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

Authors:

Mohammadreza Sadeghi (Reza.sadeghi@helsinki.fi)
Matti Aronen (moaron@utu.fi)
Tingting Chen (Tingting.chen@helsinki.fi)
Laura Jartti (lauvii@utu.fi)
Tuomas Jartti (ttjartti@utu.fi)
Olli Ruuskanen (Olli.Ruuskanen@tyks.fi)
Maria Söderlund-Venermo (Maria.Soderlund-Venermo@helsinki.fi)
Klaus Hedman (Klaus.hedman@helsinki.fi)

Version: 2 Date: 26 November 2012

Author's response to reviews: see over
Dear Editor(s),

We are thankful for the reviewers’ constructive comments that greatly helped us to improve and clarify our manuscript "Merkel cell polyomavirus and trichodysplasia spinulosa-associated polyomavirus DNAs and antibodies in blood among the elderly" by Sadeghi et al. (MS: 1493508706809805), and we appreciate the possibility to resubmit the paper to the *BMC Infectious Diseases*. We have responded to each of the points raised and hope that the revised version answers the reviewers’ concerns.

The comments by the reviewers are addressed point by point below and our responses are in red.

Thank you for considering our resubmission.

Yours sincerely,

Klaus Hedman (on behalf of all the authors)

Klaus Hedman, M.D., Ph.D.
Professor of Clinical Virology
Dept. of Virology, Haartman Institute, and
Infection Biology Research Program
PO BOX 21 (Haartmaninkatu 3)
FIN-00014 UNIVERSITY OF HELSINKI
Finland

Phone: +358-9-1912 6473
Mobile: +358 50 5249 086
Fax: +358-9-1912 6491
e-mail: klaus.hedman@helsinki.fi
Reviewer 1 (8687219338231362):

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.

As suggested, this part has now been revised (p. 6, last paragraph).

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.

All these comments are now addressed in the revised manuscript. The reason for testing 2 serum samples per patient has now been added (p. 8, Sample collection). Antibody positivity vs. negativity of all patients remained the same in follow-up (p.
Furthermore, the MCpyV DNA prevalences now apply to the percentages of patients; the same now also holds for the IgG prevalences (P. 11, MCpyV and TSPyV qPCR).

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.

As suggested, this has now been done on page 12, the Associations between clinical characteristics and MCpyV DNA positivity, and in the legend of Table 3.

-Minor Essential Revisions

4. Trichodysplasia spinulosa are two separate words.

Thank you; this has now been corrected throughout the manuscript.

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.

As suggested, this has been revised in the Abstract and Background of the revised manuscript.

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.
This has now been corrected (P. 5, Background second paragraph).

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].

As advised, this has been corrected (P. 9, second paragraph, line 3).

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.

The sensitivity of the TSPyV PCR was equal to that of the MCpyV PCR, which is now stated in Methods (page 10, first paragraph, last line).

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.

As suggested, we now briefly describe the antibody assay (P. 10, MCpyV and TSPyV serology). We also give the cutoff values, which were the same as those published.

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).

The two PCRs were equally sensitive; the information on the TSPyV PCR sensitivity has been added onto Methods, page 10, first paragraph, last line.
11. Have the authors looked for associations between M CPyV DNA positivity and seropositivity and between M CPyV DNA positivity and low and high seroresponders? Additional information on this subject would increase the value of this manuscript.

We now compare the seropositivities, as well as the antibody levels, with the viral DNA results in Results (page 12, last line) and address these findings in Discussion (p. 14, third paragraph).

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

Highly appreciating this suggestion from both of the Reviewers, the possibility of DNA contamination via needle sampling is now discussed on Page 14, third paragraph.

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).

As mentioned in comment number 8, we have now added in Methods the TSPyV PCR sensitivity. Being the same with both assays, five copies per reaction corresponding to 200 copies/mL of serum, the sensitivity is appropriate with no need of further discussion, as we trust that the Reviewer agrees.
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.

As suggested, the TSPyV DNA prevalence part has been extended (P. 6).

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.

As suggested, the Ct value range has been added (P. 11 MCpyV and TSPyV qPCR).

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?

Two published primer sets targeting short MCpyV sequences in conserved regions of the large T antigen (LT) gene and the viral capsid protein (VP1) gene (Table 1) were used according to Goh et al. [32]. The sequences of the samples showed high homology (100%) to each other and also to the previously described sequences (P. 11 MCpyV and TSPyV qPCR).

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.

Associations between clinical characteristics and MCPyV and TSPyV seroprevalences have now been added in Results (page 13).

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.

We found no significant correlation between antibody levels and increasing age (P. 12, MCPyV and TSPyV serology).

Reviewer: 21912245896821589

Both Merkel cell polyomavirus (MCPyV) and Trichodysplasia Spinulosa associated virus (TSPyV) are newly described human polyomaviruses beside also HPV6, 7, 9 and more recently HPV10 (close to Malaw Ivy). These two viruses appear to be associated to respectively MCC and Trichodysplasia Spinulosa, two rare diseases in immunocompromized patients. This was further extensively confirmed by others. However, these viruses (mainly MCPyV) may be also present as innocent bystander and there is now an abundant literature that described the presence of MCPyV DNA in various clinical situations (non melanoma skin cancer, KSV... ...). Furthermore, many studies have reported a significant detection of the viral DNA in the skin of healthy persons and serologic investigations have shown that most individuals have been infected by these two viruses with high seroprevalences as common features for MCPyV and TSPyV as well as for the other new human polyomaviruses, HPV6, HPV7 and HPV9 thus supporting the hypothesis of highly ubiquitous viruses.
The epidemiologic investigation of Sadeghi et al. is clearly in line with these above statements and does not provide significant new additional data. Serological data are confirmatory and molecular detection of MCpYV DNA in the blood of elderly is in line with the similar frequent detection in various subset of immunocompromised patients that is further ore a common feature of human polyomaviruses.

We do not fully agree with this comment. To our knowledge this is the first sizeable material of serum that has been studied for the presence of MCpYV and TSPyV among aging individuals, in order to determine whether these viruses appear in this high risk population. Furthermore according to Reviewer 1 in major compulsory comment number 1, “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”.

1- Since MCpYV is common on skin surfaces of most individuals with in some patients high viral load, could the low level of detection in blood be a consequence of the sampling venipuncture?

We thank the Reviewer for this proposal, and now bring it up on Discussion, page 14, second paragraph. Please also see our answer to point 12 of Reviewer 1.

2- Previous discrepancies described in the literature between LT and VP PCR where mostly explained regarding the likely integrated of not status of the virus in tumor samples rather than sensitivity differences. This is more unlikely with the expected episomal presentation of the virus in blood.

The discordance between LT and VP1 positivity has also been reported in non Merkel cell tumor or samples, e.g. in these references of our manuscript:


3- The link between VP1 detection and respiratory diseases is confusing and need clarification ...

As suggested, this has now been done on page 12, the Associations between clinical characteristics and MCPyV DNA positivity, and in the legend of Table 3.

4- It would have been of interest to compare antibody levels with the detection of viral DNA,

We now compare the antibody levels with the viral DNA finding in Results (page 12, last line) and address the findings in Discussion (P.14, third paragraph).

ADDITIONAL CHANGES TO THE MANUSCRIPT

In addition, we have made some improvements on the English of the manuscript.

We hope that with these revisions our manuscript is acceptable for publication.

Thank you for considering our resubmission.