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Evaluation of Torque Teno Virus DNA Load as a Predictive Biomarker in Kidney Transplant Recipients Converted from Calcineurin Inhibitors to Belatacept

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Introduction: Belatacept is a relevant alternative to calcineurin inhibitors (CNIs) after kidney transplantation (KT). Circulating Torque Teno virus (TTV) DNA load is correlated to infections and rejection risks post-KT in patients treated with CNIs. The aim of this study was to assess the TTV DNA load profile in kidney transplant recipients converted from CNIs to belatacept and explore its use as a predictive biomarker.

Methods: Sixty-eight single-center kidney transplanted recipients who were converted from CNIs to belatacept between June, 2015 and December, 2020 were included in this study. Whole blood TTV DNA load was measured before, at 3, 6, and 12 months post-belatacept conversion. Our primary end point was to assess the TTV DNA load profile and correlate the results with rejection and opportunistic infection (OPI).

Results: TTV DNA load remained stable after belatacept conversion, that is, 3.8 (3.1–4.9), 4.4 (3.2–5.4), 4.0 (3.0–5.7) and 4.2 (3.0–5.2) $\log_{10}$ copies/ml at baseline, 3, 6, and 12 months, respectively. No correlation was found between TTV DNA load and post-KT complications. Chronic allograft dysfunction at 1 year post-conversion was associated with a lower TTV DNA load after 6 and 12-months ($P = 0.014$ and $P = 0.021$, respectively). A higher TTV DNA load was found in older patients and in those with higher body mass index (BMI) ($P = 0.023$ and $P = 0.005$, respectively).

Conclusion: Conversion from CNIs to belatacept did not affect TTV DNA load. OPIs or acute rejection occurrences were not associated with TTV DNA load. However, low TTV (lTTV) DNA load after 6 months postconversion may be a promising tool to predict graft dysfunction risk at 1-year postconversion.

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Chronic kidney diseases are a major public health problem leading to various complications and a higher risk of mortality. KT is the best therapeutic option for renal replacement therapy and improves survival and quality of life in comparison to chronic dialysis.[1,2] Long-term immunosuppressive therapy posttransplantation is mandatory, but sometimes not sufficient, to prevent both T cell-mediated and antibody-mediated rejections. Currently, CNIs such as tacrolimus or ciclosporin in association to mycophenolate mofetil and in some cases steroids, are the cornerstone of maintenance immunosuppression.[3] The monitoring of immunosuppressive therapies and drug adjustments rely on therapeutic drug monitoring, using pharmacokinetic tools. High tacrolimus trough levels have been associated with the risk of nephrotoxicity but low tacrolimus trough levels do not predict the occurrence of acute rejection episodes after KT.[4,5] In addition, long-term exposure to tacrolimus is

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associated with chronic nephrotoxicity, such as interstitial fibrosis and arteriolar hyalinosis, and eventually graft loss, even if tacrolimus-targeted concentrations are within desired ranges.[6] Tacrolimus remains the cornerstone of the immunosuppressive regimen in 2023; however, there is a need to use other similarly efficient molecules, yet not nephrotoxic, to prevent rejection.

Belatacept is a fusion protein blocking the costimulation signal and the cross-talk between T and B lymphocytes. Belatacept inhibits the interaction between the clusters of differentiation CD28 and CD80 (B7-1)/86 (B7-2) corresponding to the second activation signal, which is needed for T cell activation by the antigen presenting cells. Belatacept is devoid of nephrotoxicity and i.v. infusion may increase the patient's adherence to the treatment. It has been demonstrated in the BENEFIT and BENEFIT-EXT trials that belatacept-treated KT recipients have up to 7-years post-transplantation superior glomerular filtration rate as compared to CNI-treated patients.[7-9] Metabolic benefit of belatacept has been also reported with a glycated hemoglobin decrease in patients with type 1 and type 2 diabetes and an improvement of blood pressure and lipid profiles.[10,11] However, it has been reported that belatacept is associated with a higher risk of OPIs as compared to CNI-based therapy and a higher risk of rejection postconversion.[12,13] Moreover, the therapeutic drug monitoring of belatacept and the correlation with rejection and OPI risk is not yet established.[14] A biomarker that can accurately depict the immune system's condition during immunosuppressive treatment is essential for anticipating the risks of infection and rejection.

TTV is a small, ubiquitous virus belonging to the *Anelloviridae* family with single stranded DNA genome. Twenty-nine species have been described.[15] It infects more than 90% of the population without any pathogenic role. The prevalence of TTV DNA in plasma differs across different ages and seems to be higher in the elderly.[16] It is currently investigated as a new immunological tool to assess the global level of immunosuppression under CNI, after KT. Indeed, an lTTV DNA load indicates underimmunosuppression and a high rejection risk.[17-20] Conversely, a high TTV (hTTV) DNA load indicates overimmunosuppression and a high infectious risk.[18,21] It seems to be a promising tool for risk stratification in KT patients under CNI regimen.[22] To date, TTV viral load evolution and its ability to be a predictive biomarker in KT patients treated with belatacept has never been studied.

The main objective of this study was to assess the profile of TTV DNA load in KT recipients converted from a CNI-based regimen to a belatacept-based regimen, and to correlate TTV DNA load with the risk of OPI and acute rejection. In addition, we aimed to assess the ability of TTV DNA load to be a clinical predictive biomarker.

METHODS

Patients

Patients who underwent KT between June, 1997 and December, 2020 in our university hospital, were retrospectively assessed. We included patients that underwent a late conversion (>6 months post-transplantation) from tacrolimus-based regimen to belatacept-based regimen. Conversion was motivated either by prevention of tacrolimus-induced nephrotoxicity or to limit overt tacrolimus-induced nephrotoxicity, in cases of biopsy-proven tacrolimus-induced nephrotoxicity (defined by lesions such as arteriolar hyalinosis, arteriolar myocytes vacuolization, thrombotic microangiopathy, and/or acute tubular necrosis). The decision to switch to belatacept was additionally driven by intolerance to CNI (manifested as digestive disorders, tremors, or challenges in dosage adjustments) and the presence of histological vascular lesions, which could be inherited from the donor. Blood samples were collected before the conversion, at 3, 6, and 12 months post-belatacept conversion; the samples were stored frozen at −80 °C until subsequent analyses. The dosing assessments were conducted retrospectively on whole blood from residual samples obtained during routine care. Clinical data were retrospectively collected from our clinical or biological electronic records at preconversion, at 12 months postconversion, and at the last follow-up. All patients provided written consent for the storage and reuse of their blood samples. The University Hospital review board approved this retrospective monocentric study (registration RnIPH 2023, protocol SwitchAbela; CNIL number: 2205066 v 0).

Conversion to belatacept consisted of belatacept first infusion at day 0 (5 mg/kg), day 14, day 28, and then monthly every 28 days. Tacrolimus was given without changing the dose (trough level 6–8 ng/ml) during 2 months after starting belatacept, and tacrolimus was reduced to half the dose in the third month postswitch, to be finally discontinued at the beginning of the fourth month. The conversion protocol did not include the addition of steroids. No further adjustments were made in immunosuppression during the year post-conversion, except the CNI gradual reduction. Finally, should the patients receive mycophenolate mofetil or everolimus at the time of conversion, these drug doses remained unchanged.

Viral Load Quantification

TTV DNA load quantification was measured before the conversion and at 3, 6, and 12 months post belatacept conversion using the TTV R-GENE kit (bioMérieux, Marcy 86 l'Etoile, France). TTV DNA load were quantified in whole blood samples, retrospectively. After extraction by the EMAG platform (bioMérieux, Marcy 86 l'Etoile, France) quantitative polymerase chain reaction was performed with the Lightcycler 480 II (Roche, 4, cours de l'Ile Seguin, France). TTV DNA loads were expressed in $\log_{10}$ copies/ml.

End Points

Our primary end point was to evaluate the whole blood TTV DNA load profile after belatacept conversion. Secondary end points were the correlation of whole blood TTV DNA load with clinical events, that is, renal allograft function, OPI, and biopsy-proven rejection (BPR).

Renal function was estimated by the glomerular filtration rate (eGFR) using the Chronic Kidney Disease-Epidemiology Collaboration formula; proteinuria was assessed by the ratio between proteinuria and creatininuria. Patients were classified into 2 subgroups as follows: (i) those who had a GFR decay (eGFR decrease between preconversion and month 12 >5 ml/min per 1.73 m^2) and (ii) those who presented with a stable or improved eGFR in month 12.

OPI was defined by the presence of at least 1 of the following infections occurring during the follow-up: cytomegalovirus (CMV) disease, BK virus (BKV) nephropathy, or any infection requiring hospitalization. CMV disease was defined by the detection of the virus associated with symptoms. BKV-nephropathy was biopsy-proven.

Antibody-mediated rejection was defined by histological lesions (glomerulitis and peritubular capillaritis), C4d labelling and/or presence of donor-specific antibodies (DSAs) following the last Banff classification.[23] Cellular rejection was defined by the presence of tubilitis and interstitial inflammation on biopsy. In the absence of any graft biopsy, we considered that there was no BPR. Biopsies were prompted either by the presence of proteinuria or by a decline in GFR. In addition, a screening for DSA was conducted both before the conversion and at the 12-month mark.

For other subgroup analyses, we determined 2 groups based on the TTV DNA load according to Jaksh *et al.*[24] study as follows: (i) hTTV group when there were >6.2 $\log_{10}$ copies/ml, and lTTV group when viral load was <4.6 $\log_{10}$ copies/ml.

Statistical Analysis

Means are presented with SD and medians are presented with the first and third quartile. A Wilcoxon test was used for paired comparisons and Mann-Whitney test for unpaired comparisons of 2 groups. Analysis of variance or Kruskall Wallis tests were performed for the comparisons of more than 2 groups of patients. A 2-sided *P*-value of <0.05 was considered statistically significant. Statistical analyses were conducted using the R statistical software (version 4.3.0; R Foundation for Statistical Computing, Vienna, Austria).[25]

RESULTS

Description of the Population

Seventy-two patients were converted from tacrolimus to belatacept between June, 2015 and December, 2020. Four patients were excluded from the analysis because of the absence of follow-up biological samples for TTV load. Clinical characteristics of the remaining 68 patients are presented in Table 1. The patients were mostly men, with a mean age of 58.6 years. Induction therapy consisted of antithymocyte globulin in 92.6%. Belatacept conversion was done after a median time of 48.6 (26.8–125.1) months post-KT, and mean follow-up was 93.8 $\pm$ 71 months post-KT and 16.5 $\pm$ 5.8 months post-belatacept conversion. There was no death during the follow-up period, but 4 patients lost their graft. There was no lost to follow-up. Only 1 patient developed *de novo* DSA during the year postconversion.

TTV DNA Load Profile Post-Belatacept Conversion

First, we assessed the TTV DNA load before belatacept conversion (under stable tacrolimus-based immunosuppressive regimen) and after conversion to belatacept until 1 year. Whole blood median TTV DNA loads were at 3.8 (3.1–4.9) $\log_{10}$ copies/ml, 4.4 (3.2–5.4) $\log_{10}$ copies/ml, 4.0 (3.0–5.7) $\log_{10}$ copies/ml and 4.2 (3.1–5.2) $\log_{10}$ copies/ml at preconversion, 3, 6, and 12 months postconversion, respectively (Figure 1). There was no statistical difference between preconversion (tacrolimus based-regimen) and month 12 (belatacept based-regimen) TTV DNA loads and between each time point. The general tendency was to have a higher TTV DNA load at month 3 postconversion.

TTV DNA Load and Clinical Outcomes Correlations

Glomerular Filtration Rate and Proteinuria

We then assessed eGFR during the follow-up post-belatacept conversion. Mean GFR was similar at baseline and at month 12 postconversion, that is, 45.1 $\pm$ 19.5 ml/min per 1.73 m^2 and 44.6 $\pm$ 19.4 ml/min per 1.73 m^2, respectively. At the last follow-up (38.5 [33–49] months), mean

eGFR was 43.5 ± 19.6 ml/min per 1.73 m^2. Proteinuria was significantly different at baseline and at month 12 post-conversion, that is, 540.8 ± 842.9 mg/mmol and 851.9 ± 1588.6 mg/mmol, respectively.

We then assessed the TTV DNA load according to the decrease in eGFR (as defined in the Methods section) during the first year postconversion. There were 14 patients (20.6%) in the deterioration group and 56 (82.4%) patients in the no deterioration group. At month 6 and month 12, TTV DNA load was lower in the eGFR deterioration group than in the group that did not experience a decrease their eGFR (month 6: 3.4 [2.6–4.0] log_{10} copies/ml vs. 4.3 [3.1–5.9], $P = 0.021$; month 12: 3.7 [2.5–4.2] log_{10} copies/ml vs. 4.4 [3.4-5.6], $P = 0.030$, Mann-Whitney test) whereas no significant differences were found at preconversion and at month 3 (Figure 2). We could not find a significant difference in TTV DNA load at month 3 although there was a tendency to a lower TTV DNA load in the eGFR deterioration group. No linear correlation was found between TTV DNA load at each time point and the eGFR difference between preconversion and month 12 (Supplementary Figure S1).

In addition, we looked at TTV DNA load intra-individual variability by evaluating the delta between month 12 and preconversion. Between the deterioration and no deterioration group, the difference was significant with an increase in the TTV DNA load between preconversion and month 12 for the no deterioration group, whereas it remained stable for the deterioration group (no deterioration: 0.32 [-0.11 to 0.41] log_{10} copies/ml; deterioration: 0.10 [-0.98 to 0.31] log_{10} copies/ml, $P = 0.000091$, Mann-Whitney test) (Supplementary Figure S2). In order to see the predictive effect of this variability, we assessed the delta TTV DNA load between month 6 and preconversion. The patients who had a GFR deterioration between preconversion and month 12 had a significant decrease in their TTV DNA load between preconversion and month 6, as compared to those without deterioration who tended to increase TTV DNA load (no deterioration: 0.36 [-0.09 to 1.00] log_{10} copies/ml; deterioration: -0.12 [-0.46 to 0.20], $P = 0.000012$, Mann-Whitney test) (Supplementary Figure S2).

Table 1. Clinical characteristics of kidney transplanted (KT) patients

Baseline characteristics	Total ($N = 68$)
Gender; Male, *n* (%)	41 (60.3%)
Medical history, *n* (%)	
- Hypertension	49 (76.6%)
- Diabetes	10 (15.6%)
Nephropathy, *n* (%)	
- Diabetic nephropathy	3 (4.5%)
- Vascular nephropathy[a]	6 (8.7%)
- Other glomerulopathy[b]	22 (31.9%)
- Polycystic and hereditary	21 (30.4%)
- Uropathy	2 (2.9%)
- Indetermined	11 (16.1%)
Dialysis before KT, *n* (%)	49 (74.2%)
Dialysis time (yr), median (Q1, Q3)	2.6 (0.7, 5.4)
Donor type; deceased, *n* (%)	43 (63.2%)
CMV serology; positive, *n* (%)	34 (75.6%)
KT	
Cold ischemia (min), median (Q1, Q3)	885.5 (515.8, 1188.8)
First graft, *n* (%)	55 (82.1%)
Induction therapy; ATG, *n* (%)	63 (92.6%)
Dialysis at day 7, *n* (%)	5 (8.1%)
Preconversion	
Age at the conversion (yr), median (Q1, Q3)	59.2 (51.3, 68.6)
Indication	
- Nephrotoxicity	48 (70.5%)
- Vascular lesion	8 (11.7%)
- Tac intolerance	9 (13.2%)
Maintenance therapy, *n* (%)	
- Tac MMF CS	16 (23.9%)
- Tac MMF	37 (54.4%)
- Tac mTORi CS	5 (7.4%)
- MMF mTORi CS	1 (1.5%)
- MMF mTORi	4 (6.0%)
Residual Tac concentration, median (Q1, Q3)	5.90 (4.95, 6.40)
CS at the conversion, *n* (%)	15 (22.4%)
BMI, median (Q1, Q3)	25.1 (21.8, 27.4)
Delay between KT and conversion (months), median (Q1, Q3)	48.6 (26.8, 125.1)
Rejection, *n* (%)	10 (14,9%)
eGFR, median (Q1, Q3)[c]	41.4 (31.7, 52.8)
Proteinuria, median (Q1, Q3)[d]	221 (143.2, 481.0)
DSA, *n* (%)	6 (9.8%)
Postconversion	
Maintenance therapy with belatacept, *n* (%)	
- MMF CS	17 (25.0%)
- MMF	38 (56.7%)
- mTORi CS	5 (7.5%)
- mTORi	7 (10.4%)
eGFR at 1-year, median (Q1, Q3)[c]	42.8 (32.7, 52.8)
Last follow-up eGFR, median (Q1, Q3)[c]	39 (33.1, 49.2)
Proteinuria at 1-year, median (Q1, Q3)[d]	332.8 (200.2, 659.8)
Last follow-up proteinuria, median (Q1, Q3)[d]	335.8 (189.4, 662.9)
Last follow-up rejection, *n* (%)	3 (4.4%)
Last follow-up DSA, *n* (%)	4 (6.7%)
Stop belatacept, *n* (%)	6 (9.0%)
Graft loss, *n* (%)	4 (6%)

ATG, antithymocyte globulin; BMI, body mass index; CMV, cytomegalovirus; CS, corticosteroids; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; KT, kidney transplantation; MMF, mycophenolate mofetil; MTORi, mTOR inhibitors; Q1 Q3, first and third interquartile; Tac, tacrolimus.
[a]Vascular nephropathy includes nephroangiosclerosis (4 patients) and uremic and hemolytic syndrome (1 patient).
[b]IgA nephropathy, focal and segmental glomerulosclerosis, lupus nephropathy, extramembranous glomerulonephritis were included.
[c]Expressed in ml/min per 1.73 m^2 after Chronic Kidney Disease-Epidemiology Collaboration formula.
[d]Expressed as proteinuria-to -creatininuria ratio in mg/mmol.

BPR

Of the 68 patients included, 10 were diagnosed with BPR before conversion (5 acute humoral rejections and

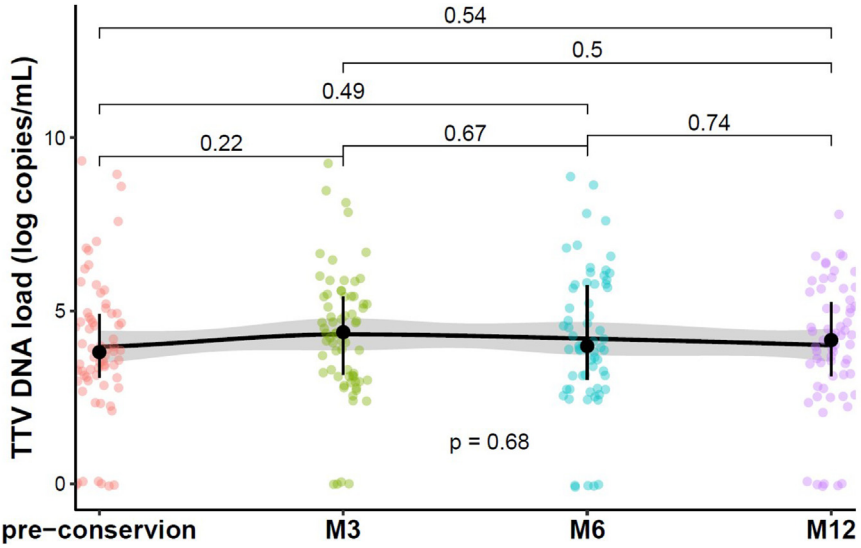
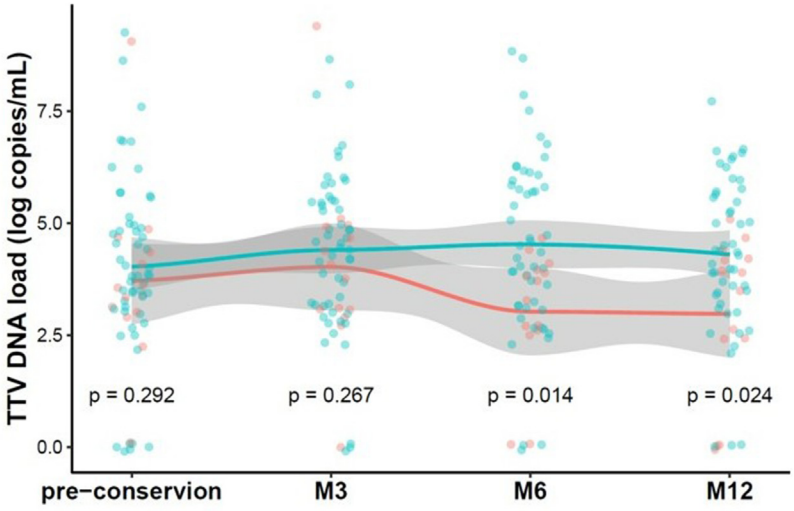


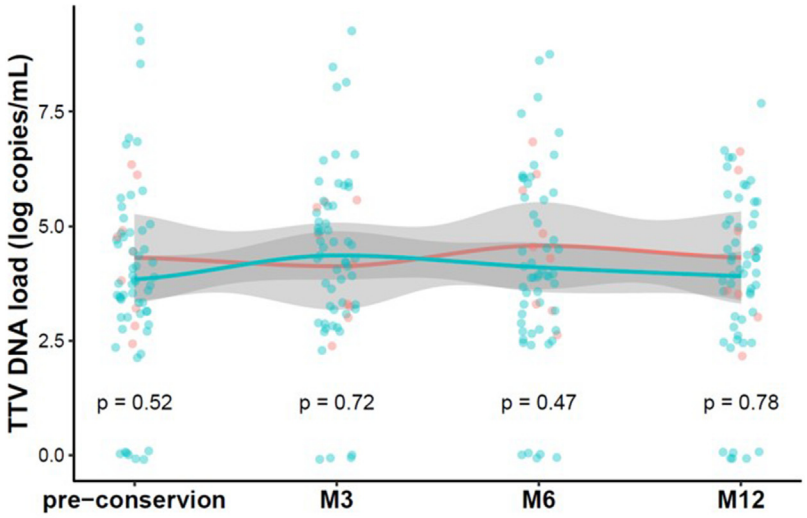
Figure 1. Profile of the whole blood TTV DNA loads after conversion to belatacept. TTV DNA load is expressed as $\log_{10}$ copies/ml. Black brackets represent quartiles and bold points represent medians. Colored points represent patient distribution, and the black line represents the evolution of mean whole blood TTV DNA load with SD in grey. *P*-values were calculated between each point with a paired Wilcoxon test (statistical significance is defined by a *P*-value < 0.05). The general analysis of variance test was not significant ($P = 0.68$). M, month; TTV, Torque Teno virus.

5 acute cellular rejections) on biopsy for cause. Overall, only 2 patients (2.9%) presented with a BPR within the first-year postconversion (acute cellular rejection at 4 months postconversion for the 2 patients). TTV DNA load for the first of these rejecting patients showed lower TTV DNA load than the other patients of the cohort. Indeed, their whole blood TTV DNA was negative at preconversion, 2.34 $\log_{10}$ copies/ml (vs. a median of 4.4 $\log_{10}$ copies/ml in the cohort) at month 3, 3.57 $\log_{10}$ copies/ml (vs. a median of 4.0 $\log_{10}$ copies/ml in the cohort) at month 6 and negative at month 12. For the second of these rejecting patients, TTV DNA load was 3.83 $\log_{10}$ copies/ml at preconversion and 4.42 $\log_{10}$ copies/ml at month 3 (data were missing for month 6 and month 12). During the whole follow-up, 2 more patients developed acute-humoral BPR. One of them had lower preconversion whole blood TTV DNA load than the cohort (3.42 $\log_{10}$ copies/ml vs. a median



Time		Pre-conversion	M3	M6	M12
Median (Q1-Q3)	Deterioration	3.49 (2.98-4.29)	4.00 (3.08-4.90)	3.45 (2.61-3.95)	3.69 (2.46-4.25)
	No deterioration	3.86 (3.10-4.98)	4.53 (3.23-5.50)	4.41 (3.15-5.94)	4.39 (3.47-5.60)

Figure 2. Outcome of TTV DNA loads before and after belatacept conversion depending on GFR deterioration. TTV DNA load is expressed as $\log_{10}$ copies/ml. Colored points represent patient distribution, and the black line represents the evolution of mean whole blood TTV DNA load with SD in grey. In blue is the no deterioration group, and in red is the deterioration group. *P*-values were calculated between the 2 groups at each point with a Mann-Whitney test (statistical significance is defined by a *P*-value < 0.05). GFR, glomerular filtration rate; M, month; TTV, Torque Teno virus.



Time		Pre-conversion	M3	M6	M12
Median (Q1-Q3)	Infection	4.47 (3.27-4.88)	4.11 (3.15-5.40)	4.75 (3.29-5.73)	3.99 (3.45-5.29)
	No infection	3.74 (3.03-4.97)	4.40 (3.17-5.42)	3.94 (2.76-5.71)	4.09 (2.96-5.11)

Figure 3. TTV DNA load profiles before and after belatacept conversion according to developing opportunistic infections. TTV DNA load is expressed as $\log_{10}$ copies/ml. Colored points represent patient distribution, and the black line represents the evolution of mean whole blood TTV DNA load with SD in grey. In blue is the no infection group and in red is the infection group. *P*-values were calculated between each point with a Mann-Whitney test (statistical significance is defined by a *P*-value <0.05). M, month; TTV, Torque Teno virus.

of 3.8 $\log_{10}$ copies/ml) but was similar at month 12 without DSA. The other one had DSA preconversion and his TTV DNA load remained stable during the 1-year follow-up (4.12 $\log_{10}$ copies/ml at pre-conversion, 4.26 $\log_{10}$ copies/ml at month 3, 4.16 $\log_{10}$ copies/ml at month 6 and 4.15 $\log_{10}$ copies/ml at month 12) (results not shown).

The patient that developed DSA during the year postconversion did not experience BPR.

OPIs

We then assessed the correlation between TTV DNA load and the occurrence of OPI (CMV disease, BKV nephropathy, hospitalization for any infectious cause). Nine patients (13.2%) developed an OPI during the follow-up. Higher but not statistically significant TTV DNA loads were found at preconversion (4.3 ± 2.1 $\log_{10}$ copies/ml vs. 3.8 ± 2.1 $\log_{10}$ copies/ml, $P = 0.52$, Mann-Whitney test), at month 6 (4.6 ± 1.5 $\log_{10}$ copies/ml vs. 4.1 ± 2.1 $\log_{10}$ copies/ml, $P = 0.47$, Mann-Whitney test), and at month 12 postconversion (4.1 ± 1.3 $\log_{10}$ copies/ml vs. 3.4 ± 1.8 $\log_{10}$ copies/ml, $P = 0.78$, Mann-Whitney test) in the OPI group than in the non-OPI group, respectively (Figure 3). At month 3, TTV DNA load was 4.1 ± 2.1 $\log_{10}$ copies/ml in the OPI group and 4.5 ± 1.9 $\log_{10}$ copies/ml in the non-OPI group ($P = 0.72$, Mann-Whitney test).

OPI consisted of 1 CMV disease, 1 BKV nephropathy, and 7 hospitalizations for various infections, including 3 for SARS-Cov2 pneumopathy. There were no BPR and no graft loss in the OPI group. Unexpectedly, the patient with CMV disease had a lower whole blood TTV DNA load than the mean cohort at baseline (2.4 $\log_{10}$ copies/ml vs. 4.0 ± 2.0 $\log_{10}$ copies/ml) and at month 12 (2.1 $\log_{10}$ copies/ml vs. 4.0 ± 1.8 $\log_{10}$ copies/ml). Regarding only the hospitalizations for infection, TTV viral load was numerically higher at pre-conversion and month 12 in patients with infection occurrences than those without infections, but the differences were not significant.

In addition, TTV DNA load intraindividual variability between month 12 and preconversion was similar between the OPI and non-OPI group (OPI: −0.08 [−0.23 to 0.21] $\log_{10}$ copies/ml; non-OPI: 0.31 [−0.20 to 0.88] $\log_{10}$ copies/ml, $P = 0.081$, Mann-Whitney test) (Supplementary Figure S3).

Factors Associated With HTTV LTTV DNA Load

We then assessed the factors associated with hTTV DNA load (hTTV >6.2 $\log_{10}$ copies/ml) and lTTV DNA load (lTTV <4.6 $\log_{10}$ copies/ml), pre-Belatacept and post-Belatacept conversion.

Preconversion

Univariate analyses of baseline results are presented in Table 2. Eight patients had an hTTV (mean TTV DNA load 7.7 ± 1.1 log10 copies/ml) and 45 had a lTTV (mean TTV DNA load 2.9 ± 1.4 log10 copies/ml) at baseline. The patient's age was higher in the hTTV group (62.0 ± 12 vs. 55 ± 13 years in the lTTV group, $P = 0.005$) as well as the BMI (26.1 ± 4 vs. 23.7 ± 4 kg/m^2, $P = 0.023$); however, the time between KT and belatacept conversion was lower in the hTTV

Table 2. Factors associated with preconversion high and low whole blood TTV DNA loads in kidney transplantation (KT) recipient before conversion from tacrolimus to belatacept

Variables	High ($N = 8$)	Low ($N = 45$)	*P* value
TTV DNA load (log_{10} copies/ml), mean (SD)	7.7 (1.1)	2.9 (1.4)	
Age at the conversion(yr), median (Q1, Q3)	66.2 (51.8, 69.1)	55.4 (45.6, 63.8)	0.005[a]
Body mass index, median (Q1, Q3)	23.7 (23.6, 28.0)	23.3 (21.0, 26.0)	0.023[a]
Delay between the graft and the conversion (months), median (Q1, Q3)	16.5 (11.8, 32.9)	53.4 (34.4, 137.4)	0.005[a]
CMV status before KT, D/R, N (%)			0.420
+/−	0 (0.0%)	3 (16.7%)	
+/+	3 (42.9%)	20 (45.5%)	
−/−	2 (28.6%)	4 (9.1%)	
−/+	1 (14.3%)	7 (15.9%)	
Dialysis before KT; yes, N (%)	6 (85.7%)	31 (72.1%)	0.748
ATG at the induction; yes, N (%)	5 (71.4%)	31 (73.8%)	0.760
Medical history, N (%)			
Diabetes	1 (14.3%)	6 (14.3%)	1.000
Hypertension	6 (85.7%)	30 (71.4%)	0.455
Graft number			0.905
1	6 (85.7%)	35 (79.5%)	
≥2	1 (14.3%)	9 (20.5%)	
Donor type; deceased, N (%)	3 (42.9%)	27 (61.4%)	0.327
Nephropathy			0.516
Without IS treatment	6 (85.7%)	28 (63.6%)	
With IS treatment[b]	1 (14.3%)	16 (36.4%)	
IS at the conversion, N (%)			
Tac MMF CS	1 (14.3%)	11 (25.0%)	
Tac mTORi CS	1 (14.3%)	2 (4.5%)	
Tac MMF	3 (42.9%)	24 (54.5%)	
MMF mTORi	0 (0.0%)	4 (9.1%)	
CS at the conversion; yes, N (%)	3 (42.9%)	15 (34.1%)	0.856
Rejection before Belatacept; yes, N (%)	2 (28.6%)	5 (4.5%)	0.383
DSA at the conversion; yes, N (%)	0 (0.0%)	6 (15.4%)	
GFR at the conversion (ml/min/1.73 m^2), median (Q1, Q3)[c]	27.1 (26.7, 42.3)	42.5 (32.5, 51.6)	0.268
Proteinuria at the conversion (mg/mmol), median (Q1, Q3)[d]	455.8 (221.0, 473.6)	221.0 (143.3, 449.4)	0.484
Lymphocyte rate (G/L), median (Q1, Q3)	0.5 (0.4,0.8)	1.1 (0.8,1.4)	0.004[a]

(Q1, Q3), interquartile range; ATG, antithymocyte globulin; CS, corticosteroids; D/R +/−, serological status of the kidney transplant donor and recipient; DSA, donor specific antibody; FGS, focal glomerulosclerosis; GFR, glomerular filtration rate; HUS, hemolytic uremic syndrome; IS, immunosuppressive; KT, kidney transplantation; MMF, mycophenolate mofetil; MTORi: mTOR inhibitors; Tac, tacrolimus; TTV, Torque Teno virus.
[a]Statistically significant (*P*-value <0.05).
[b]FGS, glomerulopathy except diabetic one, IgA nephropathy, HUS, lupus are included in this subgroup, corresponding to the nephropathy with IS treatment needed.
[c]Expressed in ml/min per 1.73 m^2 after Chronic Kidney Disease-Epidemiology Collaboration formula.
[d]Expressed as proteinuria-to-creatininuria ratio in mg/mmol.

group (23.1 ± 16 vs. 86.5 ± 69 months, $P = 0.005$). The hTTV group showed a lower lymphocyte count at baseline (0.675 ± 0.607 vs. 1.13 ± 0.457 G/l, $P = 0.011$). However, considering all sampling times, there was no correlation between the lymphocyte count and the TTV DNA load (Supplementary Figure S4).

No other factors were found to be associated with hTTV. eGFR was numerically lower in the hTTV group (37.7 ± 20 ml/min per 1.73 m^2 vs. 44.7 ± 15 ml/min per 1.73 m^2) although not statistically significant. In addition, no correlation was found between TTV DNA load and the recipient age at the time of belatacept conversion (Supplementary Figure S5).

We observed a significant decrease of TTV DNA load in the hTTV group between preconversion and month 12 (7.26 [6.89–8.67] log_{10} copies/ml at preconversion and 6.33 [4.30–5.56] log_{10} copies/ml at month 12, $P = 0.01$, Wilcoxon test) unlike in the lTTV group in which TTV DNA load remained stable (3.38 [2.75–3.79] log_{10} copies/ml at preconversion and 3.58 [2.56–4.40] log_{10} copies/ml at month 12, $P = 0.11$, Wilcoxon test) (Figure 4).

At Month-12

We considered month 12 analysis to be relevant for TTV measurement under stable belatacept therapy and away from any tacrolimus intake. Univariate analyses of factors associated with month 12 TTV DNA load are presented in Table 3. Eight patients had, at month 12, an hTTV (mean TTV DNA load 6.6 ± 0.5 log10 copies/ml) and 41 patients had an lTTV (mean TTV DNA load 3.0 ± 1.4 log10 copies/ml). The hTTV group at month 12 presented with a higher preconversion BMI (27 ± 3.6 vs. 23.4 ± 3.5, $P = 0.044$) whereas the age at conversion was similar between the hTTV and lTTV

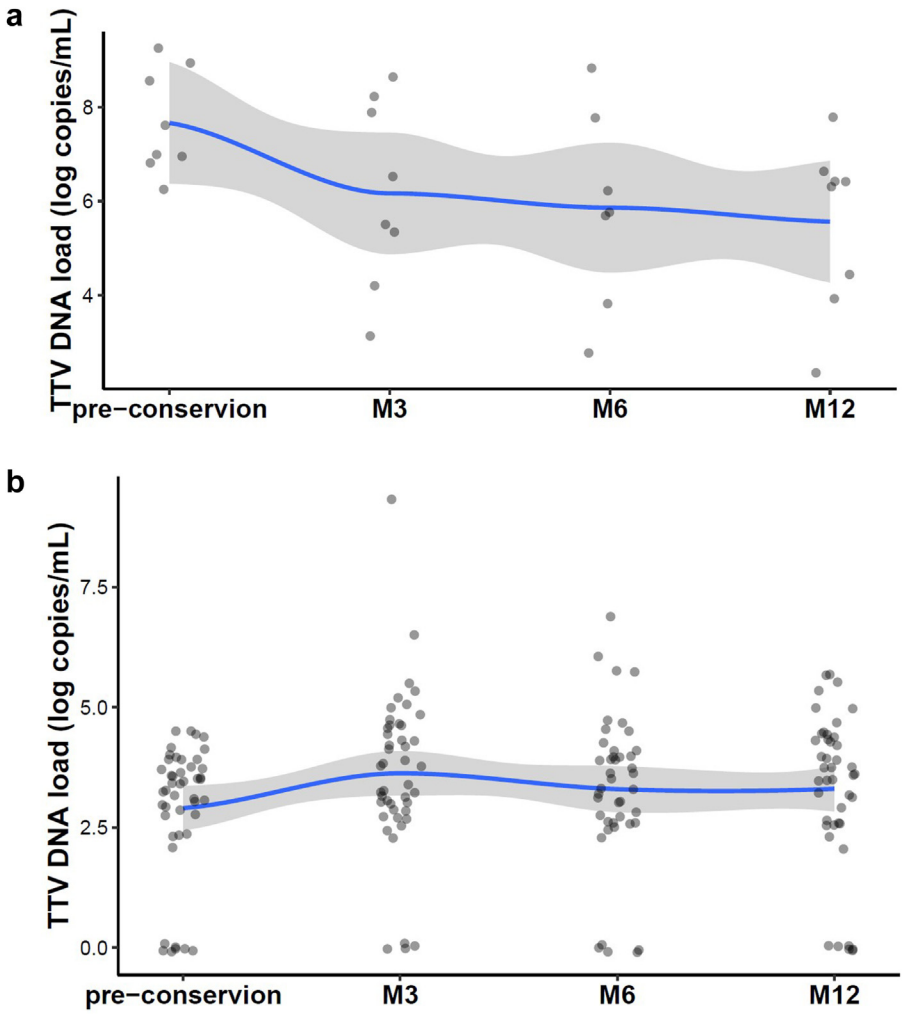


Figure 4. Profile of the whole blood TTV DNA loads after conversion to belatacept in the (a) hTTV group and (b) lTTV group. TTV DNA load is expressed as $\log_{10}$ copies/ml. The blue line represents mean TTV DNA load with SD in grey. *P*-values were calculated between each point with a Wilcoxon test (statistical significance is defined by a *P*-value < 0.05). The general analysis of variance test was not significant with $P = 0.68$. M, month; hTTV, high TTV; lTTV, low TTV; TTV, Torque Teno virus.

group ($P = 0.109$). The lTTV group presented more frequently with a GFR decay (>5 ml/min per 1.73 m^2 GFR deterioration) between preconversion and month 12 had, but the difference was not statistically significant.

There were no significant differences in the trend of TTV DNA load between patients with or without high blood pressure or between patients with or without diabetes (results not presented). However, a significant difference was observed between patients with obesity (defined as BMI >25 kg/m^2) and those without obesity at 3, 6, and 12 months (Supplementary Figure S6).

When comparing the GFR evolution in patients whose TTV DNA load increased (by more than 0.5 log10 copies/ml) during the year postconversion and those for whom it decreased (by more than 0.5 log10 copies/ml), it appears that in the decreased group, GFR tends to decrease, whereas in the increased group, GFR tends to increase (results not shown).

DISCUSSION

Our study is the first to evaluate the TTV DNA load as a predictive biomarker of OPI and acute rejection after conversion from tacrolimus to belatacept therapy in kidney transplant recipients. We did not observe any significant modification of TTV DNA loads between preconversion and months 3, 6, and 12 after belatacept conversion. However, there was a tendency for a higher TTV DNA load at month 3 than at baseline. A possible explanation is that during 3 months postconversion, patients cumulated both tacrolimus and belatacept, thereby increasing temporarily the global immunosuppression burden, thus leading to higher TTV DNA loads. This strategy, however, may explain the very low rate of rejection during the year postconversion (2 patients). In the Strassl *et al.*[17] study, a TTV DNA load peak was observed after 3-months transplantation (4.3 × 108 copies/ml as compared to pretransplantation

Table 3. Factors associated with M12 high and low whole blood TTV DNA loads in kidney transplantation (KT) recipient before conversion from tacrolimus to belatacept

Factors	High (n = 8)	Low (n = 41)	P-value
TTV DNA load (log_{10} copies/ml, mean (SD)	6.6 (0.5)	3.0 (1.4)	
Age at the conversion (yr), median (Q1, Q3)	67.8 (58.3, 67.8)	55.3 (45.6, 68.3)	0.109
Body mass index, median (Q1, Q3)	25.3 (24.9, 28.4)	23.1 (21.5, 26.2)	0.044[a]
Delay between the graft and the conversion (months), median (Q1, Q3)	27.8 (22.3, 43.2)	49.3 (27.7, 109.1)	0.267
CMV status before KT, D/R, n (%)			0.104
+/−	0 (0.0%)	10 (25.0%)	
+/+	4 (57.1%)	17 (42.5%)	
−/−	1 (14.3%)	5 (12.5%)	
−/+	0 (0.0%)	7 (17.5%)	
Dialysis before KT; yes, n (%)	6 (85.7%)	26 (66.7%)	0.387
ATG at the induction; yes, n (%)	6 (85.7%)	25 (65.8%)	0.099
Medical history, n (%)			
Diabetes	1 (14.3%)	5 (13.2%)	0.820
Hypertension	6 (85.7%)	29 (76.3%)	0.812
Graft number			0.761
1	7 (100.0%)	32 (80.0%)	
≥2	0 (0.0%)	8 (20.0%)	
Donor type; deceased, n (%)	3 (42.9%)	26 (65.0%)	0.408
Nephropathy			0.115
Without IS treatment	7 (100.0%)	26 (65.0%)	
With IS treatment[b]	0 (0.0%)	14 (35.0%)	
IS at the conversion, N (%)			
Tac MMF CS	1 (14.3%)	10 (25.0%)	
Tac mTORi CS	2 (28.6%)	2 (5.0%)	
Tac MMF	3 (42.9%)	20 (50.0%)	
MMF mTORi	0 (0.0%)	4 (10.0%)	
Steroids at the conversion; yes, n (%)	3 (42.9%)	15 (37.5%)	0.605
Rejection before Belatacept; yes, n (%)	3 (42.9%)	6 (15.0%)	0.097
DSA at the conversion; yes, n (%)	0 (0.0%)	6 (17.1%)	
GFR at the conversion (ml/min per 1.73 m^2), median (Q1, Q3)[c]	28.4 (22.9, 47.6)	39.0 (34.5, 48.71)	0.364
GFR deterioration between baseline and month 12; yes, n (%)	0 (0.0%)	12 (29.3%)	0.235
Proteinuria at the conversion (mg/mmol), median (Q1, Q3)[d]	455.8 (201.9, 1026.5)	215.2 (133.9, 445.7)	0.465
Lymphocyte rate (G/l), median (Q1, Q3)	1.2 (0.8, 1.7)	1.3 (0.8, 1.7)	0.110

(Q1, Q3), interquartile range; ATG, antithymocyte globulin; CS, corticosteroids; D/R +/−, serological status of the kidney transplant donor and recipient; DSA, donor specific antibody; FGS, focal glomerulosclerosis; GFR, glomerular filtration rate; HUS, hemolytic uremic syndrome; IS, immunosuppressive; KT, kidney transplantation; MMF, mycophenolate mofetil; MTORi: mTOR inhibitors; Tac, tacrolimus; TTV, Torque Teno virus.
[a]Statistically significant (P-value <0.05).
[b]FGS, glomerulopathy except diabetic one, IgA nephropathy, HUS, Lupus are included in this subgroup, corresponding to the nephropathy with IS treatment needed.
[c]Expressed by ml/min/1.73 m^2 after CKD-EPI formula.
[d]Expressed as proteinuria/creatininuria ratio in mg/mmol.

1.9 × 104 copies/ml) on 169 KT patients. Patients in this study were followed-up with for less than a year and TTV DNA loads decreased modestly at the end of the follow-up (4.2 × 106 copies/ml). The variations in TTV DNA load between the study conducted by Strassl *et al*. and our study could potentially be attributed to the fact that our patients underwent transplantation 4 years before the conversion. Our cohort exhibits lower infectious and rejection risks, along with significantly reduced immunosuppressive treatment. These results were confirmed by other studies.[26] We observed the same phenomenon after belatacept conversion leading us to hypothesize that during the first 3 months, immunosuppression is higher in relation to the following: (i) loading dose at belatacept introduction and (ii) CNI and mycophenolate mofetil or everolimus coprescriptions. Furthermore, correlation between belatacept concentrations (i.e., through levels) and TTV DNA load have to be assessed in future studies. Regarding CNI doses, it has been shown by Cañamero *et al*.[26] that mean tacrolimus trough levels were not correlated to TTV DNA load.

In the conversion protocol, because of over-immunosuppression, a higher OPI risk is expected. However, we did not see any difference in TTV DNA loads between the patients who underwent OPI and those who did not. In posttransplantation studies, TTV was able to predict the risk of OPI when all infections were assessed; however, the correlation between TTV load and a specific infection such as BKV is more controversial.[17,18] Indeed, Fernández-Ruiz *et al*. found that higher TTV DNA levels were significantly associated with patients developing BKV DNAemia in 215

patients followed-up with during the first-year post-transplantation,[27] whereas this correlation was not found in the Handala *et al*.[22] study. A more recent study of van Rijn *et al*.[21] showed that TTV DNA load better predicts acute rejection than OPI in CNI-treated patients. They observed an important decrease in the rejection risk every 10-fold TTV load-increase, whereas BKV and CMV viremias remained stable. In our cohort, whether we assessed OPI individually or together as a composite criterion, we did not observe any TTV DNA load difference or a significant intraindividual variability. This may be due to the small patient sample size number and a low infectious event rate. Indeed, we had only 1 case of CMV disease and 1 case of BKV nephropathy. Reactivation of CMV, BKV, and Epstein–Barr virus was also assessed in relation to TTV DNA load. There was no observed correlation between CMV and BKV reactivation and TTV DNA load. However, a weak but positive correlation was identified between Epstein–Barr virus reactivation and TTV DNA load.[28]

Recent studies show an increased risk of OPI in late-conversion from tacrolimus to belatacept.[29] The main risk factors were a low eGFR at conversion, older recipient age, and steroid use. Current recommendation for early belatacept use in KT is in association with low-dose tacrolimus.[29] Thus, we expected a higher TTV DNA load after belatacept conversion but this hypothesis was not confirmed. Two hypotheses can be made as follows: (i) TTV DNA load is not efficient to predict OPI risk under belatacept-based therapy; and (ii) belatacept leads to an infectious risk but this risk is not important enough to modify the TTV DNA load, which is reassuring regarding the use of belatacept in clinical practice.

With respect to rejection, an lTTV DNA load seemed to be associated with a high risk as described in many studies in the immediate posttransplantation period.[30-32] We could not properly assess the rejection risk because of the low number of rejection event (2 patients). However, 1 patient who presented with an acute cellular rejection during the year post-belatacept conversion had a very low TTV DNA load (lower than the mean TTV DNA load of our cohort).

TTV viruses rest in T lymphocytes, that is why we were expecting a higher T lymphocyte rate in the hTTV group. Indeed, it has been shown that TTV is a lymphotropic virus, but TTV replication is controlled by peripheral mononuclear cells, particularly T-lymphocytes.[33] This may explain why induction therapy with lymphocyte depleting agents and CNI-based therapies have been associated with higher TTV DNAemia, and why patients with a low T lymphocyte count also have a higher TTV DNA load. However, no correlation was found between TTV DNA loads and lymphocyte count.

Belatacept has proven to be beneficial for renal function as compared to CNI-based therapies.[7,9] In our study, eGFR at baseline and at 1-year after the conversion was stable. We found that TTV DNA load was not similar between patients whose eGFR decreased during the postconversion year and those in whom eGFR was stable. In addition, looking at intraindividual variability in patients with an eGFR decay, there was a decrease of the TTV DNA loads, unlike in those with a stable eGFR. This might suggest that at 6 months postconversion, TTV DNA load may be predictive of eGFR worsening after 1-year conversion. TTV DNA load variation cut-offs need to be defined; however, we suggest that a decrease of TTV DNA load between preconversion and month 6 could be predictive of eGFR worsening at month 12. This suggests a closer monitoring in patients experiencing such TTV DNA load decrease. This hypothesis needs to be confirmed but was never suggested in the literature.

Concerning the factors associated with hTTV or lTTV DNA loads, we decided to analyze them at preconversion and at month 12 in order to have an immunosuppression without CNI and stable belatacept therapy. We observed that patients in the hTTV group were older, with a higher BMI and that the conversion was earlier post-KT than in the lTTV group. It is known that immunosenescence may lead to a decrease in the ability by the immune system to control TTV reactivation, thus explaining why TTV DNA load is higher in the elderly.[15] Even though we did not find any correlation between TTV DNA load and recipient age, patients with hTTV were older. For the BMI, obesity was associated with a higher TTV DNA load than lean patients (2.39 $\log_{10}$ copies/ml [1.69–3.33] vs. 1.88 $\log_{10}$ copies/ml [1.08–2.43], $P = 0.027$) in the Herz *et al*.[34] study. This result is confirmed by our study and may reflect a compromised immune function in this particular population. The subgroup analysis cut-off of TTV DNA were based on the Jaksh *et al*.[24] study as follows: 4.6 $\log_{10}$ copies/ml for the low group and 6.2 $\log_{10}$ copies/ml for the high group. Some other teams have worked with different cut-off levels for these risks. For example, a TTV load threshold of 3.4 $\log_{10}$ copies/ml at transplantation allowed prediction of graft rejection in the Solis *et al*.[31] study, with 39% of the patients with less than 3.4 log/ml displaying graft rejection versus only 3% of the patients with higher loads. In order to establish recommendations about CNI adjustment regarding TTV DNA load, a European study is in progress.[35]

CONCLUSION

Conversion from tacrolimus to belatacept did not impact TTV DNA load. TTV DNA in belatacept-treated

recipients did not correlate with the occurrence of OPI or acute rejection postconversion. However, TTV DNA load after 6 months post-belatacept conversion may be a promising tool to predict the risk of graft dysfunction at 1 year. Further studies need to be carried out, to lead to recommendations on clinical use of the TTV DNA load as a biomarker.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

All the authors participated in research design and in the completion of the research. LC, JN and AT participated in the writing of the paper and in data analysis. LR edited the manuscript. MG contributed to data analysis.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Correlation between preconversion and month 12 GFR differences and TTV DNA load (a) pre-conversion, (b) month 3, (c) month 6, (d) month 12 after the conversion.

Figure S2. TTV DNA load intraindividual variability between preconversion and (a) month 12, (b) month 6, depending on GFR deterioration (between preconversion and month 12).

Figure S3. TTV DNA load intraindividual variability between preconversion and month 12 depending on opportunistic infections.

Figure S4. Whole blood TTV DNA load and lymphocyte rate (all time).

Figure S5. TTV DNA load and recipient age at the belatacept conversion.

Figure S6. TTV DNA load profiles before and after belatacept conversion according to obesity (body mass index < 25 kg/m^2) at preconversion.

STROBE Statement.

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