Torque Teno Virus in Children Who Underwent Orthotopic Liver Transplantation: New Insights About a Common Pathogen

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Background. Torque Teno virus (TTV) is a ubiquitous infectious agent. Transplant recipients are at risk of hepatitis E virus (HEV) infection and could be vulnerable to TTV-associated adverse effects. The aim of this study was to evaluate the influence of immunosuppression and HEV infection on TTV replication and liver injury in pediatric patients after orthotopic liver transplantation (OLT).

Methods. Pediatric recipients of liver transplants were classified into the following 2 groups: (1) those with normal serum aminotransferases levels and (2) those with persistently increased serum aminotransferases levels and histological features of chronic hepatitis of unknown etiology. The TTV load was assessed in 342 serum samples by use of TaqMan real-time polymerase chain reaction, along with TTV genogroups and coinfection with HEV.

Results. TTV DNA was detected in 96% of tested serum samples. Viral load was significantly lower in patients with features of chronic hepatitis, of whom 78% had liver fibrosis scores of ≥2. Viral load decreased during post-transplantation follow-up. Viral load and genogroups were influenced by immunosuppression. Lower viral load was observed in patients coinfected with HEV.

Conclusions. TTV infection is widespread, and its replication is closely related to immune status and viral coinfection. High TTV viremia is not associated with hepatitis after OLT, but, conversely, liver inflammatory activity impairs TTV replication.

Keywords. Torque Teno virus; orthotopic liver transplantation; hepatitis E virus; immunosuppression; chronic hepatitis.

Torque Teno virus (TTV) is a nonenveloped, circular, single-stranded DNA virus and is a member of the Alphatorquevirus genus within the Anelloviridae family [1]. The high prevalence in the general population (90%–100%) [1, 2] led to the hypothesis that TTV could be a commensal virus [3, 4]. TTV is likely acquired early in life and results in lifelong viremia, with most carriers being asymptomatic [5] and infected with >1 strain of TTV [4]. Indeed, a study of cord blood and newborns found a rapidly rising prevalence in the months following birth, with 100% of children testing positive at the age of 2 years [2].

TTV was first isolated in a serum specimen from a patient from Japan who developed hepatitis after a blood transfusion [6]. Since then, many studies have attempted to find a link between TTV and liver diseases [7], mainly because this virus replicates in the liver [8]. A potential pathogenic role has been suggested for TTV genotype 1a in children with chronic hepatitis of unknown etiology [5] and in chimpanzees [9]. An association between viral load and hepatocellular carcinoma has also been suggested [10]. However, TTV does not
fulfill the criteria of a hepatitis virus, and current opinion is that TTV does not play a pathological role under normal conditions [1].

TTV has a high genetic diversity, hampering accurate detection. According to the 2011 report of the International Committee on the Taxonomy of Viruses, 29 accepted species are now included within the Alphatorquevirus genus, with differences of ≥35% at the nucleotide level. Furthermore, the literature defines 5 genogroups, with a sequence variability of ≥50% between each [1]. In addition to the genomic diversity of TTV, the ubiquity of the virus has prompted questions about its pathogenic role. This highlights the importance of a robust detection assay and the use of viral load instead of prevalence to measure the impact of TTV infection on health. Its high prevalence in the general population does not exclude a pathological role for this agent [3]. One possibility is that the pathogenic effects of TTV are exhibited only at a high virus load and that this threshold is not attained in healthy individuals. Additional possibilities are that TTV can cause opportunistic disease in immunosuppressed individuals or that it acts as a cofactor during coinfection with another virus [3]. Children who have undergone orthotopic liver transplantation (OLT) are immunosuppressed and vulnerable to opportunistic infection such as that due to hepatitis E virus (HEV), a condition that can lead to chronic hepatitis [11]. The goal of this study was to determine the influence of immunosuppression and HEV infection on TTV replication in pediatric patients after OLT. Our secondary aim was to investigate the potential role of TTV on liver injury in this population.

SUBJECTS AND METHODS

Study Groups

Children who underwent liver transplantation were classified into the following 2 groups: (1) those with normal serum aminotransferases levels during follow-up after OLT (n = 66; hereafter referred to as the nonhepatitis group) and (2) those with persistently increased serum aminotransferases levels and histological features of chronic hepatitis of unknown etiology despite extensive screening (n = 14; hereafter referred to as the chronic hepatitis group). All patients underwent transplantation and were followed at CHU Sainte-Justine (Montreal, Canada) between 1992 and 2010. All patients received blood transfusions during transplantation (median number/patient, 16 transfusions [range, 1–324 transfusions] in the nonhepatitis group and 24 transfusions [range, 4–81 transfusions] in the chronic hepatitis group). Sixty-four liver biopsy specimens from all children were reviewed by an experienced pathologist according to METAVIR scoring system [12]. The chart of each patient was reviewed for reported clinical characteristics, such as sex, age at OLT, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, γ-glutamyltransferase (GGT) level, and immunosuppressive regimen. This study was conducted under the approbation of the ethics committee of CHU Sainte-Justine.

Nonhepatitis Group

A total of 212 serum samples (6 pre-OLT and 206 post-OLT samples) from 66 patients (37 girls; median age, 13.7 years [range, 1.8–25.5 years]) were included in this study. These patients underwent OLT at a median age of 2.4 years (range, 1 month–16.8 years) for the following conditions: biliary atresia (33/66), fulminant or subfulminant hepatitis (8/66), tyrosinemia (6/66), primary sclerosing cholangitis (4/66), cholestasis (3/66), hepatoblastoma (3/66), autoimmune hepatitis (2/66), congenital hepatic fibrosis (2/66), North American Indian childhood cirrhosis (1/66), Alagille syndrome (1/66), progressive intrahepatic familial cholestasis (1/66), COACH syndrome (1/66), and type IV glycogenosis (1/66). These patients were followed for a median time of 6 years (range, 0.5–16.9 years).

Chronic Hepatitis Group

A total of 111 serum samples (8 pre-OLT and 103 post-OLT samples) from 14 patients (8 girls; median age, 17.4 years [range, 5.9–19.8 years]) and with chronic hepatitis of unknown etiology post-OLT were included. Inclusion criteria for this chronic hepatitis group were past or current abnormal serum aminotransferase levels after OLT (defined as levels of at least 2 times the upper limit of normal) and histological features of chronic hepatitis without any defined etiology despite extensive screening. Usual infectious causes (hepatitis A, B, C, and D viruses, Epstein-Barr virus, cytomegalovirus, adenovirus, herpes group viruses, parvovirus, and echoviruses) were ruled out. Relapse of autoimmune hepatitis, primary sclerosing cholangitis, and toxic causes were excluded. These children underwent transplantation at a median age of 2.5 years (range, 10 months–5.6 years) for the following reasons: tyrosinemia (7/14), biliary atresia (5/14), North American Indian childhood cirrhosis (1/14), and Alagille syndrome (1/14). Median follow-up duration was 14.6 years (range, 5.1–17.6 years).

Healthy Control Group

Nineteen healthy children (10 females; median age, 12.1 years [range, 2.1–21 years]) were recruited as controls. They were siblings of patients. The controls did not have liver-related health problems.

Immunosuppressive Treatment

Immunosuppressive treatment was different among patients and varied through time following OLT. For analysis, patients were divided according to the number of immunosuppressants taken at the moment of blood sampling. Seventy-two percent of samples (145/201; data were missing for 5) from the nonhepatitis group and 22% (23/103) from the chronic hepatitis group were from patients who received 1 immunosuppressant: cyclosporine (CsA) or tacrolimus. Twenty-two percent of samples

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(44/201) from the nonhepatitis group and 68% (70/103) from the chronic hepatitis group were from patients who received 2 immunosuppressants: tacrolimus/mycophenolate mofetil (MMF), tacrolimus/azathioprine (AZA), tacrolimus/rapamycin, tacrolimus/steroids (methylprednisolone or prednisone), CsA/AZA, or CsA/MMF. Six percent of samples (12/201) from the nonhepatitis group and 10% (10/103) from the chronic hepatitis group were from patients who received 3 immunosuppressants: tacrolimus/AZA/rapamycin, tacrolimus/MMF/steroids, tacrolimus/MMF/AZA, or CsA/AZA/steroids. Postoperative immunosuppressive therapy consisted of 1 mg/kg/day of intravenous methylprednisolone. The treatment protocol included calcineurin inhibitors (CNIs), mainly 0.2–0.3 mg/kg/day of tacrolimus, for patients aged <12 years, or 0.1–0.15 mg/kg/day of tacrolimus, for those aged ≥12 years. Tacrolimus levels were kept at 12–15 ng/mL during month 1 after OLT, at 10–12 ng/mL during months 2 and 3, at 5–10 ng/mL during months 4–6, and at 4–5 ng/mL after month 6. Alternatively, CsA was administered to maintain levels at 300–350 ng/mL during weeks 1 and 2 after OLT, at 250–300 ng/mL during weeks 3 and 4, at 200–250 ng/mL during weeks 5 and 6, at 150–200 ng/mL during weeks 7–12, and at 100–150 ng/mL after week 12 post-OLT. When required, CNIs were combined with a second agent, namely AZA (1–2 mg/kg/day orally) or MMF (600 mg/m²/dose orally twice daily). In rare cases, we also used rapamycin (3 mg/m² orally once daily followed by 1 mg/m² once daily).

**Laboratory Analysis**

**Viral DNA and RNA Extraction and TTV Load Quantification**

Viral DNA and RNA were extracted and purified as previously described, and feline calicivirus was added as internal process control [11]. A real-time TaqMan quantitative polymerase chain reaction (PCR), amplifying the highly conserved untranslated region of TTV, was performed using the Brilliant QPCR core reagent kit (Stratagene, LaJolla, CA). Primers used were NG473, NG352, and NG369-P as previously described (Supplemental Table 1) [10]. Amplification was performed on a Stratagene Mx 3005p system (Stratagene, LaJolla, CA) under the following conditions: 95°C for 3 minutes, followed by 42 amplification cycles of 15 seconds at 95°C and 22 seconds at 60°C. To quantify viral load, a standard curve was made using 10-fold serial dilutions (10⁻⁸–10⁰ genomic equivalents) of purified DNA plasmid in a solution of 5 ng/mL salmon sperm. The detection limit of the assay with DNA from the TTV clone as a template was 1 × 10⁰ genomic equivalent copies. Viral load was expressed as copies per milliliters.

**TTV Genogroup Identification**

TTV genogroup identification was performed using nested PCR as described [13]. Primers specific for conserved sequences among genotypes (see universal primer in Supplementary Table 1), 1 primer common to all TTV genogroups (T2S; Supplementary Table 1), and 5 degenerated primers discriminating the different genogroups (T2G1A, T2G2A, T2G3A, T2G4A, and T2G5A; Supplementary Table 1) were used.

**HEV Detection**

HEV infection status was established for all samples by enzyme-linked immunosorbent assay, and HEV was detected by reverse transcription PCR of viral RNA extracted from serum as previously described [11].

**Statistical Analysis**

Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM, 2011). TTV loads have been log-transformed before analysis to ensure normal distribution. Differences were considered significant at P < .05. Only 2-tailed analyses were performed.

**RESULTS**

**OLT, TTV Prevalence, and Viral Load**

TTV DNA was detected in 96% of all tested serum samples. Among 14 samples obtained before OLT, 10 (71%) were TTV positive (5 of 6 in the nonhepatitis group, and 5 of 8 in the chronic hepatitis group). Among samples obtained after OLT, 99% (204/206) from the nonhepatitis group and 98% (101/103) from the chronic hepatitis group had detectable TTV DNA. Overall, only 1 transplant recipient (in the nonhepatitis group) did not become infected with TTV during follow-up, increasing the prevalence in these groups to near 100%. In contrast, only 13 of 19 healthy children (68%) had detectable TTV DNA in their serum (P < .0001, by the χ² test, compared with OLT recipients). No significant differences were evidenced between pre-OLT children and healthy children or between the chronic hepatitis and nonhepatitis groups.

Because the TTV prevalence is high in the general population and even higher among immunosuppressed patients, the differences in prevalence may not be sufficient to understand the influence of TTV infection on posttransplantation outcome [5]. Thus, we measured viral load. We found a significantly higher TTV load in serum samples obtained after OLT, compared with serum samples obtained before OLT (P < .001) or from healthy children (P < .0001, by 1-way analysis of variance, with the Tukey post hoc test; Figure 1A). Surprisingly, the TTV load was significantly lower after OLT among patients in the chronic hepatitis group (P < .0001; Figure 1A), of whom 11 (78%) had liver fibrosis scores of ≥2.

For all patients, viral load decreased during the posttransplantation follow-up period (Pearson correlation, −0.373; P < .0001; Figure 1B) and with increasing age (Pearson correlation, −0.457; P < 0.0001, data not shown), regardless of whether hepatitis developed after OLT. No significant relationship was found between TTV load and serum ALT level (P = .175), AST
level \((P = .880)\), or GGT level \((P = .128\), by Pearson correlation). No significant effect was found between the number of transfusions during transplantation and viral load \((P = .361\), by the \(t\) test, for patients receiving >25 transfusions vs those receiving <25 transfusions), and there was no significant correlation between the number of transfusions and viral load (Pearson correlation, 0.230; \(P = .074\)).

**Immunosuppression and TTV Load**

The difference in TTV load observed in samples obtained before OLT receipt, samples obtained after OLT receipt, and samples from healthy children suggests that immunosuppression may affect TTV viremia. Children were subdivided into groups according to the number of prescribed immunosuppressive agents. Administration of 1 immunosuppressant medication was sufficient to increase the TTV load significantly \((P < .0001; Figure 1C)\). Addition of other immunosuppressive drugs did not cause further significant increases in the TTV load. The type of immunosuppressant had no effect on the TTV load (data not shown).

**OLT and TTV Genogroups**

TTV genogroups were identified to understand whether a specific genogroup (or a combination of genogroups) was associated with chronic hepatitis following OLT or was favored by immunosuppression. Genogroups were successfully identified...

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**Figure 1.** Variation in Torque Teno virus (TTV) load among study groups (A), during follow-up (B), and by number immunosuppressive agents received (C). A, TTV load in serum specimens obtained before orthotopic liver transplantation (OLT), in those obtained after OLT from patients with chronic hepatitis or no hepatitis, and in those from healthy children. Statistically significant differences in viral load between the groups are specified. B, The TTV load diminished during the posttransplantation period regardless of hepatitis development after OLT (Pearson correlation, \(-0.373\); \(P < .0001\)). C, The TTV load increased with the number of immunosuppressive agents received (**\(P < .0001\), compared with the group with no immunosuppression), but taking multiple drugs did not lead to further increases in viral load. The group of individuals who did not take any immunosuppressive drug was composed of pre-OLT patients and healthy children. Open circles illustrate outliers. Viral load is expressed as log\(_{10}\) copies per mL.
in 95% of serum samples (312/328, including samples obtained before OLT and those from healthy controls) with detectable TTV DNA. Genogroup 3 was the most prevalent in samples from healthy children and those collected before OLT (Table 1). After OLT, the distribution spread to the other genogroups, with the exception of genogroup 4, which had not been detected in any patient. An overall change in the genogroup distribution was found among all groups (Table 1). Specifi
cally, there were significant differences between samples from the chronic hepatitis group and those from healthy children (P = .0248), samples from the chronic hepatitis group and those obtained before OLT (P = .0428), samples from the nonhepatitis group and those from healthy children (P = .0435), and samples from the nonhepatitis group and those obtained before OLT (P = .0462, by the χ² test). There were no differences in genogroup distribution between samples obtained before OLT and those from healthy children (P = .06). OLT influences genogroups infecting the host, but genogroup distribution was not associated with the presence of hepatitis after OLT (P = .7744, for samples from the chronic hepatitis group vs samples from the nonhepatitis group).

Since multiple genogroups are frequently found in individu als infected with TTV (even in healthy people [4, 14, 15]), the number of different genogroups detected in the same serum specimen were analyzed. The total number of genogroups infecting simultaneously a patient was different between children who did and those who did not undergo transplantation but was not linked to the outcome after OLT (P = .0018; Table 1). The number of transfusion did not result in a higher number of genogroups (P = .619, by χ² analysis).

There were no differences in the number of genogroups between samples obtained from patients before OLT and those from healthy children. A significant higher proportion of infections with multiple genogroups was found in samples from the chronic hepatitis group vs those obtained before OLT (P = .0150), in samples from the nonhepatitis group vs those from healthy children (P = .0212), and in samples from the nonhepatitis group vs those obtained from patients before OLT (P = .0008, by the χ² test). The number of TTV genogroups was not associated with development of hepatitis after OLT (P = .2251 for samples obtained from the chronic hepatitis groups vs those obtained from the nonhepatitis group), but a higher viral load was observed when patients were infected with multiple genogroups (P < .0001; Figure 2A). No combination of genogroups was significantly associated with development of hepatitis after OLT or with a higher viral load (data not shown).

The kinetics of TTV infection (genogroup and number of genogroups) was then examined in each OLT recipient through time. No clear pattern of infection emerged from the follow-up of TTV genogroups through time, regardless of the development of chronic hepatitis after OLT. Multiple presentations of infection were observed. Some patients were infected only after OLT and then contracted multiple TTV infections due to varying genogroups over time. Others remained infected with the same TTV genogroup throughout the follow-up period, with infection only varying in terms of viral load. The only identifiable pattern is that TTV infection was dynamic and highly variable through time among patients after OLT.

### Immunosuppression and Multiple-Genogroup Infections

Immunosuppressive drugs might be responsible for the change of distribution in genogroups and the numbers of genogroups observed after OLT. We found that taking immunosuppressive drugs led to a heterogeneous genogroup distribution (pre-OLT vs 1 drug, P = .06; pre-OLT vs 2 drugs, P = .0128; pre-OLT vs 3 drugs, P = .155 [by the χ² test for all]) and higher number of genogroups found in a single patient (pre-OLT vs 1 drug, P = .0007; pre-OLT vs 2 drugs, P = .0217; pre-OLT vs 3 drugs, P = .0079 [by the χ² test for all]) (Table 2). Similar to viral load (Figure 1C), once immunosuppressed, the number of prescribed immunosuppressive drug(s) did not influence the distribution (P = .7070) or number (P = .2200) of genogroups found.

### Influence of Coinfection With HEV

We have previously shown that patients who underwent OLT and developed chronic hepatitis of unknown etiology have a

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**Table 1. OLT Influences Diversity and Number of TTV Genogroups in Torque Teno Virus–Positive Patients**

<table>
<thead>
<tr>
<th>Genogroup</th>
<th>Samples After OLT</th>
<th>Samples From Healthy Children</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples Before OLT (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genogroup</td>
<td>Chronic Hepatitis Group (n = 94)</td>
<td>Nonhepatitis Group (n = 195)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (10)</td>
<td>48 (51)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0)</td>
<td>30 (32)</td>
<td>3 (23)</td>
</tr>
<tr>
<td>3</td>
<td>5 (50)</td>
<td>29 (31)</td>
<td>5 (38)</td>
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<td>4</td>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5</td>
<td>4 (40)</td>
<td>56 (60)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>χ²</td>
<td></td>
<td>0.0428</td>
<td>0.0462</td>
</tr>
<tr>
<td>Genogroups detected per patient, no.</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>1</td>
<td>8 (57)</td>
<td>38 (36)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>2</td>
<td>2 (1)</td>
<td>46 (45)</td>
<td>2 (10)</td>
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</tr>
<tr>
<td>χ²</td>
<td></td>
<td>0.0150</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Abbreviation: OLT, orthotopic liver transplantation.

<sup>a</sup> By χ² analysis.

<sup>b</sup> Compared with samples obtained before OLT.
high prevalence of HEV infection [11]. Liver coinfection could change the influence of TTV infection in OLT patients. Therefore, HEV coinfection status in OLT recipients was studied. Interestingly, the TTV load was significantly lower in OLT recipients positive for anti-HEV IgM and IgG antibodies ($P = .0184$, by 1-way analysis of variance with the Tukey posttest; all OLT recipients were included; Figure 2B). However, HEV status did not influence the diversity of genogroup ($P = .9850$) or the number of genogroups found in 1 individual ($P = .7590$; Supplementary Table 2). Liver coinfection after OLT, such as that due to HEV, does not influence genogroups distribution but impairs TTV replication.

Table 2. Immunosuppressive Treatment Influences Diversity and Number of Torque Teno Virus Genogroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Immunosuppressive Treatment (Before OLT; n = 14)</th>
<th>Immunosuppressive Treatment</th>
<th>$P^b$ (Between Immunosuppression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genogroup</td>
<td></td>
<td>1 (n = 168)</td>
<td>2 (n = 114)</td>
</tr>
<tr>
<td>1</td>
<td>1 (7)</td>
<td>87 (52)</td>
<td>58 (51)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0)</td>
<td>58 (35)</td>
<td>38 (33)</td>
</tr>
<tr>
<td>3</td>
<td>5 (36)</td>
<td>61 (36)</td>
<td>27 (23)</td>
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<tr>
<td>4</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>5</td>
<td>4 (29)</td>
<td>91 (54)</td>
<td>61 (54)</td>
</tr>
<tr>
<td>$P^b$</td>
<td>.0605</td>
<td>.0128</td>
<td>.1550</td>
</tr>
<tr>
<td>Genogroups detected per patient, no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8 (57)</td>
<td>45 (27)</td>
<td>45 (39)</td>
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<tr>
<td>2</td>
<td>1 (7)</td>
<td>90 (54)</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>$P^b$</td>
<td>.0007</td>
<td>.0217</td>
<td>.0079</td>
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Abbreviation: OLT, orthotopic liver transplantation.

$^a$ By $\chi^2$ analysis.

$^b$ Compared with samples obtained before OLT.
DISCUSSION

This study reports a high prevalence of infection and a high TTV load among OLT recipients. Viral load was influenced by immunosuppressive regimen, chronically increased serum aminotransferases levels, and histological hepatitis and liver viral coinfection. Although this study is not the first to investigate TTV infection in liver transplant patients [16, 17] or children with chronic hepatitis [5, 18], it is the first to report a significant negative association between TTV load and chronic hepatitis occurrence or HEV coinfection. This work stands out because of the large number of samples collected, the long follow-up of OLT patients, and the detection method.

The TTV prevalence among OLT recipients was very high, which is consistent with previous studies [1, 16]. In contrast, the TTV prevalences among samples obtained from patients before OLT and those obtained from healthy children were lower than values reported in previous studies from other parts of the world [1, 16]. This may be due to the lower viral load observed in nonimmunosuppressed children, which, in some samples, may have been below the detection threshold (1 × 10^2 copies/mL of serum). Our detection threshold was established using a TTV clone, which may not reflect the threshold for clinical samples. However, reports on TTV prevalence in healthy individuals vary, and this prevalence may reflect the actual situation in our area, especially since the TTV prevalences in samples obtained before OLT and those obtained from healthy children were comparable. The increase in the TTV prevalence between samples obtained before OLT and those obtained after OLT could be related to the blood transfusions during surgery [1] and to the immunological status of transplant recipients.

Since the TTV prevalence is globally high globally, the TTV load is more relevant than prevalence to understand TTV infection. Compared with TTV loads before OLT and those in healthy individuals, TTV loads after OLT were higher. This is most likely due to immunosuppression and cellular immunomodulation of the virus [19, 20]. The strong influence of immunosuppression has been previously shown in a study reporting that a high TTV load was inversely proportional to CD4+ T-cell counts during HIV infection, suggesting that TTV loads may increase in parallel with the impairment of cellular immune status [20]. This could be compared to the period immediately after OLT, when immunosuppressive treatment is maximized in children, leading to a higher viral load. This also coheres with our observation that immunosuppressive treatment has a dramatic effect on TTV control. The viral load diminished during the time after OLT, which can be explained by the progressive diminution of immunosuppressive drug doses during the follow-up.

Interestingly, we found a lower viral load in patients with chronic hepatitis after OLT than in those with a normal outcome of OLT. Recently, we reported a high prevalence of chronic HEV infection in these patients [11]. Having recent HEV infection correlated with a lower TTV load. Because we do not understand sufficiently the immunobiology of TTV, we can only speculate on the link between HEV infection and TTV load. Two hypotheses can be explored to explain these phenomena. First, since TTV and HEV can both replicate in hepatocytes [8, 21], there could be a competitive use of the replication machinery in the cell. To confirm this hypothesis, cultured-cell coinfection experiments could be performed. Another possibility is that inflammation triggered in the liver against HEV nonspecifically impairs TTV through hepatocyte death and increased interferon secretion. Coherent with this hypothesis, TTV is susceptible to the effects of interferon [22]. Alternatively, chronic hepatitis, which resulted in a higher METAVIR score in affected patients, could be the result of immunopathology processes caused by lymphocytes reacting against TTV. However, this is speculative and needs to be confirmed in further studies. Altogether these results suggest that, in contrast to the cooperation between TTV and Epstein-Barr virus [23], TTV and HEV do not act as a cofactors and that liver inflammatory activity could impair TTV replication in the liver.

Genogroup distribution was altered by OLT and immunosuppression. Genogroup 1, 2, and 5 infections were favored in these conditions, comparable to adult liver transplant recipients [16]. Multiple-genogroup infections resulted in higher viral loads, as described in healthy adults [14]. However, we did not observe a higher viral load with any specific combinations of TTV genogroups. Follow-up of TTV infection after OLT showed a variety of infections and reinfection patterns. This suggests that we might be continuously exposed to various strains of TTV and are frequently infected with several TTV genogroups. Follow-up of TTV infection after OLT showed a variety of infections and reinfection patterns. This suggests that we might be continuously exposed to various strains of TTV and are frequently infected with several TTV genogroups. Follow-up of TTV infection after OLT showed a variety of infections and reinfection patterns. 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investigated in these patients. In conclusion, TTV is possibly not pathogenic, but its replication is closely linked with immunosuppression and viral coinfection. Further studies exploring TTV viremia as surrogate marker of liver health after OLT could yield interesting results.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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K. B. assisted in experiments, analyzed the results, and wrote the manuscript; M. D.-N. performed most of the experiments; M.-J. G. performed the TTV load quantification; N. P. reviewed and scored the liver biopsy specimens; J. B. helped in the conception of this study; F. A. and U. H. conceived and supervised the study and, with K. B., wrote the manuscript. All authors participated in the drafting, revision, and final approval of the published version.

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