Received: 7 May 2024 | Accepted: 8 July 2024

DOI: 10.1002/jmv.29814

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

TTV and CMV viral load dynamics: Which emerges first during immunosuppression?

Piergiorgio Roberto[1,2,3] | Lilia Cinti[1,3,4] | Dario Lucente[5] | Gianluca Russo[3,6] | Quirino Lai[3,7] | Alessandra Micozzi[3,8] | Giuseppe Gentile[3,8] | Ombretta Turriziani[1,3] | Alessandra Pierangeli[1] | Guido Antonelli[1,3]

[1]Laboratory of Microbiology and Virology, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy

[2]PhD National Programme in One Health Approaches to Infectious Diseases and Life Science Research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

[3]University Hospital "Policlinico Umberto I", Rome, Italy

[4]PhD National Programme in Innovazione nella diagnosi, prevenzione e terapia delle infezioni a rischio epidemico-pandemico, Dipartimento di Biotecnologie Mediche, University of Siena, Siena, Italy

[5]Department of Mathematics & Physics, University of Campania "Luigi Vanvitelli", Caserta, Italy

[6]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy

[7]Department of Chirurgia Generale e Specialistica, Sapienza Università di Roma, Roma, Italy

[8]Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy

Correspondence
Piergiorgio Roberto, Laboratory of Microbiology and Virology, Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena, 324, 00161, Rome, Italy.
Email: piergiorgio.roberto@uniroma1.it

Funding information
EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases, Grant/Award Numbers: PE00000007, INF-ACT; Italian Ministry of University and Research, Grant/Award Number: PRIN 2022ZRNTHW

Abstract

Novel biomarkers reflecting the degree of immunosuppression in transplant patients are required to ensure eventual personalized equilibrium between rejection and infection risks. With the above aim, *Torque Teno Virus* (TTV) viremia was precisely examined in a large cohort of transplanted immunocompromised patients (192 hematological and 60 solid organ transplant recipients) being monitored for *Cytomegalovirus* reactivation. TTV load was measured in 2612 plasma samples from 448 patients. The results revealed a significant increase in TTV viral load approximately 14 days following CMV reactivation/infection in solid organ transplant (SOT) patients. No recognizable difference in TTV load was noted among hematological patients during the entire timeframe analyzed. Furthermore, a temporal gap of approximately 30 days was noted between the viral load peaks reached by the two viruses, with *Cytomegalovirus* (CMV) preceding TTV. It was not possible to establish a correlation between CMV reactivation/infection and TTV viremia in hematological patients. On the other hand, the SOT patient cohort allowed us to analyze viral kinetics and draw intriguing conclusions. Taken together, the data suggest, to our knowledge for the first time, that CMV infection itself could

Piergiorgio Roberto and Lilia Cinti contributed equally to this study.

potentially cause an increase in TTV load in the peripheral blood of patients undergoing immunosuppressive therapy.

KEYWORDS
Biomarker, *Cytomegalovirus* (CMV), Immunosuppression, SOT, *Torque Teno Virus* (TTV)

1 | INTRODUCTION

Torque Teno Virus (TTV) is a member of the *Anelloviridae* family. It is well known that it can be detected in the blood of most of the human population, although it is not associated with any specific disease.[1]

It has been widely demonstrated that TTV represents a surrogate for immune function, and, in particular, its viral load has been proposed as a biomarker of functional immunity in the management of transplant patients, especially solid organ transplant (SOT) patients.[2–4] More specifically, it has often been reported that monitoring TTV viremia could provide an additional diagnostic tool for the prediction of *Cytomegalovirus* (CMV) reactivation.[5]

Despite numerous and very recent studies in the field, to our knowledge, the precise relationship between the viral load of TTV and that of CMV or, more specifically, the kinetics of CMV appearance in peripheral blood related to TTV blood level has been only marginally addressed.[6]

Therefore, with the aim of adding some information on this (in our opinion) important aspect of the field, we carried out the present study, whose main objective was to verify whether a correlation exists between the viral loads of the two viruses and to clarify whether the increase in TTV viral load precedes or follows the reactivation of CMV in immunosuppressed transplant patients.

To obtain a statistically useful number of patients, this real-life study was performed at the Sapienza University "Policlinico Umberto I" Hospital on a population of transplant patients selected during a 9-month period (April to December) based on the request for the presence of CMV in the context of their post-transplant monitoring.

2 | MATERIALS AND METHODS

2.1 | Study of population

In this prospective study, a cohort of 448 patients of the "Policlinico Umberto I" in Rome was analyzed. From April to December 2022, a total of 2612 plasma samples were collected for molecular analysis of CMV. All patients who tested positive for CMV were included in the study, in addition to a control group of patients who tested negative. Among them, temporal monitoring was possible only for 300 patients (67%) who had at least three samples. Indeed, for the remaining 148 patients, only one (22%) or two (11%) samples were available. The overall sample population derived mostly from transplant patients (84%). Sixty-four percent belongs to hematological patients, the vast majority being allogeneic transplant recipients. The other 20% consisted of samples from SOT recipients (transplanted for at least 1 year), predominantly kidney, with a smaller portion involving liver and lung transplants. Finally, the remaining 16% of the sample population was related to heterogeneous patients, including ones with cystic fibrosis, infectious diseases, and individuals in the intensive care unit.

The Local Ethics Board of the Sapienza University of Rome approved the present study (CE 6338). Informed consent was also obtained for each patient included in the study.

2.2 | Molecular analysis

Molecular assay for CMV and TTV detection was conducted at the Microbiology and Virology unit of the University Hospital "Policlinico Umberto I" in Rome. Sample extraction was performed using the ELITe GALAXY automated system (ELITechGroup SpA) commonly used in clinical diagnosis. Amplification of CMV DNA was conducted using the ELITe CMV MGB® Kits (ELITechGroup SpA), which allows the detection of a range from 455 to 2.5×10^6 copies/mL of DNA. TTV DNA detection was accomplished through an in-house PCR protocol conducted on the CFX96 platform (Bio-Rad Laboratories Inc.) employing the method described above.[7]

2.3 | Statistical analysis

Data management, analysis, and visualization were performed in Python (https://www.python.org) using Pandas (https://pandas.pydata.org), SciPy (https://scipy.org), statsmodels (https://www.statsmodels.org), and Matplotlib (https://matplotlib.org) libraries. Null hypothesis testing was performed using the nonparametric Mann–Whitney–Wilcoxon test. *p*-Values are reported in figures according to the following notation: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3 | RESULTS

TTV load was measured in a total of 2612 plasma samples from 448 patients, with a mean age of 57 years (IQR: 47–67). CMV was measured in the same samples. TTV was detected in 88% of both samples and patients (93% of hematological patients, 95% of SOT recipients, and 76% of the heterogeneous patients). However, CMV load was revealed in 24% of the analyzed samples and in 39% of patients (31% of hematological patients, 37% of SOT recipients, and 49% of the heterogeneous population).

The correlation between CMV and TTV viral load was then examined. Initially, the correlation between TTV and CMV copies was investigated considering all samples, assuming the value < 455 CMV copies/mL as "not detected." The results of such analysis are shown in Figure 1 and basically suggest an absence of correlation between viral loads, with an R^2 value of 0.0094.

To further address the relation between TTV and CMV viral load values, the samples were divided into two macro-categories: positive and negative for CMV reactivation/infection. The mean and median TTV load was then calculated for both groups of samples. Figure 2 displays the results, demonstrating that two significantly distinct distributions ($p < 0.001$) exist. Indeed, the median number of TTV copies in negative CMV samples ($n = 1694$) is 6394 copies/mL, whereas in samples with a positive molecular CMV result ($n = 541$), the value is 21 205 copies/mL. Interestingly, no differences are evident when looking at mean values instead of median values: 2.1×10^6 copies/mL in negative CMV samples versus 2.0×10^6 copies/mL in positive CMV samples. The ambiguity in these results prompted us to further investigate the viral load data, with the objective of refining our understanding of the interrelation between the two viruses. For this purpose, only patients who could be followed over a period of time were selected. Among these, patients with a CMV-negative sample at first evaluation were distinguished from patients who began monitoring CMV-positive. Figure 3 represents the 146 patients who began monitoring with an absence of CMV viremia and subsequently tested positive during follow-up. The TTV viral load during this CMV-negative stage was then compared with that during CMV reactivation/infection. This comparison revealed a significant difference ($p < 0.05$) in TTV viral loads, which were higher during CMV positivity than during the negative stage. However, when considering patients who started monitoring as CMV-positive and later became negative, TTV loads in the two stages were comparable and not significantly different (data not shown).

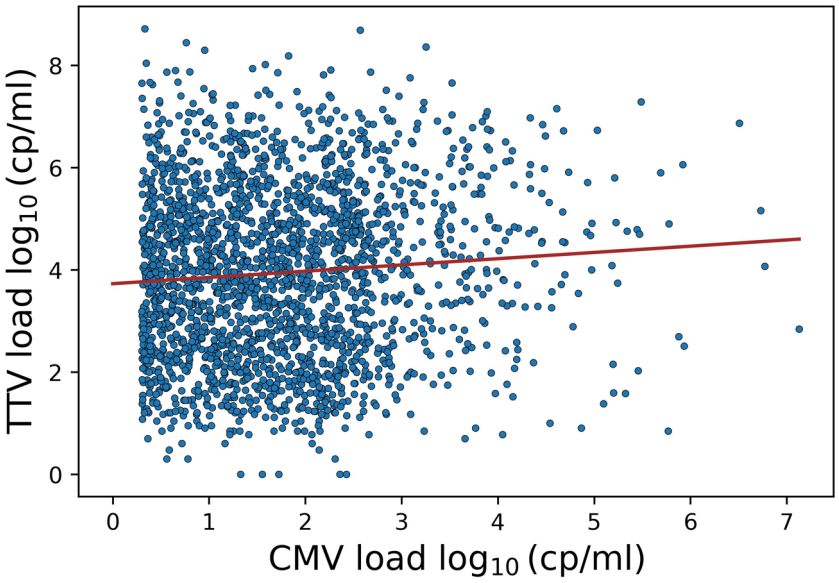

FIGURE 1 Scatter plot of TTV load (*y*-axis) versus CMV (*x*-axis) load relative to the same serum sample. Brown solid line represents the best linear fit, showing that the data are weakly correlated ($R^2 = 0.0094$). CMV, *Cytomegalovirus*; TTV, *Torque Teno Virus*.

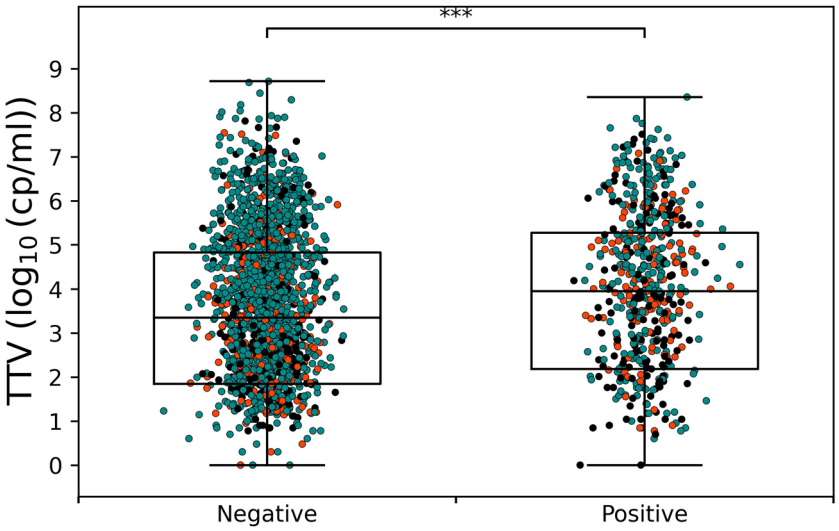

FIGURE 2 Distribution of TTV viral load with superimposed Whisker plot for CMV negative samples and CMV positive samples. SOT and hematological patients are indicated with orange and green circles, respectively, while black circles represent the rest of the population. The black solid lines inside the boxes denote the median values. Mann–Whitney–Wilcoxon test *p*-value is reported according to the scoring system described in the main text. CMV, *Cytomegalovirus*; SOT, solid organ transplant; TTV, *Torque Teno Virus*.

Moreover, an analysis was conducted to assess potential predictive signals in TTV viral load preceding CMV reactivation. For this purpose, the TTV load was analyzed 40 days before and 40 days after CMV reactivation, with the start of positivity set as "day zero."

Cumulative analysis of data that included all patients did not reveal any significant results (data not shown). On the contrary, the analysis performed on the two groups of patients (hematological and SOT) generated interesting results. Indeed, the results in Figure 4 show a clear increase in TTV viral load approximately 14 days after "day zero," observed only for the regression analysis representing SOT patients, whereas no increase in TTV viral load was observed in hematological patients during the entire timeframe analyzed.

In addition, a careful study of the relative kinetics of the viral load of both TTV and CMV viruses was planned. For this analysis, "day zero" was defined as the peak CMV load of each patient, and its temporal relationship with TTV load was assessed (Figure 5). A gap of approximately 30 days was observed between the peak loads reached by the two viruses. However, the increase in TTV viral load following the peak of CMV positivity can again be observed only in SOT patients, while the regression analysis describing hematological patients does not show any specific and clear-cut trend.

4 | DISCUSSION

Monitoring the immune system in specific categories of immunocompromised individuals is of paramount importance for a modern and proper patient management of some diseases. In recent years, there have been numerous studies aiming to correlate the viral load of TTV with the functionality of the immune system, often

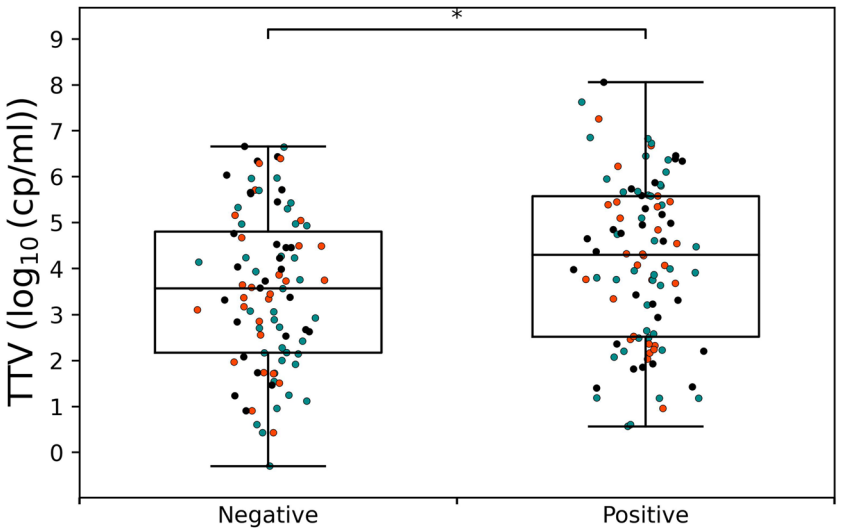

FIGURE 3 Distribution of average TTV viral load with superimposed Whisker plot for patients with no prior CMV positivity. Each circle represents the average value of TTV load per patient. Averages are performed over the period before the first CMV reactivation/infection (negative) and during the first reactivation/infection (positive). SOT patients and hematological patients are indicated with orange and green circles, respectively, while black circles represent the rest of the population. The black solid lines inside the boxes denote the median values. Mann–Whitney–Wilcoxon test *p*-value is reported according to the scoring system described in the main text. CMV, *Cytomegalovirus*; SOT, solid organ transplant; TTV, *Torque Teno Virus*.

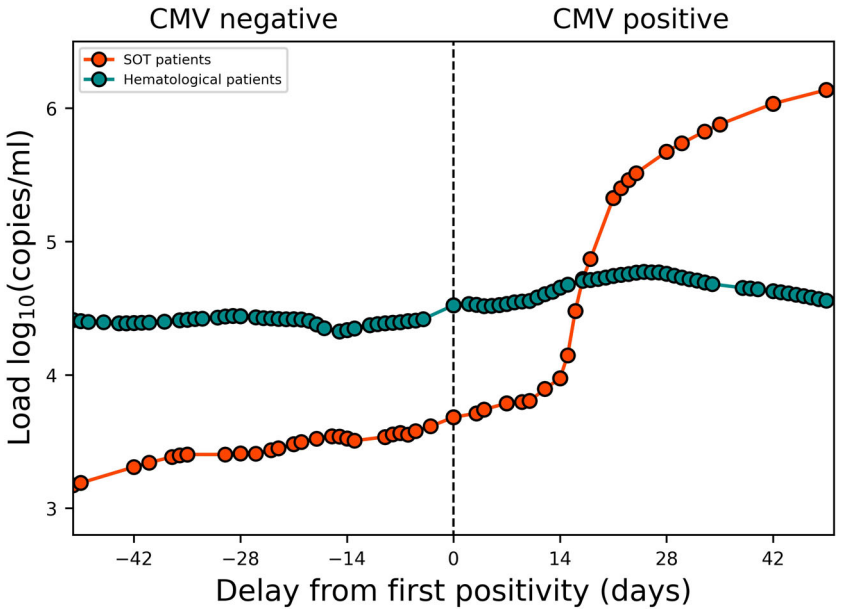

FIGURE 4 Kinetics of TTV and CMV viral loads as a function of time elapsed from first CMV reactivation/infection (Day 0). SOT and hematological patients are indicated in orange and green, respectively. The results were obtained by interpolating the data using nonparametric smoothing (lowess). CMV, *Cytomegalovirus*; SOT, solid organ transplant; TTV, *Torque Teno Virus*.

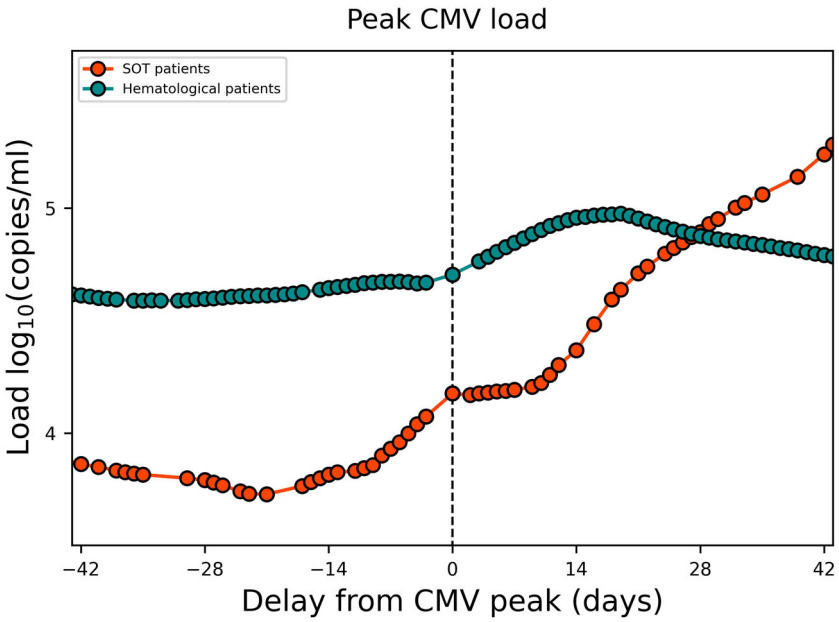

FIGURE 5 Kinetics of TTV and CMV viral loads as a function of time elapsed from maximum CMV load (Day 0). SOT and hematological patients are indicated in orange and green, respectively. The results were obtained by interpolating the data using nonparametric smoothing (lowess). CMV, *Cytomegalovirus*; SOT, solid organ transplant; TTV, *Torque Teno Virus*.

demonstrating interesting and promising results.[8–10] In this study, a large patient population was studied to investigate the correlation between TTV and CMV loads in two major vulnerable groups of transplant patients: hematological and SOT.

The significant size of the analyzed sample allowed us to strongly suggest that in SOT recipients a correlation exists between TTV load and CMV loads. Numerous articles in the literature identify TTV as a valuable immunological marker in this patient category.[11,12] However, controversies persist regarding its predictive role in CMV reactivation. Indeed, on the one hand, it seems that an increase in TTV load can very likely predict viral reactivation.[5,12] On the other hand, an increase in TTV load is correlated only with the probability of organ rejection and not with the prediction of CMV reactivation.[13]

In our study, the analysis of viral load kinetics suggests a delayed rise in TTV following a CMV load increase. Specifically, the linear regression representing TTV load diverges from the descending curve of CMV, which "persists" at higher levels for a longer period. This observation is highlighted by the significant difference in terms of TTV load between the two negative phases: that preceding the first CMV detection and that subsequent to it. In light of the analysis of these data, predicting CMV reactivation remains challenging when using TTV in the analyzed categories.

Looking at the TTV and CMV load and kinetics in hematological subjects, no correlation between the two viruses has been recorded. This is intriguing but, in some ways, expected. Indeed, first of all, it should be taken into account that when "hematological patients" are considered, we are dealing with heterogeneous diseases and related heterogeneous therapeutic protocols, and both of these factors can differently affect TTV load levels. On the other hand, TTV replication has been demonstrated in hematopoietic cells such as granulocytes,[14] and blood disorders such as chronic and acute myeloid leukemias disrupt the cellular precursors of granulocytes. This can, at least in part, justify the possibility that TTV load may vary depending on the harbored number of specific hematopoietic cells in a specific patient.[6,15] However, the study does not provide conclusive data regarding the latter aspect. Nevertheless, in our opinion it has the merit of confirming the complexity of the phenomenon and offers interesting insights for the choice of approaches and conditions to continue experimental studies on the topic.

In conclusion, taken together, the data suggest, to our knowledge for the first time, that CMV infection itself could potentially cause an increase in TTV load in the peripheral blood of patients undergoing immunosuppressive therapy. Evidence in the literature suggests an immunosuppressive activity of CMV,[16,17] indirectly supporting the above considerations. Therefore, CMV reactivation or infection in vulnerable patients could lead to significant clinical sequelae that may directly or indirectly cause immunological dysfunction, whose effect is a consequent increase in TTV plasma viral load. At the moment, such an assumption seems to apply only to SOT patients, while in hematological patients, the immunosuppressive effect of CMV reactivation may not be clearly discernible in terms of TTV load.

AUTHOR CONTRIBUTIONS

Piergiorgio Roberto and Lilia Cinti: Conceptualization; investigation; acquisition of data; data curation; formal data analysis; writing—original draft. **Dario Lucente**: Data analysis; statistical analysis; revision; interpretation of data. **Gianluca Russo, Quirino Lai, Alessandra Micozzi, and Giuseppe Gentile**: Clinical investigation; revision; visualization. **Ombretta Turriziani and Alessandra Pierangeli**: Visualization; supervision; revision. **Guido Antonelli**: Conceptualization; validation; writing—original draft; editing and revision; visualization; supervision; project administration. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The study was carried out as part of the activities of the following PhD course: national course "One health approaches to infectious diseases and Life Science research: [PhD One Health]"—University of Pavia & Sapienza the University of Rome for Piergiorgio Roberto and national course "Innovazione nella Diagnosi, Prevenzione e Terapia delle Infezioni a Rischio Epidemico-Pandemico"—University of Siena & Sapienza University of Rome for Lilia Cinti. This work was supported by the Italian Ministry of University and Research: PRIN 2022 [grant number 2022ZRNTHW] to Guido Antonelli and by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (project no. PE00000007, INF-ACT).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data cannot be publicly shared as they contain sensitive information that could compromise the privacy and consent of research participants. However, interested parties can request access to the data from the corresponding author. This restriction is in place to uphold privacy and ethical standards.

ETHICS STATEMENT

The Local Ethics Board of the Sapienza University of Rome approved the present study (CE 6338). Informed consent was also obtained for each patient included in the study.

ORCID

Piergiorgio Roberto http://orcid.org/0000-0002-9170-3654
Ombretta Turriziani http://orcid.org/0000-0001-6400-9168
Alessandra Pierangeli http://orcid.org/0000-0003-0633-360X
Guido Antonelli http://orcid.org/0000-0002-2533-2939

REFERENCES

1. Gore EJ, Gard L, Niesters HGM, Van Leer Buter CC. Understanding torque teno virus (TTV) as an immune marker. *Front Med*. 2023;10:1168400. doi:10.3389/fmed.2023.1168400
2. Focosi D, Antonelli G, Pistello M, Maggi F. Torquetenovirus: the human virome from bench to bedside. *Clin Microbiol Infect*. 2016;22(7):589-593. doi:10.1016/j.cmi.2016.04.007
3. Strassl R, Schiemann M, Doberer K, et al. Quantification of torque teno virus viremia as a prospective biomarker for infectious disease in kidney allograft recipients. *J Infect Dis*. 2018;218(8):1191-1199. doi:10.1093/infdis/jiy306
4. Kotton CN. Torque teno virus: predictor of infection after solid organ transplant. *J Infect Dis*. 2018;218(8):1185-1187. doi:10.1093/infdis/jiy384
5. Maggi F, Focosi D, Statzu M, et al. Early post-transplant torquetenovirus viremia predicts cytomegalovirus reactivations in solid organ transplant recipients. *Sci Rep*. 2018;8(1):15490. doi:10.1038/s41598-018-33909-7
6. Albert E, Solano C, Giménez E, et al. The kinetics of torque teno virus plasma DNA load shortly after engraftment predicts the risk of high-level CMV DNAemia in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant*. 2018;53(2):180-187. doi:10.1038/bmt.2017.235
7. Roberto P, Cinti L, Napoli A, et al. Torque teno virus (TTV): a gentle spy virus of immune status, predictive marker of seroconversion to COVID-19 vaccine in kidney and lung transplant recipients. *J Med Virol*. 2023;95(2):e28512. doi:10.1002/jmv.28512
8. Redondo N, Navarro D, Aguado JM, Fernández-Ruiz M. Viruses, friends, and foes: the case of torque teno virus and the net state of immunosuppression. *Tranpl Infect Dis*. 2022;24(2):e13778. doi:10.1111/tid.13778
9. Grenda R. Torque teno (TTV) viral load as a biomarker of immunosuppressive strength after kidney transplantation in children. *Pediatr Nephrol*. 2021;36(1):1-3. doi:10.1007/s00467-020-04607-2
10. van Rijn A, Roos R, Dekker F, Rotmans J, Feltkamp M. Torque teno virus load as marker of rejection and infection in solid organ transplantation: a systematic review and meta-analysis. *Rev Med Virol*. 2023;33(1):e2393. doi:10.1002/rmv.2393
11. Solis M, Velay A, Gantner P, et al. Torquetenovirus viremia for early prediction of graft rejection after kidney transplantation. *J Infect*. 2019;79(1):56-60. doi:10.1016/j.jinf.2019.05.010
12. Cañamero L, Benito-Hernández A, González E, et al. Torque teno virus load predicts opportunistic infections after kidney transplantation but is not associated with maintenance immunosuppression exposure. *Biomedicines*. 2023;11(5):1410. doi:10.3390/biomedicines11051410
13. van Rijn AL, Wunderink HF, Sidorov IA, et al. Torque teno virus loads after kidney transplantation predict allograft rejection but not viral infection. *J Clin Virol*. 2021;140:104871. doi:10.1016/j.jcv.2021.104871
14. Kosulin K, Kernbichler S, Pichler H, et al. Post-transplant replication of torque teno virus in granulocytes. *Front Microbiol*. 2018;9:2956. doi:10.3389/fmicb.2018.02956
15. de la Asunción CS, Giménez E, Hernández-Boluda JC, et al. Assessment of the potential value of plasma torque teno virus DNA load monitoring to predict cytomegalovirus DNAemia in patients with hematological malignancies treated with small molecule inhibitors: a proof-of-concept study. *J Med Virol*. 2023;95(7):e28933. doi:10.1002/jmv.28933
16. Spencer JV, Lockridge KM, Barry PA, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol*. 2002;76(3):1285-1292. doi:10.1128/jvi.76.3.1285-1292.2002
17. Poole E, Neves TC, Oliveira MT, Sinclair J, da Silva MCC. Human cytomegalovirus interleukin 10 homologs: facing the immune system. *Front Cell Infect Microbiol*. 2020;10:245. doi:10.3389/fcimb.2020.00245

How to cite this article: Roberto P, Cinti L, Lucente D, et al. TTV and CMV viral load dynamics: which emerges first during immunosuppression? *J Med Virol*. 2024;96:e29814. doi:10.1002/jmv.29814