Review

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Integrated Immunologic Monitoring in Solid Organ Transplantation: The Road Toward Torque Teno Virus-guided Immunosuppression

Peter Jaksch, MD,[1] Irene Görzer, MD, PhD,[2] Elisabeth Puchhammer-Stöckl, MD,[2] and Gregor Bond, MD, PhD[3]

Abstract. Potent immunosuppressive drugs have been introduced into clinical care for solid organ transplant recipients. It is now time to guide these drugs on an individual level to optimize their efficacy. An ideal tool simultaneously detects over-immunosuppression and underimmunosuppression, is highly standardized, and is straightforward to implement into routine. Randomized controlled interventional trials are crucial to demonstrate clinical value. To date, proposed assays have mainly focused on the prediction of rejection and were based on the assessment of few immune compartments. Recently, novel tools have been introduced based on a more integrated approach to characterize the immune function and cover a broader spectrum of the immune system. In this respect, the quantification of the plasma load of a highly prevalent and apathogenic virus that might reflect the immune function of its host has been proposed: the torque teno virus (TTV). Although TTV control is driven by T cells, other major immune compartments might contribute to the hosts' response. A standardized in-house polymerase chain reaction and a conformité européenne-certified commercially available polymerase chain reaction are available for TTV quantification. TTV load is associated with rejection and infection in solid organ transplant recipients, and cutoff values for risk stratification of such events have been proposed for lung and kidney transplantation. Test performance of TTV load does not allow for the diagnosis of rejection and infection but is able to define at-risk patients. Hitherto TTV load has not been used in interventional settings, but two interventional randomized controlled trials are currently testing the safety and efficacy of TTV-guided immunosuppression.

(*Transplantation* 2022;106: 1940–1951).

CLINICAL PROBLEM AND INTRODUCTION

Allograft transplantation (TX) is the preferred treatment for patients with end-stage solid organ disease. Immunosuppressive drugs are crucial for reducing the risk of organ rejection. Despite this desired effect, the compromised immune system of the recipient increases the risk for infectious and oncologic disease. Moreover, current immunosuppression regimens are unable to control chronic subclinical allorecognition of the graft, which leads to graft damage and loss.[1,2] Thus, the optimal management of immunosuppressive drug dosing requires a delicate balance between inadequate and excessive immunosuppression. At present, there is no diagnostic test or algorithm for the optimal guidance of immunosuppressive drugs.[3,4] Monitoring in routine post-TX care largely relies on the quantification of calcineurin inhibitor through levels in the peripheral blood, which correlate more closely with the risk of drug-related toxicity than with the effectiveness of immunosuppression.[5] Thus, there is an urgent need for tools to personalize immunosuppression to reduce the risk of infectious and oncologic disease as well as graft rejection.

A broad range of assays for immunologic monitoring has been proposed in recent decades.[6-8] However, only one noninvasive test system has been broadly accepted for routine post-TX care and implemented in diagnostic guidelines: donor-specific antibodies (DSAs).[9,10] Notably, DSAs are associated with antibody-mediated rejection (AMR) only and are not applicable for the detection of T cell-mediated rejection (TCMR), which is the dominant rejection type in the early phase after TX.[11] Moreover, DSAs are not useful to assess susceptibility to infections

Received 8 December 2021. Revision received 6 March 2022.

Accepted 7 March 2022.

[1] Division of Thoracic Surgery, Department of Surgery, Medical University of Vienna, Vienna, Austria.

[2] Center for Virology, Medical University of Vienna, Vienna, Austria.

[3] Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Vienna, Austria.

Funded by European Union Horizon2020 research and innovation programme, grant agreement number 896932.

The authors declare no conflicts of interest.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

G.B. wrote the manuscript. P.J. wrote the section Torque Teno Virus in Lung Transplantation. I.G. and E.P.-S. wrote the section Torque Teno Virus—an Introduction.

Supplemental Visual Abstract; http://links.lww.com/TP/C418.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Correspondence: Gregor Bond, MD, PhD, Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Währinger Gürtel 18-20, Vienna A-1090, Austria. (gregor.bond@meduniwien.ac.at).

ISSN: 0041-1337/20/10610-1940

DOI: 10.1097/TP.0000000000004153

or other consequences of excessive immunosuppression. Indeed, most assays analyzing blood and urine for immunologic monitoring currently under investigation—including cell-free donor-derived DNA,[12,13] chemokines, gene expression- and proteomics-based assays, and Enzyme-linked-immuno-Spot[6,8,14]—focus on the prediction of graft rejection only. However, some biomarkers—including immunoglobulins, leukocyte subsets, components of the complement system, and viral nucleic acids—have been proposed for the prediction of infections in solid organ transplantation (SOT).[7] Notably, the value of any immunologic monitoring tool—including DSA—has not been sufficiently proven in an interventional randomized controlled clinical setting.[4]

The ideal marker for the guidance of immunosuppressive drugs would simultaneously predict the consequences of both overimmunosuppression and underimmunosuppression. To address this need, two commercially available assays have been proposed: a test of leucocyte function known as the QuantiFERON Monitor (Qiagen, Germany; now T-SPOT.PRT, Oxford Immunotec, United Kingdom),[15] and the assessment of lymphocyte function using the ImmuKnow (ImmuKnow; Cylex, Germany; now Eurofins Viracor, United States).[16] The latter has been tested in an interventional randomized controlled setting, with test results suggesting a reduction of infectious events in liver TX (LTX) patients treated by ImmuKnow-guided immunosuppression. However, the trial design warrants careful interpretation of these preliminary data. Recently, a more functional and holistic assessment of the immune system has proposed the assessment of virus-specific T cells (Tvis) as a marker of immunosuppression. In a phase II trial of Tvis-guided immunosuppression in pediatric recipients of a kidney allograft, no major safety signals were noted.[17] However, the complex laboratory technique required for Tvis analysis might complicate a broad implementation in routine post-TX care.

Monitoring torque teno virus (TTV) in the peripheral blood is a promising new strategy to characterize the immune function. TTV can be detected in up to 90% of healthy individuals and has not been linked to any human disease.[18] The prevalence of TTV in immuno-compromised patients after TX is up to 100% and the virus is unaffected by conventional antiviral drug therapies used in the post-TX setting.[18] TTV copy number is directly associated with the amount and type of immunosuppressive drugs administered to TX recipients and additional major factors determining the immune function of its host (eg, age and sex); thus, it is indirectly associated with graft rejection and infectious disease.[18] Two randomized controlled interventional trials are currently investigating the value of TTV-guided immunosuppression[19-21] in kidney (KTX) and lung transplant (LuTX) recipients.

With novel and innovative tools to characterize the immune status potentially entering post-TX routine care in the next years, a review covering these assays is both timely and essential. Due to the differences in applied test systems and study designs as well as the paucity of high-quality prospective trials, it is not reasonable to perform a quantitative systemic review. We aimed to cover novel approaches for comprehensive immunologic monitoring by means of a narrative review with a clear focus on TTV.

NOVEL CONCEPTS

An ideal marker for the guidance of immunosuppressive drugs is able to simultaneously predict the consequences of both overimmunosuppression and underimmunosuppression, allows for comparability across assessment centers, and is supported by interventional studies based on biomarker-guided drug dosing.

In this respect, a commercially available assay measuring the concentration of ATP from $CD4^+$ cells after stimulation (ImmuKnow) has been proposed. Three meta-analyses, including noninterventional studies, have been published: one demonstrating a benefit for immunosuppression guided by ImmuKnow in SOT[22] and two with a negative result.[23,24] Consequently, the ImmuKnow assay was tested in a single-center randomized controlled trial including 202 LTX recipients.[16] In the intervention group, the tacrolimus dose was reduced if the ImmuKnow values were low and vice versa. Infection events after the first 2 wk post-TX were lower in the interventional group, and there was no difference in acute rejection. The trial design complicates a reliable analysis concerning safety and efficacy: (1) multiple endpoints were used, (2) no sufficient-definitions of infection and rejection were provided, (3) no assessor blinding was performed, (4) uncertainty about the impact of "clinical experience" on ImmuKnow-guided tacrolimus dosing exists, and (5) a post hoc analysis (exclusion of the first 2 weeks post-TX) was performed.

Recently, a broader assessment of the immune function—a commercially available test of interferon gamma in whole blood after T cell and Toll-like receptor stimulation, known as the QuantiFERON Monitor (Qiagen)—was tested in a single-center cohort study.[15] A total of 151 KTX, LTX, LuTx, and small bowel TX recipients were enrolled. Notably, no statement on blinding was available and no primary outcome was predefined. Patients with an infection had lower interferon gamma values detected in the samples drawn before the event compared with those without infection. However, potential confounders and effect modifiers were not considered. No differences were observed concerning rejections. Notably, such finding might have been overlooked due to the different levels of immunosuppression required to prevent rejection in the 4 types of SOT included. Despite these encouraging preliminary results, no further prospective study or interventional trial testing the value of QuantiFERON Monitor-guided drug dosing are currently registered.

A more comprehensive assessment of the immune system might be feasible by assessing the ability of the immune system to control opportunistic viruses. A single-center cohort study analyzed 383 KTX recipients.[25] Epstein-Barr virus (EBV) viremia was more frequent among patients experiencing at least 1 opportunistic infection (including zoster, *Pneumocystis jirovicii*, aspergillosis, legionellosis, tuberculosis, and nocardiosis). No primary outcome was predefined, multiple testing was performed, and no sufficient effect size adjustment was performed. Moreover, the association between EBV viremia and infections might have been disguised due to the per-protocol decrease in immunosuppression in the case of EBV polymerase chain reaction (PCR) >4 log10 copies/milliliter (c/mL) in two consecutive measurements. Notably, EBV-based monitoring might be complicated due to insufficient post-TX prevalence, reactivity towards the antiviral drugs routinely

used in the post-TX setting, and the association with disease requiring EBV infection treatment (eg, lymphoma).

Expanding upon the idea of a functional readout for the characterization of the immune system, a German 4-centered, open-label, randomized controlled trial involving 64 pediatric KTX recipients tested the steering of immunosuppressive therapy by levels of virus-specific $CD4^+$ cells (Tvis) directed towards adenovirus, cytomegalovirus (CMV), and herpes simplex virus.[17] In the active group, the dosage of immunosuppressive drugs was decreased if Tvis were low and vice versa. Tvis were analyzed in 4 steps over a total of 6 h, including (1) stimulation, (2) fixation, (3) immunostaining, and (4) flow-cytometry. No difference in the estimated glomerular filtration rate (cystatin C based on Schwartz and Filler) in the intention-to-treat population 24 mo post-TX was observed (adjusted mean difference: 1.7; 95% confidence interval [CI], −10.2 to 13.6). In the intervention group, dose reductions of cyclosporine and everolimus following Tvis levels were performed in 28 of 31 patients, while dose increases were performed for 2 patients. In the intervention group, lower drug trough levels and doses of everolimus and cyclosporine were noted. Additionally, more patients were free of glucocorticoid treatment. In the intervention group, 11 acute rejection episodes (including borderline changes suspicious for acute TCMR) were documented in comparison with 19 rejections in the control group. Moreover, fewer patients with EBV viremia were detected in the interventional group. The overall numbers of adverse events in the intervention and control groups were comparable. Considering the limitations of an open-label protocol with a surrogate endpoint in combination with a short-term follow-up, this trial provides evidence for the potential of Tvis measurements to detect patients in which a reduction in immunosuppressive therapy might be feasible without an increased risk of rejection. These unique and promising data justify a phase III randomized controlled trial to determine the efficacy of Tvis-guided immunosuppression. Notably, the complexity of Tvis monitoring might pose an obstacle for further large-scale efficacy trials and introduction into clinical routine.

TTV—AN INTRODUCTION

Recently, a highly prevalent and nonpathogenic virus has been introduced for immunologic monitoring in SOT recipients. TTV is a small nonenveloped DNA virus that was discovered in Japan in 1997.[26] TTV contains a single-stranded circular DNA of negative polarity that is approximately 3800 bases in length with at least 4 overlapping open reading frames (ORFs).[27] This small genome exhibits a strikingly high genomic diversity.[28,29] All TTV sequences known to date can be phylogenetically grouped into 21 distinct TTV species under the genus *Alphatorquevirus* of the family of Anelloviridae. Notably, a taxonomic update of the Anellovirus classification was recently published.[30]

TTV is highly prevalent in a wide variety of mammalian species.[31] In the human population, TTV-DNA is persistently detectable in the peripheral blood of up to 95% of healthy persons throughout their lifetime.[32-36] TTV, together with Beta- and Gammatorqueviruses, are considered the most abundant eukaryotic viruses in the human virome.[28,29,37] TTV was identified in blood and other samples taken from various body sites of healthy persons, as well as in environmental samples, which highlights its omnipresence.[38] The main TTV transmission routes in humans are thought to be fecal-oral and airway-mediated. Notably, initial infections seem to occur very early in life.[37] It is believed that TTV mainly replicates in T cells; however, as a polytrophic virus, its DNA was detectable in all leukocyte subsets and many other cell types.[39-42] TTV is a very actively replicating virus, and it has been estimated that >10 log10 c/mL of virions are generated per day in a healthy human body, with >90% of these being cleared by the immune system. Consequently, in cases with detectable TTV, viral load varies over time but remains at approximately 2 log10 to 8 log10 c/mL.[40,43-45] Multiple TTV strains can accumulate in a human host, which may be acquired simultaneously or serially over time, thereby leading to a mixture of TTV strains copersisting.[46-48]

In healthy immunocompetent individuals, plasma TTV levels are maintained at a well-balanced steady state that is controlled by host immunity.[49] However, only limited data exist on the specific immune response toward TTV as well as on the evasion mechanisms of TTV. Various immune compartments have been suggested to contribute to virus control: TTV-specific IgM and IgG antibodies directed against ORF1- and ORF2-encoded proteins can be found in TTV-positive individuals.[50-52] The high TTV loads detected in SOT recipients with interleukine-2 signal blocking immunosuppression suggest T cells being crucial for the virus control.[18] Evidence of the contribution of natural killer cells and antigen-presenting cells has also been provided.[53] Disease-modifying drugs (eg, anti-CD20) have been shown to increase TTV load in patients with rheumatoid arthritis, thereby suggesting that B cells contribute to TTV control.[54] Toll-like receptor TTV antigen-recognition[55] and interferon-α has been suggested to limit TTV replication.[56-58] Viral immune evasion is partly based on the ability of ORF2 to interfere with the host's inflammatory response via the suppression of NF-κB translocation.[59] TTV particles circulating in exosomes being less exposed to neutralizing antibodies might serve as another immune evasion mechanism.[60]

Due to the lack of a cell culture or well-established serological assay, the diagnosis of TTV infection is based on molecular methods. The measurement of TTV-DNA via quantitative PCR represents a sensitive and rapid method that has been applied to testing various samples, including serum and whole blood. Of note, TTV load in whole blood is >1 log level higher compared with plasma because of the high viral loads in leukocytes.[61] Currently, an in-house PCR developed by Maggi et al[62] and a commercially available PCR—both optimized and standardized for plasma—are mostly in use.[45] Despite high sequence diversity among TTV species, there is a short conserved ≈150-base sequence stretch at the 5′ nontranslated region of the TTV genome. This region is suited for the detection and quantification of most—if not all—TTV species known to date.[63] However, caution should be exercised when plasma TTV loads quantified in different laboratories and by different PCR assays, respectively, are compared.[45] TTV quantified by the in-house PCR produces results, which might differ up to 2 log10 levels between laboratories. In this respect, it is important to note that these differences are constant and thus linear across the whole range of TTV load; therefore, results from different labs

are comparable. A standardization process has been performed via the External Quality Assessment pilot study by Quality Control for Molecular Diagnostics, with accuracy in TTV quantification being observed in all participating laboratories.[64] Besides the in-house PCR, a commercially available PCR (Real-time detection and quantification kit, TTV R-GENE; bioMérieux SA, France) was introduced and conformité européenne-certified for routine clinical application in 2021.[45] In the same year, an EU-funded project (TTVguideTX) was initiated to implement, harmonize, and quality control this assay (in addition to the necessary process already performed during the conformité européenne certification) in 13 TX centers across Europe in preparation for a randomized controlled trial.[19,65] First results from the TTVguideTX project show low intercenter variability (data under review).

TTV is considered a nonpathogenic virus, and no substantial association with clinical symptoms has been detected to date.[66,67] The virus is unaffected by conventional antiviral drug therapies[68] and highly resistant to inactivation procedures.[18] Most importantly, the TTV load in peripheral blood might mirror the immune status of its host. A group at Stanford was the first to demonstrate an association between TTV load and allograft rejection in SOT.[68] Subsequently, other groups verified this finding and additionally provided evidence for the association between TTV load and infection.[69] TTV load was shown to directly associate with the amount and type of immunosuppressive drugs and thus with allograft rejection and infectious disease.[24,68] TTV is also associated with other major determinants of immune function, including age and sex of the host.[18] In addition to infectious events in SOT, peaks of TTV replication have been observed to occur during solid cancer growth, and associations have been described between TTV load and the use of chemotherapy.[70,71] In patients infected with HIV, TTV load was predictive for the course of immune recovery.[72] Recently, an association has been shown between TTV load and response to severe acute respiratory syndrome coronavirus type 2 vaccination in KTX recipients.[73] According to these findings, the central hypothesis of immune monitoring using TTV in SOT has formed: if the immune system is strong, the TTV load is low; this indicates a risk for organ rejection. If the immune system is weak, the TTV load is high; this indicates a risk for infection.

TTV IN KIDNEY TRANSPLANTATION

The majority of the reports on the value of TTV for immunologic monitoring in SOT have been focused on KTX. Reproducible evidence has been provided for an association between TTV load and rejection and infection, respectively (Table 1). Notably, all existing studies were observational and from single centers, and only half of them followed a prospective design. Moreover, some of their results must be interpreted with caution due to potential biases in study design, including selection bias, small numbers of events,

TABLE 1.

Studies that evaluated the association between TTV load and allograft rejection in kidney transplant recipients

Study design[a]	TX period	Included patients	Endpoint; timing	Total BX; BX proven rejection	PCR	Main association	Limitations[b]
Cohort[74]	2014–2016	221	Clinically overt rejection; <3 mo post-TX	10[c]	C	TTV at TX–rejection	Secondary endpoint; BX not mandatory; multiple testing; missing information on model design/some major determinants of TTV not included
Cohort[75]	2016–2018	37	Rejection (iBX); months 4–12 post-TX	39; 11	IH	TTV 2 wk before BX–TCMR, AMR, mixed	Limited number of events
Cohort[76]	2016–2018	82	Rejection (pBX); month 12 post-TX	82; 19	IH	TTV at BX–TCMR, AMR	High loss to follow-up
Cross-sectional[77]	1973–2014	715	Rejection (pBX); 6 y post-TX	86; 46	IH	TTV at BX–AMR	Cross-sectional design; possible selection bias due to missing BX in DSA-positive subjects
Case-control[78]	2012–2017	113	Rejection (iBX); months 4–12 post-TX	113; 33	IH	TTV 1 mo before BX–TCMR, AMR	Case-control design
Case-control[79]	2012–2014	63	Rejection (iBX); <2 y post-TX	12[d]	C	TTV pre-TX–TCMR, AMR, mixed	Possible selection bias; nonrejection not BX proven; multiple testing; no effect size adjustment
Case-control[21]	2003–2013	389	Clinically overt rejection; <12 mo post-TX	80; 54[e]	IH	TTV kinetic–time to rejection	Secondary endpoint; BX not mandatory; possible misclassification of rejection: nonrejection in BX categorized as rejection

[a]The studies are listed according to the design and date of their online publication.
[b]All studies followed a noninterventional and single-center design.
[c]The total number of biopsies was not stated; 11 events were scored as rejection.
[d]The total number of biopsies was not stated; 14 biopsies were available for the posttransplant month 1 evaluation.
[e]Eighty-eight events were scored as rejection.
AMR, antibody-mediated rejection; BX, biopsy; C, commercial; DSA, donor-specific antibody; iBX, indication biopsy; IH, in-house; pBX, protocol biopsy; PCR, polymerase chain reaction; TCMR, T cell–mediated rejection; TTV, torque teno virus; TX transplantation.

post hoc analyses, multiple testing, and insufficient effect size adjustments (Tables 1 and 2). However, sufficient evidence exists for a linear, robust, and independent association between TTV load and all types of clinically overt and subclinical rejection, including TCMR and AMR,[21,74-79] as well as infectious events,[21,74,75,79-85] including all common posttransplant pathogens in adult KTX recipients (opportunistic infections, CMV, BK polyomavirus [BKV], and bacterial infections), respectively. For easier interpretation of the studies presented next, we included the section Application of TTV Cutoff Values in Clinical Routine of Kidney Transplant Care and Table 3. Therein, we converted all relevant TTV cutoff values to correspond a commercially available PCR. With only two studies available in pediatric KTX,[85,86] this review will focus on adult cohorts.

TTV and Kidney Transplant Rejection

An association between TTV load and rejection in KTX recipients was first described in a cross-sectional study by the Vienna Group.[77] A total of 1165 KTX recipients were subjected to screening for AMR and TTV, and 86 DSA-positive patients were subjected to a protocol biopsy. A total of 46 were diagnosed with AMR (median AMR diagnosis: 6 y posttransplant).[87] TTV load at the time of biopsy was lower in AMR-positive recipients when compared with patients who had no AMR (6.6×10^4 c/mL, interquartile range [IQR], 3.0×10^3–7.2×10^5 versus 2.6×10^5 c/mL, IQR, 2.2×10^4–2.1×10^6). Statistical tests, including multivariate analysis, revealed a robust and independent linear association between TTV load and AMR (risk ratio, 0.94, 95% CI, 0.90-0.99).

Subsequently, the Vienna Group provided evidence for an association between acute rejection detected upon indication biopsies and TTV load in a case-control study,[78] screening 1010 consecutive renal allograft recipients. Inclusion criteria were an indication biopsy performed between months 4 and 12 post-TX and adequately stored blood samples for retrospective TTV quantification taken between month 4 post-TX and the date of the transplant biopsy. The median time between TTV quantification and biopsies was 43 d. Patients with rejection (n = 33; 14 AMR and 19 TCMR) had lower levels of TTV, with a median of 3.1×10^7 c/mL (IQR, 4.9×10^5–2.3×10^8 c/mL) compared with patients without rejection (n = 80; 2.3×10^8 c/mL, IQR, 1.4×10^7–3.6×10^9 c/mL). The risk for rejection decreased by 11% per log level increase in TTV load (risk

TABLE 2.

Studies that evaluated the association between TTV load and infection in kidney transplant recipients

Study design[a]	TX period	Included patients	Endpoint; timing	Patients with event; infectious events	PCR	Main association	Limitations[b]
Cohort[80]	2016	71	Infection leading to medical measure; months 4–12 post-TX	22; 41	IH	TTV 1 mo before event–infection	Interim analysis; secondary endpoint
Cohort[74]	2014–2016	221	Infection leading to medical measure/opportunistic infection + malignancy; <12 mo post-TX	51; 65	C	TTV 1 mo post-TX–subsequent event	Two main endpoints; multiple testing; missing information on model design/some major determinants of TTV not included
Cohort[81]	2015–2016	116	BKV viremia; <12 mo post-TX	24; 24	C	NA	Multiple testing; no effect size adjustment
Cohort[75]	2016–2018	274	Infection leading to medical measure; months 4–12 post-TX	127; 193	IH	TTV 1 mo before event–infection	Secondary endpoint
Case-control[82,c]	2011–2016	145	CMV viremia; <4 mo post-TX	35; 35	IH	TTV days 0 to 10 post-TX–CMV	Possible selection bias; main analyses include LTX; multiple testing; no effect size adjustment
Case-control[79]	2012–2014	66	BKV viremia; <2 y post-TX	50; 50	C	TTV–BKV month 6 post-TX	Possible selection bias; multiple testing; no effect size adjustment
Case-control[83]	2014–2016	215	BKV viremia; <12 mo post-TX	47; 47	C	TTV 1 mo post-TX–subsequent BKV	No data on subject selection; multiple testing; missing information on model design/some major determinants of TTV not included
Case-control[21]	2003–2013	389	BKV and CMV viremia; <12 mo post-TX	182; 105/77[d]	IH	TTV kinetic–time to infection	CMV secondary endpoint

[a]The studies are listed according to their design and date of their online publication.
[b]All studies followed a noninterventional and single-center design.
[c]Kidney and liver transplant recipients.
[d]One hundred five BKV and 77 CMV.
BKV, BK polyomavirus; C, commercial; CMV, cytomegalovirus; IH, in-house; LTX, liver transplantation; NA, not available; PCR, polymerase chain reaction; TTV, torque teno virus; TX, transplantation.

TABLE 3.

Proposed plasma TTV load cutoff values determined in kidney transplant recipients for the risk prediction of allograft rejection and infection, respectively

Citation	Event; timing	Predictor	TTV cutoff[a]	AUC	Sensitivity	Specificity	PPV	NPV
	Rejection							
77	AMR (pBX); 6 y post-TX	TTV at BX	<3.6 log10	NA	NA	NA	NA	NA
79	Rejection; <2 y post-TX (iBX)	TTV pre-TX/TTV 1 mo post-TX	<3.4 log10	NA	NA	NA	0.63	0.92
			<4.2 log10				0.48	0.92
75	Rejection (iBX); months 4–12 post-TX	TTV 2 wk before BX	<4.6 log10	0.73	0.36	0.89	0.56	0.77
76	Rejection (pBX); month 12 post-TX	TTV at BX	<4.6 log10	NA	0.63	0.51	0.27	0.82
	Infection							
82	CMV viremia; <4 mo post-TX	TTV days 0 to 10 post-TX	>3.8 log10	0.72	0.83	0.56	NA	NA
74	Infection/opportunistic infection or malignancy; <12 mo post-TX	TTV 1 mo post-TX	>3.2 log10	0.62	0.90	0.31	0.54	0.77
			>4.6 log10	0.70	0.76	0.66	0.41	0.90
83	BKV viremia; <12 mo post-TX	TTV 1 mo post-TX	>5.0 log10	0.75	0.77	0.75	0.31	0.96
84	Death due to infectious cause	TTV 5 y post-TX	>3.4 log10	NA	0.55	0.67	NA	NA
75	Infection; months 4–12 post-TX	TTV 1 mo before infection	>6.6 log10	0.62	0.41	0.76	0.36	0.80

[a]To facilitate comparison of the proposed TTV cutoffs, values have been converted to values that correspond to the commercial PCR.
AMR, antibody-mediated rejection; AUC, area under the curve; BKV, BK polyomavirus; BX, biopsy; CMV, cytomegalovirus; iBX, indication biopsy; NA, not available; NPV, negative predictive value; pBX, protocol biopsy; PPV, positive predictive value; TTV, torque teno virus; TX, transplantation.

ratio, 0.89, 95% CI, 0.82-0.96) in a multivariate model, including all potential confounders selected based on background knowledge.

More evidence on the association between TTV and rejection was provided by the Strasbourg Group in a case-control study.[79] A total of 14 patients experienced rejection: 6 TCMR, 2 AMR, and 6 mixed rejections. Patients without biopsy were categorized as rejection-negative. The TTV loads pretransplant and at month 1 posttransplant were lower in patients who subsequently developed graft rejection than in graft rejection-free patients. TTV loads of <3.4 log10 c/mL (negative predictive value [NPV] = 92%, positive predictive value [PPV] = 63%) and <4.2 log10 c/mL (NPV = 92% and PPV = 48%), respectively, were suggested as cutoff values for risk stratification.

Recently, a case-control study by the Leiden Group linked TTV load to rejection[21] screening 519 recipients. A total of 88 recipients were categorized as rejection positive within 1 y after TX: 80 had undergone a biopsy, and 54 had clear histological evidence for rejection. The patients classified as rejection-negative were not subjected to biopsy. A predictive linear mixed-effects model showed a decreased risk of rejection with increasing TTV load (hazard ratio [HR], 0.74 per logTTV c/mL, 95% CI, 0.71-0.76).

Evidence of an association between acute rejection and TTV load was first provided in a prospective setting by the Madrid Group.[74] The study was designed to test the association between TTV and infections, and organ rejection was the secondary endpoint. A cohort of 221 patients was analyzed, and 10 showed biopsy-proven rejection in the first 3 mo posttransplant. A biopsy for the diagnosis of rejection was not mandatory but relied on clinical course. The patients classified as "no rejection" were not subjected to biopsy. Multivariate analysis revealed an association between baseline TTV and rejection (HR per logTTV 0.69, 95% CI, 0.49-0.97).

The value of TTV in clinically overt rejection was also evaluated by the Vienna Group in an observational cohort study including 386 consecutive adult kidney graft recipients.[75] All TTV measurements taken after TTV load stabilization at the end of post-TX month 3 with an available subsequent for cause biopsy (ie, the primary endpoint) were analyzed. Samples for TTV quantification were taken at a median of 154 d after TX and preceded subsequent biopsies, with a median of 14 d. Of the 39 biopsies, 11 showed signs of allograft rejection: 5 TCMR, 2 AMR, and 4 mixed. Patients with allograft rejection had lower levels of TTV compared with patients without rejection (3.5×10^6 c/mL, IQR, 1.7×10^5–1.3×10^8 c/mL versus 2.5×10^8 c/mL, IQR, 5.8×10^6–9.3×10^8 c/mL) in subsequent biopsies. The odds for rejection decreased by 22% with every log level increase of TTV (odds ratio, 0.78, 95% CI, 0.62-0.97). An area under the curve (AUC) of 0.73 (IQR, 0.54–0.92) was calculated to classify rejection by TTV level. A TTV load cutoff of 1.5×10^6 c/mL corresponded to a specificity of 89%, sensitivity of 36%, NPV of 77%, and a PPV of 50%. Multivariate testing found no confounding variables.

Recently, the association of TTV and subclinical rejection was observed in a cohort study from the Vienna Group with a 1 y protocol biopsy.[76] The primary outcome (ie, allograft rejection) was diagnosed in 19 of the 82 available cases (15 TCMR and 4 AMR). Patients with rejection had lower TTV loads when compared with patients without rejection (2×10^5 c/mL, IQR, 3×10^3–2×10^6 versus 7×10^5 c/mL, IQR, 1×10^5–2×10^7). A multivariate analysis demonstrated an independent inverse association between TTV and rejection (risk ratio, 0.92, 95% CI, 0.86-0.98). The study also demonstrated that an increase in chronic graft damage between the month 3 and month 12 protocol biopsies was associated with the number of days with a TTV load $<10^6$ c/mL within the same period of time (coefficient: 0.07, 95% CI, 0.01-0.14).

TTV and Infection in Kidney Transplant Recipients

In the interim analysis[80] of the full data set described in detail previously,[75] the Vienna Group was the first to provide evidence for the association between TTV and infection in a prospective setting. Infection was the secondary endpoint of the study and defined as any bacterial, fungal, or viral infection requiring antimicrobial or

antiviral treatment, reduction of immunosuppressive drugs, hospitalization, or prolongation of hospital stay. All patients who continued to be followed after month 3 posttransplant and had a TTV infection were included in the analysis. For these patients, TTV was quantified at 785 time points. TTV measurements were followed by an infectious event in 193 of the observed periods in 127 patients, whereas no infectious event was documented 592 times. TTV was quantified over a median of 27 d before the onset of infection. The likelihood of infection increased by 11% with every log level increase in TTV (odds ratio, 1.11, 95% CI, 1.06-1.15). A comparable effect size was described for infections that did not require hospitalization. The largest effect size was calculated for BKV infections, followed by CMV disease and infections restricted to opportunistic pathogens. A smaller effect size was found for infections with extracellular bacteria. An AUC of 0.62 (IQR, 0.58–0.67) was calculated when classifying infections by TTV level. A TTV level $>5.8 \times 10^9$ c/mL corresponded to a specificity of 90%, sensitivity of 18%, NPV of 77%, and a PPV of 37% to detect infection.

In the same year, the Pisa Group provided further evidence on the association between CMV viremia and TTV in a case-control study including 145 patients.[82] CMV infection was defined as the presence of viral DNA above the detection threshold (800 viral genomes/mL of whole blood) within the first 4 mo posttransplant. TTV load between days 0 and 10 after TX was higher in the CMV-positive patients (n = 35) when compared with CMV-negative patients (n = 110). The optimal cutoff value for TTV load to detect CMV reactivation in the first 10 d was determined as 3.5 log10 c/mL (sensitivity: 83% and specificity: 56%) Similar differences were observed when subjects were stratified by the type of transplanted organ.

Additional evidence of an association between infection and TTV load was provided by a trial of the Madrid Group discussed in detail previously.[74] The authors defined two primary outcomes: infection (need for hospitalization and intravenous antimicrobial therapy) and immunosuppression-related adverse events (iRAE; occurrence of opportunistic infection: intracellular bacteria, herpesviruses [eg, CMV disease], BKV [PVAN and presumptive PVAN], invasive yeasts/molds, parasites, and posttransplant de novo malignancy). A total of 51 patients had 65 episodes of iRAE. Upon analyzing TTV at discrete time points, associations between subsequent study outcomes were detected at months 1, 3, and 6 (only iRAE) but not on day 0, day 7, and month 12 posttransplant. The AUCs for diagnosing infection and iRAE via TTV load at month 1 were 0.62 (95% CI, 0.52-0.73) and 0.70 (95% CI, 0.59-0.82). After applying cutoff values of 3.2 log10 c/mL and 4.5 log10 c/mL for infection and iRAE, respectively, a sensitivity of 90% and specificity of 31% were calculated to detect infection, while a specificity of 76% and sensitivity of 66% were calculated to detect iRAE beyond month 1 posttransplant. The association between TTV and infection/iRAE remained significant after multivariate adjustment. The authors also analyzed TTV AUCs and doubling times and described associations with the endpoints. However, the complexity of calculation-based cutoffs using longitudinal data might limit the implementation in clinical practice.

Possible limitations of TTV quantified in the early phase of post-TX were reported in a cohort study from the Amiens Group.[81] Overall, 24 of the 116 patients (21%) had positive plasma BKV during the first year after TX. TTV load at months 1, 2, and 3 post-TX were not associated with BKV viremia. Limited evidence on the association between TTV and BKV was provided from a study by the Strasbourg group, as reported in detail here previously.[79] A total of 50 patients with detectable levels of BKV and 16 without were selected. Compared with BKV-negative recipients, TTV loads were only higher at month 6 post-TX in patients with BKV replication. More evidence for an association between TTV and BKV was provided in a case-control study including 215 patients by the Madrid Group.[83] TTV load at month 1 posttransplant was higher among patients developing BKV viremia (n = 47) thereafter when compared with those who remained free from BKV viremia. For TTV loads >5.0 log10 c/mL at posttransplant month 1, a sensitivity of 77%, specificity of 75%, positive predictive value of 31%, and negative predictive value of 96% to predict subsequent BKV viremia were calculated. A study by the Leiden Group described in detail here analyzed the association between TTV and BKV (primary endpoint) and CMV viremia (secondary endpoint), respectively.[21] Of the 389 recipients, 27% (n = 105) developed BKV viremia, and 20% (n = 77) developed CMV viremia within 1 y after TX. A predictive linear mixed-effects model showed an increased risk of viremia with increasing TTV (HR, 1.03 per logTTV c/mL and 95% CI, 1.03-1.04 for BKV; HR, 1.01 and 95% CI, 1.01-1.01 for CMV).

Application of TTV Cutoff Values in Clinical Routine of Kidney Transplant Care

Several TTV cutoff values for the detection of rejection and infection have been proposed (Table 3). In this respect, it is important to note that the limited diagnostic test performance of TTV load does not allow for the prediction of subsequent rejection and infection; instead, it defines at-risk patients. Notably, the cutoff values have not been evaluated in an interventional setting.

Using an in-house PCR, a TTV load <6 log10 c/mL quantified after stabilization in month 3 post-TX was suggested as a risk factor for subsequent rejection in the first year after TX[75,76,78] by the Vienna Group. This value corresponds to a TTV load of 4.6 log10 c/mL detected by the commercial PCR (the Vienna in-house PCR quantifies TTV at 1.4 log10 c/mL higher than the commercial PCR; data under review). Specificity for prediction of rejection increased for TTV loads <5 log10 c/mL (corresponding to 3.6 log10 c/mL for the commercial PCR), whereas sensitivity decreased. For long-term, stable transplant recipients, the risk of rejection might be acceptable for a TTV load as low as 5 log10 c/mL (corresponding to 3.6 log10 c/mL for the commercial PCR).[77] Additionally, cutoff values for the risk prediction of rejection within 2 y post-TX by pre-TX (TTV load <3.4 log10 c/mL) and month 1 post-TX TTV assessment (TTV load <4.2 log10 c/mL), respectively, have been proposed by applying the commercial PCR.[79] In this respect, it is interesting to note that TTV is quantified in the plasma of healthy individuals without immunosuppression with a median of 2.3 log10 c/mL by the commercial PCR.[61] Due to the great variety of noninvasive assays for the diagnosis of graft rejection tested in a multitude of trials with a large range of designs published in current literature, our review is not able

to provide a comparison of the test performance between the proposed TTV cutoff values and these assays. However high-quality and up-to date reviews and comments are covering such assay systems in detail.[6,8,12-14]

Using an in-house PCR, the Vienna Group suggested a TTV load >8 log10 c/mL, quantified after stabilization in month 3 post-TX, as a risk factor for subsequent infection in the first year after TX. This cutoff changes to 6.6 log10 c/mL if converted to values that correspond to the commercial PCR.[75,80] Specificity for the prediction of infection increased for TTV loads >9 log10 c/mL (>7.6 log10 c/mL for the commercial PCR), whereas sensitivity decreased. Using an in-house PCR, a TTV load >3.5 log10 c/mL in the first 10 d posttransplant was associated with a risk for CMV viremia in the first 4 mo post-TX,[82] which changes to a value of 3.8 log10 c/mL if converted to values corresponding to the commercial PCR (the in-house PCR performed in Pisa quantifies TTV at 0.3 log10 c/mL below the commercial PCR[45]). TTV loads >3.2 log10 c/mL and >4.6 log10 c/mL quantified by the commercial PCR at month 1 post-TX were associated with a risk of infection and immunosuppression-related adverse events such as opportunistic infections in the first year post-TX, respectively.[74] A TTV load >5.0 log10 c/mL quantified by the commercial PCR at post-TX month 1 was proposed as a risk factor for the development of BKV viremia in the first year post-TX.[83] Taken together, a TTV load between 4.6 log10 c/mL and 6.6 log10 c/mL—corresponding to values obtained by the commercial PCR—detected after TTV stabilization in month 3 post-TX might be an optimal range to reduce rejection and infection in the first year (Figure 1).

A randomized controlled single-blinded interventional trial—TTVguideIT—involving 260 KTX recipients from 13 centers in 6 countries across Europe will test the value of TTV-guided immunosuppression based on these suggestions.[19,65] The trial is sponsored by the Medical University Vienna and financed by the European Union. Immunological low-risk and stable adult KTX recipients will be randomized at month 4 post-TX to receive either TTV-guided tacrolimus dosing or conventionally dosed immunosuppression in the first year post-TX. The primary composite outcome at month 12 post-TX includes infection and graft rejection assessed by personnel blinded to the randomization sequence. The trial will begin in 2022, and results are expected in 2026.

TTV IN LUNG TRANSPLANTATION

Convincing evidence for associations between TTV load and rejection and infection, respectively, has been provided in the LuTX setting 3 y earlier than for the KTX setting.[63,69,88-90] However, less data are available, and the same caveats concerning study design apply (Table 4).

The Vienna Group was the first to describe the association between TTV and infections in a cohort study including 31 patients.[69] TTV loads were analyzed during the steady state of TTV kinetics after month 3 post-TX in patients before the first episode of microbial infection (n = 13). TTV loads were higher when compared with the TTV load detected in patients who did not experience clinical complications (n = 11). A cutoff of 9.3 log10 c/mL was predictive for the development of infection, with a sensitivity of 54% and specificity of 91%. The same groups also exhibited an association between TTV load and chronic lung allograft dysfunction (CLAD) for the first time.[88] A case-control study included 20 patients developing CLAD (forced expiratory pressure in 1 s ≤80%) within 3 y post-TX and 27 matched controls. Recipients with CLAD showed lower TTV loads, and a cutoff of 7.0 log10 c/mL detected CLAD with a sensitivity of 65% and specificity of 82%.

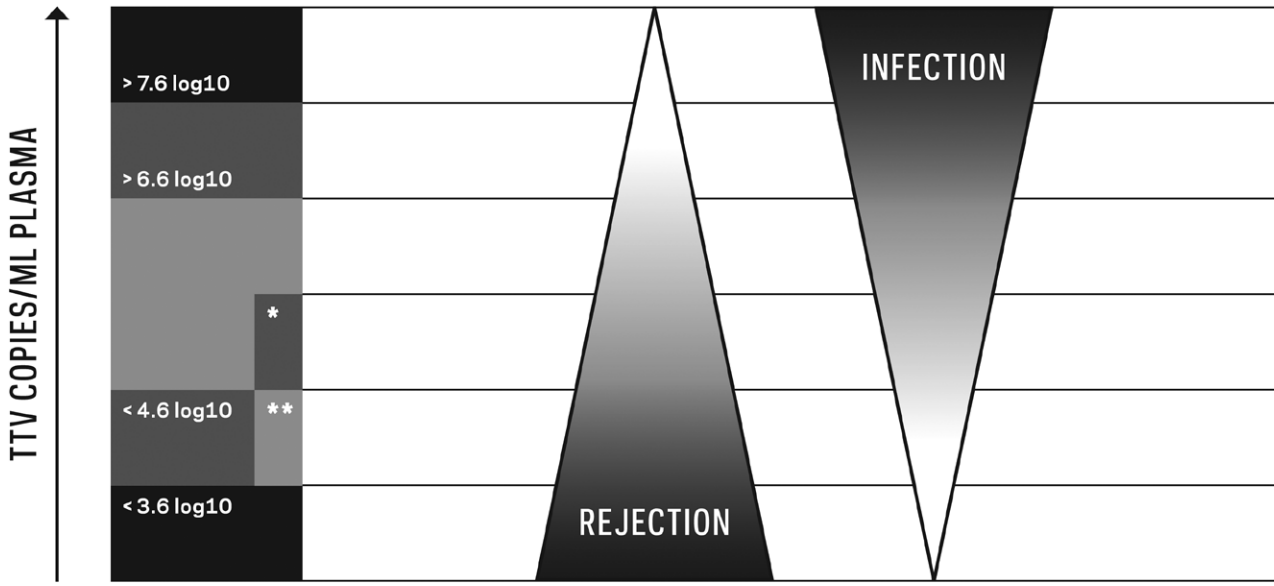

FIGURE 1. The hosts' plasma torque teno virus (TTV) load in relation to the risk of allograft rejection and infection in kidney (KTX) and lung transplant (LuTX) recipients. A high TTV load indicates a risk of infection, and a low TTV load indicates a risk of rejection. Proposed cutoff values for risk stratification have been converted to values that correspond to the commercial polymerase chain reaction (PCR) to facilitate comparison of the published data. The risk for infection increases above 6.6 log10 c/mL for both KTX and LuTX recipients. For KTX patients, the risk for rejection increases at TTV loads below 4.6 log10 c/mL. The field including an asterisk represents TTV loads below 5.6 log10 c/mL, which already indicate a risk in the LuTX setting due to the higher level of immunosuppression needed to prevent rejection compared with KTX. In KTX recipients transplanted >1 y ago, a TTV load above 3.6 log10 c/mL might indicate sufficient immunosuppression to prevent rejection (double asterisk).

TABLE 4.

Studies of lung transplant recipients' evaluations of association between TTV load detected by in-house PCR and allograft rejection and infection, respectively

Study design[a]	TX period	Included patients	Endpoint; timing	Number of events	Main association	Limitations[b]
			Rejection			
Cohort[89]	2013–2015	143	CLAD/AR (iBX); month 6–y 5 post-TX	22 CLAD 11 AR	TTV 3 mo before event–rejection	Three endpoints; some major determinants of TTV not included in the effect size adjustment
Case-control[88]	2003–2013	47	CLAD; month 4–y 3 post-TX	20	TTV at event–rejection	Possible selection bias; rejection not BX proven; no effect size adjustment
Case-control[90]	2006–2015	34	AR (pBX); months 4–12 post-TX	13	TTV before event–rejection	Possible selection bias; 2 endpoints; 3 suspected rejection episodes in the control group; no effect size adjustment
			Infection			
Cohort[69]	2008	31[c]	Infectious events; month 4–y 2 post-TX	13	TTV before event–infection	Insufficient definition of outcome; no effect size adjustment
Cohort[89]	2013–2015	143	Infections requiring hospitalization; month 6–y 5 post-TX	28	TTV 3 mo before event–infection	Three endpoints; some major determinants of TTV not included in the effect size adjustment
Case-control[90]	2006–2015	34	Infection leading to medical measure; months 4–12 post-TX	19	TTV months 4 to 12 post-TX–infection	Possible selection bias; 2 endpoints; no effect size adjustment

[a]The studies are listed according to their design and date of their online publication.
[b]All studies followed a noninterventional and single-center design.
[c]A total of 24 patients analyzed.
AR, acute rejection; BX, biopsy; CLAD, chronic lung allograft dysfunction; iBX, indication biopsy; pBX, protocol biopsy; PCR, polymerase chain reaction; TTV, torque teno virus; TX, transplantation.

The first prospective cohort study on CLAD was also published by the Vienna Group.[89] During 3 y post-TX, 28 of 143 patients developed an infection requiring hospitalization. Overall, 22 patients with CLAD and 11 with acute rejection were registered. The maximum TTV load during the 3 mo before an event was associated with infection. The risk increased within a rate of 5.05 with every log increase in TTV load (HR; 95% CI, 2.94-8.67). Moreover, the minimum TTV load during the 3 mo before an event was associated with CLAD and acute rejection. The risk for CLAD and acute rejection decreased within a rate of 0.71 and 0.48, respectively, with every log increase in TTV load (HR; 95% CI, 0.54-0.93 and 0.26-0.88). TTV levels between 7 log10 c/mL and 9.5 log10 c/mL were described cutoff values to avoid high risk of rejection or infection.

In the same year, the Freiburg Group presented a case-control study including 34 patients with 13 patients experiencing biopsy-proven rejection between 4 and 12 mo after LuTX[90] and 21 matched patients without rejection. TTV load before the event was lower in patients with rejection compared with patients without rejection. Additionally, TTV load decreased before rejection. The sensitivity of 1 log decrease in TTV load for a subsequent rejection episode within 1 mo was 74%, with a specificity of 99%. Within this cohort, 19 patients had an infection leading to medical measures (antibiotic treatment, hospitalization, or change in immunosuppression). The TTV load during months 3 and 12 post-TX was higher in the group of patients with infectious complications when compared with those of other recipients.

Based on the cutoff values determined in their noninterventional studies, the Vienna Group—together with the Hannover LuTX center—initiated a two-center, open-label, randomized, controlled, and investigator-driven trial including 144 LuTX recipients to investigate the safety and preliminary efficacy of immunosuppression guided by TTV monitoring as an add-on to conventional therapeutic drug monitoring (VIGILung).[20] The study is sponsored by the University of Marburg and financed by the Deutsche Forschungsgemeinschaft. Adult de novo LuTX recipients with tacrolimus-based immunosuppression and stable graft function are randomized 1:1 to receive either (1) tacrolimus guided by TTV monitoring in addition to drug trough level (active group) or (2) tacrolimus according to conventional therapeutic drug monitoring (control group) after month 3 post-TX. In the active group, the tacrolimus target range will be adjusted according to TTV load following predefined steps. If TTV is above the predefined optimal range, tacrolimus will be reduced by 1 step; if it is below the optimal range, tacrolimus will be increased by 1 step. Outcomes will be assessed 12 mo after randomization with the change in estimated glomerular filtration rate as the primary endpoint. Main secondary endpoints will include allograft function, allograft rejection, and infections. Trial results are expected in 2024.

TTV IN LIVER TRANSPLANTATION

Compared with KTX and LuTX, the association between TTV and immunologic events in LTX recipients is not supported by the same amount and quality of data, while the same caveats concerning study design also apply (Table 5). However, preliminary data suggest an association between TTV load and infectious events and organ rejection.[82,91-93]

TABLE 5.

Studies in liver transplant recipients' evaluations of association between TTV load detected by in-house PCR and allograft rejection and infection, respectively

Study design[a]	TX period	Included patients	Endpoint; timing	Events	Main association	Limitations[b]
			Rejection			
Cohort[91]	NA	39	BX proven rejection; <12 mo post-TX	13[c]	TTV pre-TX–rejection	Possible selection bias; insufficient endpoint definition; multiple testing; possible model overfitting
Cohort[92]	2014–2017	63	BX proven rejection; <12 mo post-TX	19[c]	TTV pre-BX–rejection	Missing data on BX without rejection; 2 endpoints; no effect size adjustment
			Infection			
Cohort[92]	2014–2017	63	CMV viremia/ disease; <12 mo post-TX	26[d]	TTV at event–CMV viremia/ disease	Two endpoints; no effect size adjustment
Cross-sectional[93]	1982–2016	136	BKV events; 10 y post-TX	23	TTV at event–urinary BKV	Possible selection bias; missing data on sampling; multiple testing; no effect size adjustment
Case-control[82,e]	2011–2016	90	CMV viremia; <4 mo post-TX	64	TTV days 0 to 10 post-TX–CMV viremia	Possible selection bias; main analysis includes KTX; multiple testing; no effect size adjustment

[a]The studies are listed according to the date of their design and online publication.
[b]All studies followed a noninterventional and single-center design.
[c]The numbers of total biopsies were not stated.
[d]Five of the cases were diagnosed with CMV disease.
[e]Kidney and liver transplant recipients.
BKV, BK polyomavirus; BX, biopsy; CMV, cytomegalovirus; KTX, kidney transplantation; NA, not available; PCR, polymerase chain reaction; TTV, Torque Teno virus; TX, transplantation.

TTV and Liver Transplant Rejection

The Swiss Transplant Cohort Study was the first to describe an association between TTV and biopsy-proven rejection in LTX recipients.[91] A total of 39 recipients were dichotomized according to TTV positivity at TX. The cumulative incidence of rejection in recipients with detectable TTV at TX was lower (21%, 95% CI, 8-37) than in patients with undetectable TTV (70%, detection limit: 25 c/mL; 95% CI, 28-90) in the first year after TX. A prospective study from Spain including 63 LTX recipients provides more data on the association between TTV and acute biopsy-proven rejection.[92] A total of 20 rejection episodes were diagnosed in 19 patients: 12 upon indication biopsy and 8 upon protocol biopsy. No differences in the TTV load of plasma obtained closest to the rejection event were observed between episodes of rejection and nonrejection. However, in the subgroup of biopsies performed upon clinical indication, TTV was lower in patients with rejection compared with patients without rejection. A cutoff value of 5.6×10^4 c/mL yielded sensitivity, specificity, NPV, and PPV values of 100%, 77%, 100%, and 38%, respectively.

TTV and Infection in Liver Transplant Recipients

A German group was the first to describe a correlation between urinary BKV and serum TTV load in 136 LTX patients in a cross-sectional study.[93] A positive correlation was described between urinary BKV DNA and serum TTV load. However, no association was found between BKV viremia and TTV. The Pisa Group provided evidence of an association between CMV viremia and TTV in a case-control study including LTX and NTX patients (as previously described in detail in the context of KTX).[82] A total of 90 subjects with LTX were included, and 64 patients (65%) showed CMV reactivation. TTV loads between days 0 and 10 after TX were higher in the CMV-positive patients when compared with the CMV-negative patients.

Within the data set described here in the section on TTV and rejection in LTX patients, the Spanish group also reported an association between TTV load and CMV.[92] During CMV disease (n = 5) and CMV infection (n = 26), TTV load was higher when compared with the remaining time points (1.6×10^8 versus 7.1×10^5 c/mL and 3.9×10^6 versus 6.2×10^5 c/mL). However, based on linear regression, no associations were observed between TTV load and CMV DNA during episodes of infection.

Taken together, existing evidence of an association between TTV and infectious events remains limited. Larger prospective studies with longitudinal monitoring are encouraged to determine whether findings concerning TTV and infectious events in other SOTs are also applicable to LTX. Moreover, such future studies could also define clinically useful cutoffs.

SUMMARY AND OUTLOOK

Novel concepts of immunologic monitoring in SOT have emerged to guide—and thus optimize—immunosuppressive drugs. The apathogenic and highly prevalent TTV represents a promising candidate in this regard. The viral copy number in the peripheral blood of its host has been shown to associate with organ rejection and infectious disease. Although TTV cutoff values for the guidance of immunosuppression have been proposed in the adult KTX and LuTX settings, more data are needed for LTX and heart TX. PCR-based assays or TTV quantification offer a simple and standardized implementation. Diagnostic test performance of TTV load does not allow for the prediction of subsequent rejection and infection; instead, it defines at-risk patients. Hitherto TTV load has not been tested in an interventional setting. Currently, two multinational, investigator-driven, randomized controlled interventional trials are testing the safety and efficacy of TTV-guided immunosuppression in KTX and LuTX recipients.

ACKNOWLEDGMENTS

The authors would like to thank Frederik Haupenthal for lecturing.

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