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Review article

Torque-Teno virus viral load as a potential endogenous marker of immune function in solid organ transplantation

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ABSTRACT

Solid organ transplantation (SOT) recipients receive immunosuppressive therapy to avoid rejection of the transplanted organ. Immunosuppressive therapy increases the risk of infections. However, no existing marker reliably reveals the status of the immune function in SOT recipients. Torque-Teno virus or Transfusion-transmitted virus (TTV) has gained attention as a possible endogenous marker of the immune function. TTV is a non-enveloped, circular single strand DNA virus, and it may be considered a part of the human virome. In a bidirectional relationship, the immune system detects TTV and TTV may also modulate the activity of immune system. These characteristics have made the virus a possible candidate indicator of immune function. In this systematic review, we describe the role and potential function of TTV viral load as an endogenous marker of the immune function and consequently the level of immune suppression in SOT recipients.

Contents

Abbreviations: ACR, Acute cellular rejection; ABMR, Antibody-mediated rejection; APCs, Antigen-presenting cells; ATG, Anti-Thymocyte globulins; AUGs, Alternative start codons; BAL, Broncho-alveolar lavage; bp, Base pairs; CCL7, Chemokine ligand 7; CD, Cluster of differentiation; CEBM, Center for evidence-based medicine; CLAD, Chronic lung allograft dysfunction; CMV, Cytomegalovirus; CNI, Calcineurin inhibitors; CpGs, Cytosine-phosphate-guanine; CTLA-4, Cytotoxic T-lymphocyte antigen 4; dd-PCR, Digital droplet PCR; EBV, Epstein Barr Virus; ELISA, Enzyme-linked immunosorbent assay; HBV, Hepatitis B virus; HCC, Hepatocellular carcinoma; HCV, Hepatitis C virus; HEV, Hepatitis E virus; HPV, Human Papilloma Virus; HSCT, Hematopoietic stem cell transplantation; IL, Interleukin; miRNAs, micrornas; mRNAs, Messenger RNAs; MS, Multiple sclerosis; mTOR, Mammalian targets of rapamycin; NPV, Negative predictive value; ORF, Open reading frame; PCR, Polymerase chain reaction; PGD, Primary graft dysfunction; PPV, Positive predictive value; SOT, Solid organ transplantation; TAIP, TTV-derived apoptosis-inducing protein; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-alpha; Tregs, Regulatory T-cells; TTV, Torque-Teno virus..

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1. Introduction

Treatment of patients with immunosuppressive drugs after solid organ transplantation (SOT) is a delicate balance between under-immunosuppression which may result in rejection and over-immunosuppression may result in severe opportunistic infections [1]. To date, no ideal marker exists that reliably reveals the status of immune function in SOT recipients [1]. Torque-Teno virus or Transfusion-transmitted virus (TTV) is a virus that has gained attention as a possible marker of immune function [2–5]. TTV is found in water, air, soil and also in human body samples. TTV rarely causes disease in healthy individuals, and TTV viral load in immunosuppressed patients is higher than in healthy controls [6].

In this systematic review, we describe the role and potential function of TTV viral load as a marker of immune function and hence level of immunosuppression in SOT recipients.

2. Description of the virus

TTV is a non-enveloped, circular single strand DNA virus first identified in a patient with acute post-transfusion hepatitis in 1977 [7]. TTV is a member of the *Anellovirus* family characterized by the presence of a circular DNA [8]. This virus is 30–50 nm in diameter, and the genome is 3.8 kb in length and contains 3739 base pairs (bp) expressing 3 messenger RNAs (mRNAs) [9]. These mRNAs use alternative start codons (AUGs) for translation and encode at least 6 open reading frame (ORF) proteins [9]. To date, 29 genotypes of TTV have been discovered and classified in 5 genogroups [10]. It is estimated that $>3.8 \times 10^{10}$ copies/ml of TTV virions are generated in the healthy human body per day and that >90% of these are cleared by the immune system. Consequently, in cases with detectable viral load, TTV viral load remains about 10^2 to 10^8 copies/ml [11].

2.1. Protein synthesis and immunobiology of TTV

In the genome of TTV, ORF1 is the longest encoded region with the highest rate of genetic mutations [12]. This gene encodes a capsid protein (ORF1 protein) that may help TTV in immune evasion and persistence [13]. The ORF2 genome region encodes the ORF2 protein that can inhibit NF-kappa-B translocation from cytosol to nucleus and as a result interrupt translation of genes and decrease production of inflammatory cytokines and enzymes such as interleukin (IL)-6, IL-8 and cyclooxygenase 2 [8,14]. The ORF3 region in genotype 1 encodes the ORF3 protein that may have a role in cell cycle and antiviral resistance [12,13]. Moreover, TTV-derived apoptosis-inducing protein (TAIP) is a protein encoded by the ORF3 region in TTV genotype 1 that induces apoptosis in hepatocellular carcinoma (HCC) cells [15].

All TTV proteins are antigenic and generate an antibody response, though the intensity of this response varies [13]. Anti-TTV IgM begins to increase 10–21 weeks after TTV infection and 5–11 weeks later it usually decreases and finally disappears. Anti-TTV IgG starts to increase about 16 weeks after infection and can be detected for at least 4 years. Despite detectable antibody titers, anti-TTV antibodies are neither protective nor neutralizing [11].

On antigen-presenting cells (APCs) such as B-lymphocytes, monocytes/macrophages and plasmacytoid dendritic cells, toll-like receptor (TLR)-9 recognizes TTV DNA as a foreign DNA and depending on the position and number of cytosine-phosphate-guanine (CpGs) motifs, this trigger or inhibits production of inflammatory cytokines [8,16]. Furthermore, TTV produces microRNAs (miRNAs) that inhibit interferon signaling, improve TTV evasion, and facilitate TTV persistence in the host [17,18].

TTV viral load increases under conditions with compromised immune response [4]. Replication of TTV is inversely correlated with number and especially functions of T-lymphocytes [3,19,20]. In addition to iatrogenic or drug induced immunosuppression, some genetic variations or polymorphisms in the co-stimulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) or CD152 may affect TTV replication after hematopoietic stem cell transplantation (HSCT) [21]. CTLA-4 is an inhibitory receptor that is expressed on the surface of activated T-cells. This receptor acts as a negative regulator of T-cell mediated immune response [21]. Polymorphisms in CTLA-4 may result in stronger inhibition of T-cell proliferation and activation and therefore a more intensified suppression of immune response to TTV [21]. TTV replication in different tissues, especially in hepatic cells, can induce production of pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumor *necrosis factor-alpha (*TNF-α*) and* Chemokine (C—C motif) ligand 7 (*CCL7)* [22]. CCL7 is a chemo-attractant for different subtypes of leukocytes that may contribute to the pathology of inflammatory diseases and cancers [22,23]. Furthermore, some studies indicate that TTV can trigger autoimmune diseases such as multiple sclerosis (MS) with antigenic mimicry or by suppression of regulatory T-cells (T_{regs}) [24–26]. Recently, it was suggested that TTV may play a role in immunosenescence as TTV viral load higher than 4.0 log copies/ml is a strong predictor of mortality in elderly subjects [27]. However, defining the exact mechanism of this correlation requires further investigations [27].

2.2. Transmission and detection methods

TTV is transmitted by parenteral, trans-placental, by breast milk, respiratory and fecal-oral routes [28,29]. TTV can be found in most tissues and cells except red blood cells and platelets [12,29]. TTV is polytrophic and replicates in liver, bone marrow and peripheral blood mononuclear cells, especially T lymphocytes [29,30]. Although one recently published study has suggested that granulocytes are the primary site for TTV replication after HSCT in children [31]. Because of the presence of lymphocytes, TTV viral load is 100 times higher in whole blood than in plasma [32]. Accordingly, TTV can be detected by quantitative or qualitative polymerase chain reaction (PCR) techniques in different clinical specimens [33–36]. Furthermore, immunoassays including enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are used to detect antibodies against ORF1 (anti-N22 polypeptide) or ORF2 proteins [37,38].

Up to now, there is no widely standardized TTV diagnostic method. PCR is an accurate method, however, applying different primers results in variable diagnostic sensitivity. Moreover, PCR is relatively expensive, requires advanced laboratory instruments and expert technicians limiting the analysis to resource strong centers [29,38]. Recently, a standardized quantitative real time PCR for detection of TTV (TTV R-GENE® assay) was introduced by a group of researchers in France [39]. Limit of detection of this assay in blood and plasma samples is 146 copies/ml (2.16 log10 copies/ml) and 167 copies/ml (2.22 log10 copies/ml), respectively. This assay can detect 12 human TTV genotypes and is compatible with different thermo-cyclers. Furthermore, the assay has been tested in healthy volunteers, donors and kidney transplant recipients and the diagnostic accuracy has been confirmed [39]. Other detection methods such as EIA and ELISA are cheaper and easily performed, but these methods have limited use since they detect antibodies, and they cannot differentiate between current and previous infections due to the large overlap in time between IgM and IgG antibodies [38].

2.3. Pathogenicity factors

Contrary to what was initially assumed, TTV is not necessarily a pathogen, and it may be considered a part of the human virome [5,12]. Pathogenicity may be genotype dependent. Thus, TTV genotype 1 has been found both in healthy carriers and in patients with elevated liver enzymes and hepatitis with unknown etiology [12,40]. TTV genotype 4 is not prevalent in healthy subjects, but it has been found in patients with inflammatory conditions such as rheumatoid arthritis and severe acute respiratory diseases [16]. The TTV viral load is higher in drug induced immunosuppressed patients compared to healthy carriers [6,41,42]. Furthermore, TTV viral load is higher in patients co-infected with other microorganisms such as Epstein Barr Virus (EBV) [24], Human Papilloma Virus (HPV) [43], Helicobacter pylori [44], human immunodeficiency virus (HIV) [45] and Cytomegalovirus (CMV) infection [46] compared to TTV mono-infected patients. Age and gender are other factors that have been associated with TTV viral load with the highest viral loads found in older men and the lowest found in young women [46]. Importantly, TTV viral load increases in patients with autoimmune or inflammatory diseases and in immunocompromised patients [25,47–50].

2.4. Prevalence of detectable TTV replication

TTV is ubiquitous and the prevalence of detectable TTV replication is about 12% in drinking water, 15% in hospital setting air and surface samples, and 38–100% in wastewater samples [29].

In humans, the prevalence of detectable TTV replication ranges from 0 to 48% in samples from cord blood or blood samples from newborns depending on the PCR technique applied. This prevalence increases during the first months after birth and reaches up to 67% in 1 to 12 months old healthy infants [35]. Breast milk is one of the important transmission routes, and TTV is detected in 23 to 67% of breast milk samples [35]. Furthermore, fecal-oral is another rout of transmission and TTV DNA is detected in 84% of saliva [51] and 25% of fecal samples [52] from healthy individuals.

The prevalence of detectable TTV replication varies in different geographical regions and populations, and TTV has in some regions been detected in as many as 95% of healthy individuals [12,53]. It should be noted that measuring prevalence of detectable TTV replication is highly dependent on the assays applied, so cross-study comparisons should be avoided [35,54–56]. Factors such as type of specimen (for example plasma or whole blood) and different PCR methods or primers can affect the prevalence of detectable TTV replication [32,35,54,55]. For example, the reported prevalence of patients with detectable TTV DNA in kidney transplant recipients by nested PCR is about 33% using primers specific for ORF1 region while it is 92% among the same patients when using primers specific for non-coding region of the TTV genome [54].

Here we will systematically review and describe the role and potential function of TTV as an endogenous marker of immune function and hence level of immune suppression in SOT recipients.

3. Methods

We searched PubMed and Scopus (up to 2018 Dec 31) using this keywords combination: ("TTV" OR "Torquetenovirus" OR "Alphatorquevirus") AND ("Transplant*" OR "reject*" OR "infect*" OR "immune*") and found 185 articles.

We excluded studies that did not refer to SOT, case reports, reviews, duplicates and papers in languages other than English (155 papers excluded).

We included 30 articles including original articles, short reports, commentaries (letter to editor) and conference proceedings that appeared to contain information relevant to the TTV and SOT.

We define the type of studies and level of evidence for each article according to guidelines of Oxford center for evidence-based medicine (CEBM) (https://www.cebm.net). This grading system has 5 levels. Studies in level 1 contain the strongest and studies in level 5 include the weakest evidences [57].

4. Results and discussion

4.1. Solid organ transplantation

In all SOT recipients immunosuppressive therapy is initiated before or at the time of transplantation in order to prevent rejection and increase organ and patient survival [58–60]. Usually, a multidrug regimen containing agents with different mechanism of action is used for induction or maintenance of immunosuppression and high-dose steroids or combination of immunosuppressive agents is used for treatment of rejection [59,60]. In the following, five main groups of immunosuppressive agents are described, and their mechanism of action is presented in supplementary table 1.

Biological agents are antibodies and due to intense and rapid action, these agents are often used for induction of immunosuppression [59]. Calcineurin inhibitors (CNI) such as Tacrolimus and Cyclosporine are the second group of immunosuppressants and the cornerstone of immunosuppressive therapy [59]. It should be noted that, as their target molecules differ, Tacrolimus is more potent than Cyclosporine in calcineurin inhibition [59]. The third group includes anti-proliferative agents such as Azathioprine and mycophenolic acid based agents. Mammalian targets of rapamycin (mTOR) inhibitors such as Sirolimus and its derivative Everolimus are a fourth group of immunosuppressants [58]. Finally, the last group is Corticosteroids such as Prednisolone [58,59].

4.2. Kidney transplantation

4.2.1. Prevalence and associated factors

Studies including data on TTV in kidney transplant recipients are presented in supplementary table 2. In kidney transplant recipients, prevalence of patients with detectable TTV DNA has been estimated to range between 10 and 100% (supplementary table 2) [55,56,61,62]. The prevalence of various TTV genotypes in kidney transplant recipients differs in different geographical areas, i.e. genotypes 2 and 5 are the most prevalent genotypes in Brazilian kidney transplant recipients [61] while genotype 1 is the most common genotype in Hungary [56].

In recent studies, it has been suggested that age and gender are associated with TTV in kidney transplant recipients [2,63,64]. TTV viral load is higher in older and male recipients and may also be higher in recipients with older donors [63,64], though data are inconsistent [54,56,65].

4.2.2. TTV viral load during the course of transplantation

TTV viral load increases rapidly from day 7 to day 30 and peaks around month 3 to 12 post-transplantation [2,64], and the viral load decreases gradually after this time [2,63].

4.2.3. Association with immunosuppressive drugs

TTV mostly replicates in T-lymphocytes and administration of T-cell depleting agents such as Anti-Thymocyte globulins (ATG) results in TTV viral load decrease on day 7 of therapy with a rebound after day 15 [3]. ATG results in a larger TTV viral load reduction than Basiliximab that inhibits proliferation and activation of T-cells [3,59], while the highest TTV viral load has been found in patients on belatacept-based immunosuppression [63]. Belatacept blocks co-stimulation of T-cells and inhibits generation of antiviral T-cells that may leads to insufficient suppression of TTV replication [59,63]. In contrary with short-term, in mid-term and during post-transplant months 1 and 3, there are negative correlations between TTV viral load and number of CD3+, CD4+ and CD8+ T lymphocytes [2]. Partial repopulation of T-cells, differences in ATG dosage or concurrent prescription of other immunosuppressive agents in different studies and induction of immunosenescence by ATG may explain this discrepancy [2].

The serum levels of CNI do not correlate with TTV viral load during the first months after kidney transplantation [3,63,64]. However, the TTV viral load is higher in patients on regimens containing tacrolimus compared to regimens containing cyclosporine or mTOR inhibitors [63]. In addition, TTV viral load is higher in patients that receive high doses (>1.5 g) of Mycophenolic acid compared to patients receiving lower doses [64].

4.2.4. Association with infection and rejection

CMV-seropositive recipients have higher TTV viral load than seronegative patients [2,66]. This may represent the impact of CMV infection on induction of immunosenescence and accordingly control of TTV replication by the immune system in transplant recipients [2]. TTV viral load is higher in recipients with primary CMV infection post transplantation compared to patients with latent or reactivation of CMV infection (positive CMV IgG before transplantation). However, CMV prophylaxis has no effect on TTV viral load [64]. In a study including 280 SOT recipients (146 kidney, 134 liver recipients), 235 (145 kidney, 90 liver recipients) had CMV reactivation. Interestingly, among these SOT recipients, TTV viral load higher than 3.45 log DNA copies/ml 10 days post-transplant was an independent predictor of subsequent CMV reactivation with sensitivity and specificity of 83.1% and 56.2%, respectively [66]. In kidney transplant recipients without a clinical diagnosis of BK viremia, *Anelloviridae* subtypes (including TTV) are the dominant viral family. However, in kidney transplant recipients, with a clinical diagnosis of BK viremia the *polyomaviridae* are the dominant viral family [67].

Antibody-mediated rejection (ABMR) is one of the major causes of graft loss in kidney transplant recipients and associated with inadequate immunosuppression [63]. In a recent study, including 715 (669 ABMR negative & 46 ABMR positive) kidney transplant recipients, it was demonstrated that higher TTV viral load was independently associated with lower ABMR risk. The risk of ABMR decreases by 0.91 with each log increase in TTV viral load [63]. Based on this finding it has been suggested that TTV viral load reflects the efficiency of immunosuppression in this group of kidney transplant recipients [63]. In agreement with this finding, higher pre-transplant TTV viral load is associated with lower rate of acute graft rejection [2].

4.3. Liver or pancreas transplantation

4.3.1. Prevalence and associated factors

Summary of studies including data on TTV in liver transplant recipients is presented in supplementary table 3. TTV has been reported to be prevalent (16–100%) in liver transplant recipients. The genogroups 2 and 5 are more prevalent among liver transplant recipients than among healthy controls, but no TTV genogroup has been reported to be particularly predominant in liver transplant recipients [6,41]. There exists no information about prevalence of detectable TTV replication in pancreas transplant recipients [3].

Neither age and sex, nor post-transplant time, number of blood transfusions and reason for transplantation are associated with TTV viral load in liver transplant recipients [6,68].

4.3.2. TTV viral load during the course of transplantation

In liver transplant recipients, TTV viral load progressively increases and peaks three months post transplantation [4,41,66,69,70]. Hereafter, the viral load starts to decline during the 6–12 month post transplantation [4,70] to reach a baseline level on average >12 years post transplantation [70].

4.3.3. Association with immunosuppressive drugs

Intensity of immunosuppression is associated with TTV viral load [4,70]. Patients that receive CNI plus azathioprine or mycophenolate mofetil have higher TTV viral loads than patients on CNI alone 3 months post transplantation [41]. The type of CNI (Tacrolimus vs. Cyclosporine) does not influence TTV viral load in liver transplant recipients [41,70].

4.3.4. Association with infection and rejection

In liver transplant recipients, hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection are not associated with an increased TTV viral load [41]. However, TTV viral load is lower in hepatitis E virus (HEV) co-infected (anti-HEV IgM and IgG positive) pediatric liver transplant recipients compared to HEV negative recipients [6]. Moreover, despite TTV viral load not being associated with HCV co-infection, HCV treatment may affect TTV viral load as this decreases in liver transplant recipients with recurrent HCV infection, when treated with pegylated interferon and ribavirin [41]. In contrast, the TTV viral load is higher during episodes of CMV infection (defined as CMV DNA >1000 copies/ml) compared to infection free time points [70]. In addition, as mentioned earlier TTV viral load higher than 3.45 log DNA copies/ml predicts subsequent CMV infection with high sensitivity and specificity [66]. In liver transplant recipients with post-transplant renal impairment, detectable TTV viral load in serum is higher in recipients without BK viruria than recipients with BK viruria. It is likely that TTV and BK virus replicate in the same urothelial cells and competitively use the same cellular factors, and/or BK virus suppress the replication of TTV virus [71].

From an immunological perspective, it is notable that the incidence of biopsy-proven acute cellular rejection (ACR) in the first post-transplantation year is lower in liver transplant recipients that are TTV-positive than in TTV-negative at the time of transplantation (21% vs. 70%, $P = .004$) [68]. Effects of confounding factors such as age, gender, concomitant viral infections and number of immunosuppressive agents have been evaluated in a proportional regression model that

found TTV-positive recipients to have lower incidence of rejection during the first year after transplantation [68]. Moreover, TTV viral load is lower in patients with clinical episodes of ACR with the lowest TTV viral loads detected in patients with moderate ACR compare to mild or non-ACR patients [70]. TTV viral load with a cut-off of 4.75 log10 copies/ml has been demonstrated to predict ACR (especially in moderate ACR) with sensitivity, specificity, and negative and positive predictive values of, 100%, 77%, 100% and 38%, respectively [70]. TTV viral load before liver transplantation or during first week and first month post transplantation cannot predict upcoming ACR accurately [70]. Thus, measuring TTV viral load before or during the first week after liver transplant has not been found to be an accurate predictor of infection or rejection [70].

4.4. Lung and/or Heart transplantation

4.4.1. Prevalence and associated factors

Summary of studies including data on TTV in lung and/or heart transplant recipients is presented in supplementary table 4. Information about prevalence of detectable TTV replication in heart transplant recipients is limited [72]. The reported prevalence of detectable TTV replication in heart transplant recipients ranges from 25 to 79%, and genogroup 2 is present in 40% of heart transplant recipients [72].

Lung transplant recipients are a group of SOT recipients with high prevalence of detectable TTV replication (up to 95%) [20]. The most prevalent genotype has not been identified [20,73–77]. However, a recent study has found that TTV strains of genogroup 3 are more prevalent in BAL and blood samples of lung transplant recipients compared to healthy controls [78].

The TTV viral load has been measured in blood samples and broncho-alveolar lavage (BAL) fluid specimens from both lung donors and recipients. Surprisingly, the TTV viral load is found to be higher in BAL fluid from donors than healthy volunteer controls. TTV viral load is inversely correlated with age, while no correlation to history of tobacco exposure, aspiration and cause of death is found [75]. Neurohumoral and inflammatory responses, lung infiltration with leukocytes and iatrogenic immunosuppression by corticosteroids in brain-dead lung donors are possible reasons for higher TTV viral load in the patients [75], but more studies are needed to determine the exact etiologic factors.

The BAL fluid TTV viral load in lung transplant recipients is not correlated with transfusion of blood products, type of transplant (single or double lung) or cause of preoperative lung disease [75].

4.4.2. TTV viral load during the course of transplantation

In lung transplant recipients, TTV DNA is detectable in BAL fluid from the first hour after lung re-perfusion [75]. In blood samples, the TTV viral load increases during the first 3 months after transplantation [20,73,77] with an incremental trend starting 15 days after initiation of immunosuppressive drugs peaking about day 60 after transplantation [20] and reaching a steady state 120 days after transplantation [79]. The TTV doubling time does not seem to be associated with age, gender or underlying disease [20]. In children, TTV viral load is associated with race and age and is higher in non-Caucasians and older lung recipients compared to Caucasians and younger recipients [80].

4.4.3. Association with immunosuppressive drugs

As noted above, the TTV viral load is associated with intensity of immunosuppression in SOT recipients [4,41]. Because of the more intensified immunosuppressive regimens in lung transplant recipients, the TTV viral load reaches higher levels compared to other SOT recipients [73]. It is controversial if there is correlation between TTV viral load and CNI serum levels. Previous studies describe that TTV viral load correlates with Tacrolimus and Cyclosporine serum levels [73,77], and the TTV viral load is lower in patients on Cyclosporine containing regimen 6–24 month post transplantation [73,77]. However, a recent study could not find any association between CNI serum level and TTV viral load [79].

4.4.4. Association with infection and rejection

Some studies have investigated the association between TTV viral load and the risk of infections in lung transplant recipients [73,77,79]. One study reported that the TTV viral load is higher in lung transplant recipients 28 to 76 days before diagnosis of an infection. The authors concluded that plasma TTV viral load higher than 9.3 $\log_{10}$ copies/ml after 90 days of transplantation could be used to predict the risk of infection with a sensitivity and specificity of about 53% and 91%, respectively [73]. In line with this study, a recent study demonstrated that an upper threshold of 9.2 log10 copies/ml and a lower threshold of 8.1 log10 copies/ml predicted infectious events with a sensitivity and specificity of 87% and 71%, respectively [81]. These results, however, was contradicted by a study concluding that the mean TTV viral load was not associated with and cannot predict infections [77]. It should be noted that in the first and second study, Tacrolimus (74% and 100%) was the predominantly used CNI, while Cyclosporine was used in 80% of cases in the third study [73,77,81]. TTV viral load is lower in patients on Cyclosporine containing regimens [73,77]. Thus, the difference in results may be due to differences in choice of CNI.

Notably, primary graft dysfunction (PGD) is associated with slower increase in BAL fluid TTV viral load compared to controls (non PGD lung transplant recipients) during the peri-transplant period [75]. In patients with biopsy-proven lung rejection, TTV viral load in serum starts to decrease by the rate of one log per month [79]. Each one log10 decrease in TTV viral load increases the risk of ACR by 2 fold [81]. Moreover, a 10-fold decrease in TTV viral load per month can predict rejection with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 74%, 99%, 83% and 97%, respectively [79].

Chronic lung allograft dysfunction (CLAD) is a type of rejection that is associated with inadequate immunosuppression. It has been reported that TTV viral load lower than 7.0 $\log_{10}$ copies/ml can predict the risk of CLAD indicating that TTV viral load lower than this cut-off is a predictor of insufficient immunosuppression [76]. Furthermore, each one log10 decrease in TTV viral load increases the risk of CLAD by 40% [81]. Although, TTV may have limited value to predict immune related events during the first 1–2 months post-transplant because its kinetics is different among individual patients. Moreover, TTV kinetics is not associated with clinical parameters and induction regimens in this 1–2 months period [81].

5. Discussion

Potential role of TTV as marker of immunosuppression or immune activation in solid organ transplantation.

Several studies found that TTV viral load and kinetics are associated with immune status i.e. presence of infection or rejection in SOT [3,4,6,63,68,75–77,82]. However, many studies were of poor quality with low level of evidence, including small studies without appropriate control groups. Thus, at present there is not sufficient evidence to support a role for TTV viral load as an accurate marker of immunosuppression or infections. At best, the current knowledge may be used to generate hypotheses regarding the role and usability of TTV.

To clarify the role of TTV viral load as a reliable predictor of the level of immunosuppression or risk of infections or rejection, a number of issues should be addressed. At first, the measurement technique should be standardized [79,83,84]. Using a unique and standard method such as digital droplet PCR (dd-PCR) [77] or TTV R-GENE® assay [39] for measurement of TTV, may result in a better reproducibility of findings.

One may speculate that the burden of immunosuppression can be tailored in each recipient by stratification according to detectable TTV viral load. However, many other factors could interfere. TTV has an affinity to T-lymphocytes and administration of anti-T lymphocyte agents

such as ATG and Basiliximab influence TTV viral loads [3,66]. Combination immunosuppressive therapy (for example CNI plus antiproliferative agents) has the greatest effects on TTV viral load [41] which may explain why TTV viral load is higher in SOT recipients with more intensified immunosuppressive regimens compared to less immunosuppressed patients [73]. Although the number of T cells does not decrease, the ability of T cells to control the virus diminishes [63]. TTV viral load is lower in kidney transplant recipients that receive mTOR inhibitors compared with Tacrolimus [63]. The antiviral potential of mTOR inhibitors could be one of the explanations for this observation [85]. In addition to drug induced immunosuppression, attention should also be paid to genetic variations or polymorphisms of immune modulating molecules as these variations may affect function of T cells and hereby change the rate or amount of TTV replication in transplant recipients [21]. Thus, future studies should focus on the impact of different types of immunosuppressive agents and preferentially include healthy controls. Such studies should focus at establishing possible associations between TTV and immune functions using deep immunological profiling including functional immunological assays.

There is sparse evidence to suggest that TTV is a predictor of infections and rejections. Most of the available studies are cross-sectional or retrospective studies and/or do not have relevant controls. In case of prospective studies or cohorts, most of previous studies are single center studies with limited number of patients. Thus, detectable TTV replication at the time of transplantation and increases in TTV viral load in the peri-transplant period may be associated with lower acute cellular rejection [68] and lower risk of PGD [75]. Moreover, higher TTV viral load is correlated with lower risk of ABMR [63] and TTV viral load with a specific cut off can predict upcoming infections [73] and the risk of CLAD [76]. These associations suggest that TTV viral load may be useful as a predictive marker for immunosuppression or immune activation in solid organ transplantation [76]. However, larger prospective studies with clinical relevant and well-defined endpoints are warranted. TTV seroprevalence is not 100% and this may introduce bias we cannot account for. Some factors can influence TTV seroprevalence including, sensitivity of assay, time since exposure to TTV, lack of exposure and cross-reactivity with other antibodies. However, in this paper we have reported TTV prevalence according to detectable TTV replication and not according to serology tests (Seroprevalence). In future studies, in parallel to PCR, it could be interesting to investigate the serology of TTV among donors and recipients before and after transplantation to differentiate TTV reactivation from acute infection as is already implemented for CMV monitoring [86].

In conclusion, it remains to be shown if TTV is an accurate marker of level of immune suppression, or if TTV is a predictor of complications such as infections or rejections. To do this, we need larger prospective cohort studies with well-defined end-points that compare different immunosuppressive regimens. Although more research is needed, the use of the TTV viral load could be an interesting tool as an add-on to the current strategies to monitor immune status and immune-related complications in SOT.

Authors' contributions

OR, CHD, SSS, AR, MP, SRO and SDN designed the study. OR performed the initial search and wrote the first draft of manuscript, CHD cross-checked the referenced articles. OR and CHD classified the articles. SSS, AR, MP, SDN and SRO supervised the review. OR, CHD, SSS, AR, MP, SRO and SDN revised and commented on the manuscript. All authors read and approved the final version of manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data are included in this published article and supplementary tables.

Competing interests

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Appendix A. Supplementary data

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