Author's response to reviews

Title: Evidence for HSV-1-induced pneumonitis in patients under standard immunosuppressive therapy for rheumatic and vasculitic autoimmune disease

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Author's response to reviews: see over
To
Dr. Melissa Norton, M.D.
Editor in Chief
BMC Pulmonary Medicine

Re: Manuscript ID CLTX-08-CR-0096

Dear Dr. Norton,

Thank you for the generally positive review and the opportunity for us to resubmit a revised version of our manuscript “Evidence for HSV-1-induced pneumonitis in patients under standard immunosuppressive therapy for rheumatic and vasculitic autoimmune disease” (Manuscript ID BMC Pulmonary Medicine MS: 6105323142346202).

Please find enclosed a point-by-point answer to each of the comments of the referees, a detailed list of changes made in the manuscript, and the revised version of the manuscript.

We have included important new statistical data as well as clinical/histologic images as requested by the three referees. We hope that the new presentation of our retrospective study and the incorporation of the considerations raised by the reviewers improved the manuscript so that it may now be acceptable for the readership of BMC Pulmonary Medicine.

Thank you for your time and consideration

Sincerely

PD Dr. med. Holger Schmid
List of changes made in the revised version of the manuscript (ID BMC Pulmonary Medicine MS: 6105323142346202):

Reviewer #1
We highly appreciate the important suggestions and have addressed them as follows:

1. A lack of stated study rationale, lack of stated study goal, and lack of focus was criticised. The rationale of the study was that while HSV-1 pneumonitis has been described in cancer, transplant, and HIV patients, no such reports and no systematically acquired data exist on rheumatologic/vasculitic patients, although this is an important patient population. The goal of the study was to retrospectively study the role of HSV-1 in ambulatory-acquired pulmonary infections of outpatients under a maintenance immunosuppressive regimen for autoimmune diseases. The questions were with regard to the frequency of HSV-1 and if HSV-1 occurs as the sole identifiable agent or as a bystander of other infections. To this end, we took advantage of the relatively large body of patients affiliated with our rheumatologic and nephrology outpatient clinic at the University of Munich, which comprises approx. 1080 patients with rheumatoid arthritis/ANCA-vasculitis, and retrospectively studied a period of 8 years. We have now stated these facts in a more succinct manner. In our view, the fact that the significance of HSV-1 in pulmonary infections is difficult to dissect is an additional take-home message that is stressed throughout the manuscript.

According to suggestions by the other two referees, we completely re-evaluated our entire set of data with respect to analyzing all admissions to all our affiliated hospitals. Thereby, we were able to identify one additional patient with Wegener’s disease and positive HSV-1 detection. We have now also included patients with systemic lupus erythematosus (approx. 320 clinic patients) in addition to patients with rheumatoid arthritis and ANCA-vasculitis, yielding another patient with SLE and HSV-1. In the years 2000-2007, 766 episodes of hospital admission of rheumatoid arthritis/ANCA-vasculitis/SLE patients are recorded, 63 of which occurred because of pneumonia/pneumonitis (in 63 patients; 1 episode/patient). 23 of these 63 patients were subjected to BAL, and in 14/63 PCR analysis for HSV-1 was performed. 6 patients had a positive HSV-1 PCR result and are presented in the revised Table 1, which now contains combined information from former Figure and Table 1. The six cases represent the etiologic spectrum from supposed isolated HSV-1 pneumonitis to HSV-1 as presumable bystander of other infections. We can conclude, that respiratory deterioration and pneumonia leading to hospital admission was a common event in outpatients with autoimmune diseases treated with current standard immunosuppressive regimens. Acute life-threatening respiratory failure associated with the detection of HSV-1 in the lower respiratory tract was a rare, but significant finding. As requested, we have eliminated the statement that immunosuppression for autoimmune disease was a risk factor for HSV-1 pneumonitis.

We agree that condensation of the manuscript in order to achieve more focus was necessary. Therefore, we have tried to reduce redundancies and to eliminate unnecessary information throughout the manuscript.

2. More information on pathological findings on bronchoscopy was requested. According to these useful suggestions, the bronchoscopic/histologic findings are now stated in more detail in Table 1. We have included a representative bronchoscopic image from Case 1 (Figure1C). In addition, an image of HSV-1 antigen staining in a BAL cytospun from Case 2 is shown. Unfortunately, our Pathology archive could not retrieve photographic documentation or tissue featuring inclusion bodies from one of our 6 HSV-1-positive cases.
Reviewer #2
We highly appreciate the constructive suggestions and have addressed them as follows:

1. As suggested, we have completely re-evaluated our entire set of data with respect to analyzing all admissions to all our affiliated hospitals (see also Comment 1 to Reviewer #1). Thereby, we were able to identify one additional patient with Wegener’s and positive HSV-1 detection on BAL. We have now also included patients with systemic lupus erythematosus (approx. 320 clinic patients) in addition to patients with rheumatoid arthritis and ANCA-vasculitis, yielding another patient with SLE and HSV-1 detection on BAL. In the years 2000-2007, 766 episodes of hospital admission of autoimmune patients are recorded, 63 of which occurred because of pneumonia/pneumonitis (in 63 patients; 1 episode/patient). 23 of these 63 patients were subject to BAL, and in 14/63 PCR analysis for HSV-1 was performed. PCR for HSV-1 in BAL was a routinely performed diagnostic procedure for evaluation of viral pneumonitis in this cohort. Together, we now describe 6 patients with a positive HSV-1 PCR result.

2. We agree, that Reference 28 is a report concerning an immunocompetent patient. It was initially given to make the point that an inverse mechanism, i.e. viral HSV-1 tracheobronchitis leading to microaspiration and subsequent bacterial pneumonia rather than HSV-1 becoming reactivated in the course of a bacterial pneumonia has been postulated by some authors. We agree that the citation was misleading and have eliminated it in the revised version of the manuscript.

3. We agree that the original phrasing concerning the average viral loads was not clear enough. We have clarified this section: “…showed a viral load (9.750.000 Geq/ml) that was 3 and 13-fold greater than the detected average (3.700.000) and median (775.000) of all cases, respectively,…”

4. In the autoimmune outpatient cohort that was admitted to our hospital and subsequently diagnosed to have pneumonia/pneumonitis (63 patients in the study period), acute life-threatening respiratory failure associated with the detection of HSV-1 in the lower respiratory tract was a rare, but significant finding. In approximately 10% of these patients, pulmonary infection was associated with the detection of HSV-1. All subsequent statements are qualified accordingly.

5. According to this useful suggestion, we merged all data from former Figure 1 and Table 1 into new Table 1 in the revised manuscript.

6. The textual remarks were followed and the spelling errors corrected. The references were corrected according the BMC Pulmonary Medicine reference style, using EndNote.
Reviewer #3
We highly appreciate the constructive suggestions and have addressed them as follows:

1. The question was raised on how many patients out of 1080 had respiratory deterioration, and how many were tested for HSV-1 in total (see also Comment 1 to Reviewer #1). As suggested by this useful remark, we have completely re-evaluated our entire set of data with respect to analyzing all admissions to all our affiliated hospitals. Thereby, we were able to identify one additional patient with Wegener’s disease and positive HSV-1 detection. We have now also included patients with systemic lupus erythematosus (approx. 320 clinic patients) in addition to patients with rheumatoid arthritis and ANCA-vasculitis, yielding another patient with SLE and HSV-1 detection in BAL. Now, 766 episodes of hospital admission of autoimmune patients are recorded, 63 of which occurred because of pneumonia/pneumonitis (in 63 patients; 1 episode/patient). 23 of these 63 patients were subject to BAL, and in 14/63 PCR analysis for HSV-1 was performed. PCR for HSV-1 in BAL was a routinely performed diagnostic procedure for evaluation of viral pneumonitis in this cohort. Together, we now describe 6 patients with a positive HSV-1 PCR result.

2. The question was raised on what basis decisions for viral testing (influenza, adenovirus) were made. Indeed, influenza testing was mainly based on seasonal plausibility, i.e., it was performed for Cases 3 and 5 (January and February, respectively), while it was not performed for Cases 1, 2, 4, and 6 (May, April, September, and June, respectively). Microbiological/virological testing was requested by the physician who performed bronchoscopy and BAL. Therefore not always an identical selection of pathogens was tested for, as illustrated for adenovirus. We have included the seasonal information in Table 1 at the segment of influenza. We have also included a footnote for adenovirus commenting on the irregularity of testing.

3. The reviewer mentioned that there are a lot of confounding variables that can influence detection sensitivity for bacterial infections in BAL. Indeed, our analysis of the spectrum of agents detected in the 63 patients with pneumonia/pneumonitis in our study revealed that in 60% of cases no pathogen could be identified (see Figure 3A). This stresses the remarks made by the reviewer. In addition to this, we are now commenting on the possibility of false-negative detection of another bacterial/fungal/viral agent in Cases 1 and 2 which would alter the conclusion that they represent an entity of isolated HSV-1 pneumonitis.

4. We have expanded the written documentation on BAL and cytology-findings in Table1. We have also included a picture of immunohistochemical HSV-1 staining of an infected cell from a BAL cytospun that was taken from Case 2. Unfortunately, our Pathology archive could not retrieve photographic documentation or tissue featuring inclusion bodies from one of our 6 HSV-1-positive cases.

5. We completely agree, that it is highly doubtful, that the perianal ulcer in Case 2 was linked to HSV-1 reactivation. Therefore we have eliminated this information from the revised manuscript.