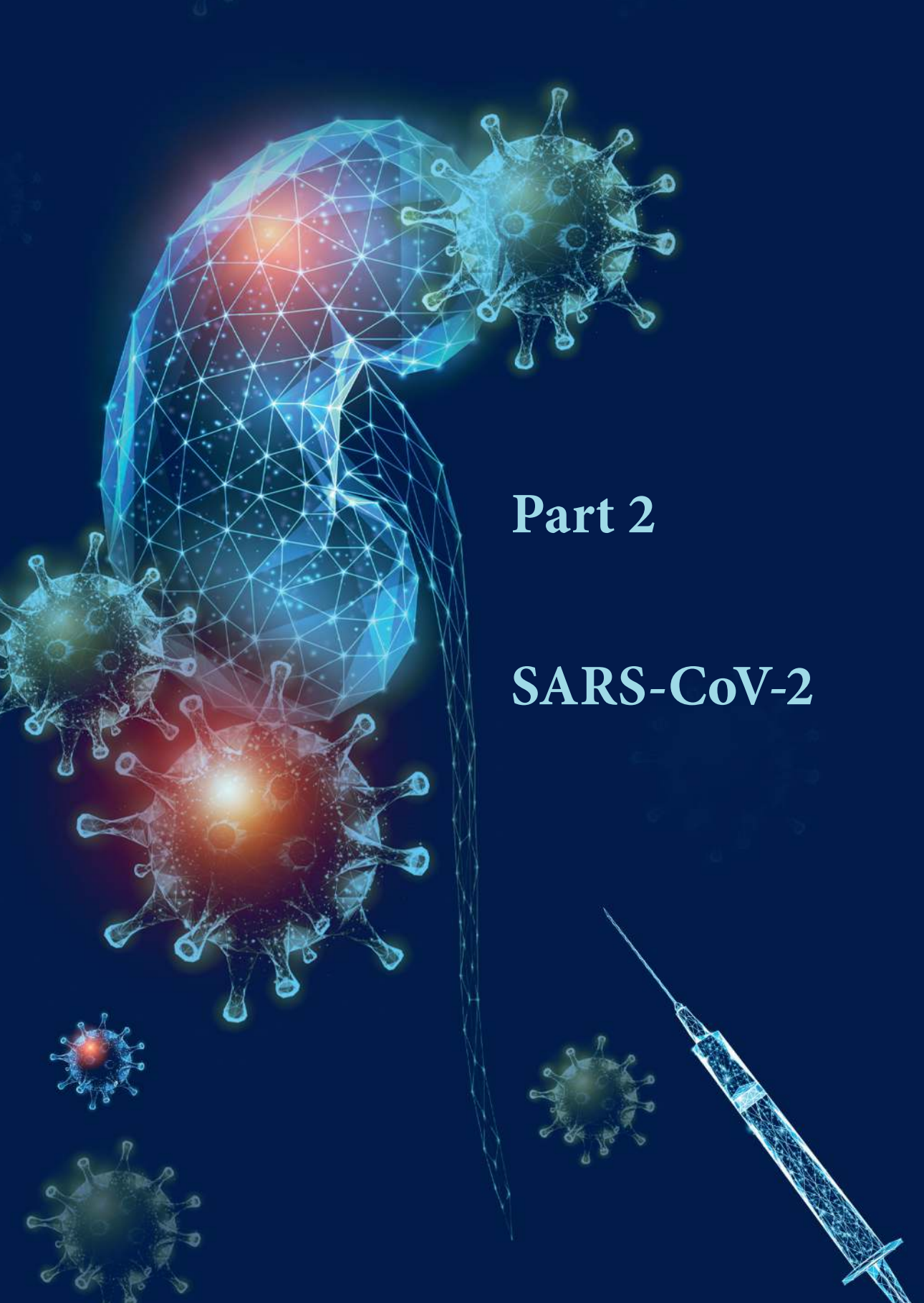
			CD8 TM pre	CD8 TM M3	CD8 TM M12	CD8 CM pre	CD8 CM M3	CD8 CM M12	CD8 EM pre	CD8 EM M3	CD8 EM M12
Donors	CMV IgG neg	median	0,063	0,040	0,047	0,104	0,024	0,065	0,056	0,070	0,043
		25% percentile	0,022	0,000	0,000	0,044	0,000	0,001	0,000	0,012	0,000
		75% percentile	0,138	0,106	0,216	0,169	0,105	0,270	0,177	0,168	0,245
	CMV IgG pos	median	0,019	0,026	0,019	0,028	0,002	0,029	0,006	0,029	0,025
		25% percentile	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
		75% percentile	0,072	0,155	0,057	0,070	0,217	0,342	0,039	0,070	0,099
	CMV neg vs. pos	P-value	0,211	0,659	0,596	0,126	0,930	0,659	0,375	0,285	0,536

			B cell pre	B cell M3	B cell M12	VZV-IgG T=0 vacc	VZV-IgG T=1 mo	VZV-IgG T=3 mo	VZV-IgG T=12 mo
Donors	CMV IgG neg	median	0,460	0,820	0,775	1239,0	2499,0	2175,0	1381,0
		25% percentile	0,325	0,650	0,568	825,3	1747,0	1466,5	1308,5
		75% percentile	0,875	1,018	1,123	1648,0	3787,5	3087,5	1811,5
	CMV IgG pos	median	0,470	0,870	0,870	1146,7	2989,5	2393,0	2091,0
		25% percentile	0,320	0,570	0,558	659,3	2097,5	2012,0	1703,0
		75% percentile	0,720	1,155	1,150	1748,8	3866,5	3162,8	2516,0
		P-value	1,000	0,762	0,818	0,877	0,369	0,600	0,051

Table S4. VZV-specific T cell responses in patients with and without a kidney transplant <1 year post-vaccination and in donors

			CD4 TM M12	CD4 CM M12	CD4 EM M12	CD8 TM M12
Recipients	**KT<1y: no**	**median**	0,164	0,253	0,056	0,020
		25% percentile	0,054	0,069	0,022	0,004
		75% percentile	0,271	0,673	0,268	0,073
	KT<1y: yes	**median**	0,224	0,298	0,096	0,075
		25% percentile	0,083	0,111	0,000	0,005
		75% percentile	0,458	0,413	0,168	0,299
Donors		**median**	0,106	0,117	0,070	0,038
		25% percentile	0,050	0,028	0,024	0,000
		75% percentile	0,332	0,374	0,145	0,183
	KT<1y no vs. yes	**P-value**	0,351	0,904	0,887	0,168
	KT<1y no vs. Donors	**P-value**	0,872	0,467	0,989	0,357
	KT<1y yes vs. Donors	**P-value**	0,233	0,221	0,873	0,327





Part 2

SARS-CoV-2

5

The RECOVAC IR study: the immune response and safety of the mRNA-1273 COVID-19 vaccine in patients with chronic kidney disease, on dialysis, or living with a kidney transplant.

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Nephrol Dial Transplant. 2021 Aug 27;36(9):1761-1764

Coronavirus disease 2019 (COVID-19) is associated with severe morbidity and mortality in patients with chronic kidney disease (CKD), on dialysis and kidney transplant recipients.[1,2] Although effective COVID-19 vaccination would lead to great clinical benefit, most studies with the presently available vaccines have excluded aforementioned patients. The resulting lack of data is a problem, because vaccine efficacy is known to be considerably lower in patients with CKD and renal replacement therapy.[3] Recent reports suggested that only a minority of kidney transplant recipients developed anti-severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) antibodies after messenger RNA (mRNA) COVID-19 vaccination.[4,5]

The REnal patients COVID-19 VACcination Immune Response (RECOVAC IR) study (ClinicalTrials.gov NCT04741386) aims to assess immunogenicity and safety of COVID-19 vaccination in these specific patient groups up to 12 months post-vaccination (Figure 1). This prospective, controlled multicenter study includes 4 different cohorts: (A) 175 patients with CKD stages 4/5 (CKD4/5) (estimated glomerular filtration rate <30 ml/min/1.73m^2), (B) 175 on dialysis, (C) 300 kidney transplant recipients and (D) 200 controls (family or household members) in 4 university medical centers across the Netherlands. Included are people >18 years of age, without previously known COVID-19, active malignancy or immune deficiency (Supplementary data, Table S1). Participants receive two doses of the mRNA-1273 COVID-19 vaccine (Moderna Biotech Spain, S.L.) with a 28-day interval.

The primary endpoint is the SARS-CoV-2 spike S1-specific IgG antibody concentration on day 28 after second vaccination, measured by a validated fluorescent bead based multiplex-immunoassay.[6] Classification as responders or non-responders is based on seroconversion. The threshold for seropositivity based on receiver operating characteristics curve analysis was set at 1,04 AU/mL or 10,08 binding antibody units (BAU)/ml according to the recently adopted National Institute for Biological Standards and Control/World Health Organization COVID-19 reference serum 20/136 in individuals without measurable anti-S antibodies at baseline.[7] The percentages of responders in cohorts A-C are compared with cohort D, as well as quantitative levels within and between cohorts to define groups that respond suboptimal to vaccination. Individuals who appear seropositive at baseline will be analysed separately.

Secondary endpoints are antibody longevity up to one year post vaccination and SARS-CoV-2-specific T and B cell responses. Neutralizing capacity of SARS-CoV-2-specific antibodies is determined by a plaque reduction neutralization assay in a subgroup of participants, guided by S1-specific IgG level outcome.[8] SARS-CoV-2 specific T cell response is measured by an interferon (IFN)-γ release assay (IGRA) on freshly collected whole blood and IFN-γ enzyme-linked immunosorbent spot assay (ELISpot) on cryopreserved peripheral blood mononuclear cells (PBMCs; Mabtech IFN-γ antibody pairs with alkaline phosphatase development). Results are expressed as IU IFN-γ per ml plasma (IGRA) or number of IFN-γ producing SARS-CoV-2 specific T cells per million PBMCs. Any spot

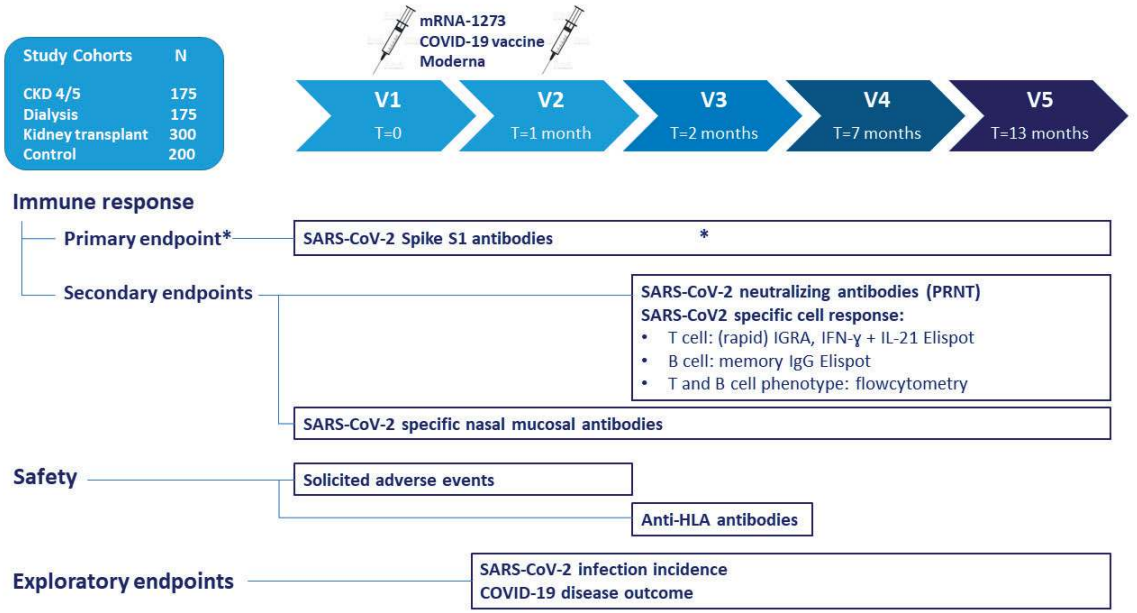

Figure 1.

Four cohorts of study participants attend 5 study visits. At V1 and V2 participants receive the mRNA-1273 COVID-19 vaccine (Moderna). SARS-CoV-2 spike S1 antibodies are measured at all time points, including baseline. **Primary endpoint is the antibody response at V3**. Secondary endpoints are SARS-CoV-2 neutralizing antibodies and specific T and B cell responses, measured at V3-5 and SARS-CoV-2 specific nasal mucosal antibodies, measured at all time points. Safety is monitored by questionnaires to register solicited adverse events during 7 days after every vaccination. In immunized patients, anti-HLA antibodies are monitored after vaccination. SARS-CoV-2 infection incidence and disease outcome from first vaccination to end of study are exploratory endpoints.

Visit 1: 1st vaccination. Visit 2: 2nd vaccination.

PRNT: plaque reduction neutralization assay. IGRA: interferon-γ release assay. IFN-γ: interferon-γ. IL-21: interleukin-21. HLA: human leucocyte antigen.

above the medium control is considered positive. The number and phenotype of SARS-CoV-2 specific T cells will be studied by flow cytometry with human leucocyte antigen (HLA) class I tetramers as previously described.[9,10] In-depth flow cytometric analyses for functional and phenotypical characterization of SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cell responses will be performed in a subset of patients by staining for typical phenotypic markers in combination with assessment of activation-induced markers (AIMs) and cytokine production after specific stimulation with overlapping peptide pools from the complete SARS-CoV-2 protein divided over 2 subpools (S1 and S2).[11,12] SARS-CoV-2 specific B cells will be enumerated and phenotyped by flow cytometry as previously published.[13] The frequency of SARS-CoV-2 specific memory B cells will be determined by ELISPOT.[14] Infection with SARS-CoV-2 occurs via the mucosal surface of the respiratory tract. To understand if and how antibody concentrations in serum correlate with those on the mucosal surface[15], nasal mucosal lining fluid is collected by non-invasive sampling (nasosorption) in a subset of patients. Induction, persistence and neutralizing capacity of mucosal antibodies against SARS-CoV-2 will be assessed and correlated to immune responses in the blood.

Solicited local and systemic adverse events, are reported during 7 days after each vaccination (Supplemental data, Questionnaire S1). The incidence and severity of COVID-19 is monitored for 1 year. The number of participants who underwent diagnostic testing, and the number and results of the tests are reported, as well as information about disease severity for participants with a positive test (Supplemental data, Questionnaire S2). In immunized patients, anti-HLA antibodies will be measured after vaccination.

Sample size calculation is based on the primary endpoint: induction and levels of SARS-CoV-2-specific antibodies. Based on published data, we expect a vaccine efficacy of 90% seroconversion in controls, while we assume a lower efficacy rate of 80% in both CKD4/5 and dialysis patients and of 65% in kidney transplant recipients, due to use of immunosuppressive medication and impaired kidney function. With a non-inferiority limit of 20%, alpha 0.05 and beta 0.2, 155 participants in the CKD4/5 and dialysis groups and 172 kidney transplant recipients are required. Assuming a drop-out rate of approximately 10% we include 200 participants in the control cohort, and 175 participants in the CKD4/5 and dialysis cohorts each. To allow analyses of the effects of time after transplantation and type of immunosuppressive medication, the number of kidney transplant recipients is expanded to 300.

As mRNA vaccines lead to endogenous antigen production and presentation, they are expected to induce balanced immune responses. Previous trials showed that mRNA-1273 vaccination leads to neutralizing antibody responses and induction of S-specific T cells. However, the exact correlates of protection against COVID-19 are still unknown. Moreover, larger scale and long-term measurements of both humoral and cellular immune responses to COVID-19 vaccination have not yet been performed in kidney disease patients.

Harmonization of methodology is crucial to enable the international scientific community to compare efficacy of different SARS-CoV-2 vaccines. We hope that our study design can serve as a reference and model for other studies in specific risk populations.

To study the "correlate of protection" of kidney disease patients after COVID-19 vaccination, as reflected by SARS-CoV-2 infection incidence and severity, additional large population-based studies are needed. Such studies should disclose which immunological test provides the best surrogate for protection against the presently most abundant variant and different variants of SARS-CoV2.

In conclusion, the results of the RECOVAC-IR study will reveal whether CKD patients, those on dialysis and kidney transplant recipients can be adequately protected against COVID-19 by vaccination, or whether other measures, like booster vaccinations, are required.

ETHICS APPROVAL

Approval was obtained from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL76215.042.21) and the local ethics committees of the participating centres (University Medical Center Groningen, Radboud University Medical Center, Amsterdam University Medical Center and Erasmus Medical Center).

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SUPPLEMENTAL MATERIALS

Table S1. Inclusion and exclusion criteria

Inclusion criteria
1. Eligible for COVID-19 vaccination as described by the instructions of the manufacturer
2. Age of 18 years or older
3. Capable of understanding purpose and risks of the study, given written informed consent
4. Either
A. CKD stages 4/5, with an eGFR <30 ml/min/1.73m^2 by CKD-EPI
B. Hemodialysis or peritoneal dialysis
C. Kidney transplant recipient at least 6 weeks after transplantation
D. Partner, sibling or household member of participating patient
Exclusion criteria
1. History of severe adverse reaction associated with a vaccine and/or severe allergic reaction (eg, anaphylaxis) to any component of the study intervention(s)
2. Multi-organ transplant recipients
3. Previous or active COVID-19 disease
4. Active (hematological) malignancy
5. Inherited immune deficiency
6. Infection with Human Immunodeficiency Virus (HIV)
7. Bleeding diathesis or condition associated with prolonged bleeding that would, in the opinion of the investigator, contraindicate intramuscular injection
Additional criterion for patients with CKD stages 4/5, on dialysis and controls:
- Maintenance treatment with immunosuppressive therapy in the 6 months before inclusion, including cytotoxic agents or systemic corticosteroids
Additional criterion for kidney transplant recipients:
- Administration of alemtuzumab, ATG, or rituximab in the 3 months before inclusion
Additional criterion for controls:
- Severely impaired kidney function, eGFR < 45 ml/min/1.73m^2 by CKD-EPI

CKD: chronic kidney disease. eGFR: estimated glomerular filtration rate. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.
ATG: anti-thymocyte globulin.

Questionnaire S1. Sollicited AEs

Participant number: ……. Day: ……..(1-7) after vaccination:…(1 or 2)

Date: ……..-…….-……

Questionnaire side effects RECOVAC study

Please complete this questionnaire every day, from the day of each vaccination up to and including 7 days after each vaccination.

We are interested in possible side effects that you have had in the last 24 hours.

Please answer all the questions by selecting the answer that applies most to you. There are no "correct" or "incorrect" answers. The information will be treated strictly confidentially.

Arthralgia (for example wrist or knee)	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity
Fatigue	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity
Fever	☐ None ☐ Between 38,0 en 38,4 ◦C ☐ Between 38,5 en 38,9 ◦C ☐ 39,0 ◦C of higher
Chills	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity
Headache	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity
Myalgia	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity

Participant number: ……. Day: ……..(1-7) after vaccination:...(1 or 2)

Date: .....-……-…….

Nausea	☐ None ☐ Some, but no interference with activity ☐ Moderate, interference with activity ☐ Significant, prevents daily activity
Redness at injection site	☐ None ☐ Yes, 2,5 to 5,0 cm ☐ Yes, 5,1 to 10 cm ☐ Yes, more than 10 cm
Swelling at injection site	☐ None ☐ Yes, 2,5 to 5,0 cm ☐ Yes, 5,1 to 10 cm ☐ Yes, more than 10 cm
Pain at injection site	☐ None ☐ Some, but no interference with activity ☐ Moderate: - interference with activity, or - needed analgesic more than once, but NO maar GEEN strong analgesic such as morfine or oxycodon ☐ Significant: - prevents daily activity, or - needed strong analgesic such as morfine or oxycodon

Questionnaire S2. Incidence and outcome COVID-19

Corona Questionnaire RECOVAC study

Participant number	
Date	
Time of completion	☐ Prior to 2nd vaccination ☐ 28 days after 2nd vaccination ☐ 6 months after 2nd vaccination ☐ 12 months after 2nd vaccination

Please complete this questionnaire within 7 days of receipt and return it.

If you have any questions, please contact:

Version 3, 26-01-2021

Instructions how to answer the questions

For most questions you can choose from a number of answers. Tick the box with the answer of your choice. Example: you have a black car. With the question below, tick the box with answer 5 and fill in "black" on the dotted line.

What color is your car?

1 ☐ Not applicable; I don't have a car

2 ☐ Red

3 ☐ White

4 ☐ Blue

5 ☒ A different color, namely: **Black**……

If you have made a mistake: Example: you have a black car but accidentally ticked answer 2 Red, then tick the box of your choice and put an arrow in front of the correct answer. Blot out the wrong answer.

What color is your car?

1 ☐ Not applicable; I don't have a car

~~2 ☒ Red~~

3 ☐ Wit

4 ☐ Blauw

→ 5 ☒ A different color, namely: **Black**……

Usually you have to answer every question. Only if it is clearly stated, you may skip answers.

- You may only tick one answer per question, unless the question states that you can give multiple answers.
- If there are dotted lines, you are expected to fill in the requested information accordingly.
- Use a black or dark blue pen.
- To be clear: this is not a test; there are no 'right' or 'wrong' answers.

The following questions are about **the period between your last study appointment and the current appointment.**

1. Did you take a corona test?
   - ☐ No
   - ☐ Yes, actually ….. (number) times

2. On what date was the test taken? …./…./…. (day/month/year)

3. What was the result of the corona test?
   - ☐ **Negative**, so I did **NOT** a coronavirus infection, proceed to question 10
   - ☐ **Positive**, so I **DID** have a coronavirus infection, proceed to question 4
   - ☐ Unknown

4. Where was your corona test taken? (multiple answers are possible)
   - ☐ At the Municipal Health Department
   - ☐ In the hospital
   - ☐ At a commercial test location
   - ☐ At hom, by the general practitioner
   - ☐ Somewhere else, namely ……..
   - ☐ Unknown

5. Have you been hospitalized **due to a coronavirus infection**?
   - ☐ No
   - ☐ Yes
   - ☐ I was admitted for something else but also appeared to have corona

6. Have you had oxygen treatment **due to a coronavirus infection**?
   - ☐ No
   - ☐ Yes
   - ☐ I already had oxygen treatment before I got corona

7. Have you been admitted to the intensive care unit **due to a coronavirus infection**?
   - ☐ No
   - ☐ Yes
   - ☐ I was admitted to intensive care for something else but also appeared to have corona

8. Have you been on life support (kept in artificial sleep with a breathing tube in your throat) **due to a coronavirus infection?**
   - ☐ No
   - ☐ Yes

9. Have you used prednisone or dexamethasone for more than 7 days in a row **due to a coronavirus infection**?
   - ☐ No
   - ☐ Yes
   - ☐ Unknown

10. Date of questionnaire completion: ……/……/..... (day/month/year)

6

Alternative strategies to increase the immunogenicity of COVID-19 vaccines in kidney transplant recipients not responding to two or three doses of an mRNA vaccine. A randomized clinical trial.

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Both authors contributed equally as first* and last ** authors

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ABSTRACT

Background An urgent need exists to improve the suboptimal COVID-19 vaccine response in kidney transplant recipients (KTRs). We aimed to compare three alternative strategies with a control single dose mRNA-1273 vaccination: a double vaccine dose, heterologous vaccination, and temporary discontinuation of mycophenolate mofetil or mycophenolic acid.

Methods This open-label randomised trial, done in four university medical centres in the Netherlands, enrolled KTRs without seroconversion after two or three doses of an mRNA vaccine. Between Oct 20, 2021, and Feb 2, 2022, 230 KTRs were randomly assigned block-wise per centre by a web-based system in a 1:1:1 manner to receive 100 μg mRNA-1273, 2 × 100 μg mRNA-1273, or Ad26.COV2-S vaccination. In addition, 103 KTRs receiving 100 μg mRNA-1273, were randomly assigned 1:1 to continue (mycophenolate mofetil+) or discontinue (mycophenolate mofetil–) mycophenolate mofetil or mycophenolic acid treatment for 2 weeks. The primary outcome was the percentage of participants with a spike protein (S1)-specific IgG concentration of at least 10 binding antibody units per mL at 28 days after vaccination, assessed in all participants who had a baseline measurement and who completed day 28 after vaccination without SARS-CoV-2 infection. Safety was assessed as a secondary outcome in all vaccinated patients by incidence of solicited adverse events, acute rejection or other serious adverse events. This trial is registered with ClinicalTrials.gov, NCT05030974 and is closed.

Findings Between April 23, 2021, and July 2, 2021, of 12 158 invited Dutch KTRs, 3828 with a functioning kidney transplant participated in a national survey for antibody measurement after COVID-19 vaccination. Of these patients, 1311 did not seroconvert after their second vaccination and another 761 not even after a third. From these seronegative patients, 345 agreed to participate in our repeated vaccination study. Vaccination with 2 × mRNA-1273 or Ad26.COV2-S was not superior to single mRNA-1273, with seroresponse rates of 49 (68%) of 72 (95% CI 56–79), 46 (63%) of 73 (51–74), and 50 (68%) of 73 (57–79), respectively. The difference with single mRNA-1273 was −0·4% (−16 to 15; p=0·96) for 2 × mRNA-1273 and −6% (−21 to 10; p=0·49) for Ad26.COV2-S. Mycophenolate mofetil– was also not superior to mycophenolate mofetil+, with seroresponse rates of 37 (80%) of 46 (66–91) and 31 (67%) of 46 (52–80), and a difference of 13% (−5 to 31; p=0·15). Local adverse events were more frequent after a single and double dose of mRNA-1273 than after Ad26.COV2-S (65 [92%] of 71, 67 [92%] of 73, and 38 [50%] of 76, respectively; p<0·0001). No acute rejection occurred. There were no serious adverse events related to vaccination.

Interpretation Repeated vaccination increases SARS-CoV-2-specific antibodies in KTRs, without further enhancement by use of a higher dose, a heterologous vaccine, or 2 weeks discontinuation of mycophenolate mofetil or mycophenolic acid. To achieve a stronger response, possibly required to neutralise new virus variants, repeated booster vaccination is needed.

INTRODUCTION

Kidney transplant recipients (KTRs) are at risk for a severe course of COVID-19 with a high mortality rate.[1] Although effective COVID-19 vaccination is therefore of great importance, the humoral and cellular immune response after two primary mRNA-based vaccinations is severely diminished in KTRs, especially when their immunosuppressive regimen contains mycophenolate mofetil or mycophenolic acid.[2] Consequently, administration of additional vaccine doses to KTRs has become common practice. However, even after a third or fourth vaccination, a considerable proportion of organ transplant recipients remains a serological non-responder.[3] It is therefore imperative to investigate whether alternative vaccination strategies could be more immunogenic.[4]

A potential option to increase immunogenicity of repeated COVID-19 vaccination is to increase vaccine dose, as is also applied for hepatitis B vaccination in patients receiving haemodialysis and for influenza vaccination in organ transplant recipients.[5] Applying a multisite injection regimen could provide additional stimulation of the immune system. [6] A second option could be to use different combinations of vaccines, so-called heterologous vaccination. Studies have suggested that heterologous prime-boost vaccination regimes (vector-based followed by mRNA) could result in a stronger immune response compared with homologous regimes.[7] Finally, the strong negative association between the use of mycophenolate mofetil–mycophenolic acid and vaccine immunogenicity[2] suggests that temporary discontinuation of the use of these drugs might improve the immune response to vaccination.

Based on these considerations, we designed a randomised clinical trial to compare the immunogenicity of a double dose of the mRNA-1273 vaccine, heterologous vaccination with Ad26.COV2-S, and temporary discontinuation of mycophenolate mofetil or mycophenolic acid to the immunogenicity of a control single dose mRNA-1273 vaccination. This trial was done in KTRs who were serological non-responders after two or three doses of an mRNA-based vaccine.

METHODS

Study design

This prospective, open-label, randomised, controlled trial was done between Oct 20, 2021, and Feb 5, 2022, in four university medical centres in the Netherlands (Amsterdam UMC, UMC Groningen, Radboudumc Nijmegen, and Erasmus MC Rotterdam), as part of the Dutch Renal patients COVID-19 VACcination (RECOVAC) study. Ethical approval was obtained from the Dutch Central Committee on Research Involving Human Subjects, the

central ethics committee at the UMC Groningen, and the local ethics committees of the participating centres.

Patients

Between April 23, 2021, and July 2, 2021, all adult patients with a functioning kidney transplant in the Netherlands were asked to participate in a study for antibody measurement after COVID-19 vaccination. Patients who had given informed consent, either electronically or in writing, were sent a finger prick package to collect a blood sample at home between 14 and 56 days after COVID-19 vaccination.[8] A central laboratory did the anti-SARS-CoV-2 RBD IgG ELISA assay. For this assay, which was used to identify seronegative patients from our national survey, the validated cutoff concentration for seropositivity is ≥50 binding antibodies units (BAU)/mL.[8,9]

For the present study, we invited patients without seroconversion at 14–56 days after the second or third dose of an mRNA-based COVID-19 vaccine, either mRNA-1273 (Moderna Biotech Spain, Madrid, Spain) or BNT162b2 (BioNTech/Pfizer, Mainz, Germany), or a combination of both (Figure 1). Patients who had COVID-19 (defined as a reported positive SARS-CoV-2 PCR-test or presence of nucleocapsid-specific antibodies) before or during this study were excluded. Detailed inclusion and exclusion are provided in the appendix (Table S1).

Randomisation

The study was done in two different cohorts. In cohort one, KTRs receiving any combination of immunosuppressive drugs were included. These patients were randomly assigned in a 1:1:1 manner to receive either a single dose of the mRNA-1273 vaccine (100 μg, intramuscularly), two doses of mRNA-1273 simultaneously in both upper arms (2 × 100 μg, intramuscularly), or the Ad26.COV2-S vaccine (Janssen Biologics, Leiden, The Netherlands; 5×10^{10} viral particles, intramuscularly). This cohort is referred to as the alternative vaccination study group. In cohort two, only patients receiving triple immunosuppressive therapy consisting of a calcineurin inhibitor, mycophenolate mofetil or mycophenolic acid, and steroids were included. These patients were randomly assigned to either continuation of mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil+) or discontinuation of mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil–) from 1 week before until 1 week after vaccination with a single 100 μg intramuscular dose of the mRNA-1273 vaccine. This cohort is referred to as the mycophenolate mofetil– mycophenolic acid discontinuation study group. In both study groups, randomisation was done block-wise per centre, by means of the web-based randomisation system ALEA (FormsVision, Abcoude, Netherlands). Patients could only participate in one cohort. Masking was infeasable as a proportion of patients was assigned to receive 2 × mRNA-1273 in both upper arms or to temporarily discontinue mycophenolate mofetil or mycophenolic acid.

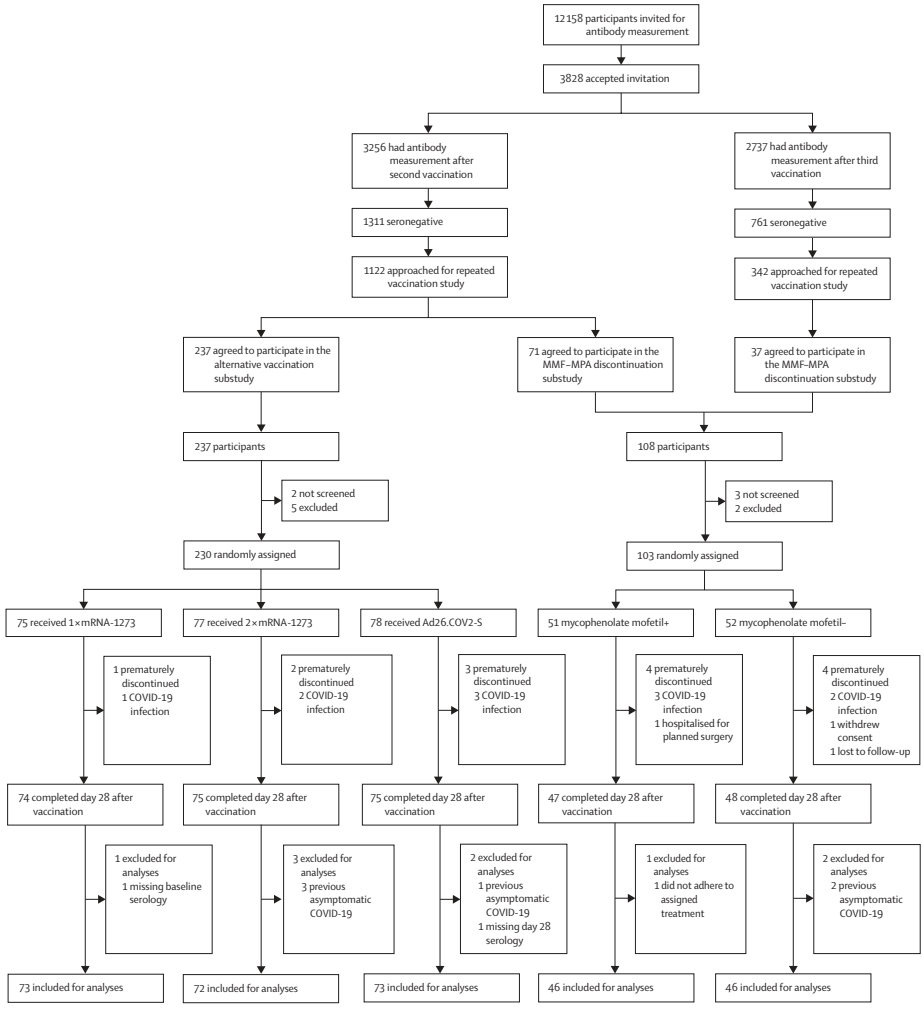

Figure 1. Flowchart of the vaccination study
Kidney transplant recipients with available antibody measurements after COVID-19 vaccination; study enrolment and outcomes in alternative vaccination study group; study enrolment and outcomes in the MMF/MPA discontinuation study group. MMF/MPA=mycophenolate mofetil–mycophenolic acid.

Procedures

In both study groups, blood samples were collected at baseline (ie, before vaccination) and at 28 days after vaccination. In cohort two, an additional blood sample was collected at 1 and 2 weeks after discontinuing mycophenolate mofetil or mycophenolic acid mainly to monitor kidney transplant function. Questionnaires were used to report solicited local and systemic adverse events for 7 days after vaccination and to monitor occurrence of SARS-CoV-2 infections. A detailed overview of study visits and assessments is provided in the appendix (Table S2).

Outcomes

Primary outcome was the percentage of participants with a spike protein (S1)-specific IgG concentration of at least 10 BAU/mL at 28 days after vaccination, assessed in all participants who had a baseline measurement and who completed day 28 after vaccination without SARS-CoV-2 infection. As a post-hoc sensitivity analysis, we also assessed the percentage of responders to vaccination after exclusion of the patients who appeared to be anti-S1 IgG positive at time of repeated vaccination. Secondary outcomes were the serum concentration of S1-specific IgG, the presence of virus neutralising antibodies, SARS-CoV-2 specific T-cell response and safety, all collected at 28 days after vaccination. Exploratory outcomes were the association between baseline clinical and immunological parameters on the one hand and the primary outcome on the other. Post-hoc added exploratory outcomes were the correlation between neutralising activity against the ancestral, delta, and omicron strains and S1-specific IgG concentration, the correlation between S1-specific IFN-γ spot-forming cells (SFCs) and the concentration of S1-specific antibodies, and the correlation between the results of both T-cell assays. The prespecified outcomes anti-SARS-CoV-2 antibodies in nasal fluid (secondary) and SARS-CoV-2 reactive $CD4^+$ and $CD8^+$ cells and RNA-seq analysis (exploratory) will be reported separately.

S1-specific IgG was measured in serum samples by a validated fluorescent bead-based multiplex-immunoassay as described previously[10,11] and expressed as BAU/mL. Patients were classified as seropositive or seronegative based on a threshold for seropositivity for this specific assay, defined by a receiver operator curve analysis at a S1-specific IgG concentration of at least 10 BAU/mL.[11,12]

To identify patients who had a SARS-CoV-2 infection before study entry, nucleocapsid-specific antibodies were measured at baseline by multiplex immunoassay, as previously described,[10] and classified as positive or negative (cutoff for positivity set at ≥22 arbitrary units per mL).[13]

Plaque reduction neutralisation tests against the ancestral, delta, and omicron SARS-CoV-2 variants were done as previously described.[2,12,14] For feasibility, it was a priori decided to measure neutralising antibodies only in a random sample of 25 KTRs in each study group.

SARS-CoV-2-specific T-cell responses were measured in subsets of patients by means of an interferon-gamma (IFN-γ) ELIspot assay and a commercially available IFN-γ release assay (IGRA) as previously described.[12,15] The ELISpot assay was done in the same random sample of patients selected for the measurement of neutralising antibodies. IGRA was done in 95 KTRs included in the alternative vaccination study group at one participating centre (Erasmus MC).

Safety was assessed in all vaccinated patients in terms of incidence of solicited local and systemic adverse events within 1 week after vaccine administration graded according to severity. Participants reported these adverse events daily on a specific form. The incidence of acute rejection and other serious adverse events was monitored until 28 days after vaccine

administration. Information on SARS-CoV-2 infection and outcome of COVID-19 was collected by means of a questionnaire, completed at 28 days after vaccination.

Statistical analyses

The sample size was established to test the superiority of alternative vaccination strategies. In cohort one, assuming a response rate of 45% with the two alternative strategies (ie, 2 × mRNA-1273 and Ad26.COV2.S) compared with the 20% that was expected with a single dose of 1 × mRNA-1273, and a superiority margin of 5%, a group size of 89 was required to achieve a power of 80% and a level of significance of 2·5% (corrected from 5% because of multiple testing). To account for dropouts, we aimed to include 100 patients in each group. In cohort two assuming a superior response rate of 45% in patients with temporary discontinuation of mycophenolate mofetil or mycophenolic acid compared with the 20% that was expected with continuation of mycophenolate mofetil or mycophenolic, and a superiority margin of 5%, a group size of 71 was required to achieve a power of 80% and a level of significance of 5%. To account for dropouts, we aimed to include 80 patients in each group.

Continuous data are presented as mean SD or as median IQR in case of non-normal distribution. Categorical data are presented as percentages. Differences between groups were tested by means of an independent t test, Mann-Whitney-U test, Wilcoxon Singed Rank test (for within-group comparisons), or Pearson χ^2 test, depending on data type and distribution. Correlations were tested by means of the Pearson correlation with log transformation of data in case of non-normal distribution. In post-hoc subgroup analyses, the effect of vaccination strategies was compared after participants were stratified for sex (male or female), age (≥60 or <60 years), estimated glomerular filtration rate (eGFR; ≥45 or <45 mL/min per 1·73 m^2), time after last kidney transplantation (≥6·5 or <6·5 years), first kidney transplantation (yes or no), and in the alternative vaccination study group, the use of mycophenolate mofetil or mycophenolic acid (yes or no). The association between baseline clinical parameters and the seroresponse at 28 days after vaccination was assessed by means of multivariable logistic regression analyses. All analyses were done with IBM SPSS statistics version 23·0 (SPSS, Chicago, IL). Figures were created with GraphPad Prism version 5·00 (GraphPad Software, San Diego, CA). A two-sided p value of less than 0·05 was adopted to denote significance, and corrected in case of multiple testing by means of Bonferroni correction unless stated otherwise. The study is funded by The Netherlands Organization for Health Research and Development and the Dutch Kidney Foundation, and is registered with www.ClinicalTrials.gov, NCT05030974.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

From April 23, 2021, until July 2, 2021, of 12 158 invited Dutch KTRs, 3828 with a functioning kidney transplant were included in a national survey for antibody measurement after COVID-19 vaccination. Of these patients, 1311 did not seroconvert after their second vaccination and another 761 not even after a third. From these seronegative patients, 345 participated in our repeated vaccination study. A detailed flow chart is provided as Figure 1.

In the alternative vaccination study group, 230 patients were randomly assigned and in 218 patients, analysis of S1-specific antibody concentrations was done at 28 days after vaccination: 73 received a regular single dose mRNA-1273 (control group), 72 received double dose mRNA-1273 and 73 received Ad26.COV2-S (Figure 1).

In the mycophenolate mofetil–mycophenolic discontinuation study group, 103 patients were randomly assigned and in 92 patients analysis of S1-specific antibody concentrations was done at 28 days after vaccination: 46 continued mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil+), and 46 discontinued mycophenolate mofetil or mycophenolic acid from 1 week before to 1 week after vaccination (mycophenolate mofetil–; Figure 1). Baseline characteristics of all participants were similar between the groups (Table 1).

In the alternative vaccination study group, the differences in seropositivity rate at day 28 after vaccination were −0·4% (95% CI−16 to 15; p=0·96) for the 2 × mRNA-1273 group and −6% (−21 to 10; p=0·49) for the Ad26.COV2-S group compared with the 1 × mRNA-1273 group. The corresponding seropositivity rates were 50 (68%) of 73 (57 to 79) in the 1 × mRNA-1273 control group, 49 (68%) of 72 (56 to 79) in the 2 × mRNA-1273 group, and 46 (63%) of 73 (51 to 74) in the Ad26.COV2-S group (Figure 2A, left panel). The median concentration of S1-specific antibodies at day 28 after vaccination was not significantly different: 156 BAU/mL (2·47 to 797) in the 1 × mRNA-1273 control group, 92·2 BAU/mL (1·77 to 648; p=0·94) in the 2 × mRNA-1273 group, and 74·7 BAU/mL (1·60 to 250; p=0·10) in the Ad26.COV2-S group (Figure 2B, left panel). The increase from baseline in antibody concentration was significant in each of the three groups, and these increases did not differ between groups (p=0·85 and p=0·11 vs control, respectively). 20 patients in the 1 × mRNA-1273 control group, 16 in the 2 × mRNA-1273 group, and 11 in the Ad26.COV2-S group had S1-specific antibodies of at least 10 BAU/mL at baseline. When these patients were excluded in a sensitivity analysis, seroconversion rate was 31 (58%) of 53 (44 to 72) in the 1 × mRNA-1273 control group, 33 (59%) of 56 (45 to 72) in the 2 × mRNA-1273 group, and 35 (56%) of 62 (43 to 69) in the Ad26.COV2-S group, again not significantly different (p=0·96 and p=0·83 vs control, respectively; appendix Figure S1A). S1-specific antibody concentration at day 28 also did not significantly differ between these groups (p=0·88 and p=0·76; appendix Figure S1B).

In the mycophenolate mofetil–mycophenolic acid discontinuation study group, the difference in seropositivity rate at day 28 after vaccination was 13% (−5 to 31) for the my-

Table 1. Baseline characteristics

	Alternative vaccination study group			MMF/MPA discontinuation study group	
	1x mRNA-1273 (n=73)	**2x mRNA-1273 (n=72)**	**Ad26.COV2-S (n=73)**	**MMF+ (n=46)**	**MMF- (n=46)**
Female, n (%)	25 (34)	27 (38)	25 (34)	24 (52)	17 (37)
Ethnicity, n (%)					
Caucasian	68 (93)	68 (94)	65 (89)	46 (100)	45 (98)
Asian	5 (7)	1 (1)	7 (20)	0	1 (1)
Black	0	2 (3)	1 (1)	0	0
Age (years)	57·3 ± 13·5	58·5 ± 11·6	60·1 ± 12·4	59·0 ± 11·8	60·5 ± 12·0
BMI (kg/m^2)	26·7 ± 5·64	26·0 ± 3·90	26·6 ± 4·97	26·4 ± 4·72	26·6 ± 3·75
SBP (mmHg)	149 ± 24	145 ± 18	146 ± 22	141 ± 14	145 ± 20
DBP (mmHg)	85 ± 11	84 ± 11	83 ± 12	85 ± 9	84 ± 11
Number of comorbidities	2 (1-2)	1 (1-2)	1 (1-2·5)	1 (1-2)	1 (1-2)
Comorbidities, n (%)					
Hypertension	65 (89)	58 (81)	64 (88)	36 (78)	35 (76)
Diabetes Mellitus	25 (34)	16 (22)	22 (30)	9 (20)	11 (24)
History of coronary artery disease	9 (12)	5 (7)	14 (19)	4 (9)	4 (9)
Heart failure	0	2 (3)	5 (7)	2 (4)	1 (2)
Chronic lung disease	3 (4)	8 (11)	8 (11)	3 (7)	2 (4)
History of malignancy[1]	11 (15)	15 (21)	7 (10)	3 (7)	10 (22)
Auto-immune disease	2 (8)	5 (7)	3 (4)	6 (13)	3 (7)
Lymphocytes (10^9/L)	1·4 (1·1-2·1)	1·5 (1·0-1·9)	1·3 (0·8-1·6)	1·3 (0·9-1·5)	1·2 (1·0-1·6)
eGFR (ml/min/1·73m^2)	49·7 ± 18·8	48·9 ± 18·8	49·0 ± 19·1	48·4 ± 16·0	50·4 ± 19·0
Primary renal diagnosis, n (%)					
Primary glomerulonephritis	11 (15)	12 (17)	11 (15)	8 (17)	4 (9)
Pyelonephritis	2 (3)	3 (4)	0	0	0
Interstitial nephritis	1 (1)	3 (4)	4 (5)	1 (2)	1 (2)
Familial/hereditary renal diseases	15 (21)	20 (28)	13 (18)	7 (15)	8 (17)
Congenital diseases	8 (11)	2 (3)	5 (7)	1 (2)	2 (4)
Vascular diseases	6 (8)	5 (7)	8 (11)	6 (13)	2 (4)
Secondary glomerular/systemic disease	8 (11)	9 (13)	9 (12)	0	1 (2)
Diabetic Kidney Disease	7 (10)	1 (1)	4 (5)	3 (7)	10 (11)
Other	5 (7)	4 (6)	6 (8)	14 (30)	14 (30)
Unknown	10 (14)	13 (18)	13 (18)	6 (13)	9 (20)
Transplant characteristics					
First kidney transplant, n (%)	64 (88)	55 (76)	58 (80)	40 (87)	39 (85)
Time after last transplantation (years)	5·8 (3·0-10·5)	7·3 (2·7-12·5)	6·9 (2·5-12·2)	4·1 (2·0-8·0)	4·5 (1·9-7·3)
Last transplant					
Living, n (%)	51 (70)	54 (75)	55 (75)	37 (80)	30 (65)
Pre-emptive, n (%)	31 (42)	33 (46)	28 (38)	27 (59)	16 (35)
Number of immunosuppressive agents	2 (2-3)	2 (2-3)	2 (2-3)	3 (3-3)	3 (3-3)

Table 1. Baseline characteristics *(Continued)*

	Alternative vaccination study group			MMF/MPA discontinuation study group	
	1x mRNA-1273 (n=73)	**2x mRNA-1273 (n=72)**	**Ad26.COV2-S (n=73)**	**MMF+ (n=46)**	**MMF- (n=46)**
Immunosuppressive treatment, n (%)					
Steroids	42 (58)	38 (53)	46 (63)	46 (100)	46 (100)
Azathioprine	4 (5)	4 (6)	1 (1)	0	0
Mycophenolate mofetil	57 (78)	60 (83)	58 (79)	46 (100)	46 (100)
Calcineurin inhibitor	61 (84)	60 (83)	60 (82)	46 (100)	46 (100)
mTor inhibitor	3 (4)	2 (3)	1 (1)	0	0
Other	0	2 (3)	2 (3)	0	1 (2)
Induction therapy, n (%)					
Basiliximab	52 (71)	54 (75)	45 (62)	42 (91)	37 (80)
Alemtuzumab	1 (1)	1 (1)	2 (3)	1 (2)	1 (2)
Antithymocyte globulin	1 (1)	0	3 (4)	1 (2)	2 (4)
Rituximab	12 (16)	8 (11)	12 (16)	1 (2)	1 (2)
None	7 (10)	7 (10)	11 (15)	0	2 (4)
Unknown	1 (1)	2 (3)	2 (3)	2 (4)	4 (9)
Number of previous SARS-CoV-2 vaccinations, n (%)					
2	73 (100)	72 (100)	73 (100)	33 (72)	31 (67)
3	0	0	0	13 (28)	15 (33)
Time since last SARS-CoV-2 vaccination (days)	198 (189-205)	198 (187-217)	198 (194-220)	180 (115-193)	179 (109-195)
Seropositive at baseline[2], n (%)	20 (27)	16 (22)	11 (15)	14 (30)	14 (30)

Variables are presented as mean ± SD, or as median (IQ interval) in case of non-normal distribution.

Abbreviations are: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.

[1] Including melanomas, excluding all other skin malignancies

[2] Seropositivity was defined as S1-specific IgG ≥ 10 BAU/mL

cophenolate mofetil– group compared with the mycophenolate mofetil+ group (p=0·15). The corresponding seropositivity rates were 31 (67%) of 46 (52 to 80) in the mycophenolate mofetil+ group and 37 (80%) of 46 (66 to 91) in the mycophenolate mofetil– group (Figure 2A, right panel). The median concentration of S1-specific antibodies at day 28 after vaccination was 143 (4·58–966) BAU/mL and 119 (23·0–1279) BAU/mL, respectively (p=0·29; Figure 2B, right panel). The increase in antibody concentration did not differ between the two groups (p=0·24). Fourteen patients in the mycophenolate mofetil+ group and 14 in the mycophenolate mofetil– group had S1-specific antibodies of at least 10 BAU/mL at baseline. When these patients were excluded, seroconversion rate was 17 (53%) of 32 (95% CI 35 to 71) in the mycophenolate mofetil+ group and 23 (72%) of 32 (53 to 86) in the mycophenolate mofetil– group (p=0·12; appendix Figure S2A) and again, also the median concentration of S1-specific antibodies at day 28 was not significantly different (p=0·17; appendix Figure S2B).

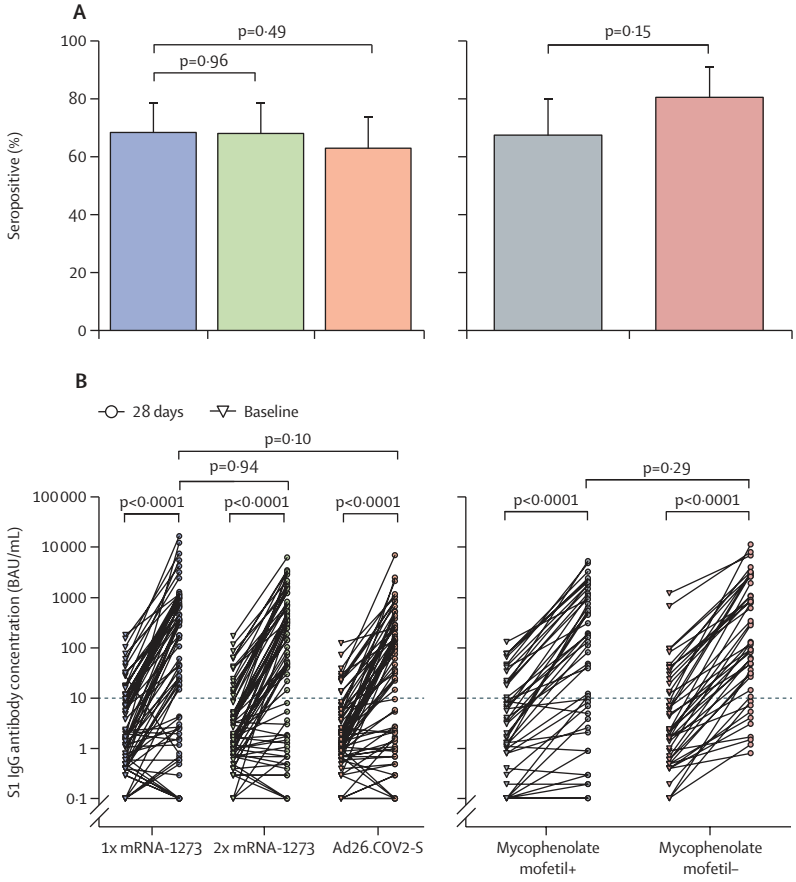

Figure 2. Serological response in the alternative vaccination study group (left panel) and the mycophenolate mofetil–mycophenolic acid discontinuation study group (right panel)

Proportion (95% CI) of seroresponders per randomisation group at 28 days after vaccination; responders were defined as participants with a S1-specific IgG antibody concentration ≥10 BAU/mL after vaccination; p values were calculated by means of the χ^2 test (A). SARS-CoV-2 Spike S1-specific serum IgG concentrations at baseline and 28 days after vaccination; depicted are dots representing each patient; dotted line indicates cutoff value for seropositivity; p values between groups were calculated by means of the Mann-Whitney U test and within groups with the Wilcoxon Signed Rank test (B). BAU= binding antibody units.

In a random selection of 25 patients per group from each study group, the neutralising activity of serum against the ancestral SARS-CoV-2 and the delta and omicron (BA.1) variants was assessed. In both the alternative vaccination study group and the mycophenolate mofetil–mycophenolic acid discontinuation study group, neutralising antibody concentrations at day 28 after vaccination were not significantly different between the groups (Figures 3A and 3B). Neutralising activity against the delta and especially against the omicron variant was lower than against the ancestral variant.

In the alternative vaccination study group, the proportion of patients with a positive response in the ELISpot assay at 28 days after vaccination was 11 (52%) of 21 (95% CI

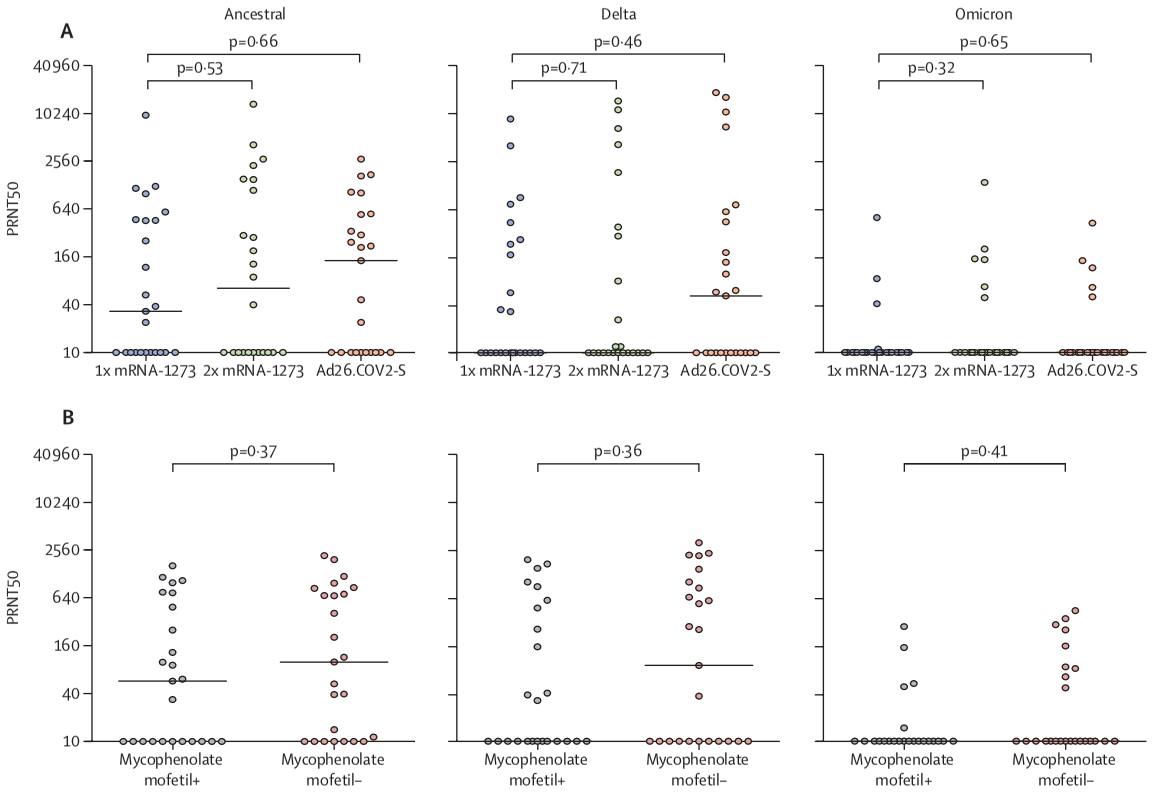

Figure 3. Neutralising antibody titres for the ancestral, delta, and omicron (BA.1) strain of SARS-CoV-2 at 28 days after vaccination in the alternative vaccination study group (A) and the mycophenolate mofetil– mycophenolic acid discontinuation study group (B)

p values were calculated by means of the Mann-Whitney U test. PRNT50=50% plaque reduction neutralisation test.

30–74) in the 1 × mRNA-1273 control group, 11 (52%) of 21 (30–74) in the 2 × mRNA-1273 group, and six (29%) of 21 (11–52) in the Ad26.COV2-S group (p=0·99 and p=0·12 vs control, respectively; Figure 4A, left panel). Median S1-specific IFN-γ SFCs/10^6 peripheral blood mononuclear cells (PBMCs) at 28 days did not differ between the three study groups (Figure 4B, left panel). At baseline, T-cell reactivity was found in a proportion of patients in the three groups: ten (45%) of 22 (24–68), seven (37%) of 19 (16–62), and nine (47%) of 19 (24–71), respectively (not significant). After exclusion of these patients, the proportion of patients with a positive response was five (42%) of 12 (15–72) in the 1 × mRNA-1273 control group, six (50%) of 12 (21–79) in the 2 × mRNA-1273 and 0 of nine in the Ad26.COV2-S group (p=0·68 and p=0·03 vs control, respectively).

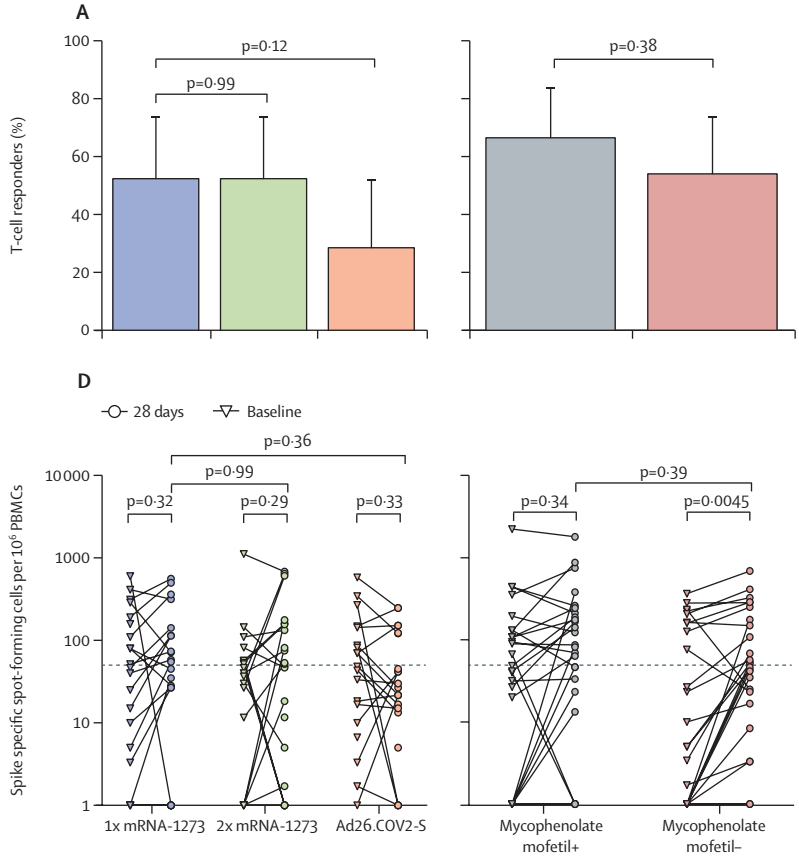

Figure 4. T-cell response measured by ELISpot in the alternative vaccination study group (left panel) and the mycophenolate mofetil–mycophenolic acid discontinuation study group (right panel)

Proportion (95% CI) of participants with response per randomisation group at 28 days after vaccination; p values were calculated by means of the χ^2 test (A). Spike specific IFN-γ SFCs/10^6 PBMCs at baseline and 28 days after vaccination; dotted line indicates threshold for T-cell response (≥50 spot forming cells/10^6 PBMCs); p values between groups were calculated by means of the Mann-Whitney U test and within groups with the Wilcoxon Signed Rank test (B). PBMCs=peripheral blood mononuclear cells.

In the mycophenolate mofetil–mycophenolic acid discontinuation study group, a positive response in the ELISpot assay at 28 days after vaccination was observed in 16 (67%) of 24 (95% CI 45–84) and 13 (54%) of 24 (32–74) of the mycophenolate mofetil+ and mycophenolate mofetil– groups, respectively (Figure 4A, right panel). Median S1-specific IFN-γ SFCs/10^6 PBMCs at 28 days did not differ between the mycophenolate mofetil+ and mycophenolate mofetil– groups (Figure 4B, right panel). T-cell reactivity at baseline was found in 12 (50%) of 24 (29–71) of the mycophenolate mofetil+ group and in nine (36%) of 25 (18–57) of the mycophenolate mofetil– group. After exclusion of these patients, the proportion of positive response was six (50%) of 12 (21–79) in the mycophenolate mofetil+ and six (40%) of 15 (16–68) in the mycophenolate mofetil– group (p=0·60).

In participants of the alternative vaccination study group included in one of the centres (Erasmus MC), the T-cell response was also assessed by an IGRA assay. The proportion of patients with a SARS-CoV-2-specific T-cell response at 28 days was five (17%) of 30 (6–35) in the 1 × mRNA-1273 control group, five (17%) of 29 (6–36) in the 2 × mRNA-1273 group, and five (17%) of 29 (6–36) in the Ad26.COV2-S group (p=0·95 and p=0·95 vs control, respectively; appendix Figure S3A). Median IFN-γ concentration at 28 days was not different between the three groups (appendix S3B).

Safety analysis was done in all patients who received a vaccination. In the alternative vaccination study group, the percentage of patients who reported any solicited adverse event after vaccination was significantly lower in patients who received the Ad26.COV2-S vaccine than in patients who received a single dose of the mRNA-1273 vaccine (60 [79%] of 76 vs 68 [96%] of 71; p=0·0024). This difference was mainly due to a lower percentage of patients with pain at the injection site in the Ad26. COV2-S group (Table 2). Only four serious adverse events (dehydration, diarrhoea, pneumonia, and COVID-19) were reported, three in the 1 × mRNA-1273 group and one in the Ad26.COV2-S group (Table 2). These serious adverse events were considered not related to vaccination. In the mycophenolate mofetil–mycophenolic acid discontinuation study group, the percentage of patients who reported any solicited adverse event after vaccination was not different between the mycophenolate mofetil+ and mycophenolate mofetil– groups (Table 2). Only two serious adverse events (cellulitis and COVID-19) were reported, one in the mycophenolate mofetil+ group and one in the mycophenolate mofetil– group. Serum creatinine at baseline and 28 days after vaccination was 133 (SD 46) μmol/L and 136 (48) μmol/L in the mycophenolate mofetil+ group (p=0·23), and 138 (60) μmol/L and 142 (55) μmol/L in the MMF– group (p=0·076).

For the exploratory outcomes, we first analysed the correlation between neutralising activity and S1-specific IgG concentration in each treatment group from both study groups (n=123). Neutralising activity against the ancestral, delta, and omicron strains correlated well with the concentrations of S1-specific IgG antibodies at 28 days after vaccination (ancestral R=0·88, p<0·0001; delta R=0·78, p<0·0001; omicron R=0·62, p<0·0001; appendix Figure S4). Notably, much higher S1-specific IgG concentrations were required for neutrali-

Table 2. Incidence of solicited adverse events up to 7 days after vaccination and serious adverse events until 28 days after vaccination.

	Alternative vaccination study group					MMF/MPA discontinuation study group		
	1x mRNA-1273	2x mRNA-1273	p-val[1]	Ad26.COV2-S	p-val[1]	MMF+	MMF-	p-val
Adverse events*	N=71	N=73		N=76		N=51	N=50	
Any adverse event, n (%)	68 (96)	72 (99)	0·30	60 (79)	0·0024	49 (96)	47 (94)	0·63
Any systemic symptom, n (%)	48 (68)	60 (82)	0·043	54 (71)	0·65	37 (73)	40 (80)	0·38
Arthralgia, n (%)	21 (30)	19 (26)	0·63	24 (32)	0·79	15 (29)	15 (30)	0·95
Fatigue, n (%)	36 (51)	44 (60)	0·25	37 (49)	0·81	28 (55)	23 (46)	0·37
Fever, n (%)	2 (3)	5 (7)	0·26	1 (1)	0·52	3 (6)	3 (6)	0·98
Chills, n (%)	15 (21)	27 (37)	0·036	13 (17)	0·54	18 (35)	12 (24)	0·21
Headache, n (%)	25 (35)	31 (42)	0·37	40 (53)	0·034	22 (43)	20 (40)	0·75
Myalgia, n (%)	32 (45)	43 (59)	0·10	31 (41)	0·60	19 (37)	22 (44)	0·49
Nausea, n (%)	13 (18)	16 (22)	0·59	12 (16)	0·68	10 (20)	9 (18)	0·84
Any local symptom, n (%)	65 (92)	67 (92)	0·96	38 (50)	<0·0001	49 (96)	45 (90)	0·23
Erythema, n (%)	5 (7)	10 (14)	0·19	3 (4)	0·41	12 (24)	10 (20)	0·67
Induration, n (%)	8 (11)	17 (23)	0·057	5 (7)	0·32	15 (29)	15 (30)	0·95
Pain at injection side, n (%)	64 (90)	67 (92)	0·73	38 (50)	<0·0001	47 (92)	45 (90)	0·70
Serious adverse events	N=75	N=77	-	N=78	-	N=51	N=51**	-
Any serious adverse event, n (%)	3 (4)	0	0·076	1 (1)	0·29	1 (2)	1 (2)	-
Related to vaccination, n (%)	0	-	-	-	-	0	0	-
Not related to vaccination, n (%)								
Total	3 (4)	-	-	1 (1)	-	1 (2)	1 (2)	-
Dehydration	1 (1)	-	-	-	-	-	-	-
Diarrhoea	1 (1)	-	-	-	-	-	-	-
Bacterial pneumonia	1 (1)	-	-	-	-	-	-	-
COVID-19	-	-	-	1 (1)	-	-	1 (2)	-
Cellulitis	-	-	-	-	-	1 (2)	-	-

Variables are given as number and percentage. P-values were calculated using Chi-squared test.

Abbreviations are: P-val, P-value.

[1]P-values are given for the comparisons versus control groups. In case of multiple testing, a p-value<0.025 was considered as statistically significant

*Missing data for 11 subjects (N=4 1x mRNA-1273, N=4 2x mRNA-1273, N=2 Ad26.COV2-S and N=1 MMF-)

**Number not equal to number randomized as one subject withdrew consent before receiving vaccination

sation of the omicron variant as compared with the delta and ancestral variant (appendix Figure S4). Second, at 28 days there was a moderate correlation between S1-specific IFN-γ SFCs and the concentrations of S1-specific antibodies (R=0·37, p<0·0001; appendix Figure S5). Third, in 28 participants T-cell responses were measured both by ELISpot and IGRA. There was a significant correlation between the results of both assays, both at baseline and at 28 days (R=0·42, p=0·027 and R=0·40, p=0·042, respectively; appendix Figure S6). Fourth,

also in subgroup analyses, the effect of the various vaccination strategies did not differ significantly in either study group (appendix Figure S7). Fifth, in multivariable stepwise backward logistic regression analysis, diabetes and lower eGFR were significantly associated with the risk of being a non-responder in the alternative vaccination study group. In the mycophenolate mofetil– mycophenolic acid discontinuation study group, continuing mycophenolate mofetil or mycophenolic acid, higher age, lower eGFR, lower lymphocyte count, and hypertension were associated with the risk of being a non-responder (appendix Table S3). Lastly, we compared baseline characteristics between patients who previously received two versus three SARS-CoV-2 vaccinations in the mycophenolate mofetil–mycophenolic acid discontinuation study group (appendix Table S4). There were no significant differences, except from a higher proportion of patients with a history of malignancy in those who had received three vaccinations (appendix Table S4).

DISCUSSION

In this prospective, randomised trial we assessed the immunogenicity of a double dose of an mRNA vaccine, heterologous vaccination, or temporary discontinuation of mycophenolate mofetil or mycophenolic acid as compared with standard dose mRNA vaccination against COVID-19 in KTRs who were serological non-responders after two or three doses of an mRNA vaccine.

The major finding of our study is that none of the investigated alternative vaccination strategies was more immunogenic than administering a single dose of the mRNA-1273 vaccine. Notably, in the two study groups, 63 to 80% of patients were seropositive after a repeated single dose vaccination. These figures are higher than the seroconversion rates of 39 to 54% reported in other studies assessing the response to third vaccination in seronegative KTR.[16,17] This discrepancy might in part be related to the fact that 24% of all participants who were seronegative during screening, appeared to be seropositive at the time of repeated vaccination, which took place at a median interval of about 6 months after the preceding vaccination. Seroconversion due to COVID-19 was excluded as well as possible on the basis of the reporting of patients and the measurement of SARS-CoV-2 nucleocapsid-specific antibodies, but asymptomatic cases could have gone unnoticed since a regular screening with PCR tests was not done. Alternatively, vaccination induced seroconversion could have occurred later than the time of assessment (14–56 days) after the second (or third) vaccination. Such a delayed humoral response after COVID-19 mRNA vaccination in KTRs has been described previously.[18,19] After exclusion of patients who were seropositive at time of vaccination, the response rate after repeated vaccination in our control group was only slightly higher than described in the literature. In addition, the longer time interval between the repeated and preceding vaccination in our patients (median 196 days) as compared with

that in other studies (median 80–109 days)[16,17] might also have contributed to a relatively high seroconversion rate.[20] In any case, the fact that multiple studies have reported a considerable increase in seroresponse rate after each additional booster vaccination[21] underscores the importance of a high uptake in new booster vaccination campaigns for all KTRs.

The presence of neutralising antibodies probably represents a major mechanism of protection against COVID-19.[22] We therefore also assessed serum neutralising activity against different SARS-CoV-2 variants in randomly selected subgroups of study participants. Although increasing concentrations of S1-specific antibodies were required to achieve neutralisation of newer SARS-CoV-2 variants, there were no significant differences between the various vaccination strategies with regard to neutralising antibody titres.

It has been shown that organ transplant recipients in whom antibodies are not detectable can still have developed cellular immunity.[23] We therefore also evaluated T-cell responses, in particular IFN-γ production by T-cells, as assessed by ELISpot and IGRA. Again, no significant effect of the type of vaccination strategy was observed. Notably, in a considerable proportion of patients a T-cell response was already detectable at baseline, suggesting that the T-cells of these patients had been primed before. This confirms the observation that the humoral and cellular immune response after COVID-19 vaccination can be discordant.[24] Unexpectedly, we observed a decrease in T-cell response between baseline and 28 days after vaccination in some participants. This suggests that ex vivo measured reactivity of T-cells isolated from peripheral blood might vary over time and can be influenced by factors unrelated to vaccination. The fact that these variations in time were observed with both ELISpot and IGRA, as well as the observed correlation between the results of both assays, argues against a major technical issue with one or both of these assays.

Previously, a stronger effect of higher vaccine doses has been shown for influenza vaccination in elderly adults and organ transplant recipients, and for hepatitis B vaccination in patients infected with the human immunodeficiency virus.[5,25] Moreover, in a phase one study with the mRNA-1273 vaccine, a dose of 250 μg was associated with increased antibody titres at 1 month after vaccination compared with a dose of 100 μg.[26] However, our data indicate that in the context of repeated COVID-19 vaccination in patients using immunosuppressive drugs, increasing the dose of the mRNA-1273 vaccine has no beneficial effect.

Several studies have suggested a stronger or longer lasting immunogenic effect of heterologous versus homologous vaccination schedules.[7,27] However in this study, we could not show an advantage on antibody response or T-cell reactivity at 28 days after heterologous vaccination with Ad26.COV2-S, which was corroborated by another randomised clinical trial.[17] However, in a non-randomised cohort study in organ transplant recipients who remained seronegative after two mRNA vaccines, percentages of seropositive patients were similar at 1 month but higher at 3 months and 6 months after administration of Ad26.COV2-S as compared with an mRNA vaccine.[28] Although the percentage of seropositive patients at 28 days after Ad26.COV2-S vaccination in our RCT was 63%, similar to that

in the observational study, the design of our study did not allow us to investigate the presence of a delayed beneficial effect of heterologous vaccination. A remarkable finding with administration of Ad26.COV2-S was the lower incidence of pain at the injection site, which was also observed in the earlier comparisons with the mRNA vaccines.[17,28]

Our rationale for temporary cessation of mycophenolate mofetil or mycophenolic acid around the time of vaccination was the strong negative association between immunogenicity of COVID-19 vaccination in KTRs and the use of these drugs in the current and previous studies.[2] Since interruption of treatment with mycophenolate mofetil or mycophenolic acid might increase the risk of graft rejection, we opted for a relatively short duration of discontinuation (2 weeks). Risks were furthermore mitigated by restricting this strategy to patients who used triple immunosuppressive therapy with sufficient exposure to the other two drugs, exclusion of patients with a higher immunological risk of rejection, and close monitoring of kidney function. We found no beneficial effect of suspending the use of mycophenolate mofetil or mycophenolic acid on the immunogenicity of repeated vaccination. Interestingly, it has been reported that a relatively high seroconversion rate (76%) was obtained after a fourth vaccine dose (BNT162b2) in 29 KTRs without a humoral immune response after previous vaccinations in whom mycophenolate mofetil, or azathioprine in one patient, was discontinued from 4–7 days before to 28–35 days after the fourth vaccination.[29] Unlike our study, this study did not include a control group, which hampers the interpretation of the results. Moreover, 20% of their patients were left on single immunosuppressive therapy during discontinuation of mycophenolate mofetil or mycophenolic acid whereas all our patients remained on double immunosuppressive therapy. Finally, the mean time since transplantation was longer than in our study (9.9 years vs 4.3 years). It remains therefore to be established whether longer duration of mycophenolate mofetil or mycophenolic acid discontinuation or replacement by another drug can be helpful, and if so, how this should be timed in relation to the repeated vaccination.

Since none of the approaches investigated here appeared to augment the response to vaccination, alternative strategies should be considered to protect immunocompromised patients who remain persistently seronegative against the consequences of COVID-19. One such strategy could be pre-exposure prophylaxis with monoclonal antibodies,[30] although the efficacy of this treatment might decline with the emergence of newer virus variants.

The main strength of this study is the prospective, randomised design. We evaluated three alternative vaccination strategies in KTRs who remained seronegative after two or three doses of a COVID-19 mRNA vaccine and included control groups that received a standard dose of mRNA vaccine. In addition to S1-specific IgG antibodies, we measured serum virus neutralising activity and T-cell reactivity at 28 days after vaccination. Lastly, our findings are relevant for other patients using immunosuppressive drugs, and useful for the design of vaccination strategies against other viruses in immunosuppressed patients.

Our study also has limitations. First, the number of patients analysed was lower than the predefined sample size in both study groups (82% and 65% of targets achieved, respectively). When we started recruitment of patients, some patients had already accepted an invitation for a third vaccination via the national vaccination programme. Moreover, patients were often reluctant to discontinue mycophenolate mofetil or mycophenolic acid temporarily for fear of rejection. Although there was a slight trend for a higher seroconversion rate in patients who temporarily suspended the use of mycophenolate mofetil or mycophenolic acid, it remains a matter of speculation whether an increase of the sample size would have changed the results essentially. Second, the sample size and duration of follow-up do not allow any conclusion on clinical efficacy against infection or disease. Nonetheless, S1-specific IgG concentrations and neutralising activity are considered the best surrogate measure for clinical outcome. Finally, we studied only one of the two available mRNA vaccines. Although increasing the dose of the mRNA-173 vaccine did not enhance the immunogenicity of vaccination, this might be different for the BNT162b2 vaccine which appears to be somewhat less immunogenic than the mRNA-1723 vaccine in the currently used dosages.

In conclusion, administering a double dose of mRNA-1273, heterologous vaccination with Ad26.COV2-S, or 2 weeks discontinuation of mycophenolate mofetil or mycophenolic acid did not increase the immunogenicity as compared with a single dose of mRNA-1273 in KTRs who remained seronegative after two or three mRNA vaccinations. Repeated vaccinations are therefore the most successful strategy to achieve seropositivity.

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RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed for COVID-19 vaccination studies in kidney transplant recipients published between May and August, 2021 using terms such as "COVID", "vaccine*", "booster", "third dose", "immunogenicity", "humoral response", and "cellular response". We found observational studies and only one randomised trial reporting that a third dose of SARS-CoV-2 mRNA vaccine resulted in a seroconversion rate of only 25–44% in patients who were seronegative after two doses of an mRNA vaccine. Alternative vaccination strategies to increase the immunogenicity of COVID-19 vaccination are therefore needed. Although increased immunogenicity of higher doses has been shown for hepatitis B and influenza vaccination in immunocompromised patients, the effect of a higher SARS-CoV-2 vaccine dose has not been studied in such patients. There are conflicting results with respect to heterologous vector based–mRNA vaccination compared with homologous regimens with an observational study showing a higher T-cell response in healthy adults, whereas one clinical trial showed no advantage in kidney transplant recipients. Lastly, a strong association between reduced vaccination efficacy and the use of mycophenolate mofetil or mycophenolic acid has repeatedly been reported, suggesting that temporarily withdrawing this medication might increase the immunogenicity of COVID19 vaccination.

Added value of this study

In this prospective randomised trial we compared the immunogenicity of three alternative vaccination strategies to that of a control single dose of mRNA-1273 in kidney transplant recipients who remained seronegative after two or three previous mRNA-based vaccinations. Even with a broad spectrum of immunological parameters we did not find superiority of a double dose of mRNA-1273 at two anatomical locations, heterologous vaccination, or temporary withdrawal of mycophenolate mofetil or mycophenolic acid. To our knowledge, we are the first to report on the effect of different vaccination strategies in patients using immunosuppressive drugs in a randomised trial including a proper control group.

Implications of all the available evidence

Repeated vaccinations are the most successful strategy to achieve a humoral immune response in kidney transplant recipients. We think that our results are directly useful for doctors caring not only for kidney transplant recipients but also for other patients on immunosuppressive drugs. Additionally, these data are important for the design of future vaccination strategies for immunosuppressed patients against other pathogens.

SUPPLEMENTARY MATERIAL

Table S1. Inclusion and exclusion criteria

Inclusion criteria
Eligible for COVID-19 vaccination as described by the instructions of the manufacturers
Age of 18 years or older
Capable of understanding purpose and risks of the study, given written informed consent
At least 6 months after kidney transplantation
Received 2 doses of mRNA-1273 and the last administration within the last 9 months
Negative seroresponse 14 to 56 days after vaccination, measured by a validated anti-spike IgG assay
Additional inclusion criteria to be eligible for MMF/MPA discontinuation study arm
Received 2 doses of BNT162b2 and/or a third dose with an mRNA vaccine (mRNA-1273 or BNT162b2) within the last three months*
Maintenance immunosuppressive therapy consisting of a calcineurin inhibitor (tacrolimus or cyclosporine), MMF/MPA, and prednisone
In case of tacrolimus treatment: last tacrolimus pre-dose level while on current dosage above 4 µg/l
In case of cyclosporine treatment: last cyclosporine pre-dose level while on current dosage above 75 µg/l
Prednisone dose at least 5 mg/day
First or second transplantation
Calculated level of panel reactive antibodies prior to last transplantation below 85%
No signs of acute rejection during the preceding year
Exclusion criteria
History of severe adverse reaction associated with a vaccine and/or severe allergic reaction (eg, anaphylaxis) to any component of the study intervention(s).
Multi-organ transplant recipient
Previous or active COVID-19 disease
Active malignancy, except non-melanoma skin cancer
Inherited immune deficiency
Infection with Human Immunodeficiency Virus (HIV)
Administration of T-cell, B cell, or plasma cell depleting antibodies during the last 6 months
Any vaccination within a week before enrolment
Bleeding diathesis or condition associated with prolonged bleeding that would, in the opinion of the investigator, contraindicate intramuscular injection.
Additional exclusion criteria for alternative vaccination study arm
History of recurrent venous thrombosis or venous thrombosis <2 years before baseline
Immune-mediated diseases associated with thrombocytopenia such as immune thrombocytopenia and atypical hemolytic uremic syndrome

* Since inclusion rate lagged behind schedule in the MMF/MPA discontinuation arm, a protocol amendment was made to extend the inclusion to patients who were seronegative after three doses of an mRNA vaccine.

Table S2. Study visits and assessments

	V1	V2	V3[2]	V4
	Day -14/-7[1]	Day 0[1]	Day 7	Day 28 ±2
Informed Consent	x			
Inclusion/exclusion criteria	x			
Medical history	x			
Immunosuppressive medication	x	x	x	x
Concomitant medication	x			
Height/weight	x			
Vital signs	x			
Safety lab	x	x	x	x
SARS-CoV-2 antibodies	x			x
Solicited adverse events		x		
COVID-19 questionnaire				x
SARS-CoV-2 vaccination		x		

[1] In patients included in the alternative vaccination study group, visits 1 and 2 can coincide on the same day

[2] Only in patients included in the mycophenolate mofetil-mycophenolic acid discontinuation study group

Vital signs: blood pressure, heart rate, temperature

Safety lab: Hemoglobin (Hb), leucocytes and differentiation, platelets, creatinine, alanine aminotransferase (ALAT), C-reactive protein (CRP), Creatinine Kinase (CK)

Table S3. Associations of patient characteristics with being a seroresponder versus non-responder at 28 days after vaccination.

	Alternative vaccination study group				MMF/MPA discontinuation study group			
	Univariable		Multivariable[1]		Univariable		Multivariable[1]	
	OR (95% CI)	p-value	aOR (95% CI)	p-value	OR (95% CI)	p-value	aOR (95% CI)	p-value
MMF+ (ref)								
MMF-					1·99 (0·77-5·17)	0·16	3·63 (1·03-12·8)	0·046
Vaccination								
1x mRNA-1273 (ref)								
2x mRNA-1273	0·98 (0·49-1·97)	0·96						
Ad26.COV2-S	0·78 (0·40-1·56)	0·49						
History of malignancy (yes vs. no)					4·93 (0·61-40·1)	0·14		
Age (years)					0·97 (0·92-1·01)	0·13	0·95 (0·90-0·99)	0·03
Hypertension (yes vs. no)	0·54 (0·22-1·31)	0·17			0·10 (0·01-0·83)	0·03	0·06 (0·01-0·67)	0·02
Lymphocytes (10^9/L)	1·21 (0·84-1·73)	0·30			3·56 (1·26-10·1)	0·02	4·53 (1·35-15·2)	0·01
eGFR (ml/min/1.73m^2)	1·03 (1·01-1·04)	0·002	1·03 (1·01-1·04)	0·002	1·06 (1·02-1·10)	0·002	1·07 (1·02-1·12)	0·005
DBP (mmHg)	1·02 (0·99-1·04)	0·19						
Number of comorbidities	0·83 (0·63-1·10)	0·19						
Diabetes (yes vs. no)	0·47 (0·25-0·85)	0·01	0·47 (0·25-0·87)	0·02				
Heart failure (yes vs. no)	0·19 (0·04-1·01)	0·05						
Mycophenolate mofetil (yes vs. no)	0·54 (0·25-1·17)	0·12						

(a)OR's (adjusted Odds Ratio) and p-values were calculated using logistic regression analysis. Dependent variable is responder versus non-responder (S1 IgG antibody level at 28 days after vaccination ≥10 BAU/mL versus <10 BAU/mL).

Baseline characteristics with a p-value of <0.2 between responders and non-responders were included in these analyses.

[1]Results of a stepwise backward analysis, including all variables from univariable regression analysis until only variables remained with p-value <0·05

Abbreviations are: OR, Odds Ratio; aOR, adjusted Odds Ratio; CI, Confidence Interval.

Table S4. Baseline characteristics of patients included in the MMF/MPA discontinuation study group that received 2 versus 3 previous COVID-19 vaccinations.

	MMF/MPA discontinuation study group		
	2 previous vaccinations (n=64)	**3 previous vaccinations** (n=28)	**P-value**
Female, n (%)	29 (45)	12 (43)	0.83
Caucasian, n (%)	63 (98)	28 (100)	0.51
Age (years)	58·4 ± 11·0	63·0 ± 13·2	0.08
Number of comorbidities	1 (1-2)	2 (1-2)	0.09
Comorbidities, n (%)			
Hypertension	48 (75)	23 (82)	0.45
Diabetes Mellitus	12 (19)	8 (29)	0.29
History of coronary artery disease	6 (9)	2 (7)	0.73
Heart failure	2 (3)	1 (4)	0.91
Chronic lung disease	4 (6)	1 (4)	0.60
History of malignancy[1]	6 (9)	7 (25)	0.048
Auto-immune disease	6 (9)	3 (11)	0.84
Lymphocytes (10^9/L)	1·3 (1·0-1·5)	1·1 (0·8-1·6)	0.24
eGFR (ml/min/1·73m^2)	50.9 ± 18.8	46.1 ± 14.0	0.23
Primary renal diagnosis, n (%)			0.48
Primary glomerulonephritis	10 (16)	2 (7)	
Pyelonephritis	0	0	
Interstitial nephritis	1 (2)	1 (4)	
Familial/hereditary renal diseases	8 (13)	7 (25)	
Congenital diseases	3 (5)	0	
Vascular diseases	4 (6)	4 (14)	
Secondary glomerular/systemic disease	1 (2)	0	
Diabetic Kidney Disease	5 (8)	3 (11)	
Other	20 (31)	8 (29)	
Unknown	12 (19)	3 (11)	
Transplant characteristics			
First kidney transplant, n (%)	53 (83)	26 (93)	0.20
Time after last transplantation (years)	3·8 (1·6-7·6)	5·4 (3·0-7·5)	0.11
Last transplant			
Living, n (%)	48 (75)	19 (68)	0.48
Pre-emptive, n (%)	31 (48)	12 (43)	0.62
Number of immunosuppressieve agents	3 (3)	3 (3)	0.51
Seropositive at baseline[2], n (%)	19 (30)	9 (32)	0.81

Variables are presented as mean ± SD, or as median (IQ interval) in case of non-normal distribution.

Abbreviations are eGFR, estimated glomerular filtration rate.

[1] Including melanomas, excluding all other skin malignancies

[2] Seropositivity was defined as S1-specific IgG ≥ 10 BAU/mL

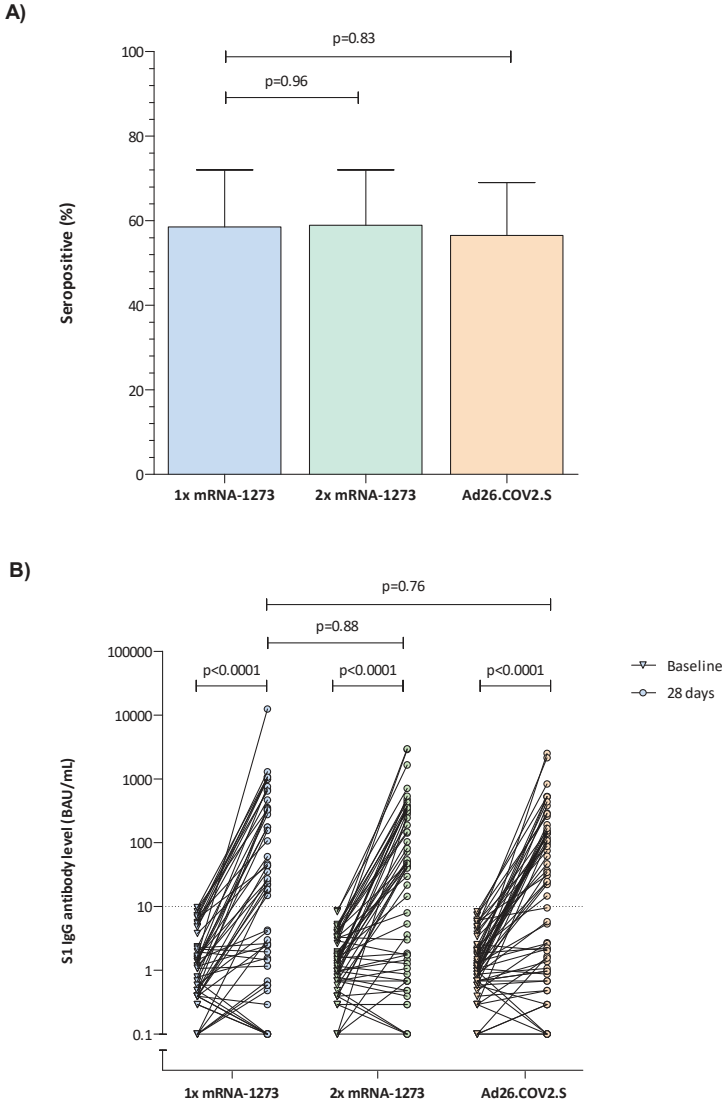

Figure S1. Serological response after exclusion of participants with S1-specific IgG ≥10 BAU/mL at baseline in the alternative vaccination study group.

A) Proportion (95% CI) of responders per randomization group at 28 days after vaccination. Responders were defined as subjects with a S1 specific IgG antibody level ≥10 BAU/mL at 28 days after vaccination; P values were calculated using X^2 test. B) SARS-CoV-2 Spike S1-specific serum IgG concentrations. Depicted are dots representing each patient, dotted line indicates cut-off value for seropositivity; p-values between groups were calculated using Mann-Whitney U test and within groups with Wilcoxon Signed Rank test.

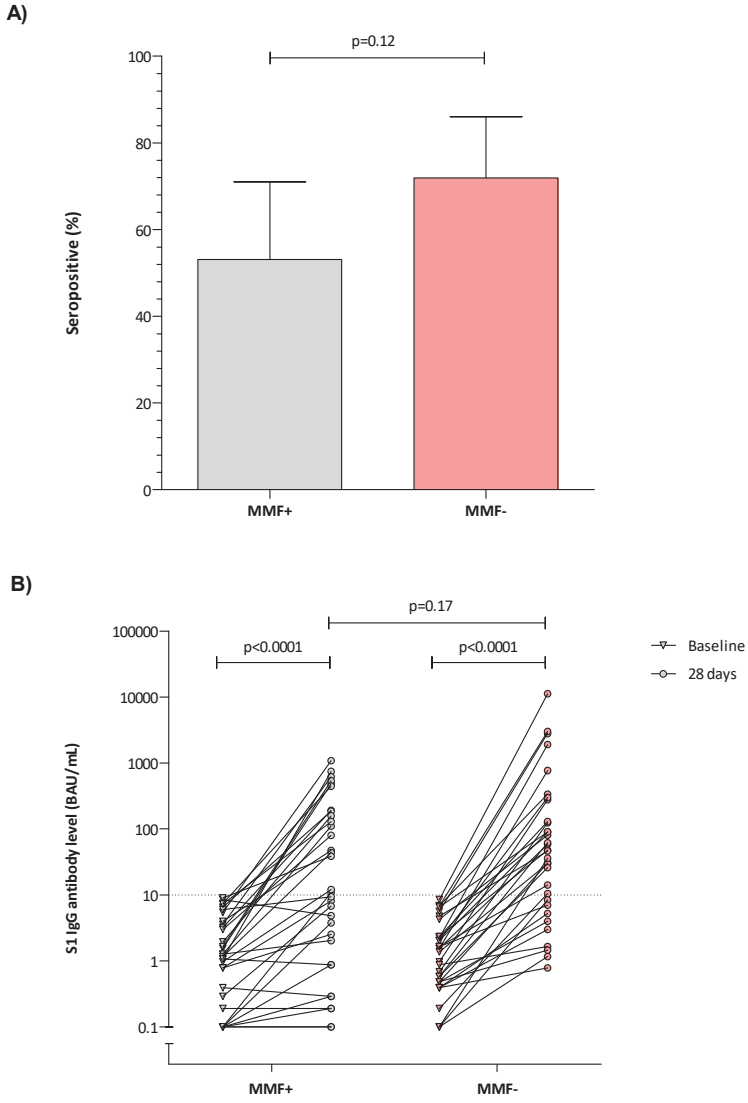

Figure S2. Serological response after exclusion of participants with S1-specific IgG ≥10 BAU/mL at baseline in the MMF/MPA discontinuation study group.

A) Proportion (95% CI) of responders per randomization group at 28 days after vaccination. Responders were defined as subjects with a S1 specific IgG antibody level ≥10 BAU/mL at 28 days after vaccination; P values were calculated using X^2 test. B) SARS-CoV-2 Spike S1-specific serum IgG concentrations. Depicted are dots representing each patient, dotted line indicates cut-off value for seropositivity; p-values between groups were calculated using Mann-Whitney U test and within groups with Wilcoxon Signed Rank test.

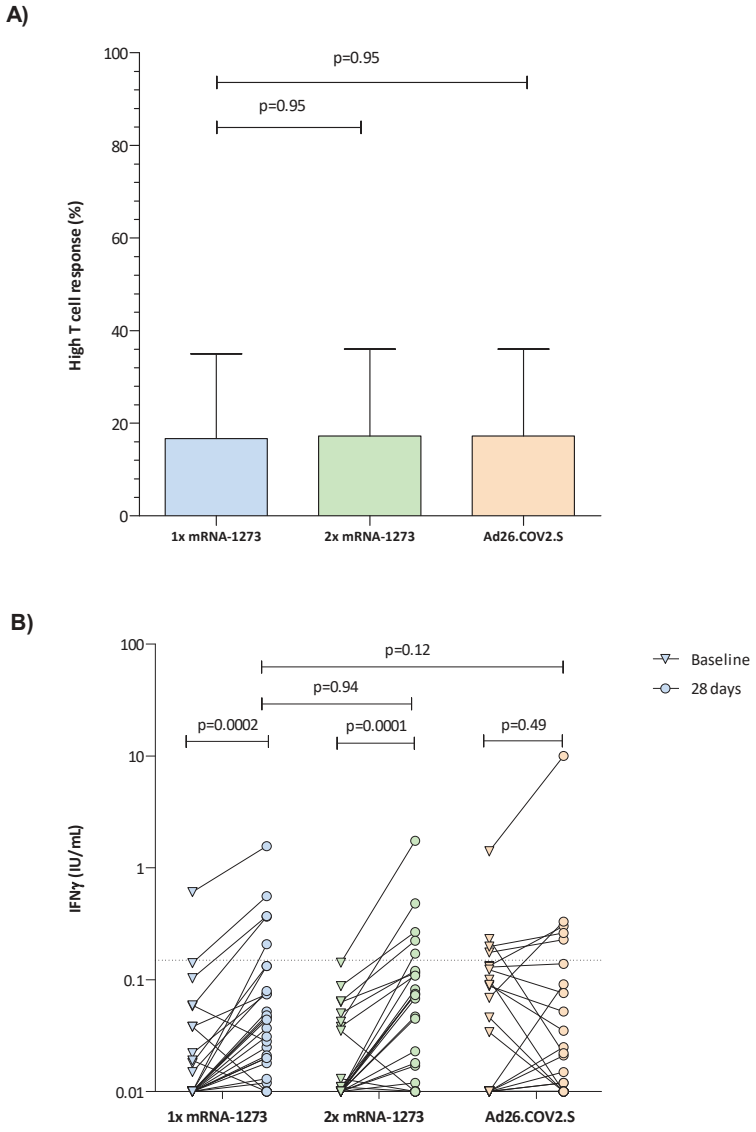

Figure S3. T-cell response measured by IFN-γ in whole blood (IGRA) in the alternative vaccination study group.

A) Proportion (95% CI) of subjects with response at 28 days after vaccination; p-values were calculated with X^2 test. B) IFN-γ concentration at baseline and 28 days after vaccination; dotted line indicates threshold for high T-cell response (≥0·15 IU/mL); p-values between groups were calculated using Mann-Whitney U test and within groups with Wilcoxon Signed Rank test.

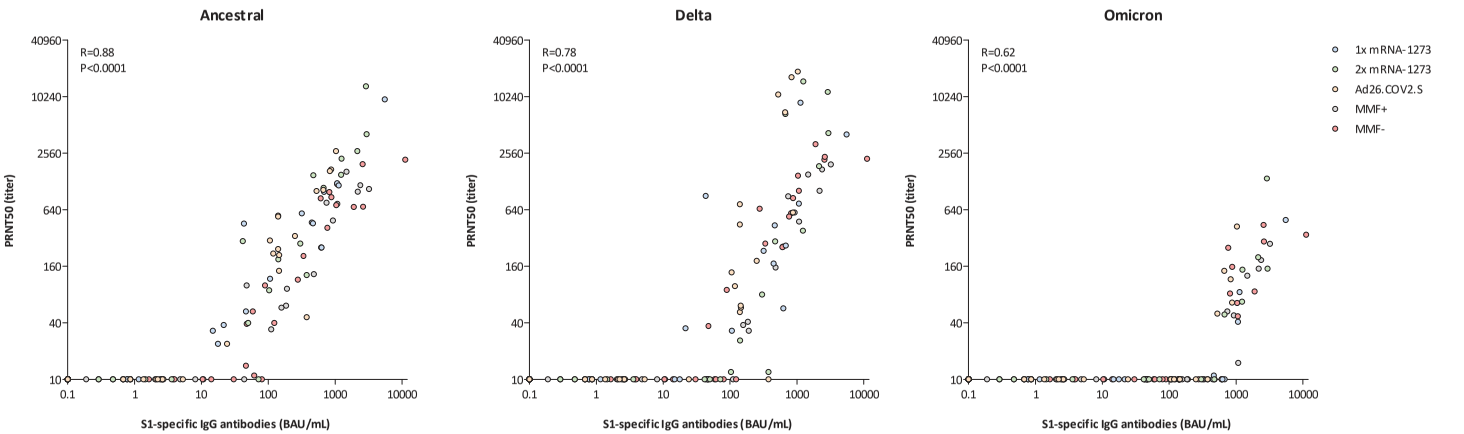

Figure S4. Association between S1-specific IgG antibody level and neutralizing titers for the ancestral, Delta, and Omicron strain of SARS-CoV-2 at 28 days after vaccination in combined random selections of all treatment groups (n=123).

Correlation was calculated using Pearson Correlation.

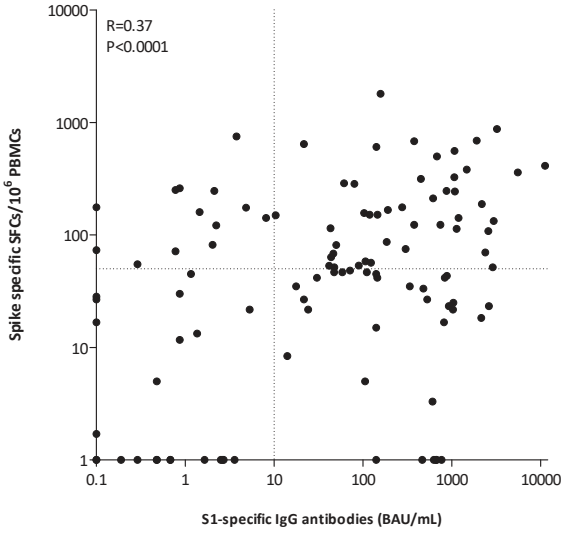

Figure S5. Association between spike specific SFCs and S1-specific IgG antibody levels at 28 days after vaccination in combined random selections of all treatment groups (n=111).

Correlation was calculated using Pearson Correlation. Dotted horizontal line indicates threshold for T-cell response and dotted vertical line indicates threshold for seropositivity.

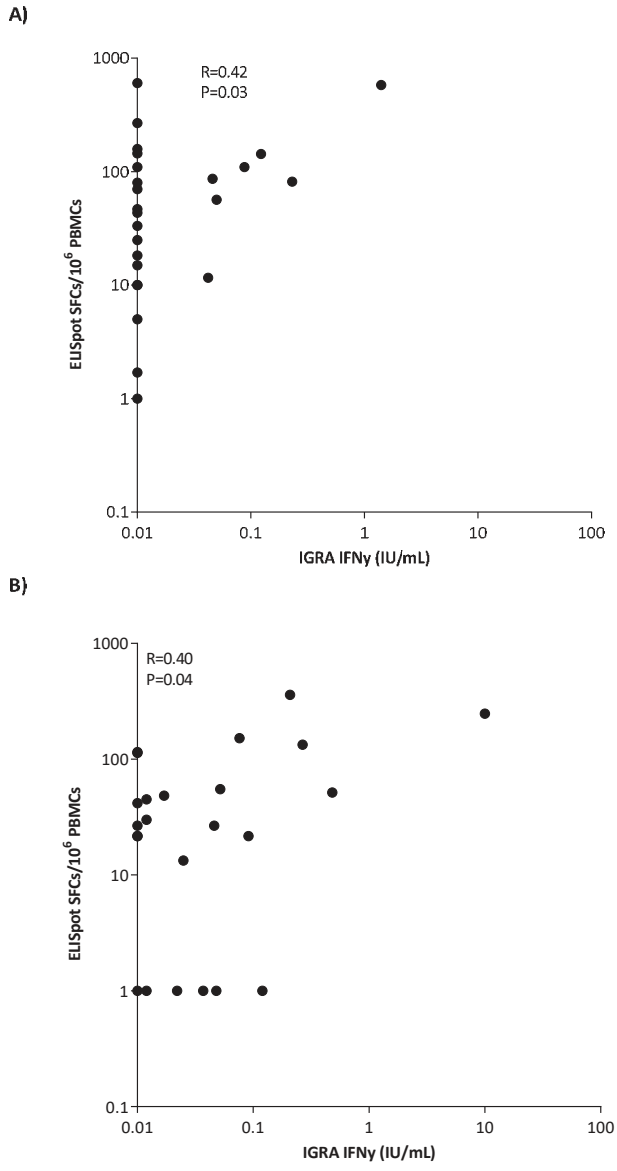

Figure S6. Association between T-cell response measured by ELISPOT versus IGRA (n=28)
A) at baseline and B) 28 days after vaccination. Correlation was calculated using Pearson Correlation.

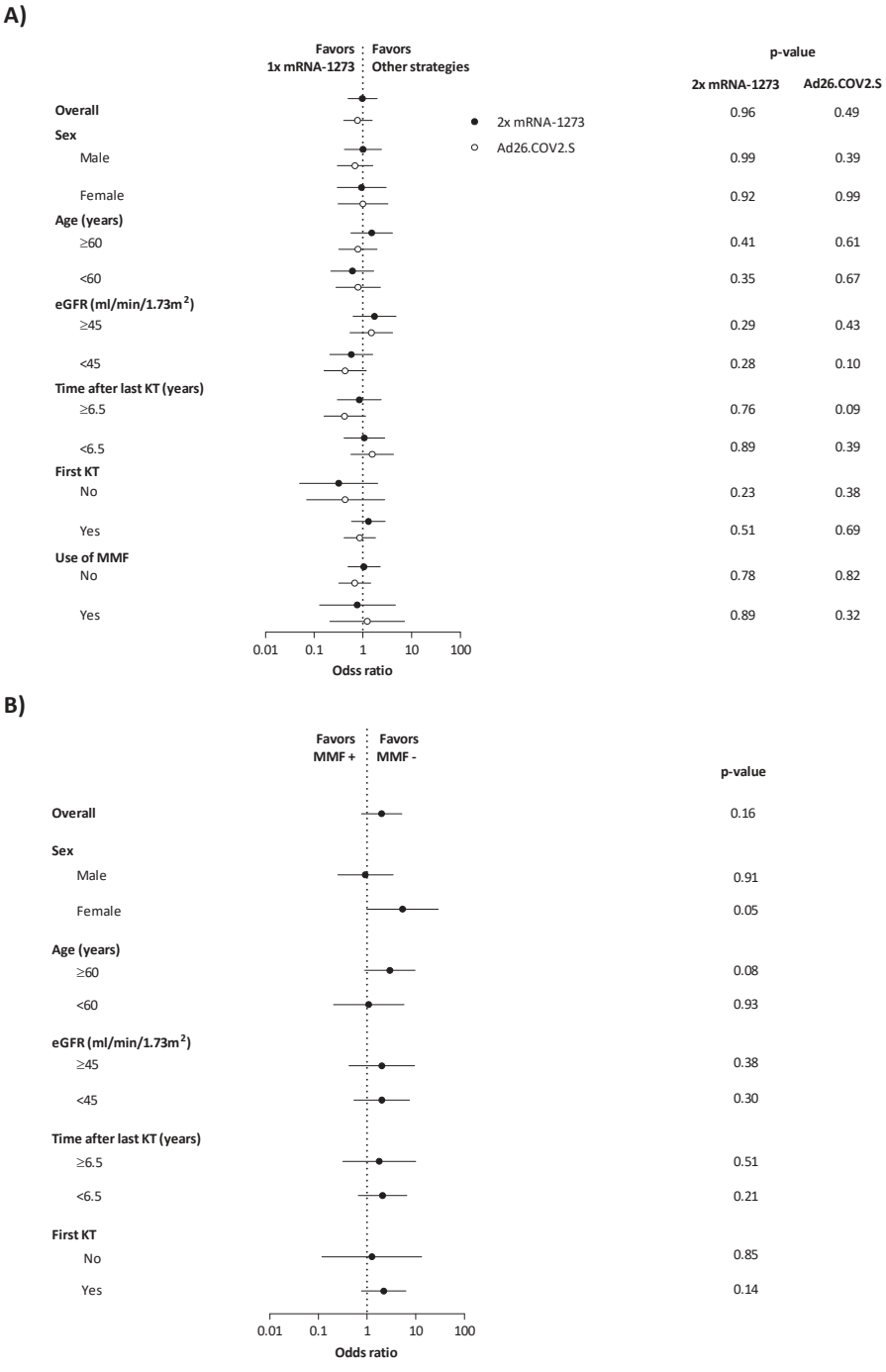

Figure S7. Subgroup analyses for differences between treatment effect of the various vaccination strategies for A) the alternative vaccination study group and B) the MMF/MPA discontinuation study group.

7 Summary, general discussion and conclusions

SUMMARY

Patients with chronic kidney disease (CKD), patients on dialysis, and kidney transplant recipients have a high risk of severe morbidity and mortality from viral infections. Vaccination is an important means of preventing severe disease, but immunocompromised individuals are less able to mount an adequate immune response. This thesis focusses on the immunogenic effects of vaccination against two viruses, varicella zoster (VZV) and SARS-CoV-2, in patients with CKD, patients on dialysis and recipients of a kidney transplant.

Part 1 Varicella zoster virus

In **Chapter 2**, we investigated the humoral and cellular response to a two-dose regimen of live attenuated VZV vaccine in VZV-seronegative kidney transplant candidates. We found seroconversion in 77% of patients. Of the patients with a follow-up of one year after vaccination, 67% still had positive anti-VZV IgG concentrations at that time. There was no difference in seroconversion rates between patients who received a kidney transplant within one year after vaccination and those who did not. Both central and effector VZV-specific $CD4^+$ memory T cells increased in 82% of patients, but VZV-specific $CD8^+$ T cells did not. No severe vaccine-related adverse events occurred. One non-responder developed mild varicella but recovered quickly without anti-viral treatment. None of the responders developed varicella, even after kidney transplantation. Two responders developed mild herpes zoster, years after vaccination and kidney transplantation. We conclude that primary VZV vaccination before kidney transplantation induced humoral and memory T cell responses, higher than reported in CKD patients after hepatitis B and influenza, and is safe. This may prevent severe varicella disease after kidney transplantation.

Most primary varicella infections occur in childhood or adolescence, so the majority of adults is VZV seropositive. However, VZV establishes lifelong latency and upon reactivation, causes herpes zoster (HZ), which occurs more often when the immune system is waning or suppressed. In **Chapter 3**, we assessed the incidence and complications of HZ in recipients of a heart, lung, liver or kidney transplant in the Erasmus MC Rotterdam. We analysed risk factors for developing HZ, as well. The HZ incidence rate was higher in all solid organ transplant (SOT) groups compared to the general population aged >80 years. Multivariable analysis showed that type of organ transplant, age ≥50 years at the time of transplantation, the use of CMV prophylaxis, and the type of anti-rejection therapy were factors that significantly influenced the risk of developing HZ. One-third of the patients with HZ suffered from complications such as post-herpetic neuralgia or disseminated HZ. Our conclusion is that in SOT recipients, HZ incidence and morbidity are highest after heart and lung transplantation, in older patients, and in those not receiving CMV prophylaxis. Therefore, a significant proportion of our patients, would benefit from prevention of HZ. This study highlights the need for an effective VZV booster vaccination.

In **Chapter 4**, we studied VZV-specific antibody, B and T cell memory responses to a live attenuated virus booster vaccine in patients awaiting kidney transplantation and aged ≥50 years. We compared these kidney transplant candidates to a gender- and age-matched control group with normal kidney function (potential living kidney donors). The median VZV-specific antibody response in the patients was significant and comparable to that of the controls' up to one year after vaccination. The patients who received a kidney transplant within one year after vaccination had a smaller increase in antibody concentration compared to the patients who did not. VZV-specific memory B cells increased equally in patients and controls at three months after vaccination, but had declined in patients at one year. The percentages of VZV-reactive $CD4^+$ T cells and central memory $CD4^+$ cells were significantly increased in both patients and controls at one year. No difference was found between patients with and without a kidney transplant. No severe vaccine-related adverse events occurred. One patient had an uncomplicated HZ episode, 16 months after vaccination. The acute rejection incidence in the patients who received a kidney transplant was comparable to the general acute rejection incidence in the same time period in our centre. In conclusion, a booster vaccination in patients with kidney failure aged ≥50 years significantly increased VZV-specific antibody and $CD4^+$ memory T cell responses and to comparable levels as in controls. These responses persisted for at least one year, even in the kidney transplant recipients.

Part 2 SARS-CoV-2 virus

In **Chapter 5**, we described the design of our prospective, controlled, multicentre study of humoral and cellular immune responses to primary COVID-19 vaccination in four different cohorts: patients with CKD stage 4/5, patients on dialysis, kidney transplant recipients, and healthy controls/individuals. The primary endpoint was the SARS-CoV-2 spike S1 specific IgG concentration on day 28 after the second vaccination. Participants were classified as responder or non-responder based on seroconversion. Secondary endpoints were antibody longevity, neutralising capacity of antibodies, SARS-CoV-2-specific T and B cell responses and induction of SARS-CoV-2-specific nasal mucosal antibodies. The T cell response was measured by two methods based on IFN-γ production: IFN-γ release and ELISpot assay. The frequency of SARS-CoV-2-specific memory B cells was determined by ELISpot assay. Functional and phenotypic characterisation of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell responses was performed by flow cytometric analyses. Safety was assessed in terms of solicited local and systemic adverse events (questionnaires), monitoring of incidence and severity of COVID-19 and, in immunised patients, measurement of anti-HLA antibodies after vaccination. This study design reports the possible correlates of protection after COVID-19 vaccination and will facilitate harmonisation of methodology for similar studies in other high-risk populations.

The effects of alternative approaches to increase the immunogenicity of SARS-CoV-2 (booster) vaccination were investigated in **Chapter 6**. We conducted a randomised, multicentre trial, comparing both alternative vaccination strategies and temporary reduction of immunosuppression to standard repeated vaccination in kidney transplant recipients who had not seroconverted after two or three mRNA vaccinations. We found that repeated vaccination does increase SARS-CoV-2-specific antibodies in previously non-responder kidney transplant recipients. However, the three alternative strategies (double dose of mRNA vaccine, heterologous vaccination with Ad26.COV2-S, and temporary discontinuation of mycophenolate mofetil) did not significantly improve seroconversion rate, T cell response or neutralisation of the ancestral, delta, and omicron strains, compared to standard dose mRNA vaccination. Importantly, no rejections and no vaccination-related serious adverse events occurred and kidney function remained stable. We conclude that to achieve a stronger immune response in kidney transplant recipients, repeated vaccination is the most successful strategy.

GENERAL DISCUSSION

Varicella zoster disease in solid organ transplant recipients

Primary varicella zoster virus infection, causing chickenpox, occurs in more than 90% of people during childhood.[1] Accordingly, 3 to 4% of the adult candidates for kidney transplantation in the Erasmus MC have no antibodies against varicella zoster. Consequently, primary varicella disease is rare in adult kidney transplant recipients. However, when primary infection does occur, very severe to even lethal disease has been observed in our centre and by others.[2-6]

Varicella zoster virus reactivation, causing herpes zoster (HZ), is more common in people with waning or suppressed immunity, such as SOT recipients.[7-9] Chapter 3 shows that in our centre, HZ incidence was significantly higher after heart or lung transplantation than after liver or kidney transplantation. Moreover, all solid organ transplant groups had a higher HZ incidence compared to the general population even when aged >80 years. In multivariable analysis, we found that age ≥50 years at time of transplantation and type of organ transplant significantly increased the risk of HZ. The negative effect of increasing age on immunity is not negated by the effect of immunosuppressive medication, but comes on top of it.[8-10] An obvious explanation for the influence of organ transplant type is that heart and lung transplant recipients are treated with a more intense immunosuppressive regimen compared to liver and kidney transplant recipients. At our centre, the maintenance immunosuppressive regimen of heart and lung transplant recipients consists of triple therapy and higher calcineurin inhibitor trough levels compared to liver and kidney transplant recipients. Whether the type of organ transplant may reflect other differences, such as degree of frailty or co-morbidity has not yet been studied. An example of a co-morbid condition after heart and lung transplantation with additional immunocompromising effect is deterioration of kidney function. Surprisingly, we found that HZ incidence was lower in patients treated with methylprednisolone for acute rejection than in patients without any rejection therapy. We found that 66% of these methylprednisolone treated rejections occurred early after transplantation, during the standard CMV prophylaxis period. Patients receiving CMV prophylaxis, in almost all cases oral valganciclovir, had a significantly lower HZ incidence. Valganciclovir inhibits replication of herpes viruses,[11] so it might have suppressed VZV reactivation at an early, asymptomatic, stage. Asymptomatic virus reactivations go undetected, but may have boosted the immune system. Another explanation for the lower HZ incidence in methylprednisolone treated patients, could be that patients who experienced acute rejection were less immunocompromised than patients who did not. Complications such as post-herpetic neuralgia (PHN), disseminated disease and cranial nerve involvement, occurred in 31% of all patients. In comparison to other studies in immunocompromised patients,[12-14] we had a lower incidence of PHN, probably due to our stricter definition of PHN, including requirement of strong analgesics. The definition

of disseminated disease is not specified in many reports, making it difficult to compare incidence rates of this severe complication. In retrospective studies, reporting bias is always a pitfall. Of our four patient cohorts, the kidney transplant recipients were the most likely to consult general practitioners in case of mild HZ. We made an effort to reach a reliable and complete statement of HZ incidence and complications by questioning our kidney transplant recipients by letter and telephone. In general, differences in incidence of HZ and complications between our study and reports from other research groups[13-21] could be explained by differences in immunosuppressive regimen, use and duration of antiviral prophylaxis, registration of cases and symptoms, and definition of complications, e.g. post-herpetic neuralgia. Regional differences in HZ incidence might also influence differences between research groups, but to what extent is very difficult to ascertain.

In conclusion, the incidence of HZ after any solid organ transplantation is higher than the incidence in the elderly general population and is mostly influenced by the degree of immunosuppression and antiviral prophylaxis. However, immunosuppression cannot be avoided in transplant recipients, and long-term use of antiviral drugs is limited by side effects such as bone marrow toxicity. Vaccination is an additional means of protection against severe disease, but whether its efficacy is sufficient in immunocompromised patients is the next subject of our investigations.

Primary and booster varicella zoster vaccination in kidney transplant candidates

Since 2003, adult VZV seronegative kidney transplant candidates have been vaccinated with live attenuated VZV vaccine (Oka strain, ≥2700 pfu/ml, 0.5 ml, Varilrix/Provarivax®, GlaxoSmithKline Beecham) at our centre. Only patients using immunosuppressive medication other than low-dose prednisolone are excluded. Chapter 2 shows the immunogenicity and safety in 52 vaccinated patients, with seroconversion in 77% at 3 months after vaccination and persistence of antibodies in 67% of the patients with a follow-up of 1 year, even if they received a kidney transplant within that same year. For comparison: in healthy seronegative adolescents and adults, seroconversion rates of 82-100% at 4-6 weeks after 2 vaccine doses have been reported.[22-24] VZV IgG levels years after vaccination are usually lower in people who were vaccinated only, compared to those with a history of VZV infection.[25,26] To our knowledge, there is only one other study of primary VZV vaccination in adult CKD patients. Crespo *et al.* found a VZV-IgG response in 12 of 17 patients (71%) at 4 weeks after a single dose of Varilrix®, and in 4 patients after a second dose, which results in an overall seroconversion rate of 94%.[27] They did not assess cellular response, nor did they measure VZV-IgG over time. In our study, 4 patients had a low level of VZV-IgG at 6 weeks after the first vaccine dose, but no detectable antibodies after the second dose. We considered them non-responders, which resulted in a lower seroconversion rate than in the study of Crespo *et al.* Other studies on vaccination response in seronegative CKD patients concerned paediat-

ric patients. In those studies, seroconversion rates of 85-100% were found, with persistence of antibodies in 62-100% of responders at a mean of 2 years after vaccination,[28-31] and in one study even after kidney or liver transplantation.[32]

The cell-mediated immune response to primary varicella vaccination has rarely been studied in adults. Two recent studies reported persistence of VZV-specific $CD4^+$ T cells in blood,[26,33] and even in bone marrow[33] of healthy adults who were vaccinated in childhood. The number of VZV-reactive $CD4^+$ T cells was lower after vaccination than after infection. $CD8^+$ T cell responses could not be detected.[26] In our study, some patients had VZV-specific $CD4^+$ memory T cells before vaccination. It is possible that they were not truly VZV naïve, but that they had lost their VZV-specific antibodies over time. Nonetheless, after vaccination the percentage VZV-specific $CD4^+$ memory T cells had increased in 82% of patients, even despite the use of immunosuppressive medication after kidney transplantation.

A safety concern of live attenuated virus vaccine is that the vaccine strain might cause severe varicella or zoster disease. It was demonstrated *in vitro* that the vaccine strain is able to establish latency.[34] In reports concerning paediatric CKD patients, no severe adverse events were observed. A few cases of HZ were seen, but all were mild.[28-31] One study reported a lower incidence of varicella and less severe disease after kidney transplantation in immunized compared to non-immunized children.[31] However, there are a few case reports of fatal VZV infection after varicella vaccination in immunocompromised patients.[35-38] In our study, one patient developed varicella at 18 days after vaccination and two patients had a HZ episode, several years after vaccination and kidney transplantation. It was not determined whether the vaccine strain or a wild type strain caused these diseases. All disease episodes were mild and the patients recovered quickly, the patient with varicella even without anti-viral treatment.

Although the live attenuated virus vaccine is contra-indicated in patients on immunosuppressive medication, Chaves *et al.* vaccinated a small number of paediatric kidney transplant recipients and showed an antibody response in 4 of 6 children.[39] The recombinant subunit zoster vaccine (RZV) (Shingrix®) can be used in patients with immunosuppressive medication, but is only licensed for vaccination of seropositive people. L'Huillier *et al.* vaccinated 23 seronegative SOT patients with RZV and showed that 55% had a positive antibody response. They also found a significant increase in VZV-reactive $CD4^+$ T cells. No transplant rejections occurred and adverse events were mild.[40]

In summary, in adult CKD patients, the immunogenicity of primary varicella vaccination has hardly been studied and its efficacy even less. From our study it can be concluded that vaccination of seronegative CKD patients elicits a reasonable humoral response. Regarding cell-mediated immune responses, an increase in VZV-reactive $CD4^+$ T cells was shown, but VZV-reactive $CD8^+$ T cell responses could not be demonstrated. Comparison of cellular immune responses between studies is difficult because of differences in assays. Nevertheless, primary vaccination is safe and may provide long-term protection against severe primary

varicella disease after kidney transplantation. The efficacy of RZV in seronegative CKD patients with and without immunosuppressive medication, should be investigated more thoroughly.

In our second study, we administered a booster vaccination to VZV-seropositive kidney transplant candidates and healthy controls, all aged ≥50 years. We again used a live attenuated vaccine (Oka strain, ≥ 29846 pfu/ml, 0.65 ml, Zostavax®, Sanofi Pasteur MSD NV), because at that time, it was the only licensed herpes zoster vaccine in the Netherlands. Notably, the booster vaccine contains ten times more plaque forming units than the primary vaccine. Chapter 3 shows that in our patients, VZV-specific IgG titres and the number of $CD4^+$ memory cells increased significantly compared to baseline for at least one year after vaccination, even to comparable levels as in the healthy controls. The increase in number of VZV-specific memory B cells was of shorter duration in patients than in controls.

In the general elderly population, immunology substudies of large phase III studies found significantly increased VZV-specific IgG titres and T cell responses at 6 weeks after vaccination. With increasing age, the responses were less high: Geometric mean fold rise (GMFR) 2.3 in 50-59 year-olds, GMFR 1.7 in 60-79 year-olds, no IgG increase in subjects >79 years. The cellular responses (responder cell frequency and IFN-γ ELISpot count) in the individuals aged >60 years had declined at one year after vaccination, but stabilized during year two and three.[41,42] The immunogenicity results parallel the greater efficacy for HZ prevention in younger compared to older vaccinees during 1-4 years after vaccination: 70%, 64% and 38% for the 50-59, 60-69 and ≥70 year age groups, respectively.[43,44] Retrospective cohort studies showed similar declining vaccine efficacy with increasing age and time after vaccination.[45-48] Adverse events were mild (mostly pain at the injection site) and percentage serious adverse events (SAEs) was similar in vaccine and placebo groups.[43,44]

Regarding patients with CKD or dialysis, the only other immunogenicity study in end-stage kidney disease (ESKD) patients showed a significant increase in VZV-specific antibody titres at 5 weeks after vaccination, but not thereafter.[49] GMFR of the antibody titres was higher in our patients than in theirs at 1 year after vaccination. They did not assess cellular immunity. Two retrospective cohort studies on booster vaccine efficacy found that in vaccinated CKD and dialysis patients aged ≥60-65 years, the risk of developing HZ was about 50% lower compared to unvaccinated matched patients.[50,51] A greater risk reduction was seen in patients who were vaccinated within 2 years after dialysis onset.[51] In our study, dialysis duration before vaccination was shorter (median 12 months, range 5-48). Pooled analyses with data of CKD patients from several study populations also showed HZ risk reductions of about 55%.[52,53]

After kidney transplantation, VZV-specific T cell numbers remained stable, but VZV IgG titres were significantly lower in our transplant recipients compared to our patients without a transplant and our healthy controls. This is consistent with reports in vaccinated lung transplant recipients[54] and in unvaccinated kidney transplant recipients.[55] No increase

in acute rejection was seen after vaccination in our patients nor in the lung transplant patients.[54] However, three cases of fatal zoster vaccine infections have been described by others.[56]

Since the approval of the adjuvant recombinant subunit zoster vaccine (RZV, Shingrix®), more studies have been described, showing an efficacy for HZ prevention in the general population aged 50-70 years of 97% at one year to 73% at ten years after vaccination, with specific anti-IgE antibodies and $CD4^+$ T-cell frequency remaining >5-fold and >6-fold over pre-vaccination levels. Adverse events consisted of local (pain) and systemic (shivering, fever, myalgia) symptoms of short duration.[57,58] In people older than 70 years, efficacy was 90% in the 4 years after vaccination.[59] Data in CKD and SOT patients are still scarce. In one post-hoc analysis of patients with undefined renal disease, and one randomized clinical trial in kidney transplant recipients, RZV efficacy was 87% and 57%, respectively.[60,61] Humoral and cellular responses were lower than in immunocompetent adults of the same age. No increase in allograft rejections or immune mediated diseases was seen in the vaccinated patients.[61] Immunogenicity and safety were reported in 4 other immunocompromised groups.[62,63] Based on its efficacy in the general population and immunogenicity in several immunocompromised populations, RZV is currently the recommended booster vaccine for elderly and immunocompromised persons in national and international guidelines.[64-66]

In summary, booster vaccination with the live attenuated virus vaccine elicits comparable antibody and T-cell responses in CKD patients as in the general elderly population and is well-tolerated. Booster vaccination with the recombinant subunit vaccine results in higher antibody titres and specific T-cell frequencies, but data in CKD and SOT recipients are still scarce. Persistence of VZV-specific T cell numbers after SOT seems feasible. This is important because adequate cell mediated immunity, not antibody titres, is needed to prevent viral spread (lower HZ incidence) and to recover from disease (less morbidity).[67-69] However, no clear threshold or correlate of protection has yet been defined. Regardless the vaccine type, administration before introduction of immunosuppressive medication yields better results.

SARS-CoV-2 vaccination in patients with chronic kidney disease, on dialysis and kidney transplant recipients

In the beginning of the pandemic, patients with chronic kidney disease (CKD), on dialysis and living with a kidney transplant suffered severely from COVID-19. Mortality risk in these patient groups was two- to four-fold higher than in the general population.[70-72] Several types of vaccines were developed at extraordinary high speed, but the pivotal trials included only few patients with CKD and excluded kidney transplant recipients.[73-77] After approval by the regulatory authorities, patients at high risk of severe COVID-19 were prioritised to receive vaccination, but vaccine efficacy still needed to be assessed in specific risk populations. The first reports of dialysis and kidney transplant patients, presented low antibody

responses in small numbers of patients.[78] To be able to provide thorough evidence on vaccine immunogenicity, efficacy and safety, large patient numbers, proper control groups and standardised immune response platforms were needed. With this purpose, the RECOVAC (REnal patients COVID-19 VACcination) consortium was started in January 2021. This consortium consists of all university medical centres in The Netherlands and collaborates with dialysis departments of Dutch hospitals, national and European nephrology organisations and registries, the Dutch kidney foundation, the Dutch kidney patient organisation, the National Institute for Public Health and the Environment and ZonMw. The first study that started was the Immune Response (IR) study: a prospective, controlled, multicentre study with three patient cohorts (CKD stage 4/5, dialysis and kidney transplant) and a control cohort. Humoral and cellular responses as well as safety were investigated. We published the study design to contribute to harmonisation of methodology, which enables comparison of efficacy of SARS-CoV-2 vaccines in different populations. In the same time period, clinical trials with similar study designs were performed in solid tumour patients (VOICE),[79,80] lung transplant patients,[81] patients with inborn errors of immunity (VACOPID),[82] and HIV patients (COVIH).[83]

The results of the RECOVAC IR study, reported by Sanders *et al.*[84,85] showed that in kidney transplant recipients the seroconversion rate after two mRNA-1273 vaccinations was significantly lower, whereas in patients with CKD 4/5 and those on dialysis it was almost comparable to controls. Also median S1-specific IgG titres were significantly lower in kidney transplant recipients and dialysis patients than in controls. Neutralizing antibody levels against the ancestral and Delta strains correlated with the S1-specific IgG antibody titres. The Omicron variant was only neutralized at high S1-specific IgG titres and T cell response rate was also significantly lower in kidney transplant recipients and dialysis patients. T cell responses correlated with the S1-specific IgG antibody titres, as well. Of interest, binding antibodies, neutralizing antibodies and T cell responses had significantly waned at six months after second vaccination and the slope of decay was similar among all patient groups and controls. Higher age, lower lymphocyte count, lower eGFR, not using steroids, shorter time after transplantation, and use of mycophenolate mofetil or mycophenolic acid (MMF/MPA) were significantly associated with the risk of being a non-responder. Vaccination was safe and dialysis and kidney transplant recipients experienced less systemic vaccination related side effects compared to CKD patients and controls. The findings of our studies led to the extension of the basic COVID-19 vaccination series in the national vaccination programme with a third dose for immunocompromised patients,[86,87] and highlighted the need for additional booster vaccinations.

Thereafter, many studies proved that vaccine immunogenicity remained low in immunocompromised patients, and especially in solid organ transplant recipients, compared to the general population,[88,89] even after a third or fourth vaccination.[90] Designing modified vaccination strategies to improve response to vaccination was urgently needed. In our

randomised controlled repeated vaccination study, we reported that of kidney transplant recipients who remained seronegative after two or three vaccinations, more than 50% became seropositive after an additional repeated vaccination. However, the three alternative strategies were not superior to standard single mRNA-1273 vaccination. All participants were seronegative at 14-56 days after second or third vaccination. Repeated vaccination took place about six months after the preceding vaccination. At the time of repeated vaccination, 24% of all participants appeared seropositive and 43% of the participants in whom T cell responses were measured had a positive T cell response. Although some asymptomatic cases could have gone unnoticed, previous COVID-19 was excluded as well as possible. Both delayed humoral response and cellular response without detectable humoral response, have been described by others in dialysis and kidney transplant patients,[91-94] but the underlying mechanisms are not yet clearly examined.

Higher doses of vaccines elicited a stronger immune response in phase one studies of mRNA vaccines[95,96] and in influenza and hepatitis B vaccination in immunocompromised patients.[97,98] In CKD and kidney transplant patients, highest antibody titres were reported after mRNA-1273 compared to BNT162b2 and virus vector vaccines,[94,99-101] but we found no other reports of higher vaccine doses in these patients.

Whether heterologous vaccination results in a better immunogenic effect compared to homologous vaccination remains debatable. In general populations, stronger humoral and cellular immunogenicity were measured at 14-30 days after vaccination,[102,103] and in a cohort of organ transplant recipients a higher seroconversion rate was reached at three and six months after heterologous third vaccination.[104] However, alike ours, another large randomised controlled trial in kidney transplant recipients did not find an advantage of heterologous vaccination on antibody or T cell response.[105]

Intensity of immunosuppression and in particular the use and intensity of mycophenolate mofetil (MMF) or mycophenolic acid (MPA) has a strong negative association with a lower immune response to COVID-19 vaccination,[84,90,94,99-101,106] and influenza vaccination.[107] The effect of MMF dose reduction on seroconversion was reported in an observational cohort study in kidney transplant recipients. MMF dose was reduced from three weeks before until one week after a third vaccination. In a cohort of 24 seronegative patients, dose reductions of 50%, 33% and 25% were observed, each in a third of the patients. Propensity scores were used to identify 24 matching patients who did not have an MMF reduction before third vaccination. All patients had a calcineurin inhibitor and most had also steroids as other immunosuppressive drugs. Seroconversion at three weeks after vaccination was seen in 29% of patients with MMF dose reduction, compared to an unusually low rate of 4% of patients without dose reduction. Those with a dose reduction of 33% or more were significantly more likely to seroconvert than the matched controls.[108] The effect of MMF/MPA discontinuation on immunogenicity in previously seronegative kidney transplant recipients was reported in a few non-randomised studies. In two observational studies, MMF/MPA was discontinued

about one week before until four to five weeks after a fourth or fifth vaccination. One study found that 76% of 29 patients had seroconverted at one month after a fourth BNT162b2 vaccination, but they did not include a control group. Mean time since transplantation was relatively long: 9.9 years and 20% of their patients had just a single immunosuppressive drug left during MPA discontinuation. They found an increase in Spike receptor binding domain (RBD)-specific B cell frequency, and signs of increased in vivo activation of spike-reactive $CD4^+$ T cells, but no increase in cell frequencies or cytokine production. No rejection, no increase in anti-HLA antibodies or donor-derived cell free DNA occurred and kidney function remained stable.[109] The other observational study found that 47% of 38 patients seroconverted at three to four weeks after a fourth or fifth mRNA-1273 vaccination in the MPA discontinuation group, compared to 13% of 24 patients continuing MPA plus a calcineurin inhibitor and steroids. However, MPA discontinuation was not randomised.[110] A follow-up report of the latter study, excluding patients with a breakthrough infection, showed that at three months after vaccination 50% of 28 MPA withdrawal patients and 13% of 13 MPA continuation patients remained seropositive. T cell response (IFN-γ release) at month three was not different between patients with and without MPA withdrawal. No comparison was made of breakthrough infections in the two groups. Of the patients who discontinued MPA, two developed de novo donor specific anti-HLA antibodies (DSA) and seven showed an increase in DSA, albeit transient in three patients. One patient had a deterioration in kidney function, but a kidney biopsy showed no rejection.[111] In one non-randomised study, MPA or azathioprine (AZA) was paused in 18 patients from one week before until one week after a third or fourth vaccination with BNT162b2. In the control group of 22 patients, the triple immunosuppressive treatment was not modified. In the MPA/AZA pause group, seroconversion rate at one month was 33% after a 4^{th} vaccination and 20% after a 5^{th}. In the control group, seroconversion rate was 32% (all controls received a 4^{th} vaccination). No difference was found in antibody levels. No de novo DSA or donor-derived cell-free DNA were detectable and kidney function remained stable.[112] So, temporarily discontinuation of MMF/MPA may increase the immune response to vaccination, but the optimal duration is uncertain and the longer the interruption, the more the risk of rejection remains a matter of concern.

Instead of discontinuing MMF/MPA another approach could be replacing MMF/MPA by an mTOR-inhibitor. In a small cohort of elderly kidney transplant patients, as part of the OPTIMIZE study[113] randomised to triple immunosuppressive therapy with either everolimus or MMF, the everolimus group reached significantly higher antibody titres after COVID-19 vaccination.[114] Maintenance therapy with everolimus was associated with less CMV and BK virus infections and was non-inferior regarding rejection risk compared to therapy with MMF/MPA in the TRANSFORM study, consisting of de novo kidney transplant recipients.[115]

Finally, there is an anecdotal report of a reduced risk of severe COVID-19 after recombinant zoster vaccination, possibly due to induction of nonspecific immunity.[116] Further research whether combining or sequentially administering different vaccines might enhance their immunogenicity, seems interesting.

All things considered, repeating vaccinations is at present the most successful strategy to improve vaccine immunogenicity. Whether temporary reduction or adjustment of immunosuppressive medication also enhances the immune response to vaccination has to be thoroughly investigated, as the risk of acute or chronic rejection remains a matter of concern in kidney transplant recipients.

CONCLUSIONS

The research as described in this thesis has led to the following conclusions:

- Vaccination is an important method to increase immunity against infectious diseases in immunocompromised patients..
- After VZV booster vaccination and COVID-19 vaccination, CKD and dialysis patients without immunosuppressive medication are able to reach an immune response which is almost comparable to the response in healthy people.
- Vaccine immunogenicity is more impaired in kidney transplant recipients than in CKD and dialysis patients without immunosuppressive medication.
- The immune response to vaccination can be increased by vaccine enhancement, vaccine administration before start of immunosuppressive medication, and repeat vaccinations.
- Further research into the effect of adjustments in immunosuppressive regimes on vaccine immunogenicity and efficacy is necessary to better cope with existing and emerging infectious diseases.

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8 Nederlandse samenvatting

SAMENVATTING

Patiënten met chronische nierschade (CNS), dialysepatiënten en niertransplantatiepatienten hebben een hoog risico op ziekte en sterfte door virale infecties. Vaccinatie is een belangrijk middel om ernstige ziekte te voorkomen. Echter, personen met een verzwakt afweersysteem zijn minder goed in staat een adequate afweerreactie op te wekken na vaccinatie. Dit proefschrift richt zich op de effecten van vaccinatie op de afweerreactie tegen twee virussen, varicella zoster virus (VZV) en SARS-CoV-2, bij patiënten met chronische nierfalen, dialyse patiënten en niertransplantatie patiënten.

Deel 1 Varicella zoster virus

Een eerste varicella zoster virus infectie veroorzaakt waterpokken en treedt bij meer dan 90% van de mensen op tijdens de kindertijd. Primaire varicella ziekte is dus zeldzaam bij volwassen niertransplantatie patiënten, maar kan zeer ernstig, tot zelfs dodelijk, verlopen. Dit is zowel in ons eigen centrum als elders in de wereld beschreven.

In **hoofdstuk 2** onderzochten we de ontwikkeling van antilichaam en cel-gemedieerde immuniteit na vaccinatie met twee doses van een levend verzwakt VZV-vaccin in niertransplantatiekandidaten die geen VZV-antilichamen hadden (seronegatief). In 77% van de patiënten ontstonden VZV-antilichamen (seroconversie). Van degenen met een follow-up van één jaar na vaccinatie had 67% op dat moment nog steeds positieve anti-VZV IgG concentraties. Er was geen verschil in de mate van seroconversie tussen patiënten die binnen een jaar na vaccinatie een niertransplantatie kregen en patiënten die dat niet kregen. Zowel centrale als effector VZV-specifieke $CD4^+$ geheugen T cellen namen toe bij 82% van de patiënten, maar VZV-specifieke $CD8^+$ T cellen niet. Er traden geen ernstige vaccingerelateerde bijwerkingen op. Eén patiënt zonder seroconversie ontwikkelde milde varicella (waterpokken), maar herstelde snel zonder antivirale behandeling. Geen van de patiënten met seroconversie ontwikkelde varicella, zelfs niet na niertransplantatie. Twee patiënten met seroconversie ontwikkelden milde herpes zoster (gordelroos), jaren na vaccinatie en niertransplantatie. We concluderen dat primaire VZV-vaccinatie vóór niertransplantatie een antilichaam en geheugen-T cel reactie induceert, hoger dan beschreven na hepatitis B en griep vaccinatie in CNS patiënten, en veilig is. Dit kan ernstige ziekte door varicella na niertransplantatie voorkomen.

De meerderheid van de volwassenen is VZV-seropositief. VZV blijft echter levenslang latent aanwezig in het lichaam en veroorzaakt bij reactivering herpes zoster (gordelroos). Herpes zoster komt vaker voor wanneer het immuunsysteem zwakker wordt of onderdrukt wordt. In **hoofdstuk 3** beoordeelden we de incidentie en de complicaties van herpes zoster bij patiënten met een hart-, long-, lever- of niertransplantaat in het Erasmus MC Rotterdam. We analyseerden ook risicofactoren voor het ontwikkelen van herpes zoster. De incidentie was hoger in alle orgaantransplantatie groepen vergeleken met de algemene bevolking van

ouder dan 80 jaar. Multivariabele analyse toonde aan dat het type orgaantransplantatie, een leeftijd van ≥50 jaar op het moment van transplantatie, het gebruik van profylactische medicatie tegen het cytomegalovirus (CMV) en het type anti-afstotingstherapie allen factoren waren die het risico op het ontwikkelen van herpes zoster aanzienlijk beïnvloedden. Eén derde van de patiënten ontwikkelde complicaties, zoals post-herpetische neuralgie of gedissemineerde herpes zoster. Onze conclusie is dat bij patiënten met een orgaantransplantaat de incidentie en morbiditeit het hoogst zijn na hart- en longtransplantatie, bij oudere patiënten en bij degenen die geen CMV-profylaxe krijgen. Daarom zou een aanzienlijk deel van deze patiënten baat hebben bij preventie van herpes zoster. Deze studie benadrukt de noodzaak van een effectieve VZV booster vaccinatie.

In **hoofdstuk 4** bestudeerden we de reactie van VZV-specifieke antilichamen en geheugen B- en T cellen op een levend verzwakt virus boostervaccin bij VZV-seropositieve patiënten van ≥50 jaar die wachtten op een niertransplantatie. We vergeleken deze niertransplantatiekandidaten met een controlegroep met gelijke leeftijd en man/vrouw verhouding en een normale nierfunctie (potentiële nierdonoren bij leven). De mediane VZV-specifieke antilichaam reactie tot een jaar na vaccinatie was bij de patiënten significant gestegen en vergelijkbaar met die bij de controlepersonen. De patiënten die binnen een jaar na vaccinatie een niertransplantatie kregen, hadden een kleinere toename van de antilichaamconcentratie in vergelijking met de patiënten die dat niet hadden gekregen. VZV-specifieke geheugen B cellen waren drie maanden na vaccinatie in gelijke mate toegenomen bij patiënten en controlepersonen, maar waren na één jaar afgenomen bij patiënten. De percentages van alle VZV-reactieve $CD4^+$ T cellen en van centrale geheugen $CD4^+$ T cellen waren op één jaar na vaccinatie significant verhoogd bij zowel patiënten als controlepersonen. Er werd geen verschil gevonden tussen patiënten met en zonder niertransplantatie. Er traden geen ernstige vaccin-gerelateerde bijwerkingen op. Eén patiënt had een ongecompliceerde herpes zoster episode, 16 maanden na vaccinatie. De incidentie van acute afstoting bij de patiënten die een niertransplantatie kregen, was vergelijkbaar met de algemene acute afstotingsincidentie in ons centrum in dezelfde tijdsperiode. Concluderend verhoogt een boostervaccinatie met dit levend verzwakt virus vaccin bij patiënten met chronische nierschade in de leeftijd van ≥50 jaar de VZV-specifieke antilichaam- en $CD4^+$ geheugen-T cel reacties significant en tot een gelijk niveau als bij controlepersonen. Deze reacties hielden minstens een jaar aan, zelfs bij de ontvangers van een niertransplantatie.

Deel 2 SARS-CoV-2

In het begin van de SARS-CoV-2 pandemie leden patiënten met chronische nierschade, dialysepatiënten en niertransplantatiepatiënten ernstig onder COVID-19. Het sterfterisico in deze patiëntengroepen was twee tot vier keer hoger dan in de algemene bevolking. Verschillende soorten vaccins werden met buitengewoon hoge snelheid ontwikkeld, maar de cruciale onderzoeken omvatten slechts enkele patiënten met chronische nierschade en

sloten niertransplantatiepatiënten uit. Na goedkeuring van de vaccins door de regelgevende instanties, werden patiënten met een hoog risico op ernstige COVID-19 met voorrang gevaccineerd, maar de werkzaamheid van het vaccin in specifieke risicopopulaties was nog niet bekend. De eerste onderzoeken bij kleine aantallen dialyse- en niertransplantatiepatiënten toonden lage antilichaamreacties. Om grondig bewijs te kunnen leveren over het vermogen om immuniteit op te wekken en over de veiligheid van vaccins, waren grote aantallen patiënten, goede controlegroepen en gestandaardiseerde immuunreactie analyses nodig. Met dit doel is in januari 2021 het RECOVAC (REnal patients COVID-19 VACcination) consortium gestart. Dit consortium bestaat uit alle universitair medische centra in Nederland en werkt samen met dialyse centra van Nederlandse ziekenhuizen, nationale en Europese nefrologie organisaties en dataregisters, de Nierstichting, de Nederlandse nierpatiënten vereniging, het Rijksinstituut voor Volksgezondheid en Milieu en ZonMW.

In **hoofdstuk 5** beschreven we het protocol van de RECOVAC Immuunrespons studie, een prospectief, gecontroleerd onderzoek in meerdere centra, naar humorale en cellulaire immuunreacties op primaire COVID-19-vaccinatie in vier verschillende cohorten: patiënten met chronische nierschade stadium 4/5, dialysepatiënten, niertransplantatiepatiënten en gezonde controlepersonen. Het primaire eindpunt was de SARS-CoV-2 spike S1 specifieke IgG-concentratie op dag 28 na de tweede vaccinatie. Deelnemers werden geclassificeerd als "responder" of "non-responder" op basis van seroconversie. Secundaire eindpunten waren de levensduur van antilichamen, de neutraliserende capaciteit van antilichamen, SARS-CoV-2-specifieke T en B cel reacties en inductie van SARS-CoV-2-specifieke antilichamen in het neusslijmvlies. De T cel reactie werd gemeten met twee methoden op basis van interferon gamma (IFN-γ) productie: de "IFN-γ release" en de "ELISpot" analyse. Het voorkomen van SARS-CoV-2-specifieke geheugen B cellen werd bepaald door ELISpot analyse. Functionele en fenotypische karakterisering van SARS-CoV-2-specifieke $CD4^+$ en $CD8^+$ T cel reacties werd uitgevoerd door flowcytometrische analyses. De veiligheid werd beoordeeld door het registreren van optreden van lokale en systemische bijwerkingen (via vragenlijsten), monitoring van de incidentie en ernst van COVID-19 en, bij geïmmuniseerde patiënten, meting van anti-HLA-antilichamen na vaccinatie. Dit protocol artikel benoemt een aantal immuunreacties na COVID-19 vaccinatie die mogelijk correleren met bescherming tegen ziekte en kan het voor andere onderzoekers gemakkelijker maken studies met een vergelijkbare methodologie in andere hoog risico populaties op te zetten.

De resultaten van deze studie zijn beschreven in twee artikelen van ons RECOVAC consortium. De belangrijkste bevinding was dat het percentage niertransplantatiepatiënten dat antilichamen maakte na twee mRNA-vaccinaties significant lager was dan dat percentage bij patiënten met chronische nierschade en dialysepatiënten. De laatste twee groepen patiënten deden het ongeveer net zo goed als de controlepersonen. De mediane S1-specifieke IgG-concentraties waren lager in niertransplantatie- en dialysepatiënten. De factoren hogere leeftijd, lager aantal lymfocyten, lagere eGFR, het niet gebruiken van corticosteroïden, kor-

tere tijd na transplantatie en gebruik van mycofenolaat mofetil of mycofenolzuur (MMF / MPA) waren significant geassocieerd met het risico om een non-responder te zijn. Vaccinatie was veilig en dialyse- en niertransplantatiepatiënten hadden minder systemische vaccinatie gerelateerde bijwerkingen vergeleken met patiënten met chronische nierschade en controlepersonen. De bevindingen van onze studies leidden tot de uitbreiding van de basis COVID-19-vaccinatiereeks in het nationale vaccinatieprogramma met een derde dosis voor immuungecompromitteerde patiënten en benadrukten de noodzaak van extra boostervaccinaties.

Alternatieve strategieën die het vermogen van SARS-CoV-2 (booster) vaccinatie om immuniteit te veroorzaken zouden kunnen verhogen werden onderzocht in de RECOVAC Herhaalde Vaccinatie studie, beschreven in **hoofdstuk 6**. We voerden een gerandomiseerde studie uit in meerdere centra, waarbij we zowel alternatieve vaccinatiestrategieën als tijdelijke vermindering van immunosuppressieve medicatie vergeleken met de standaard herhaalde vaccinatie bij niertransplantatiepatiënten die na twee of drie mRNA-vaccinaties geen antilichamen hadden gevormd. We ontdekten dat herhaalde vaccinatie SARS-CoV-2-specifieke antilichamen verhoogt bij niertransplantatiepatiënten die eerder "non-responder" waren. De drie alternatieve strategieën (dubbele dosis mRNA-vaccin, heterologe vaccinatie met het Ad26.COV2-S vaccin en tijdelijke stopzetting van mycofenolaat mofetil) gaven echter geen significante verbetering van het percentage patiënten dat antilichamen vormde, de T cel reacties of de neutralisatie van de originele, delta- en omicron virusstammen, in vergelijking met de standaard dosis herhaalde mRNA-vaccinatie. Belangrijk is dat er geen transplantaat afstotingen en geen vaccinatie gerelateerde ernstige bijwerkingen optraden en dat de nierfunctie stabiel bleef. We concluderen dat om een sterkere immuunreactie te bereiken bij niertransplantatiepatiënten, herhaalde vaccinatie de meest succesvolle strategie is.

CONCLUSIES

Het onderzoek zoals beschreven in dit proefschrift heeft geleid tot de volgende conclusies:

- Vaccinatie is een belangrijke manier om bij immuungecompromitteerde patiënten de immuunrespons tegen bestaande en nieuwe infectieziekten te versterken.
- Na VZV boostervaccinatie en COVID-19 vaccinatie kunnen patiënten met chronische nierschade en dialysepatiënten zonder immunosuppressieve medicatie een immuunrespons bereiken die bijna vergelijkbaar is met de respons in gezonde mensen.
- Het vermogen van vaccins om immuniteit op te wekken is bij niertransplantatiepatiënten lager dan bij patiënten met chronisch nierfalen en dialysepatiënten die geen immunosuppressieve medicatie gebruiken.
- De immuunrespons op vaccins kan verbeterd worden door vaccins krachtiger te maken, vaccins toe te dienen voordat immuunsuppressieve medicatie gestart wordt en vaccinaties te herhalen.
- Verder onderzoek naar het effect van aanpassingen in immunosuppressieve regimes op de immunogeniciteit en effectiviteit van vaccins is nodig om bestaande en opkomende infectieziekten beter het hoofd te kunnen bieden.

Appendices

List of abbreviations
List of publications
PhD portfolio
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Dankwoord

LIST OF ABBREVIATIONS

ABO-I	ABO-blood type incompatible
APC	allophycocyanin
AU	arbitrary units
BAU	binding antibodies units
BMI	body mass index
°C	degrees Celsius
CCR7	C-C chemokine receptor type 7
CD	cluster of differentiation
CKD	chronic kidney disease
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
DBP	diastolic blood pressure
DCD	donation after cardiac death
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunoassay
ELISpot	enzyme-linked immunosorbent spot
ESKD	end stage kidney disease
ESRD	end stage renal disease
FITC	fluorescein isothiocyanate
GMFR	geometric mean fold rise
GMT	geometric mean titer
HLA	human leucocyte antigen
HTx	heart transplantation
HZ	herpes zoster
IGRA	interferon-γ release assay
IFN-γ	interferon gamma
IL-21	interleukin-21
IgG	immunoglobulin G
IVIG	intravenous immunoglobulin
KTR/KTx	kidney transplantation
LiTx	liver transplantation
LuTx	lung transplantation
MMF	mycophenolate mofetil
ml	millilitre

MPA	mycophenolic acid
moDC	monocyte-derived dendritic cell
mRNA	messenger ribonucleic acid
PBMC	peripheral blood mononuclear cells
PE	phycoerythrin
PerCP	peridinin chlorophyl protein
pfu	plaque forming units
PHN	post-herpetic neuralgia
PRA	panel reactive antigen
PRNT	plaque reduction neutralization assay
PY	person years
r-ATG	rabbit anti-thymocyte globulin
RBD	receptor binding domain
RZV	recombinant zoster vaccine
SBP	systolic blood pressure
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SOT	solid organ transplantation
Tx	transplantation
VZV	varicella zoster virus

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1. Alternative strategies to increase the immunogenicity of COVID-19 vaccines in kidney transplant recipients not responding to two or three doses of an mRNA vaccine (RECOVAC): a randomised clinical trial.
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Erasmus MC
Universitair Medisch Centrum Rotterdam

PHD PORTFOLIO

M.M.L. Kho

Description	Organizer	EC
1 **Required**		
Erasmus MC - BROK® (Basic course Rules and Organisation for Clinical researchers) (2012)	Erasmus MC	1.50
Teach the teacher III (2015)	Erasmus MC	0.45
Vaccination Masterclass 2014-2015 (2015)	Virology Education	1.00
BROK re-certification (2016)	NFU	0.25
Workshop How to develop an e-module (2016)	Erasmus Medical University	0.15
BROK re-certification (2020)	NFU	0.25
Scientific Integrity (2021)	Erasmus MC Graduate School	0.30
2 **Optional**		
Bootcongres 2014 (2014)	Nederlandse Transplantatie Vereniging	1.30
ERA-EDTA 2014 (2014)	European Renal Association / European Dialysis and Transplant Association	0.60
Advancements in the Understanding of atypical Hemolytic Uremic Syndrome (aHUS) 2014 (2014)		0.10
Klinisch Review Symposium 2014 (2014)	Nederlandse Transplantatie Vereniging	0.30
Bootcongres 2015 Joint NTV/BTS Congres (2015)		1.00
Nederlands Congres Volksgezondheid 2015 (2015)		0.50
ATC 2015: American Transplant Congress (2015)		1.70
Regionale Nierbiopsie Avond (2015)		0.10
European Society for Organ Transplantation (ESOT 2015) (2015)		1.20
Advancements in the Understanding of atypical Hemolytic Uremic Syndrome (aHUS) 2015 (2015)		0.10

Klinisch Review Symposium 2015 (2015)		0.30
3de Regionale nascholing: Niertransplantatie anno 2016: nieuwe inzichten en uitdagingen! (2016)		0.30
Hypertensie door de jaren heen (2016)		0.20
XXVI International Congress of the Transplantation Society (TTS 2016) (2016)		1.90
Regionale nefro pathologie nascholing (2017)		0.10
Klinisch Review Symposium 2017 (2017)		0.30
Joint NTV-BTS Transplantation Congress 2018 (2018)		0.60
TTS 2018: 27th International Congress of the Transplantation Society (2018)		1.50
Praktische nefropathologie (2018)		0.60
Bootcongres 2019 Wetenschappelijk voorjaarscongres NTV (2019)		0.30
Reference meeting Geriatric Medicine (2019)	Geriatric Medicine Erasmus MC	0.30
Nephsap meeting: Primary Glomerular Diseases (2019)		0.10
ESOT 2019: European Society for Organ Transplantation (2019)		1.20
Klinisch Review Symposium 2019 (2019)		0.30
RPS Endorsed Educational Meeting Rotterdam 2019 (2019)		0.30
Coordinator and teacher Master Internal Medicine, subject nephrology (2020)	Erasmus Medical University	12.00
5e Regionale nascholing Nefrologie en Transplantatie anno 2020: op koers! (2020)		0.40
Bootcongres 2020 Wetenschappelijk voorjaarscongres NTV (2020)		0.60
ATC 2020 VIRTUAL: American Transplant Congress (2020)		0.90
ERA-EDTA 2020 (VIRTUAL): Congress European Renal Association / European Dialysis and Transplant Association (2020)		1.00
Bootcongres 2021 Wetenschappelijk voorjaarscongres NTV - Online (2021)		0.40
Reference meeting Urology (2021)	Urology Erasmus MC	0.30
ATC Open Air (2021)		0.40
Reference meeting Dermatology (Skintermezzo) (2021)	Dermatology Erasmus MC	0.30

ESOT 2021: European Society for Organ Transplantation (2021)		1.20
Klinisch Review Symposium 2021 (2021)		0.20
6e Regionale nascholing Nefrologie en Transplantatie: nefrologische zorg op maat (2022)		0.80
ATC 2022: American Transplant Congress (2022)		1.50
Bootcongres 2022 Wetenschappelijk voorjaarscongres NTV (2022)		0.60
Teaching residents Internal Medicine (2022)		10.00
Teacher in Minor Solid Organ Transplantation (2022)	Erasmus Medical University	10.00
Teaching fellows internal medicine and nephrology, nurses, transplant coordinators (2022)		7.00
		------ +
Total EC		**66.70**

CURRICULUM VITAE

Marcia Mu Lan Kho werd geboren op 5 november 1974 te Rotterdam. In 1993 behaalde zij cum laude het gymnasium diploma aan de Stedelijke Scholengemeenschap in Maastricht. Zij studeerde geneeskunde aan de Universiteit Maastricht en behaalde in 1999 met genoegen het artsexamen. Van 1999 tot en met 2002 werkte zij als arts-assistent Interne Geneeskunde in het Maxima Medisch Centrum te Eindhoven (opleider Prof.dr. H.R. Haak). Vanaf 2002 heeft zij haar opleiding Interne Geneeskunde voortgezet in het Erasmus MC (opleiders Prof. J.H.P. Wilson, Prof.dr. H.A.P. Pols, Prof.dr. J.L.C.M. van Saase) en in 2005 startte haar differentiatie Nefrologie (opleider Prof.dr. R. Zietse). Vanaf 2007 is zij werkzaam als internist-nefroloog in het Erasmus MC. De afgelopen jaren heeft zij onder andere onderzoek gedaan naar de immuunrespons van nierpatiënten op vaccinatie tegen varicella zoster en SARS-CoV-2, dat heeft geleid tot dit proefschrift.

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