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Early warning signals in psychopathology

Schreuder, Marieke J; Hartman, Catharina A; George, Sandip V; Menne-Lothmann, Claudia; Decoster, Jeroen; van Winkel, Ruud; Delespaul, Philippe; De Hert, Marc; Derom, Catherine; Thiery, Evert

Published in:
BMC Medicine

DOI:
[10.1186/s12916-020-01742-3](https://doi.org/10.1186/s12916-020-01742-3)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Schreuder, M. J., Hartman, C. A., George, S. V., Menne-Lothmann, C., Decoster, J., van Winkel, R., Delespaul, P., De Hert, M., Derom, C., Thiery, E., Rutten, B. P. F., Jacobs, N., van Os, J., Wigman, J. T. W., & Wichers, M. (2020). Early warning signals in psychopathology: what do they tell? *BMC Medicine*, 18(1), 1-11. [269]. <https://doi.org/10.1186/s12916-020-01742-3>

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Research paper



Immune response to varicella-zoster virus before and after renal transplantation

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ARTICLE INFO

Keywords:

Herpes zoster
 Cellular immunity
 Humoral immunity
 Renal transplant

ABSTRACT

Background: Herpes zoster (HZ) risk is high in renal transplant recipients. Vaccination prior to transplantation may provide a useful strategy for the prevention of HZ in the posttransplantation period. However, it is not known whether immunity to varicella-zoster virus (VZV) is affected due to treatment surrounding transplantation.

Methods: Both humoral and cellular immunity to VZV were determined prior to and 2–3 years after renal transplantation in 60 adult patients, and 62 matched healthy controls. VZV-specific cellular immunity was measured by an interferon gamma (IFN γ) enzyme-linked immunospot (ELISpot) assay and by analyzing T-cell functionality using flowcytometry. VZV-IgG levels were measured using an in-house glycoprotein enzyme-linked immunosorbent assay (gpELISA).

Results: Using paired analysis, it was determined that numbers of IFN γ -producing cells did not change after transplantation, but were significantly lower in transplant recipients after transplantation than in controls ($p = 0.028$). Patients in whom the post-transplant period was complicated by rejection or any acute infection (excluding HZ) had a lower number of IFN γ -producing cells than patients who did not. VZV IgG levels did not differ from controls, but a significant decrease was observed after transplantation ($p < 0.0001$).

Conclusions: VZV-specific cellular immunity, which is essential in the prevention of HZ, did not markedly change in patients following renal transplantation. This suggests that preventive vaccination before transplantation may be beneficial. Our results extend knowledge on VZV immunity after transplantation, vital when considering strategies for the prevention of HZ in these patients.

1. Introduction

Herpes zoster (HZ, shingles), characterized by neuralgia and a vesicular rash, is due to reactivation of a latent varicella-zoster virus (VZV) infection. Neuralgia can last for months or even years, known as post-herpetic neuralgia (PHN) (Gershon et al., 2010). The pain can have a major effect on a patients' quality of life, and is often difficult to treat (Drolet et al., 2012). After VZV vaccination or a primary VZV infection the virus remains latently present for life in sensory neurons of the dorsal root ganglia (Gershon et al., 2010; Zerboni et al., 2014). Being intensively treated with immunosuppressive medication, renal transplant recipients are known to be at increased risk of HZ. Incidence is estimated

to be 28 to 37 per 1000 person years, which is 6–11 times higher than in the general population (Johnson et al., 2015). Also, a high prevalence of PHN of up to 48.7% has been reported (Pavlopoulou et al., 2015). Disseminated disease and visceral involvement are rare complications of HZ that occur mainly in immunocompromised patients and may have a lethal outcome (Yawn et al., 2007).

Cellular immunity to VZV is considered to be essential in the immune response to VZV and prevention of HZ. Number and functionality of VZV-specific CD4⁺ T cells have been shown to be impaired in immunocompromised patient groups at increased risk of HZ (Schub et al., 2015).

Currently, two types of zoster vaccine are licensed for the prevention

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<https://doi.org/10.1016/j.antiviral.2020.104938>

Received 27 May 2020; Received in revised form 29 August 2020; Accepted 18 September 2020

Available online 6 October 2020

0166-3542/© 2020 The Author(s).

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of HZ. The first is a live attenuated vaccine, containing the same virus strain as the childhood varicella vaccine, but 14 times more potent (Levin et al., 2008). It was shown to reduce incidence of HZ by 51% and of postherpetic neuralgia by 67% in healthy people above 60 years of age (Oxman et al., 2005). It also was shown to be effective in patients with end-stage renal disease (Tseng et al., 2016) and to increase VZV-specific humoral immunity in a small group of patients immunized prior to renal transplantation (Miller et al., 2018). However, the risk of live zoster vaccination in transplant recipients is demonstrated by a case report of an infection with the vaccine VZV strain shortly after vaccination, in a 49-year old woman 4 years after receiving a renal transplant (Ortiz-Brizuela et al., 2019).

More recently, a recombinant subunit zoster vaccine has been introduced, containing VZV envelope glycoprotein E and adjuvant system AS01B. Studies in healthy persons above 50 years of age demonstrate a higher efficacy than the live attenuated zoster vaccine (Lal et al., 2015; Cunningham et al., 2016). Based on the limited available evidence on safety of zoster vaccination in patients on moderate to high doses of immunosuppressant therapy, the United States Advisory Committee on Immunization Practices (ACIP) in 2018 did not recommend either of the zoster vaccines (Dooling KL, Guo A, Patel M et al., 2018). Since then, Vink et al. published results of a study including 132 renal transplant recipients vaccinated using the recombinant subunit vaccine 4–18 months after transplantation, and an equal number receiving placebo. Cellular immunity was analysed in 36 vaccinated and 32 controls, with satisfying results. There were no safety concerns, but patients with any autoimmune or potential immune-mediated disease were excluded (Vink et al., 2020). Although this novel vaccine seems promising, even following transplantation, the optimal timing of administration of zoster vaccination remains to be determined.

To date, it is unclear how VZV-specific immunity evolves surrounding renal transplantation and consequently, whether zoster vaccination prior to transplantation could be efficacious to prevent HZ in transplant recipients. Aim of the current study therefore was to investigate VZV immunity before and after renal transplantation, in order to increase understanding of VZV-specific immune responses in this patient group.

2. Methods

2.1. Study population

Eligible patients received a renal transplant in the University Medical Center Groningen (UMCG) between 2 and 3 years before inclusion and were still under supervision in this center. Blood was drawn at outpatient clinic visits. Peripheral blood mononuclear cells (PBMC) and serum samples of the same patients, collected before administering induction therapy, immediately prior to the most recent transplantation, were retrieved from diagnostic archives. Healthy control subjects were age and sex-matched to patients.

Immediately before and 4 days after renal transplantation standard induction therapy using basiliximab (anti-CD25; 2 doses of 20 mg) was administered. Immunosuppression after transplantation consisted of tacrolimus or cyclosporine, in combination with mycophenolate mofetil and prednisolone. Prednisolone dose was gradually tapered until a fixed dose of 5 mg. Anti-rejection therapy in 11 of 60 included patients generally consisted of intravenous prednisolone (1000 mg intravenously on three consecutive days, possibly repeated). Three of 11 patients received alemtuzumab (anti-CD52) and one received anti-thymocyte globulin next to prednisolone for treatment of acute rejection.

The study was approved by the institutional review board of the University Medical Centre Groningen (METc 2014/305 and 2012/375). All patients and controls gave written informed consent.

2.2. Clinical data, including occurrence of varicella, HZ and other infections

Patient characteristics and medical history were retrieved from medical records. Furthermore, medical records were reviewed for occurrence of acute infections in the period between transplantation and study inclusion (2–3 years after transplantation). Seropositivity for herpes viruses, hepatitis viruses or BK virus were not regarded as acute infections.

In addition to review of medical records, patients and controls were asked about their history of varicella and HZ using a questionnaire (in Dutch). In contrast to the United States, routine vaccination to prevent varicella in children is not practiced in The Netherlands (Wutzler et al., 2017), and VZV immunity through natural infection is experienced in childhood (de Melker et al., 2006).

2.3. Isolation, storage and thawing of PBMC and serum

Immediately after collection of venous blood in lithium heparin containing tubes, PBMC were isolated according to standard protocols and stored in liquid nitrogen until use. Upon thawing, cell viability was evaluated by trypan blue staining, and was between 85 and 100%. Serum was stored at -20°C until use.

2.4. Interferon- γ (IFN γ) ELISpot assay

Interferon- γ (IFN γ) ELISpot assay was performed as previously described (Rondaan et al., 2014). In short, 2×10^5 PBMC (in 100 μl) were stimulated in duplicate using 10 μl UV-inactivated varicella vaccine (Provarivax; MSD, 1350 PFU/0.5 ml) in 200 μl endvolume or 5 $\mu\text{g}/\text{ml}$ of concanavalin A (positive control), while a negative control consisted of PBMC in culture medium alone. Spots were counted using an automated reader (AID EliSpot Reader; Autoimmun Diagnostika GmbH). Results were only accepted when concanavalin A yielded a positive result (see Supplemental Fig. S1). Mean number of spots in VZV-stimulated wells was corrected for mean number of spots in negative control wells. Results are referred to as number of IFN γ spot-forming cells per 2×10^5 PBMC.

2.5. Flowcytometric analysis

PBMC ($1.2 \times 10^6/\text{tube}$) were stimulated using 20 μl UV-inactivated varicella vaccine, as positive control with 5 $\mu\text{g}/\text{ml}$ staphylococcal enterotoxin B (SEB; Sigma-Aldrich) plus 1 $\mu\text{g}/\text{ml}$ anti-CD28/CD49d (Beckton Dickinson (BD) or left unstimulated (negative control) in a total volume of 200 μl . PBMC were stimulated for 18 h, of which the last 16 h in presence of 10 $\mu\text{g}/\text{ml}$ brefeldin A (Sigma-Aldrich). Fluorescent T cell barcoding staining and immunostaining with anti-CD3 (clone SK7, BD), anti-CD8 (clone SK1, BD), anti-CD69 (clone FN50, BD), anti-IFN γ (clone B27, BD), anti-tumour necrosis factor alpha (TNF α) (clone Mab11, eBioscience) and anti-interleukin 2 (IL-2) (clone 599334, BD) was performed as described previously (Rondaan et al., 2018), with addition of anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (clone C3D10, Biologend). Data were collected for 1.0×10^6 events for each sample and plotted using Kaluza v1.2 (Beckman Coulter) (Supplemental Figs. 4 and 5 for gating examples). CD4 $^+$ T cell populations were gated as CD3 $^+$ CD8 $^-$. Results were expressed as percentage of CD69 $^+$ cytokine/CTLA-4/PD-1 expressing CD4 $^+$ T cells within total CD4 $^+$ T cell population.

2.6. Antibody levels to VZV

VZV-specific IgG antibodies were quantified using an in-house glycoprotein (gp) enzyme-linked immunosorbent assay (ELISA), which was previously developed and validated using a quantitative Serion

classic VZV IgG ELISA (Institut Virion/Serion) and the institution's standard diagnostic test for VZV serology (Rondaan et al., 2014). As antigen, VZV purified glycoproteins (EastCoastBio) were used. Pooled human serum with known levels of anti-glycoprotein VZV was used as standard. According to recommendations of Institut Virion/Serion, VZV-IgG levels above 100 mIU/ml were considered positive.

2.7. Statistical analysis

Wilcoxon signed rank test, intended for paired analyses, was used to compare continuous variables before and after transplantation. Results and characteristics of subgroups of patients were compared using a Mann-Whitney test or Fisher's exact test. Comparisons between transplant recipients (post-transplantation) and healthy control groups were done using Mann-Whitney *U* test or Fisher's exact test when appropriate.

For correlations, Spearman's rho was used. P-values ≤ 0.05 (2-sided) were considered significant. Statistical analysis was performed using IBM SPSS Statistics 23 (IBM) and GraphPad Prism 7.02 (GraphPad Software, USA).

3. Results

3.1. Study population

Characteristics of 60 renal transplant recipients and 62 healthy control subjects are summarized in Table 1. At time of inclusion, 2–3 years after transplantation, only 1 (2%) transplant recipient was in need of renal replacement therapy, following rejection of the transplant kidney. There were no significant differences in gender and age between patient and control group and between patients who experienced rejection or infection after transplantation and those who did not.

3.2. Occurrence of HZ and other acute infections

Questionnaire results, asking about history of varicella and HZ, were available for 53 (88%) of transplant recipients. Combined with results from medical record review, it was determined that 13 patients (22%) had a history of HZ. In four of these patients, timing was not known. In three HZ occurred before and in six within the 2–3 years after most recent transplantation. One patient experienced HZ only three weeks after transplantation, and another patient immediately after rejection therapy (with methylprednisolone and leflunomide). No HZ episodes occurred during prophylactic use of valganciclovir.

Of the healthy control group, questionnaire results were available for 32 (52%) persons. Four (13%) stated to have experienced HZ, of which one subject a few months prior to study participation, and the other three at least 15 years prior to study inclusion.

Occurrence of acute infections in the 2–3 year period between transplantation and study inclusion is presented in Table 1. A more detailed summary is provided in Supplementary Table S2.

3.3. Cellular immunity to VZV

Number of IFN γ spot-forming cells in response to VZV stimulation did not significantly change following renal transplantation, but was lower in patients after transplantation than in healthy controls ($p = 0.028$) (Fig. 1A). Of note, before transplantation no significant difference compared to healthy controls was seen ($p = 0.106$). CMV status and history of more than one transplantation was found not to be of influence. The same held true for patients who were transplanted preemptively versus patients treated with renal replacement therapy prior to transplantation (data not shown). Interestingly, transplant recipients in whom post-transplantation period was complicated by a rejection episode were found to have a lower number of IFN γ spot-forming cells in response to VZV stimulation, which was significant prior to transplantation. Patients with a HZ history were excluded from this analysis

Table 1

Characteristics of healthy controls and transplant recipients.

	HC n = 62	Tx n = 60
Female gender, no. (%)	33 (53)	30 (50)
Age, median (range) years	58.4 (25.3–72.6)	55.6 (25.7–72.5)
Time since transplantation, median (range) months	NA	33.7 (25.6–38.4)
Cause of renal failure, no. (%)	NA	
Glomerulonephritis		25 (42)
IgA nephropathy		14 (23)
MPGN		2 (3)
FSGS		3 (5)
Anti-GBM		1 (2)
SLE		2 (3)
AAV		2 (3)
Cause unknown		1 (2)
Genetic		7 (12)
Vascular		4 (7)
Diabetic nephropathy		5 (8)
Congenital		2 (3)
Urologic		3 (5)
Chronic TIN		3 (5)
Unknown		9 (15)
Other		2 (3) ^a
History of >1 transplant, no. (%)	NA	15 (25) ^b
RRT pre-Tx, no.	NA	
None/HD/PD		17/29/14
VZV serostatus		
Negative/positive	0/62	0/60
CMV serostatus		
Negative/positive	31/15 ^c	43/17
Seroconversion in post-Tx period, no. (%)	NA	11 (19)
History of herpes zoster, no. (%)	4 of 32 (13)	13 (22) ^d
Temporary (val)ganciclovir, no. (%) ^e	NA	14 (23)
No. (%) of patients with post-Tx complications ^f	NA	
Rejection	NA	11 (19)
Infection, any/bacterial/viral/fungal ^g	NA	40/25/27/4

No.: number, Tx: transplantation, MPGN: membranoproliferative glomerulonephritis, FSGS: focal segmental glomerulosclerosis, GBM: glomerular basement membrane, SLE: systemic lupus erythematosus, AAV: ANCA-associated vasculitis, TIN: tubulointerstitial nephritis, RRT: renal replacement therapy, HD: haemodialysis, PD: peritoneal dialysis, VZV: varicella-zoster virus.

^a One patient developed renal failure because of acute tubulus necrosis (after acute aorta rupture surgery) and one patient underwent nephrectomy of his one functional kidney because of transitional cell carcinoma.

^b Two patients received a transplant organ other than kidney before their most recent renal transplantation; one received a heart transplant and one received multiple liver transplants.

^c Information on CMV status is missing for 16 persons in the HC-group.

^d In six patients, timing of herpes zoster was known to have occurred after most recent transplantation (within 2–3 years before study inclusion).

^e CMV-load was evaluated after transplantation. Patients with a positive load received (val)ganciclovir until CMV-load was <100 copies/ml twice.

^f Missing information on rejection in 2 patients and on infection (other than herpes zoster) in 1 patient.

^g More detailed information on the occurrence of infections in the 2–3 years after transplantation is provided in Supplementary Table S2.

(Fig. 1B).

Transplant recipients who experienced any acute infection other than HZ in 2–3 years post-transplantation (excluding those with a HZ history) also had lower numbers of IFN γ spot-forming cells in response to VZV stimulation than those who did not. This difference was not yet present in the same patients prior to transplantation (Fig. 1C). Analyzing data separately for viral or bacterial infections yielded similar results (data not shown).

Occurrence of HZ within 2–3 years before blood drawing was not of influence on number of IFN γ spot-forming cells in response to VZV stimulation, but number of subjects was low ($n = 6$; Supplemental Fig. S3).

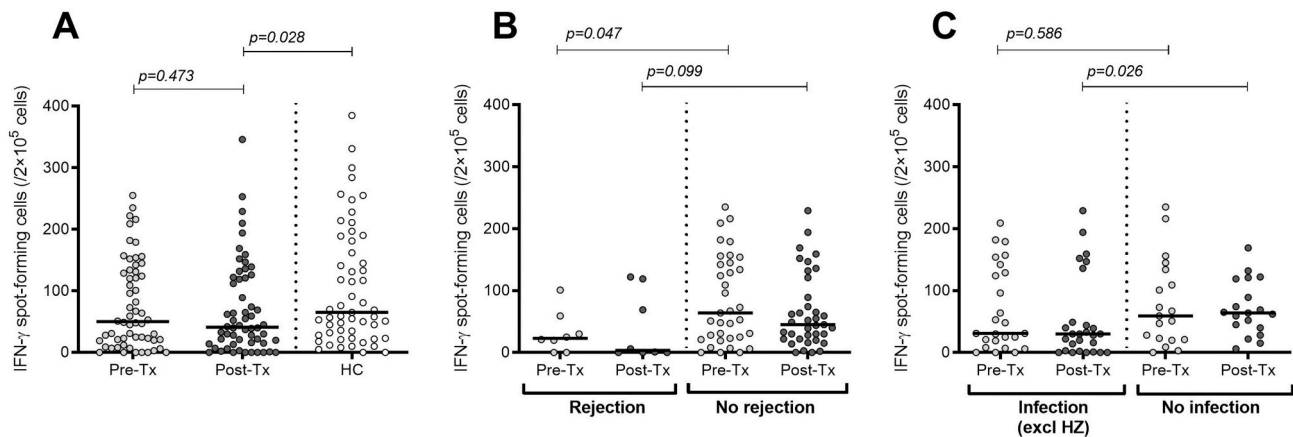


Fig. 1. Numbers of interferon- γ (IFN γ) spot-forming cells in response to VZV stimulation in (A) 55/62 healthy control (HC) subjects and 58/59 patients before (pre) and 2–3 years after transplantation (Tx), (B) 8 patients that experienced a rejection episode and 35–37 patients who did not, before (pre) and 2–3 years after transplantation (Tx), (C) 25/27 patients that experienced an acute infection (excluding herpes zoster) in the post-transplantation period and 19 patients who did not, before (pre) and 2–3 years after transplantation (Tx). Patients with a history of herpes zoster were excluded from analysis. Lines show the median.

3.4. Humoral immunity to VZV

All patients and controls were VZV seropositive. After transplantation VZV-IgG level was significantly lower than before transplantation ($p < 0.0001$). There was no significant difference between healthy controls and transplant recipients ($p = 0.149$) (Fig. 2A). CMV status was found not to be of influence (data not shown). In contrast to cellular immunity results, humoral immunity to VZV was not found to be different in transplant recipients who experienced rejection of their transplant kidney (Fig. 2B). Although not statistically significant, patients that experienced an acute infection other than HZ tended to have higher antibody levels to VZV than patients without any documentation of acute infections in their records (Fig. 2C).

3.5. Increased percentages of cytokine-expressing T cells prior to renal transplantation

Upon stimulation with VZV, significantly higher percentages of CD4⁺ T cells producing TNF α and IL-2 were found in patients prior to transplantation. This was also seen for IL-2 upon polyclonal stimulation using SEB (Fig. 3A and B). TNF α expression was significantly higher compared to HC after SEB stimulation.

Similar percentages of IFN γ -producing CD4⁺ T cells after VZV and

SEB stimulation were found before and after transplantation. Also, no difference in percentage of T cells producing this cytokine were found between transplant recipients and control subjects (Fig. 3A and B).

3.6. Expression of PD-1 and CTLA-4 before and after transplantation

Mean fluorescence intensity (MFI) of programmed cell death protein 1 (PD-1) was higher in VZV-specific cells than in polyclonal stimulated cells. No differences between transplant recipients and control subjects were observed for PD-1 expression (Fig. 4A). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) MFI on CD4⁺ T cells was significantly increased in patients after transplantation, both after VZV and SEB stimulation (Fig. 4C). Percentages of T cells expressing PD-1 were also higher after VZV stimulation compared to SEB stimulation, and increased compared to HC after SEB stimulation (Fig. 4B). Percentages of CTLA-4 positive cells increased after transplantation, but remained lower compared to HC (Fig. 4D).

3.7. Correlations

An inverse correlation between age and cellular immunity to VZV (number of IFN γ spot-forming cells) ($\rho = -0.389$, $p = 0.002$) was present in transplant recipients (post-Tx), which was not seen in controls or

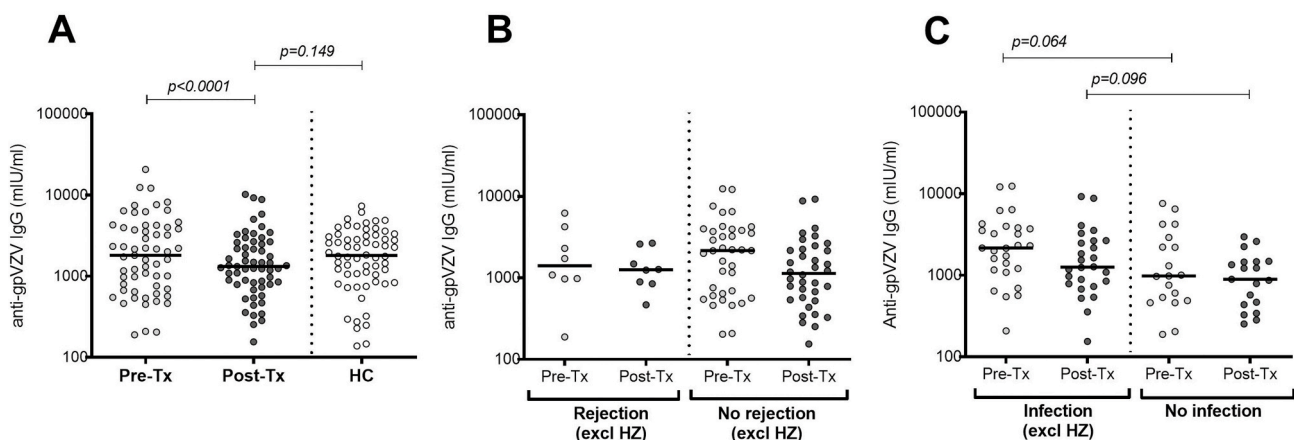


Fig. 2. Levels of anti-glycoprotein (gp)VZV IgG presented on a log-scale in (A) 55/62 healthy control (HC) subjects and 58/59 patients before (pre) and 2–3 years after transplantation (Tx), (B) 8 patients that experienced a rejection episode and 35/37 patients who did not, before (pre) and 2–3 years after transplantation (Tx), (C) 27 patients that experienced an acute infection (excluding herpes zoster) in the post-transplantation period and 19 patients who did not, before (pre) and 2–3 years after transplantation (Tx). Patients with a history of herpes zoster were excluded from analysis.

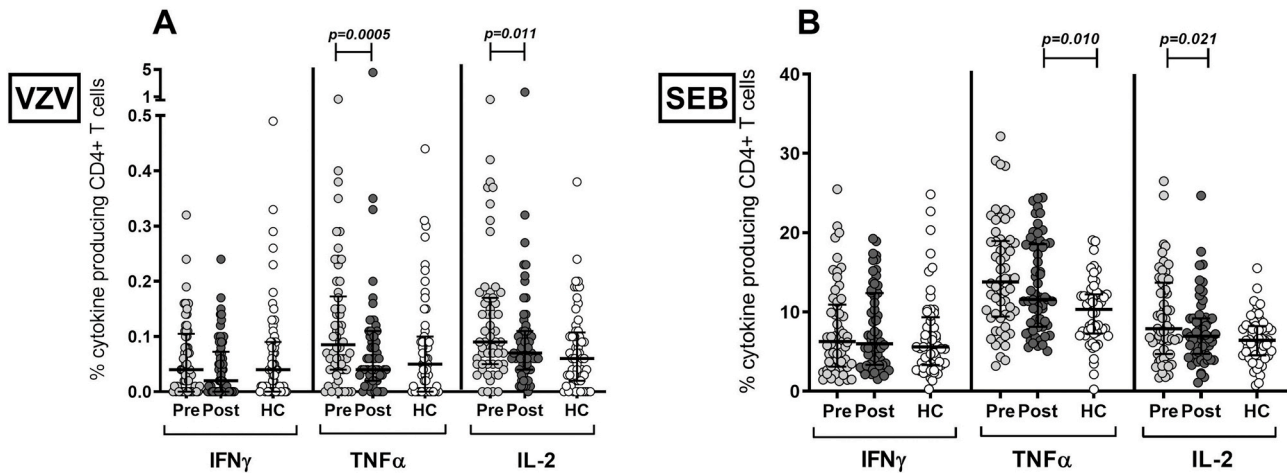


Fig. 3. Frequencies of cytokine-producing CD4⁺ T cells per total CD4⁺ T cells upon stimulation with varicella-zoster virus (VZV) (3A), and upon stimulation with staphylococcal enterotoxin B (SEB, positive control) (3B) in 56 healthy control subjects (HC) and 59 patients before (pre) and 2–3 years after (post) receiving a renal transplant. Bars show the median and interquartile range. IFN γ = interferon gamma; TNF α = tumor necrosis factor alpha; IL-2 = interleukin-2.

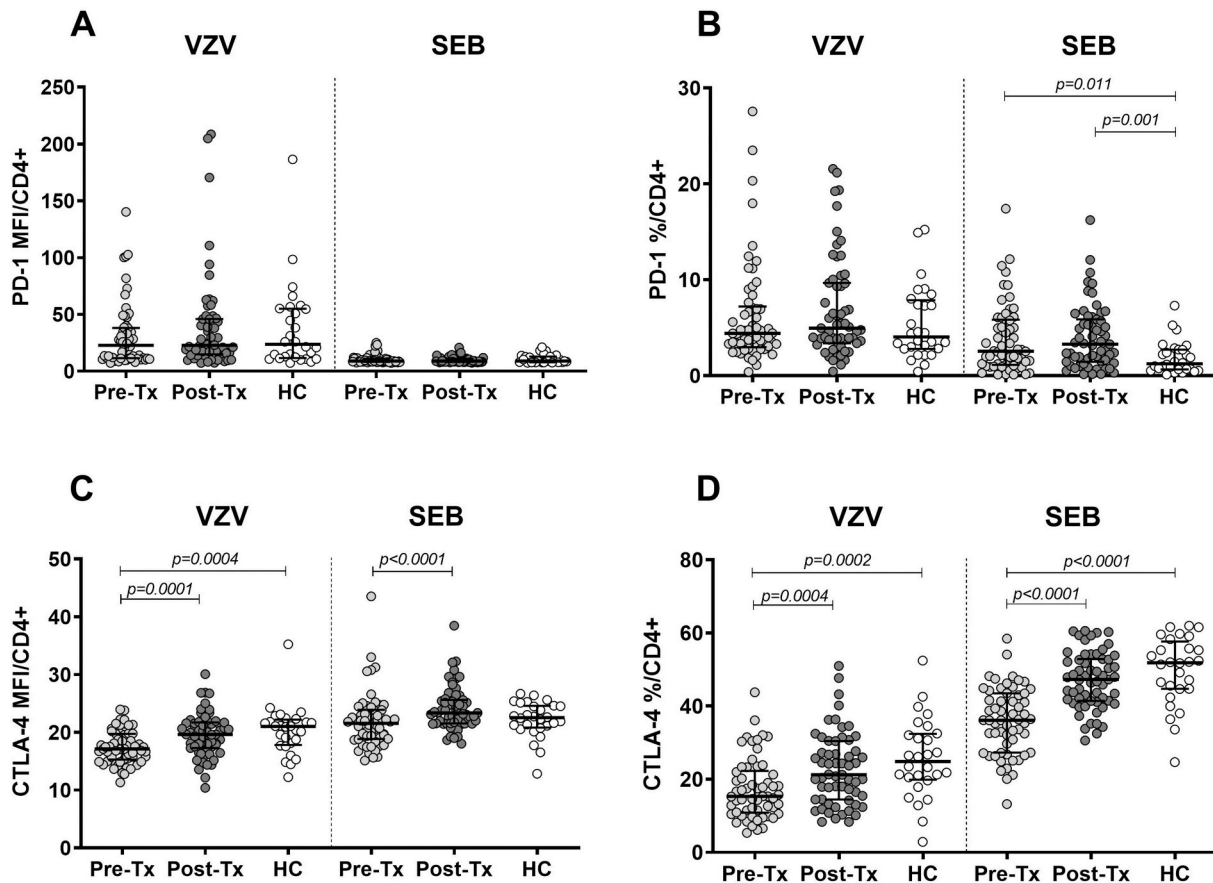


Fig. 4. Mean fluorescence intensity (MFI; A,C) and percentages of CD4⁺ T cells positive expressing (%; B, D) programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) upon stimulation with varicella-zoster virus (VZV) and staphylococcal enterotoxin B (SEB, positive control) in 28 healthy control subjects (HC) and 59 patients before (pre) and 2–3 years after (post) receiving a renal transplant. Bars show the median and interquartile range.

prior to transplantation. No significant correlations were found between age and humoral immunity to VZV, or between humoral and cellular immunity to VZV (data not shown).

4. Discussion

In this study VZV-specific immunity was evaluated in 60 patients

before and after renal transplantation. Cellular immunity to VZV, as assessed by IFN γ ELISpot assay, did not significantly change after transplantation, but was significantly lower in patients after transplantation than in control subjects. VZV-specific humoral immunity in transplant recipients did not differ from controls, but a significant decrease was observed after transplantation.

Cellular immunity to VZV, and especially CD4⁺ T cells, is considered

to be essential in immune response to VZV and prevention of HZ (Schub et al., 2015). Responding cells in IFN γ ELISpot assay consist mostly of CD4⁺ T cells. This assay, which tests functionality of antigen-specific cells, is generally considered to be a reliable method for assessment of virus-specific cellular immunity (Karlsson et al., 2003). Previously, decreased numbers of IFN γ spot-forming cells upon VZV stimulation have been demonstrated in elderly persons and different immunocompromised patient groups at increased risk of HZ (Rondaan et al., 2014; Shirane et al., 2017). Our finding of a lower number of IFN γ spot-forming cells upon VZV stimulation in transplant recipients compared to controls is in line with the reported increased HZ incidence in this group (Arness et al., 2008; Pavlopoulou et al., 2015). Interestingly, in response to VZV stimulation a lower number of IFN γ spot-forming cells was present in transplant recipients in whom post-transplant period was complicated by rejection or acute infections other than HZ. Anti-rejection therapy was previously identified to be a significant risk factor for development of HZ in renal transplant recipients (Pavlopoulou et al., 2015), but as the difference was also present before transplantation this could indicate that a suboptimal immune system is already present in these patients prior to transplantation. The lower number of IFN γ spot-forming cells in response to VZV stimulation in patients who experienced acute infection in the years following transplantation may be a reflection of a defective cell-mediated immunity in general, and therefore a higher susceptibility to infection. The finding was accompanied by a slightly higher (non-significant) VZV-IgG level in patients who experienced infection. We speculate this might be caused by subclinical reactivations of VZV in immunocompromised hosts, as already described by Ljungman et al., in 1986 (Ljungman et al., 1986).

Age is a well-known risk factor for HZ, and has been shown to be associated with impaired cellular immunity to VZV (Tang et al., 2012). This is in line with our finding of an inverse correlation between number of IFN γ spot-forming cells and age of transplant recipients.

Next to evaluating cellular immunity to VZV using an IFN γ ELISpot assay, we evaluated cytokine production of CD4⁺ T cells in response to stimulation with VZV. Cytokine production was shown to be high prior to transplantation, but did not differ between transplant recipients and control subjects. As this phenomenon was also seen upon polyclonal stimulation, it was not VZV-specific. Hypercytokinaemia could be an explanation of our findings, which is known to occur in chronic kidney disease patients, and is associated with uraemia, which causes activation and decreased functions of all immune cells (Betjes, 2013).

Upregulation of inhibitory receptors such as PD-1 and CTLA-4 has been identified as an important feature of exhausted T cells. Exhausted T cells, resulting from a persistent (viral) infection, are less able to exert their effector functions (Kahan et al., 2015). As we found expression of inhibitory receptor PD-1 to be similar in transplant recipients and controls, we could not confirm the finding of an increased PD-1 expression on CD4⁺ T cells in transplant recipients upon polyclonal stimulation, as reported by Schub et al. Notably PD-1 expression during active HZ however. (Schub et al., 2015). Mean fluorescence intensity of PD-1 was higher upon VZV stimulation than upon polyclonal stimulation. This may be explained by the physiologic role of PD-1 in the regulation of immune responses towards a specific antigen (Teigler et al., 2017). Expression and percentage of CTLA-4 however was increased after transplantation both after VZV and polyclonal stimulation. Checkpoint inhibitors are currently considered as immunomodulators in the case of tolerance induction in transplantation (Mardomi et al., 2020).

Our results are in line with those of a study by Kho et al. (2017), in which adult renal transplant candidates with undetectable VZV-IgG levels, were vaccinated using a live attenuated varicella vaccine. Cellular immunity to VZV was evaluated in 11 patients before vaccination and after transplantation (median 7.2 months after transplantation). While the total number of leukocytes decreased, the percentage of VZV-specific CD4⁺ memory T cells was shown to significantly increase, suggesting that VZV-specific cellular immunity is able to

persist during renal transplantation.

This study has limitations. Firstly, information regarding HZ and other infections could have been missing from medical records as these do not necessarily come to attention of a hospital specialist. Questionnaire results on HZ occurrence may not be completely reliable. Furthermore, differences in VZV-immunity between diagnostic subgroups could not be reliably evaluated because of the limited number of patients in the different subgroups. Medium alone is not the optimal negative control as UV-inactivated varicella vaccine that was used for stimulations contains substances other than VZV that might lead to VZV-independent T cell stimulation. Using varicella vaccine as an in vitro stimulus may lead to different results than when using the more physiologic, wild type varicella virus. Lastly, T cell tests are not standardized and results differ greatly between publications.

In conclusion, this study has added to the knowledge on the influence of transplantation on VZV immunity, which is important to understand the high HZ risk in renal transplant recipients and when considering strategies for prevention of HZ in these patients.

Funding

This work was financially supported by a Healthy Ageing grant from the University Medical Center Groningen (2014-2/222).

Ethics approval

The study was approved by the institutional review board of the University Medical Centre Groningen (METc 2014/305 and 2012/375).

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank dr. Bouke Hepkema for generously providing pre-transplant PBMC from the Transplantation Immunology archives and Annelien Hooijsma for her laboratory assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.antiviral.2020.104938>.

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