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1 **No correlation between Torque Teno virus viral load and**
2 **BK virus replication after kidney transplantation**

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Abstract

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34 **Background:** Assessment of the intensity of immunosuppression in transplant
35 recipients to estimate the risk of rejection and infection is not entirely satisfactory at
36 the present time. Determination of Torque teno virus (TTV) viral load appears to be a
37 promising tool in this setting.

38 **Objectives:** We evaluated the level of replication and kinetics of TTV during the first
39 three months after kidney transplantation compared to BK virus replication.

40 **Results:** In a retrospective cohort of 116 renal transplant recipients, TTV viral load
41 gradually increased during the first three months post-transplantation with no
42 significant difference or discriminatory threshold between patients with and without
43 BK virus replication. However, the level of TTV replication appeared to be indirectly
44 related to the risk of BK virus replication, particularly according to the induction
45 treatment used (antithymocyte globulin: ATG or basiliximab). Among patients
46 receiving ATG, those receiving cyclosporine had significantly lower TTV viral loads
47 ($p < 0.01$) with threefold lower reactivation of BKPyV (13 vs 37%) 3 months post-
48 transplantation. Similarly, among the women in our cohort, TTV viral load was
49 significantly higher in women receiving ATG (6.58 ± 1.57 versus $4.62 \pm 2.0 \log_{10}$
50 copies/mL for basiliximab: $p < 0.01$), also with threefold higher BKPyV reactivation
51 frequencies (40 vs 13,3%).

52 **Conclusion:** The multiparametric variation of TTV viral load does not appear to be
53 individually appropriate for the early detection or monitoring of possible post-
54 transplant BKPyV virus reactivation in renal transplant recipients.

55 Keywords: kidney transplantation, BK virus, Torque Teno Virus, immunosuppression

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Background

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The Anelloviridae family, comprising the Alphatorquevirus genus, includes 29 species. Alphatorqueviruses are highly prevalent in humans (90-100%) with low persistent viraemia in immunocompetent subjects (Focosi et al., 2016). These viruses have not been linked to any human disease at the present time. Data have been recently accumulated concerning the Torque Teno Virus (TTV), the most prevalent of the Alphatorqueviruses and the most abundant virus of the human virome, indicating that TTV viral load could be a useful marker to assess immunosuppression (Fernández-Ruiz et al., 2018). It has now been clearly demonstrated that an imbalance in immune status has a significant impact on TTV replication. In an individual, the TTV viral load varies between 10^2 and 10^9 copies per milliliter of plasma (Maggi and Bendinelli, 2009). Since there is currently no universal marker to assess the intensity of immunosuppression in a transplant recipient treated with an immunosuppressive cocktail, a promising strategy would be to monitor blood TTV levels.

Various recent studies have evaluated the TTV viral load after heart, lung and kidney transplantation with the primary endpoint being the presence of signs of rejection and/or infection (bacterial, viral, parasitic) in the transplant recipient (Fernández-Ruiz et al., 2018). Studies evaluating TTV viral load during follow-up after kidney transplantation have been conducted in order to assess the risk of either rejection or Cytomegalovirus disease (Maggi et al., 2018) (Schiemann et al., 2017). However, since the early 2000s, an increase in the prevalence of nephropathy caused by human BK polyomavirus (BKPyV) in renal transplant recipients has resulted in a renewed interest in this pathogen. The BKPyV may induce progressive disease,

83 mainly during the first three to six months after transplantation in three successive
84 stages: viraemia, viraemia and if viral replication persists nephropathy (Brochet et al.,
85 2019). However, it is difficult to predict post-transplant patients who could replicate
86 BKPyV.

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Objectives

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90 We therefore wanted to evaluate whether TTV viral load monitoring in the first three
91 months after kidney transplantation could have clinical value to predict an increased
92 risk of BK virus replication.

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Study design

109 Study population and specimen

110 All 116 consecutive adult renal transplant recipients transplanted between 1st
111 January 2015 and the 31th December 2016 in Amiens hospital, who had completed
112 at least one year of follow-up were enrolled. The exclusion criteria were non-adult
113 patients and those who did not have a minimum one-year follow-up. Induction
114 immunosuppressive therapy included either antithymocyte globulin (ATG) or
115 basiliximab and maintenance therapy included corticosteroids, mycophenolate mofetil
116 and tacrolimus or cyclosporine. This retrospective project was conducted in
117 accordance with the reference methodology (MR-004) and is included in the CHU
118 Amiens - Picardie internal registry of data processing activities, in accordance with
119 Article 30 of the GDPR .

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121 Quantification of BKPyV and TTV viral load

122 All samples were stored at -80°C prior to analysis. BKPyV measurements is
123 performed in our centre every month for the first six months and then every three
124 months thereafter. Total nucleic acids were extracted and amplified in a quantitative
125 real-time PCR assay kit (R-gene®;Argene, France), as previously described in detail
126 (Descamps et al., 2015). TTV DNA load was quantified by the TTV R-gene® kit (R-
127 gene®; Argene, France).

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Results

131 A total of 139 patients were transplanted at Amiens University Hospital in 2015 and
132 2016. Longitudinal plasma TTV viral load was measured in these patients at month 0
133 (M0), M1, M2 and M3 post-transplantation in order to compare these results with the
134 risk of post-transplant BKPyV replication. Samples were available for 116 patients,
135 whose characteristics are listed in Table 1 with no significant differences between
136 plasma BKPyV-positive and plasma BKPyV-negative patients and patients not
137 included (data not shown). Twenty-four of these 116 patients (20.7%) had a positive
138 plasma BKPyV viral load during the first year after transplantation and 17 (14.7%)
139 had a positive BKPyV viral load during the first three months. Longitudinal monitoring
140 of TTV viral load is shown in Figure 1A for BKPyV-positive (17 patients) and BKPyV
141 negative patients. A gradual increase in TTV viral load was observed over the first
142 three months, with no significant difference between both groups ($p>0.05$). Similarly,
143 we were unable to determine a TTV viral load threshold to distinguish between
144 BKPyV-positive patients and BKPyV-negative patients at any time point. In addition,
145 for 13/17 patients for whom matched plasma BKPyV and TTV viral loads were
146 available at 3 months, we failed to demonstrate any correlation between these two
147 parameters (Figure 1B). Determination of TTV viral load during the first three months
148 after kidney transplantation therefore does not appear to be a useful parameter to
149 predict possible BKPyV replication. In order to highlight a potential indirect role of
150 TTV viral load on BKPyV replication, we studied the influence of known BKPyV
151 replication risk factors on TTV viral load during the first three months after
152 transplantation (Demey et al., 2018). As shown in Figure 1C, among these factors,
153 immunosuppressive induction appears to play a role in TTV viral load, as a

154 significantly different viral load was observed 2 and 3 months post-transplant
155 between patients who received ATG or basiliximab with tacrolimus as calcineurin
156 inhibitor. The mean M3 TTV viral loads were 6.03 and 4.41 \log_{10} copies/mL in the
157 ATG/tacrolimus and basiliximab/tacrolimus groups, respectively ($p < 0.01$).
158 Furthermore, 37% and 17.3% of transplant recipients were tested positive for BKPyV
159 during the first three months after transplantation for these same groups respectively.
160 This difference was not observed for cyclosporine, which generally induced a lower
161 level of BKPyV reactivation. The second known risk factor for BKPyV reactivation
162 associated with differences in TTV viral load was the gender of transplant recipients
163 associated with ATG induction therapy (Figure 1D). It has now been demonstrated
164 that the rate of BKPyV reactivation is much lower in women than in men, but the
165 proportion of women of our cohort treated with basiliximab, who tested positive for
166 BKPyV was 13.3% versus more than 40% of women treated with ATG. These
167 differences between both groups could be explained by mean differences of TTV viral
168 load of 0.5 \log_{10} at month 2 and 1.96 \log_{10} at M3 (4.62 ± 2.0 versus $6.58 \pm 1.57 \log_{10}$
169 copies/mL: $p < 0.01$) (Figure 1D). Other factors known to be associated with TTV
170 replication, such as age, donor CMV serology (Fernández-Ruiz et al., 2018) (Kosulin
171 et al., 2018) and granulocyte count, were compared with BKPyV data in transplant
172 recipients, but no obvious link was demonstrated (data not shown).

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Discussion

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This study, which was designed to analyse a possible link between the level of TTV, the most abundant component of the virome, and post-renal transplantation development of BKPyV replication clearly illustrates the difficulty of using this popular and promising tool in the kidney transplant community in this setting. As already demonstrated by other teams, this test appears to have a higher positive predictive capacity for the risk of developing episodes of rejection. As in this study, most other studies have failed to demonstrate a clinically predictive TTV viral load threshold.

We observed high variation in viral load levels with high variation between populations. Many factors seem to influence the level of TTV replication, such as individual factors (age, sex, ...) although many of these factors probably remain unidentified, factors related to the immunosuppressive regimen and probably also factors specific to the virus given its great diversity and the existence of many subtypes (Rani et al., 2016). We also tried to identify variables that could be directly correlated during the first three months following transplantation with TTV replication intensity and indirectly correlated with BKPyV replication. For example, we evaluated calcineurin inhibitor concentrations, calculated their areas under the curve, lymphocyte and granulocyte counts at each time-point, pre-graft serology, a transfusion episode during transplantation, but failed to demonstrate any solid correlation.

Although there is no gold standard for monitoring the level of immunosuppression, the measurement drug through levels of calcineurin inhibitors should not be supplanted in the monitoring of post-renal transplantation.

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248 **Table 1:** Patient characteristics

Characteristics	BKPyV negative (n=99)	BKPyV positive (n= 17)	P value
Female gender	29	4	NS
Age mean (\pm SD)	48.1 (\pm 13.5)	47 (\pm 16)	NS
Positive Donor CMV serology	38	6	NS
Transfusion	10	1	NS
ATG induction	39	10	NS
Cyclosporine	33	4	NS
Tacrolimus	66	13	
Mean TTV viral load (log ₁₀ copies/ml (\pm SD))			
J0	1.85 \pm 1.45	1.60 \pm 1.43	NS
M1	2.98 \pm 1.72	2.68 \pm 1.86	NS
M2	4.71 \pm 1.58	4.23 \pm 0.60	NS
M3	5.16 \pm 2.07	4.73 \pm 2.20	NS

249 NS: not significant

250 **Figure legends**

251 **Figure 1**

252 TTV viral load 3 months post-renal transplantation and BKPyV replication

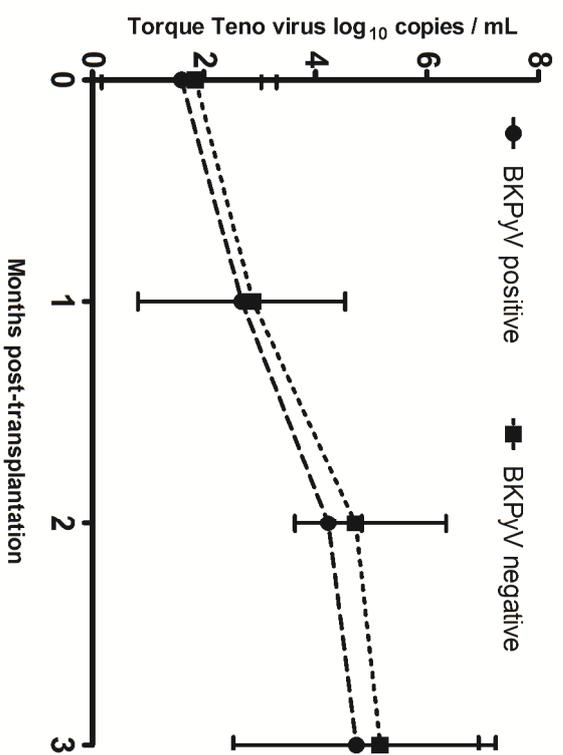
253 **A.** Longitudinal monitoring of the level of TTV replication according to the
254 presence or absence of BKPyV.

255 **B.** Month 3 matched plasma TTV and BKPyV viral loads.

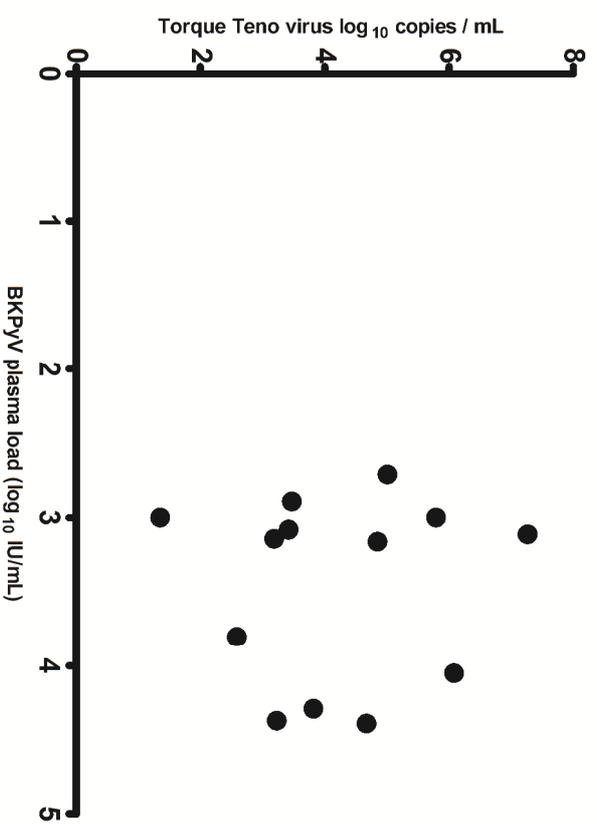
256 **C.** Longitudinal follow-up of TTV during the first three months after transplantation
257 according to immunosuppressive treatment

258 **D.** Longitudinal follow-up of TTV during the first three months post-transplantation
259 according to sex and induction immunosuppressive treatment

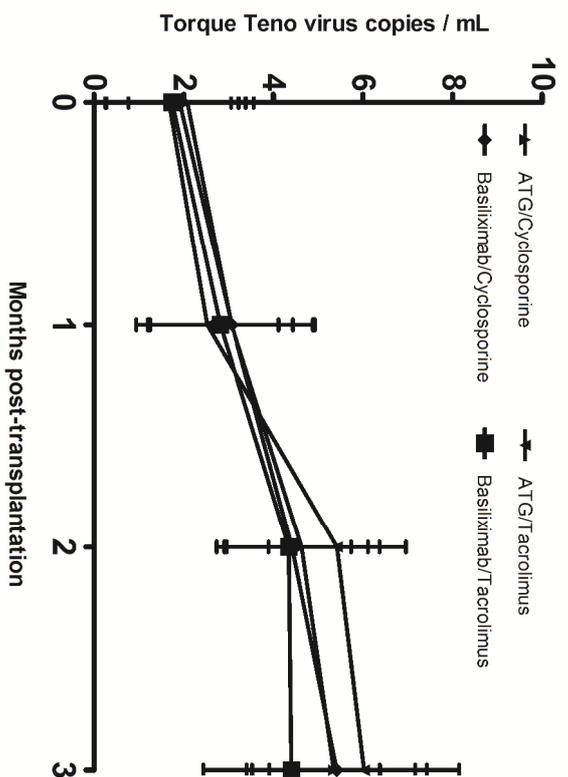
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