Journal of Clinical Virology 165 (2023) 105501

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Prospective cohort study of Torque Teno Virus (TTV) viral load kinetics and the association with graft rejection in renal transplant patients

N.S. Reyes [a,*], G. Laham [b], N. Boccia [b], G. García [b], R. Jara [a], E. Hermida [a], C. Ricarte [a], C. Diaz [b], G. Soler Pujol [b], F.A. Poletta [d], M. Echavarria [a,c]

[a] Virology Unit (CEMIC-CONICET), Centro de Educación Médica e Investigaciones Clínicas University Hospital (CEMIC), Argentina
[b] Nephrology section, CEMIC University Hospital, Argentina
[c] Virology Laboratory, CEMIC, Argentina
[d] Genetic Epidemiology Laboratory (CEMIC-CONICET), Argentina

ARTICLE INFO

Keywords:
Torque Teno Virus
Viral load kinetics
Renal transplantation
Graft rejection

ABSTRACT

Introduction: Graft survival is mainly determined by rejections and infectious complications in transplant recipients. Torque Teno Virus (TTV), a nonpathogenic and ubiquitous single-stranded DNA virus, has been proposed as a biomarker of the immune status in transplant patients. This study aimed to determine the correlation between a Home-Brew TTV PCR and R-GENE®PCR; the TTV viral load kinetics in renal transplant recipients and the association with graft rejection.

Materials and methods: Prospective cohort study on 107 adult renal transplant recipients. TTV viral load was determined in 746 plasma samples collected before and after renal transplantation by a Home-Brew PCR and a commercial PCR (R-GENE®PCR). Associations of TTV viral load with graft rejections were analyzed.

Results: Agreement of both PCR assays was 93.2% and Pearson correlation coefficient was r: 0.902 (95%CI: 0.8881–0.9149, $p < 0.0001$). TTV viral load kinetics showed an initial gradual increase reaching a peak at 3 months. This highest value was followed by a slight decrease, reaching a plateau significantly higher than the initial baseline at 6 months ($p < 0.0001$). Between (181–270) days post-transplantation, TTV median viral load in patients with graft rejection was significantly lower, 3.59 Log_{10} copies/mL (by Home-Brew PCR) and 3.10 Log_{10} copies/mL (by R-GENE®PCR) compared to patients without graft rejection (6.14 and 5.96 Log_{10} copies/mL, respectively).

Conclusions: Significantly lower TTV viral load was observed in patients with renal rejection occurring at a median of 243 days post-transplantation. Given the dynamic behavior of TTV viral load post-transplantation, cut-off values for risk stratification to predict rejection might be determined in relation to the post-transplant period.

1. Introduction

Renal transplantation is the standard treatment for patients with end-stage renal disease. Immunosuppressive drugs are crucial for graft and patient survival. However, the optimal pharmacological range requires a delicate balance between inadequate and excessive immunosuppression to avoid the rejection or infections risk [1–3].

Follow-up post-renal transplantation is largely based on calcineurin inhibitor quantification through peripheral blood levels, which correlates more with the risk of drug-related toxicity than with immunosuppression effectiveness. Tacrolimus, a calcineurin inhibitor, shows a large inter and intra-patient pharmacokinetic variability. Additionally, acute rejection can occur when the tacrolimus concentration is within the pharmacological range [4].

Biomarkers could allow a more complete characterization of the immune status of solid organ transplant (SOT) recipients. The ideal biomarker must predict the consequences of inadequate immunosuppression, be comparable across different health facilities, and should be supported by interventional studies based on biomarker-guided drug dosing.

The Torque Teno Virus (TTV) viral load has been proposed as a biomarker candidate [5]. TTV is a small, non-enveloped virus with a single-stranded circular DNA genome (3.8 Kb), member to the *Anelloviridae* family [6,7]. TTV was first described in 1997 and has not been

* Corresponding author at: Virology Unit, CEMIC University Hospital CONICET, *Av.* Galván 4102 (C1631FWO), Buenos Aires, Argentina
E-mail address: noeliasoledadreyes@gmail.com (N.S. Reyes).

https://doi.org/10.1016/j.jcv.2023.105501
Received 30 January 2023; Received in revised form 13 April 2023;
Available online 15 June 2023

associated with any human disease [8]. TTV is highly prevalent; in healthy individuals reaches up to 90%[9,10] and can reach 100% in patients after SOT [11–13].

Retrospective studies described an association between low TTV viral load and graft rejection in SOT recipients [14–16]. Conversely, high TTV levels were associated with an increased risk of infections [17, 18].

Recently, Görzer et al.[19] reported the validation of TTV viral load for risk stratification of rejection and infection using different PCR assays. Cut-off values were determined in a retrospective manner using one sample per patient obtained at a single time (median days post-transplantation: 166).

Establishing TTV viral kinetics throughout time is critical to determine the most appropriate time point for TTV screening as a biomarker for rejection or infection. For our prospective study, seven sequential plasma samples were collected during the first 3 months and one every 3 months thereafter.

The objectives of this study were to determine: a- the correlation between two PCR assays: a Home-Brew PCR and a commercial PCR (R-GENE®PCR), b- the TTV viral load kinetics in renal transplant recipients, and c- the association between TTV viral load and graft rejection.

2. Materials and methods

2.1. Patients and study design

A prospective cohort study on renal transplant recipients (>18 years old), who received a graft from living or deceased donor between November 2018 and April 2021, is being conducted at CEMIC University Hospital, Buenos Aires, Argentina.

Patients were followed over one year and 3 months post-transplantation. Demographic and clinical data were collected.

The inclusion criteria comprised patients who received thymoglobulin or basiliximab for induction therapy and an immunosuppression maintenance scheme based on tacrolimus, mycophenolic acid and steroids.

The exclusion criteria represented patients with primary graft failure, or those who changed the ambulatory care center before the third month post-transplantation.

This study was approved by the CEMIC Ethics Committee (qualified by the Department of Health and Human Services, HHS no. 1242).

2.2. Clinical outcomes

All patients were screened for rejection by renal biopsies (protocol) at predefined times to detect subclinical events. Additional biopsies (indication) were taken with renal dysfunction.

Graft rejection details are provided in Supplementary material.

2.3. Samples

Plasma samples were obtained before and after transplantation at different times. Specifically, samples were obtained on day +3, +7, +14, +21, months 1, 3, 6, 9 and 12. Samples were cryopreserved at −70 °C until tested.

Total nucleic acids were extracted from 200 μL of plasma and eluted in 50 μL using a MagNA Pure Compact System (Roche, Basel, Switzerland), following the manufacturer's recommendation.

2.4. TTV quantification

TTV detection was performed by real time PCR with Home-Brew PCR and a commercial PCR (TTV R-GENE® Kit, bioMérieux, Marcy-lEtoile, France).

The Home-Brew PCR uses specific primers and probe previously described [20], amplifying a highly conserved region of 63 nt from the 5UTR in all TTV species. R-GENE®PCR's specific primers amplify a fragment of 128 nt from the 5UTR. Details are described in Supplementary material.

2.5. Statistical analysis

Mann-Whitney test was used to compare TTV viral load from patients who had graft rejection and patients without adverse events (negative renal biopsy, negative PCR results for CMV and BKPyV and same immunosuppressive scheme).

Graft rejection events and their association with TTV viral load were analyzed according to the time post-transplantation.Only the first event was considered in each patient and TTV viral load was determined in the sample from the closest previously available date from this event.

Correlation between PCR assays was calculated using the Pearson correlation coefficient (r). Bland-Altman difference plot analysis was used to evaluate the level and limits of agreement between both PCR. Details are described in Supplementary materials.

3. Results

A total of 107 renal graft recipients were transplanted from November 2018 to April 2021 at CEMIC University Hospital. All patients, regardless of their clinical outcome, remained with a functional graft. Patient survival during the first year post-transplantation was 96.3%. Four patients died because of infections during this period. Specifically, one developed measles encephalitis, one had bacterial septicemia and two developed severe COVID-19.

Mean age among the 107 renal transplant patients was 49.6 ± 13.4 years old and 60% (65/107) were male. A total of 82 (76.6%) patients received a graft from deceased donors, 86.9% received thymoglobulin and 13.1% received basiliximab as immunosuppression induction. Most

Table 1
Clinical and demographic characteristics in renal transplant patients (n = 107).

Characteristics	
Age, years old [mean ± SD]	49.6 ± 13.4
Gender (male) [n (%)]	65 (60.7)
Number of kidney transplantation [n (%)]	
First	96 (89.7)
Second	9 (8.4)
Third	2 (1.9)
Cause of end-stage renal disease [n (%)]	
Diabetes Mellitus	12 (11.2)
Glomerular disease	28 (26.2)
Hypertensive kidney disease	7 (6.5)
Polycystic kidney disease	14 (13.1)
Other specified disorders of kidney and ureter	46 (43.0)
CMV serostatus [n (%)]	
D+/R+	77 (72.0)
D-/*R+*	16 (15.0)
D+/R-	8 (7.5)
D-/R-	3 (2.8)
Pre-transplant renal replacement therapy [n (%)]	
Hemodialysis	93 (86.9)
Time on dialysis, months [median (IQR)]	30.0 (13.4 - 52.6)
Donor age, years old [mean ± SD]	43.5 ± 17.7
Donor gender (male) [n (%)]	58 (54.2)
Type of donor [n (%)]	
Deceased donor	82 (76.6)
Living donor	25 (23.4)
Number of HLA mismatches [median (IQR)]	4 (3 - 5)
Induction therapy [n (%)]	
Thymoglobulin	93 (86.9)
Basiliximab	14 (13.1)
Initial maintenance immunosuppression scheme [n (%)]	
Tacrolimus, mycophenolic acid and steroids	107 (100)

SD: standard deviation; IQR: interquartile range.
D: donor R: recipient.

recipients (89.7%) were receiving their first renal transplant (Table 1).

3.1. Home-Brew PCR and R-GENE®PCR

Correlation of both PCR performed using 746 samples from 107 recipients showed a 93.2% agreement. Pearson correlation coefficient was r: 0.902 (95% Confidence Interval (CI): 0.8881–0.9149, $p < 0.0001$) (Fig. 1A). Sensitivity and specificity of R-GENE®PCR compared to Home-Brew PCR were 94.7% and 79.2%, respectively (Fig. 1B).

The Bland–Altman method indicated a mean difference between both PCRs of 0.35 Log_{10} copies/mL with 95% limits of agreement of −1.43 and 2.13 Log_{10} copies/mL. The Bland-Altman plot showed 689/746 (92.4%) values falling inside the limits of agreement (Fig. 2).

TTV viral load was consistently higher using Home-Brew PCR compared to R-GENE®PCR. Median viral load difference among 746 samples was 0.26 (range 0.06–0.71 Log_{10}) (Table 2). Specifically, 567/746 (76%) samples had less than 1 Log_{10} difference between both PCR assays. Only 7/746 (1%) samples had TTV viral load difference >3 Log_{10} (Table S1). In particular, one sample gave 8.92 Log_{10} copies/mL with R-GENE®PCR and 3 Log_{10} copies/mL with Home-Brew PCR. Sequencing analysis on this sample (kindly performed by Dr. F. Maggi and P. Spezia) demonstrated the presence of Torque Teno Mini Virus (TTMV).

Among 746 samples, 51 (6.8%) gave discordant results and most of them, 36/51 (70.6%) were only positive with Home-Brew. Specifically, 34/36 (94.4%) samples had a TTV viral load <3Log_{10} copies/mL and 2/36 (5.6%) samples had a viral load >3Log_{10} copies/mL (Table S2).

In contrast, 15/51 (29.4%) were only detected by R-GENE®PCR. Most of them, 14/15 (93.35) had a TTV viral load <3Log_{10} copies/mL and only 1/15 (6.7%) had a viral load >3Log_{10} copies/mL (Fig. 2).

3.2. TTV prevalence and viral load kinetics

Pre-transplant TTV prevalence was determined in 102 recipients. Of them, 83 (81.4%) and 80 (78.4%) were positive with Home-Brew PCR and R-GENE®PCR, respectively. Median viral load pre-transplant were 2.98 (2.35–3.66) and 2.79 (2.04–3.40) Log_{10} copies/mL, respectively.

Post-transplant TTV prevalence was determined in 107 recipients. All patients became TTV positive at some point post-transplantation. The highest TTV prevalence was on day 90 (98%).

TTV kinetics showed a gradual increase in viral load during the first months, reaching a peak on day 90 (median 6.78 and 6.13 Log_{10} copies/mL with Home-Brew PCR and R-GENE®PCR, respectively). Following this peak, TTV viral load slightly decreased, reaching a plateau at day 180. One year post-transplantation, median viral load were 5.65 and 5.46 Log_{10} copies/mL with Home-Brew PCR and R-GENE®PCR, respectively (Fig. 3, Figure S1).

Median viral load observed at 6, 9 and 12 months post-transplantation were significantly higher than at baseline ($p < 0.0001$ in each point).

3.3. TTV viral load and graft rejection

A total of 135 renal biopsies (protocol and indication) were performed in 92 patients during the first year and 3 months post-transplantation. Most patients, 51/92 (55.4%) had one biopsy, 33/92 (35.9%) patients had two biopsies and 6/92 (6.5%) patients had three biopsies. Median time from transplantation to the first graft rejection event was 98.5 days (51.0–223.5).

Biopsies from 15/107 (14%) patients were unavailable during the first 15 months due to the lack of follow up because of the COVID pandemic.

Graft rejection (cellular, humoral and borderline changes) occurred in 28/92 (30.4%) patients during the study period. Of them, 10/28 (35.7%) had subclinical graft rejection. All 28 patients were treated using antirejection therapy and none developed a graft loss. Most rejection occurred between 30 and 90 days post-transplantation in 13/28 (46.4%) patients. Same rejection rates (21.4%) were observed between (91–180) and (181–270) days. During (271–365) days no rejections were documented. Lower rejection rate (10.7%) was observed between (366–450) days.

Most patients 21/28 (75%) had a T cell mediated rejection (TCMR), 5/28 (17.8%) patients showed borderline change (BL), 2/28 (7.1%) patients had antibody mediated rejection (ABMR) (Table S3).

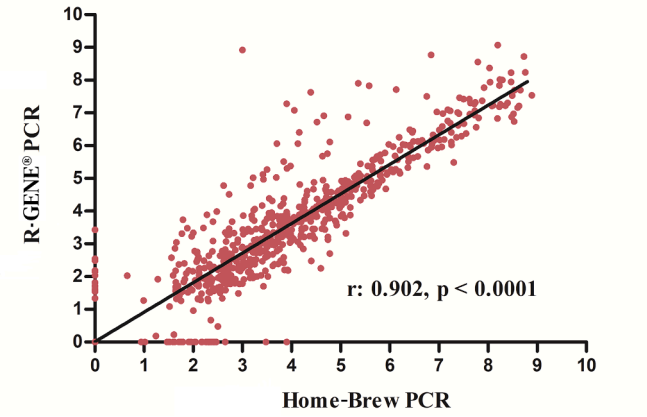
Median time between samples for TTV quantification and subsequent biopsies proven graft rejection was 40 (12.5–59.3) days.

TTV viral load was significantly lower in 6 patients with graft rejection occurring between (181–270) days post-transplantation. TTV median viral load were 3.59 Log_{10} copies/mL (by Home-Brew PCR) and 3.10 Log_{10} copies/mL (by R-GENE®PCR) compared to 6.14 (by Home-Brew PCR) and 5.96 (by R-GENE®PCR) from 74 patients without graft rejection ($p = 0.047$ and $p = 0.012$) (Table 3).

Between (30–90), (91–180) and (366–450) days, TTV viral load was not significantly different between patients with or without graft rejection (Table 3).

When analyzing only subclinical rejections, patients showed a lower TTV viral load at all points (90, 180 and 365) compared to patients without rejection. Specifically, at day 180, TTV viral load was 3.12 Log_{10} copies/mL (by Home-Brew PCR) and 2.45 Log_{10} copies/mL (by R-

A-



B-

Home-Brew PCR	Commercial PCR (R-GENE®PCR) POS	NEG	Total
POS	638 (85.5%)	36 (4.8%)	674
NEG	15 (2.0%)	57 (7.6%)	72
Total	653	93	**746**

Fig. 1. Comparison between Home-Brew PCR and commercial PCR (R-GENE®PCR)
A- Correlation between Home-Brew PCR and commercial PCR in 746 samples from 107 renal transplant patients analyzed in parallel with both PCR assays.
B- Sensitivity and specificity of the commercial PCR compared to Home-Brew PCR.

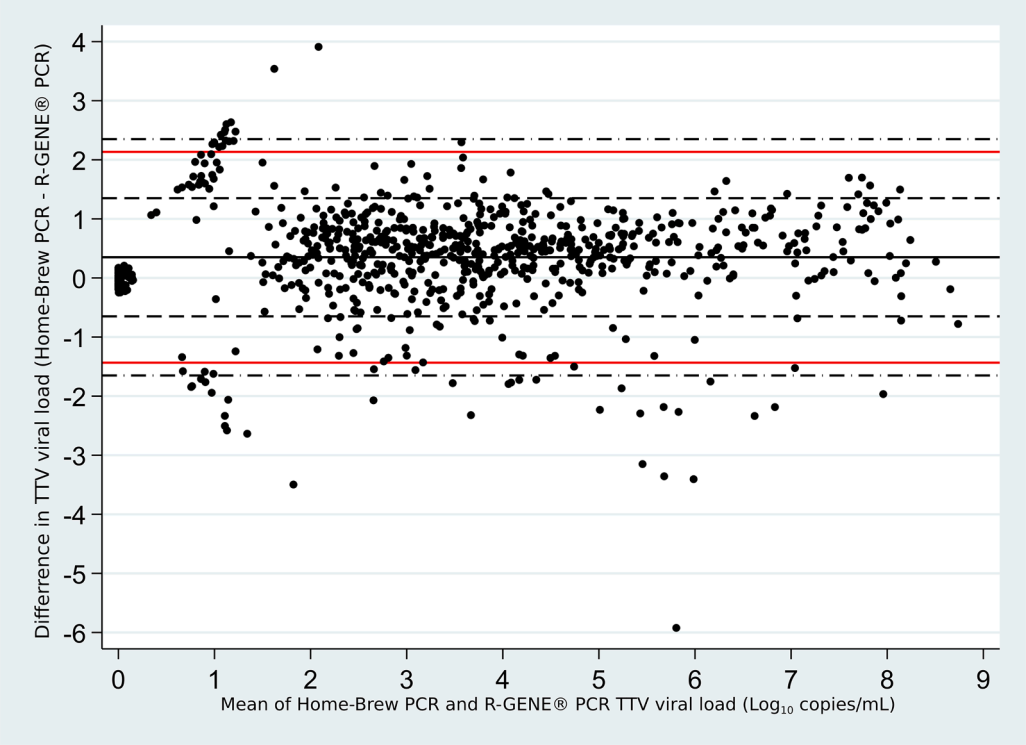


Fig. 2. Differences in TTV viral load vs. average values using Bland-Altman analysis in 746 plasma samples.
Mean difference is indicated by a black solid line, the mean +/- 1 Log_{10} by black dashed lines, and the mean +/- 2 Log_{10} by black dash-dotted lines. Red solid lines describe the limits of agreement.

Table 2
TTV median viral load kinetics in renal transplant patients by Home-Brew PCR and commercial PCR (R-GENE®PCR) before and after transplantation.

Days after transplantation	TTV median viral load (Log_{10}copies/mL) **Home-Brew PCR**	**R-GENE®PCR**	**Δ median**
Pre-transplant (0)	2.98	2.79	0.19
+3	2.65	2.26	0.39
+7	2.95	2.75	0.20
+14	3.60	3.20	0.40
+21	3.97	3.68	0.29
+30	4.59	3.88	0.71
+90	6.78	6.13	0.65
+180	5.82	5.76	0.06
+270	4.89	4.67	0.22
+365	5.65	5.46	0.19
TTV median viral load difference			0.26

GENE®PCR) compared to 6.14 Log_{10} copies/mL (by Home-Brew PCR) and 5.96 Log_{10} copies/mL (by R-GENE®PCR) ($p = 0.042$ and $p = 0.017$, respectively) (Table 4).

4. Discussion

The potential application of TTV viral load as a functional biomarker of immune status has increased in recent years, supported by its non-pathogenic nature and high prevalence [21,22]. It has been suggested that TTV kinetics could be a valuable tool to identify an increased risk of graft rejection, when SOT recipients present lower TTV viral loads [23–25]. In addition, a direct association between higher TTV viral load and infections has also been reported [17,18].

In this prospective study, we followed a cohort of renal transplant recipients for more than one year post-transplantation to evaluate TTV kinetics and its correlation with renal rejection. Simultaneously, two real time PCR assays were validated and compared.

A Home-Brew PCR amplifying a conserved 5UTR region showed adequate amplification of all 22 TTV species [20]. In our study, same primers were used to set up the Home-Brew PCR. An available commercial kit was developed and CE-marked for clinical use (R-GENE®PCR). It allows the quantification of TTV and occasionally of Torque Teno Mini Virus (TTMV) and high viral loads of Torque Teno Midi Virus (TTMdV) -members of the human *Anelloviridae* family[7]- as described by the manufacturers.

In our study, the comparison between both PCR assays showed a high agreement (93.2%), demonstrating R-GENE®PCR can detect the circulating TTV species in America. Viral load was consistently higher using Home-Brew PCR compared to R-GENE®PCR and this may be mostly related to the standard curve employed in each assay. The median difference between both PCR was 0.26 Log_{10} copies/mL. This value was similar to that previously reported [26].

The number of discordant results between both PCR was 6.8% and most of them showed differences below 2 Log_{10} or had a low viral load (R-GENE®PCR reported limit of detection is 2.4 Log_{10} copies/mL).

The one sample that showed a higher viral load difference between both PCR (5.9 Log_{10}), showed the presence of TTMV by sequencing analysis. This patient with concomitant presence of TTV and TTMV did not show graft rejection or viral infection. The clinical significance of TTMV in this population should be further investigated.

Data on TTV prevalence are very limited and no prospective studies on TTV are available in the Americas. A qualitative PCR study on TTV presence among healthy blood donors in Brazil, showed a 69% prevalence [27]. In our study, pre-transplant TTV prevalence was 80%. Interestingly, all patients became TTV positive during the study period suggesting that TTV can be broadly used as biomarker post-transplantation.

Our study represents one of the first protocols prospectively evaluating TTV viral load kinetics in renal transplant patients at 10 time points during the first year. TTV viral load showed a dynamic pattern post-transplantation, probably related to the immune status of the

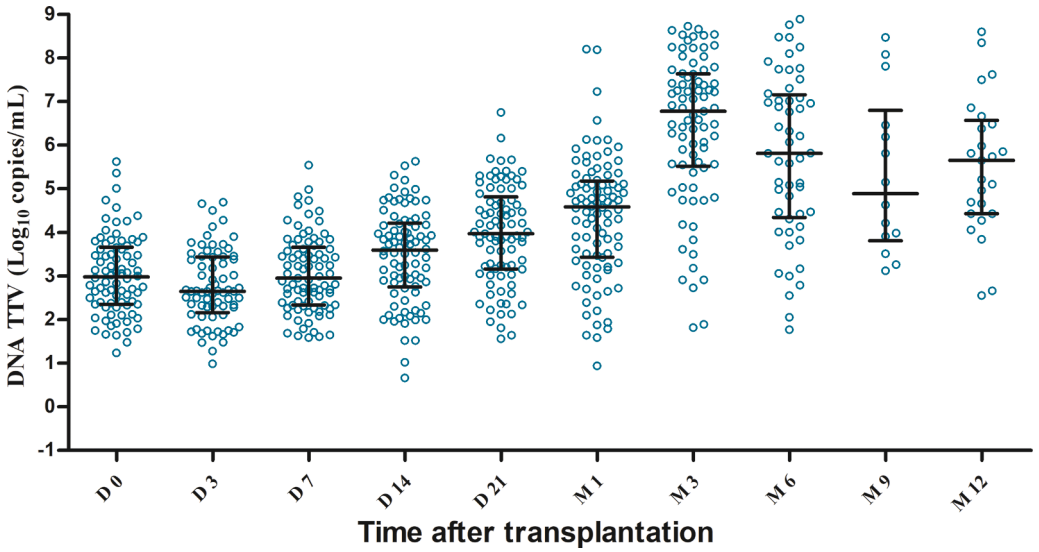


Fig. 3. TTV viral load kinetics in plasma samples from 107 renal transplant recipients detected by Home-Brew PCR.
A total of 746 plasma samples from 107 recipients were tested. At each timepoint, median viral load and IQR are presented.
D: days, M: month.

Table 3
Association between TTV viral load and graft rejection in renal transplant recipients ($n = 107$).

TTV MEDIAN VIRAL LOAD (IQR)								
	n patients		Home-Brew PCR			R-GENE®PCR		
Days after transplantation	Graft rejection	No graft rejection and no viral infection	Graft rejection	No graft rejection and no viral infection	p value	Graft Rejection	No graft rejection and no viral infection	p value
30–90	13	74	5.08 (3.25–5.49)	4.54 (3.29–5.06)	0.418	3.96 (2.26–4.77)	3.87 (2.95–4.88)	0.882
91–180	6	70	6.74 (5.07–8.31)	6.74 (5.51–7.48)	0.736	6.09 (4.21–7.33)	6.12 (4.96–6.97)	0.900
181–270	6	44	3.59 (2.81–6.06)	6.14 (4.46–7.27)	0.047	3.10 (1.60–5.40)	5.96 (4.14–7.02)	0.012
366–450	3	23	5.21 (2.66–6.86)	5.10 (4.27–6.30)	0.999	4.63 (2.56–6.03)	5.11 (3.95–6.16)	0.630

TTV viral load is expressed in Log10 copies/mL.

Table 4
Association between TTV viral load and subclinical graft rejection in renal transplant recipients ($n = 107$).

TTV MEDIAN VIRAL LOAD (IQR)								
	n patients		Home-Brew PCR			R-GENE®PCR		
Days after transplantation	Graft rejection	No graft rejection and no viral infection	Graft rejection	No graft rejection and no viral infection	p value	Graft Rejection	No graft rejection and no viral infection	p value
91–180	4	70	5.78 (4.24–7.71)	6.74 (5.51–7.48)	0.550	4.84 (3.68–6.75)	6.12 (4.96–6.97)	0.370
181–270	4	44	3.12 (2.30–5.16)	6.14 (4.46–7.27)	0.042	2.45 (0.53–4.84)	5.96 (4.14–7.02)	0.017
366–450	2	23	3.94 (2.66–5.21)	5.10 (4.27–6.30)	na	3.59 (2.56–4–63)	5.11 (3.95–6.16)	na

TTV viral load is expressed in Log10 copies/mL.; na: Not applicable.

patient (directly associated with host response to immunosuppression). In our population, TTV viral load presented a steady baseline level at pre-transplant time, with a median of 2.98 Log_{10} copies/mL. Later, viral load showed a gradual increase during the first months, reaching a peak on day 90. This characteristic peak was followed by a decrease that achieved a plateau after day 180. Interestingly, median viral load observed at this point was significantly higher ($p<0.0001$) than at baseline. Similar viral load kinetics were previously reported [23,25].

Given the dynamic nature of TTV viral load and its fluctuation throughout time, the use of TTV viral load cut-off values for risk stratification to predict rejection might be determined in relation to the post-transplantation period.

Graft rejection is one of the most serious complications. During the first year post-transplantation the rate of TCMR is consistently reported around 10% [28,29]. For the same period, the incidence of ABMR can reach up to 40% in recipients donor specifics antibody (DSA) positive, but is low in pre-transplant DSA-negative patients [30].

Most of these studies are based on renal biopsies obtained when a renal dysfunction occurs. In our study, protocol renal biopsies were obtained at predefined times to detect subclinical events. Therefore, our rejection rate (30.4%) was higher than that reported by others [23,24].

Rejection rate analyzed in periods showed that most of them

occurred before 3 months post-transplantation.

A significant association between low TTV viral load and rejection was observed between (181–270) days post-transplantation.

Doberer et al. [19,23], proposed a TTV viral load cut-off value (below 6 Log_{10}) by Home-Brew PCR as a predictor of graft rejection based on the analysis at a single period time (a median of 154 days post-transplantation). Considering this cut-off value, we demonstrated that 75% of our patients with graft rejection and 80% of those with subclinical rejection had TTV viral load below 6 Log_{10} by Home-Brew PCR.

For R-GENE®PCR assay, Solis[25] determined that renal transplant patients with TTV viral load ranging from 4.6 to 6.2 Log_{10} had a lower risk for graft rejection or viral infection. In our study, 57.1% of patients with graft rejection and 70% of those with subclinical rejection had a viral load below 4.6 Log_{10} by R-GENE®PCR.

The limitations of this study were the low number of patients and the single center design.

However, the large number of plasma samples analyzed not only contributed to the validation of R-GENE®PCR, but also was relevant to determine TTV kinetics throughout time.

This is the first study evaluating TTV viral load as a biomarker of the immunosuppression state of renal transplant patients in Argentina. A significant association between low TTV viral load and graft rejection was demonstrated at 180 days post-transplantation.

Time stratification analysis highlights the importance of evaluating TTV viral load at several points post-transplantation to determine time-specific cut-off values that may vary with the PCR assay.

Prospective multicenter studies will be needed to establish and validate optimal TTV viral load ranges to predict clinical and subclinical graft rejections.

Financial support

This work was partially supported by several institutions; including CEMIC and Fundación “Norberto Quirno” (Young Investigator Award 2019, given to Reyes N.S.), Fundación Allende (2019, given to Boccia N., MD) and Roemmers Foundation (2019, given to Echavarria M., PhD).

Declaration of Competing Interest

The authors declare no conflicts of interests.

Acknowledgments

We are very grateful to Philippe Bourgeois and Carole Janis PhD for all the support and efforts in providing TTV R-GENE® Kits (bioMérieux, Marcy-lEtoile, France). We also want to thank Fabrizio Maggi PhD and Pietro Giogio Spezia for sample sequencing.

From our hospital, we want to thank Pamela E. Rodriguez PhD, technicians Victoria Ceñal, Walter Tripelhorn and Karina Insua for sampling assistance; and Gonzalo Schirloh, MD for clinical data collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2023.105501.

References

[1] K.E. Lamb, S. Lodhi, H.U. Meier-Kriesche, Long-term renal allograft survival in the United States: a critical reappraisal, Am. J. Transpl. 11 (2011) 450–462.
[2] A. Loupy, G.S. Hill, S.C Jordan, The impact of donor-specific anti-HLA antibodies on late kidney allograft failure, Nat. Rev. Nephrol. 8 (2012) 348–357.
[3] J.A. Fishman, Infection in solid-organ transplant recipients, N. Engl. J. Med. 357 (2007) 2601–2614.
[4] L.M. Andrews, et al., Pharmacokinetic considerations related to therapeutic drug monitoring of tacrolimus in kidney transplant patients, Expert Opin. Drug Metab. Toxicol. 13 (2017) 1225–1236.
[5] O. Rezahosseini, et al., Torque-Teno virus viral load as a potential endogenous marker of immune function in solid organ transplantation, Transplant. Rev. J. 33 (2019) 137–144.
[6] M. Bendinelli, et al., Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans, Clin. Microbiol. Rev. 14 (2001) 98–113.
[7] A. Varsani, T. Opriessnig, V. Celer, F. Maggi, H. Okamoto, Taxonomic update for mammalian anelloviruses (family Anelloviridae), Arch. Virol. 166 (2021) 2943–2953.
[8] T. Nishizawa, et al., A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology, Biochem. Biophys. Res. Commun. 241 (1997) 92–97.
[9] D. Focosi, et al., Assessment of prevalence and load of Torque Teno Virus viraemia in a large cohort of healthy blood donors, Clin. Microbiol. Infect. 26 (2020) 1406–1410.
[10] E.V. Vasilyev, et al., Torque Teno Virus (TTV) distribution in healthy Russian population, Virol. J. 6 (2009) 8–11.
[11] S. Chattopadhyay, S. Rao, B.C. Das, N.P. Singh, P. Kar, Prevalence of transfusion-transmitted virus infection in patients on maintenance hemodialysis from New Delhi, India, Hemodial. Int. 9 (2005) 362–366.
[12] D. Rivanera, M.A. Lozzi, C. Idili, D Lilli, Prevalence of TT virus infection in Italian-dialysis patients, Pathol. Biol. 57 (2009) 97–100.
[13] I. Görzer, et al., Pre-transplant plasma Torque Teno virus load and increase dynamics after lung transplantation, PLoS ONE 10 (2015) 1–10.
[14] Iwijn De Vlaminck, et al., Temporal response of the human virome to immunosuppression and antiviral therapy, Cell 23 (2013) 1–7.
[15] M. Schiemann, et al., Torque Teno Virus load-inverse association with antibody-mediated rejection after kidney transplantation, Transplantation 101 (2017) 360–367.
[16] F. Simonetta, et al., Torque Teno Virus load and acute rejection after orthotopic liver transplantation, Transplantation 101 (2017) e219–e221.
[17] F. Maggi, et al., Early post-transplant Torquetenovirus viremia predicts cytomegalovirus reactivations in solid, Organ Transpl. Recipients. Sci. Rep. 8 (2018) 6–13.
[18] R. Strassl, et al., Quantification of torque teno virus viremia as a prospective biomarker for infectious disease in kidney allograft recipients, J. Infect. Dis. 218 (2018) 1191–1199.
[19] I. Gorzer, et al., Validation of plasma Torque Teno viral load applying a CE-certified PCR for risk stratification of rejection and infection post kidney transplantation, J. Clin. Virol. 158 (2023).
[20] F. Maggi, et al., TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity, J. Virol. 77 (2003) 2418–2425.
[21] S. Spandole, D. Cimponeriu, L.M. Berca, G. Mihăescu, Human anelloviruses: an update of molecular, epidemiological and clinical aspects, Arch. Virol. 160 (2015) 893–908.
[22] G. Freer, et al., The virome and its major component, Anellovirus, a convoluted system molding human immune defenses and possibly affecting the development of asthma and respiratory diseases in childhood, Front. Microbiol. 9 (2018) 1–7.
[23] K. Doberer, et al., Torque Teno Virus for risk stratification of graft rejection and infection in kidney transplant recipients — A prospective observational trial, Am. J. Transpl. (2020) 2081–2090, https://doi.org/10.1111/ajt.15810.
[24] R. Strassl, et al., Torque teno virus for risk stratification of acute biopsyproven alloreactivity in kidney transplant recipients, J. Infect. Dis. 219 (2019) 1934–1939.
[25] M. Solis, et al., Torque Teno Virus viremia for early prediction of graft rejection after kidney transplantation, J. Infect. 79 (2019) 56–60.
[26] L. Macera, et al., Comparative evaluation of molecular methods for the quantitative measure of Torque Teno Virus viremia, the new surrogate marker of immune competence, J. Med. Virol. (2019) 491–498, https://doi.org/10.1002/jmv.25488.
[27] J.C. Mazzola, et al., Prevalence of Torque Teno Virus in healthy donors of Paraná State, southern Brazil, Rev. Bras. Hematol. Hemoter. 37 (2015) 336–340.
[28] D. Seron, et al., Proposed definitions of T cell-mediated rejection and tubulointerstitial inflammation as clinical trial endpoints in kidney transplantation, Transpl. Int. 35 (2022) 1–10.
[29] C. Wehmeier, et al., Acute rejection phenotypes in the current era of immunosuppression: a single-center analysis, Transpl. DIRECT (2017) 1–10, https://doi.org/10.1097/TXD.0000000000000650.
[30] C. Roufosse, et al., Proposed De fi nitions of antibody-mediated rejection for use as a clinical trial endpoint in kidney transplantation, Transpl. Int. 35 (2022) 1–18.