Short Communication

Individuals infected with JC polyomavirus do not present detectable JC virus DNA in oropharyngeal fluids

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JC virus (JCV) is ubiquitous in the human population. Primary infection normally occurs during childhood and is followed by a lifelong persistent infection. The main mode of transmission remains unknown. Several authors have hypothesized that JCV transmission occurs through the respiratory route, and that respiratory secretions could represent a possible source of viral particles. The present study intended to evaluate oropharyngeal fluids from patients infected with JCV, in order to ascertain if respiratory secretions could indeed constitute a source of exposure to this polyomavirus. Oropharyngeal washing samples from 25 patients co-infected with JCV and human immunodeficiency virus type 1 were evaluated for the presence of JCV DNA. Regardless of the titre of antibodies or the presence of viral urinary excretion, JCV genome was not detected in oropharyngeal samples collected from any of the patients infected with JCV included in this study, which may suggest that oropharyngeal fluids are an unlikely source for JCV infection.

JC polyomavirus (JCV) is ubiquitous among the human population worldwide. Seroepidemiological studies revealed that by adulthood, more than 70% of individuals present JCV-specific antibodies (Andrews et al., 1988; Chang et al., 2002; Matos et al., 2010; Rollison et al., 2003; Stolt et al., 2003). Primary infection, typically asymptomatic, occurs during childhood and is usually followed by a lifelong latent infection in immunocompetent subjects (Andrews et al., 1988; Knowles et al., 2003; Sundsfjord et al., 1994a; Taguchi et al., 1982). Under situations of severe immune suppression, JCV may reactivate and induce a fatal demyelinating disease of the central nervous system known as progressive multifocal leukoencephalopathy (PML). In spite of the worldwide distribution of JCV infection, PML remains a very rare disease that mainly affects seriously immunosuppressed patients, in particular those infected with human immunodeficiency virus (HIV) (Berger & Major, 1999).

Asymptomatic JCV shedding in urine has been demonstrated as a common occurrence in a great part of adult individuals (Ling et al., 2003; Matos et al., 2010; Zhong et al., 2007), suggesting that asymptomatic reactivation may also occur. Though JCV urinary excretion frequently occurs in healthy individuals, higher excretion rates have been reported in patients infected with HIV (Ferrante et al., 2001; Matos et al., 2010; Schaffer et al., 2006).

The route of transmission of JCV remains unknown. The occurrence of JCV primary infection in the absence of defined and obvious clinical symptoms (Berger & Major, 1999; Berger et al., 2006) has hampered the identification of its route of transmission.

The widespread nature of JCV, along with the occurrence of the primary infection during childhood, strongly suggests a common route of transmission. The hypothesis of vertical transmission was excluded by several studies (Boldorini et al., 2008, 2011; Coleman et al., 1980; Daniel et al., 1981; Kitamura et al., 1994).

The presence of JCV DNA in tonsillar tissue from both children and adult individuals (Monaco et al., 1996, 1998) combined with the frequency with which several ubiquitous viruses are spread by the oro-respiratory route points towards the respiratory transmission of JCV (Bofill-Mas et al., 2003). The recent identification of two new human polyomaviruses, Karolinska Institutet and Washington University virus (Allander et al., 2007; Gaynor et al., 2007) in respiratory secretions of young patients, further supports the hypothesis of the respiratory route for transmission of polyomaviruses.
Respiratory secretions exhaled during the acute phase or during the reactivation of JCV infection have been proposed as a possible source of viral particles for transmission (Berger, 2000; Imperiale, 2000).

Despite the limited studies available, JCV has failed to be detected in saliva or oropharyngeal washings either from healthy or HIV-infected individuals (Berger et al., 2006; Bialasiewicz et al., 2009; Patel et al., 2008; Sundsfjord et al., 1994b); to our knowledge, the individuals included in those studies were selected irrespective of their serostatus for JCV infection, which may represent a limitation for drawing reliable conclusions. Some of the individuals, especially the youngest ones, might not yet been infected with JCV at the moment of sample collection, and thereby would not be expected to have JCV in their oropharyngeal samples.

In an attempt to try and overcome such limitation, the present study was undertaken in patients with JCV infection confirmed by serology, aiming to clarify if respiratory secretions could be a source of JC viral particles for transmission.

Twenty-five individuals seropositive for JCV were selected for this study from a group of HIV-1-infected patients attending to the Department of Infectious Diseases of the University Hospital of Coimbra, between November 2006 and June 2007. All subjects were >18 years old, and gave their informed consent for the collection and analysis of samples, prior to inclusion in this study. This study was approved by the Ethics Committee of the Coimbra University Hospital, Portugal.

Table 1 contains information on age, sex, titre of JCV antibodies, presence or absence of JCV urinary excretion, CD4+ T-cell count and plasma HIV viral load for each studied individual, referent to the date of oropharyngeal sample collection.

None of the patients showed clinical signs or symptoms suggestive of PML during the length of the study.

Single oropharyngeal washing samples were obtained from each patient. All patients were asked to gargle with 10 ml sterile saline for 15 s, collected in a sterile container. Samples were maintained on ice, homogenized, separated into 2 ml aliquots and stored at −70 °C until analysis.

Two millilitres of each oropharyngeal washing sample was centrifuged at 10 000 g for 10 min, at room temperature. The supernatant was discharged and viral DNA extraction was performed from the pellet by using the commercial kit QIAamp DNA Mini kit (Qiagen) according to the manufacturer’s instructions. Viral DNA was eluted with 100 µl elution buffer and stored at −20 °C until required for PCR.

The detection of JCV DNA in oropharyngeal samples was performed with real-time PCR, a protocol described previously, using a set of two amplification primers and two hybridization fluorescence resonance energy transfer probes, specific for the sequence of the JCV major capsid protein gene (Matos et al., 2010). Each sample was assayed in duplicate.

One positive and two negative controls were included in each batch of PCRs. One negative control consisted of the eluent obtained from nucleic acid extraction protocol performed on sterilized water instead of the oropharyngeal sample. The other negative control consisted of the PCR mixture containing water instead of the DNA template. Tissue culture supernatant of JCV Mad-4-infected SVG cells, with a concentration of 128 haemagglutination units per 50 µl, was used as the positive control.

The analytical sensitivity was evaluated by testing 10-fold serial dilutions of a quantified plasmid containing the full-length JCV genome (Advanced Biotechnologies Inc.). The detection limit was found to be 1.4–14 genome copies per reaction.

In order to evaluate a possible enzymic inhibition of the PCR due to the natural composition of the biological samples analysed, the analytical sensitivity of the PCR protocol was also evaluated using dilutions of the quantified plasmid prepared in nucleic acid extract of oropharyngeal samples instead of sterile water. The results obtained were identical to those obtained with dilutions in sterile water, thus excluding the hypothesis of a possible PCR enzymic inhibition.

Statistical analysis was performed using the $\chi^2$ test or Fisher’s exact test for comparison of categorical variables between groups. The Mann–Whitney U test was used for comparisons between two unrelated groups. For all statistical analysis, differences were considered statistically significant when $P<0.05$.

The 25 individuals enrolled in this study comprised 19 men and 6 women, aged from 27 to 61 years old (mean age of 41 years) (Table 1). The selection criterion was the presence of JCV infection confirmed by serology. The titre of JCV antibodies, assessed by a VLP-based ELISA, varied from 1 : 640 to 1 : 163 480 (median of 1 : 10 240).

All the selected patients were also infected with HIV-1, and had a wide range of HIV-1 viral load in plasma and CD4+ T-cell count ml$^{-1}$. At the time of sample collection the mean value of plasma HIV-1 viral load was 2.72 log copies ml$^{-1}$ (range 1.6–5.53 log copies ml$^{-1}$), and the mean CD4+ T-cell count was 521 cells µl$^{-1}$ (range 10–1325 CD4+ T-cells µl$^{-1}$). Such selection was made on the basis that JCV reactivation, either symptomatic or asymptomatic, is more frequently described in patients infected with HIV (Duque et al., 2010; Ferrante et al., 2001; Jiang et al., 2009; Matos et al., 2010; Schaffer et al., 2006), and thereby the probability to detect JCV DNA in oropharyngeal samples of these patients could be higher than in healthy individuals.

Thirteen of the 25 patients (52 %) had detectable JCV DNA in urine, with a mean viral load excreted of 4.81 log copies of JCV DNA ml$^{-1}$ of urine (range 2.77–6.29 log copies...
Table 1. Demographic characteristics of individuals co-infected with JCV and HIV-1 selected for the present study

The data recorded are referent to the collection date of the oropharyngeal washing samples. NA, Not applicable.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age</th>
<th>JCV antibody titre</th>
<th>CD4 ml⁻¹</th>
<th>HIV-1 plasma viral load (log copies ml⁻¹)</th>
<th>Urinary excretion of JCV</th>
<th>Urinary load of JCV (log copies ml⁻¹)</th>
<th>JCV DNA in oropharyngeal fluids</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>50</td>
<td>1 : 163 840</td>
<td>493</td>
<td>&lt;1.60</td>
<td>Detectable</td>
<td>5.44</td>
<td>Undetectable</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>33</td>
<td>1 : 10 240</td>
<td>675</td>
<td>&lt;1.60</td>
<td>Detectable</td>
<td>6.29</td>
<td>Undetectable</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>1 : 40 960</td>
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</tr>
<tr>
<td>4</td>
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<td>4.62</td>
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</tr>
<tr>
<td>5</td>
<td>M</td>
<td>40</td>
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<td>Undetectable</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>38</td>
<td>1 : 2 560</td>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<td>11</td>
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<tr>
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<tr>
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<td>NA</td>
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<td>324</td>
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<td>Undetectable</td>
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<tr>
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<tr>
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<td>1 : 640</td>
<td>675</td>
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<td>Undetectable</td>
<td>NA</td>
<td>Undetectable</td>
</tr>
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<td>1 : 40 960</td>
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<tr>
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<td>41</td>
<td>1 : 2 560</td>
<td>870</td>
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<td>NA</td>
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<tr>
<td>23</td>
<td>M</td>
<td>34</td>
<td>1 : 2 560</td>
<td>352</td>
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<tr>
<td>24</td>
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<td>1 : 10 240</td>
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<td>NA</td>
<td>Undetectable</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>27</td>
<td>1 : 10 240</td>
<td>294</td>
<td>&lt;1.60</td>
<td>Undetectable</td>
<td>NA</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>
ml⁻¹ of urine). The inclusion of patients excreting JCV in urine at the moment of sample collection, intended to represent individuals experiencing active viral replication (Berger & Major, 1999; Major et al., 1992), and are thus more likely to transmit the infection. There were no significant differences in the age (P=0.462), sex (P=0.378), CD4⁺ T-cell count (P=0.460) or plasma HIV-1 viral load (P=0.629) between patient excreting and non-excreting JCV in urine, but patients excreting JCV in urine showed significantly higher titres of JCV antibody than those who did not excrete JCV in urine (P=0.004).

The real-time PCR used for the detection of specific sequences of the JCV genome was unable to detect JCV DNA in the oropharyngeal washing samples obtained from any of the individuals included in this study.

Although individuals theoretically more prone to transmit JCV infection were enrolled in this study, no JCV DNA was detected in oropharyngeal samples collected from any of the patients studied. These data suggest that JCV is not usually found in oropharyngeal fluid samples from patients infected with JCV, irrespective of the titre of antibodies, or the presence of active viral excretion in urine.

In spite of other authors who also failed to detect JCV in ororespiratory samples with similar methodologies (Berger et al., 2006), identical protocols were suitable to detect Epstein–Barr virus genome in throat washing samples (Jeng et al., 1994; Bazzichiet al., 1998).

The findings obtained with the present study corroborate those from previous studies (Berger et al., 2006; Białasiewicz et al., 2009; Patel et al., 2008; Sundsjord et al., 1994b), and suggest that oropharyngeal fluids are an unlikely source of viral particles for JCV transmission.

However, this assumption does not rule out the hypothesis that tonsils represent the site of initial JCV infection (Monaco et al., 1996, 1998). Irrespective of the origin of viral particles, JCV may enter the organism through the respiratory route, primarily replicating in the tonsil tissue, and thereafter being disseminated throughout the organism until reaching target organs and tissues.

Recent studies reported the detection of high quantities of JCV in urban sewage samples from different geographical areas (Ahmed et al., 2010; Bofill-Mas et al., 2001, 2010; Bofill-Mas & Girones, 2001, 2003). The human JCV in urban sewage probably came from excreted virus in urine, since JCV has been detected in the urine of more than 50% of adult individuals (Ling et al., 2003; Matos et al., 2010; Zhong et al., 2007). Furthermore, recent studies also reported that 1.8–9.1% of individuals excrete JCV in stool samples (Białasiewicz et al., 2009; Vanchiere et al., 2009), which could also contribute to the frequency and quantity at which JCV is present in sewage. Some authors argue that the presence of JCV in sewage (Bofill-Mas et al., 2001) along with the relative stability of this virus under environmental conditions (Bofill-Mas & Girones, 2001; Bofill-Mas et al., 2000) may point to contaminated water and food as the vehicles of JCV transmission through the oral route, followed by initial replication in the tonsil tissue (Bofill-Mas & Girones, 2003; Hamza et al., 2009).

The results of the present study are in agreement with those previous observations. In summary, this study points towards the absence of JCV DNA from the oropharyngeal samples of patients infected with JCV, suggesting that JCV viral particles from different origins, other than ororespiratory secretions, are implicated in the virus transmission.

However, the true prevalence of JCV DNA may be underestimated by this analysis, once only a single oropharyngeal washing sample was obtained from each subject, which may not represent the exact period of JCV excretion by this route. As it has been reported for CMV infection (de Mello et al., 1996; Zanghelli et al., 1999; Stackhouse et al., 1991), the excretion of JCV in respiratory secretions by infected patients, might also be transient, and not related to antibody titre or excretion of the virus in urine.

Therefore, subsequent studies should be performed including a greater number of patients infected with JCV, either infected with HIV or immunocompetent healthy individuals, during a longer follow-up period, and with multiple samples collected. Moreover, a major contribution would be the inclusion of PML patients, once they represent the group of individuals with confirmed active viral replication, therefore more likely to excrete viral particles. Also, the evaluation of both children and adults with detectable IgM for JCV, or experiencing a rise in antibody titre, which could represent the primary infection or the reactivation of JCV, could be of interest, in the attempt to try and elucidate the transmission route of this cosmopolitan polyomavirus.

**Acknowledgements**

The authors are grateful to the Center for Pharmaceutical Studies of the Portuguese Foundation for Science and Technology through POCTI (FEDER) for financial support.

**References**


