Reviewer’s report

Title: Merkel cell polyomavirus and trichodysplasia-spinulosa-associated polyomavirus DNAs and antibodies in blood among the elderly

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Reviewer: Els van der Meijden

Reviewer’s report:

- Major Compulsory Revisions

1. The question posed by the authors in the Background section is not well defined for TSPyV. The authors state that additional data on MCPyV and TSPyV epidemiology are needed in the population at risk for MCC, elderly persons, regarding serum antibody responses and genome prevalence. For TSPyV epidemiology on elderly people, the population at risk for MCC, is not as important as it is for MCPyV. TSPyV is associated with trichodysplasia spinulosa (TS) and the described TS cases are immunosuppressed transplant patients or leukemia patients, often children. Nevertheless, this study is of interest for TSPyV, because of the large sample tested for TSPyV DNA. By my knowledge this is the first manuscript showing (negative) TSPyV DNA data of such a big population. So, the authors should mention the latter as an additional aim of this study.

2. The study population comprised 394 patients with respiratory symptoms or suspected pneumonia. 621 blood samples were collected from these patients and all of these samples were tested for the presence of MCPyV and TSPyV DNA, whereas 481 serum samples were tested for MCPyV and TSPyV serology. The reason for testing 2 serum samples per patient is not well described and, while doing so, information about virus persistence is missing. Furthermore, the seroprevalences and the MCPyV DNA prevalences are only presented as percentage of the total amount of blood samples tested but it would be equally informative to show the percentages for the total number of patients. Especially because the authors mention in the Discussion that MCPyV DNA appeared in low copy numbers in many aging individuals. The way the data are presented now, it is not clear who these individuals are.

3. In the Associations between patient characteristics and MCPyV DNA positivity section and in Table 3 it is not clear what the reference group is for odds ratio measurement. This should be indicated in the main text or in the legend. Furthermore, the authors should include a paragraph in the Methods section describing the statistical calculations and tests used, and the used software package.

- Minor Essential Revisions

4. Trichodysplasia spinulosa are two separate words.
5. In the Abstract the authors state that, like TS, MCC is associated with immunosuppression. However, for MCC the immunosuppression status is not as strict as it is for the development of TS. This subtle difference should be addressed in the Background.

6. In the Background section the authors mention that MCPyV DNA is detectable in cutaneous swabs from clinically healthy subjects at a prevalence of 40 to 100% [13, 14]. This range is too broad, in most MCPyV DNA prevalence studies, a prevalence between 40-60% is described in healthy individuals. And even in references 13 and 14, a prevalence of 100% is not found.

7. The following sentence in the Methods section is not correct: Two published primer sets targeting conserved sequences in the MCPyV genome, the large T antigen (LT) gene, and the viral capsid protein (VP1) gene (Table 1) were performed according to Goh et al [28]. The sentence should be: Two published primer sets.......were used [28].

8. In the Methods section the authors say that the MCPyV PCR could detect 200 copies/ml. Include information about sensitivity of the TSPyV PCRs.

9. The MCPyV and TSPyV serology part in the Methods section is limited, the authors refer to references 21 and 26. Are the cut-off values used in this study to determine MCPyV and TSPyV seroprevalences the same as the described cutoff values in reference 21 and 26? Please describe the cutoff values if they differ to the published ones.

10. In the MCPyV and TSPyV qPCR section the authors mention that they couldn't detect any TSPyV DNA in the blood samples. It would be interesting to know what the sensitivity level is of the TSPyV VP1 and LT PCR (see comment 8).

11. Have the authors looked for associations between MCPyV DNA positivity and seropositivity and between MCPyV DNA positivity and low and high seroresponders? Additional information on this subject would increase the value of this manuscript.

12. 6.2% of the blood samples were positive for MCPyV LT DNA and 5.5% for MCPyV VP1 DNA with low copy numbers. The authors must keep in mind that a proportion of the blood samples might be contaminated with MCPyV DNA present on the skin when taking a blood sample. This should be added to the Discussion.

13. In the Discussion section the authors explain the negative TSPyV DNA findings as the result of short duration of TSPyV viremia. The authors should also discuss the negative TSPyV DNA findings as the result from too low TSPyV DNA loads to be picked up. This brings me back to the unknown sensitivity of the TSPyV PCRs (see comments 8 and 10).

- Discretionary Revisions
14. In the Background section MCPyV DNA prevalence is extensively described but the TSPyV DNA prevalence part could be extended. The presence of TSPyV DNA in 4% of 69 renal transplant patients was found in DNA isolated from eyebrow hairs. A recent publication (Kazem et al.: Trichodysplasia spinulosa is characterized by active polyomavirus infection, J Clin Virol Mar;53(3):225-30. Epub 2011 Dec 22) describes high TSPyV DNA prevalences and loads in TS patients compared to low prevalence and loads in skin swabs from healthy individuals.

15. In the MCPyV and TSPyV qPCR section it is written that 73 blood samples are positive for MCPyV DNA with a low load. The average Ct value is depicted but it would also be informative to include the range in Ct values.

16. In the MCPyV and TSPyV qPCR section the authors mention that the sequenced MCPyV products have a high similarity to published MCPyV sequences. Does this also mean that all sequenced MCPyV products from the patients are identical or could different MCPyV strains be identified among the positive patients?

17. In the Results section associations were calculated between MCPyV DNA positivity and 3 patients groups (table 3). It would also be informative to show the serology data for these 3 groups as well. It would be interesting to see whether the seroprevalence in the respiratory disease group is increased compared to the other groups.

18. In the MCPyV and TSPyV serology section the seroprevalences for MCPyV and TSPyV are showed in the different age-groups (Figure 1). Measured antibody levels, however, are not shown, whereas they might tell something about possible waning immunity of the elderly, especially when a decrease in seroresponses is observed with age.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests