Author's response to reviews

Title: Burden of severe RSV disease among immunocompromised children and adults: a 10 year retrospective study.

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Author's response to reviews:

Replies to the reviewers' comments

We thank the editor and the reviewers for his/her comments and suggestions. The reviewers' comments are indicated in bold with their page and line numbers (from the original manuscript) retained. However, please note that the page and line numbers in our responses to reviewers' comments refer to our numbering in the revised manuscript. Please note that we have done our best to include all information requested by all reviewers, which unabled us to bring the word count down to 2500 words (2936 words).

Reviewer 1

▪ MAJOR COMMENTS

1) ABSTRACT P.2, LINES 28-32

“Authors should state clearly in the manuscript text that inclusion of both outpatient and inpatient individuals in the study. “

We have followed this suggestion in the revised manuscript (p. 2, ll. 10).

2) TABLE 1:

“To emphasize more on such category, consider re-segregation of Table 1 into outpatient and inpatient.”

We have and re-segregated Table 1 into in and out-patients within each subgroup: children and adults. For clarity purposes we have provided the information on inpatients for ALL variables.

“There are a lot of information in Table 1 that were not even described raising concerns on their importance/significance to the study (e.g. ANC, ALC). “

Table 1 refers to patient-baseline characteristics. As such, we provided detailed information on the population such as type of immunosuppression, GVHD, ANC. Given our small sample size and thus the limited number of variables we could possibly include in our regression models, we only included those variables that were significant in univariable analyses and a priori of clinical significance such as age, bacterial co-infection, nALC and immunosuppressive categories. The remaining variables such as ANC or GVHD were not significant in univariable analyses. Therefore, we believe that the non-inclusion of these variables did not affect our findings.

3) VIROLOGY:

“Results from the virology studies seem to be under appreciated. Authors concluded that bacterial co-infection was a significant determinant of associated LRTI and pneumonia but failed to acknowledge the role of viral co-infection in such conditions or in the exacerbation of the disease burden. Table 1 also shows that there are more viral than bacterial co-infections overall. Immunocompromised patients are also known for prolonged shedding of pathogens (e.g., virus shedding) requiring extended antibiotic treatments. Were these sought for in the patients? “

We absolutely agree that the role of viral co-infections should be accounted for disease severity such as hospital admission and LRTI. Although a recent systematic review with meta-analysis (Asner et al; PLos One, 2014) did not support differences in severity between viral co-infections and single infections, another observational study (Asner et al, Clin Microbiol Infect, 2015) supported that children co-infected with RSV and another virus, presented with increased rates of pneumonia, likely as a result of increased inflammatory markers induced by the presence of multiple viruses thus favoring progression to pneumonia.

In the present study, viral co-infections were not included in multivariable analyses as it was not significantly associated with the outcomes of interest in univariable analyses. Furthermore we performed post hoc sensitivity analyses excluding all 52 patients with viral co-infections, in order to evaluate the robustness of the estimates related to the potential differences in disease severity. As summarized in our supplementary Addendum Table 4, the exclusion of viral co-infections did not affect our risk estimates. Therefore, we believe that the association between our predictors and the outcomes was not affected by viral co-infections.

However, this important suggestion has been added in the revised manuscript (p. 10, ll. 23-28 and p.11, ll 1-2) and referred to as a potential limitation (p.12, ll.13-16). Please also refer also to supplementary Addendum Table 4

“Immunocompromised patients are also known for prolonged shedding of pathogens (e.g., virus shedding) requiring extended antibiotic treatments. Were these sought for in the patients?”

We also agree that immunocompromised patients shed viruses for a prolonged period. Persistence of viral co-infection was only documented among patients with persistent respiratory conditions. Furthermore, the retrospective nature of our study limited the number and the quality of the information obtained. Finally, we limited the collected information to 30 days of the diagnosis of RSV infection.

4) RSV-ATTRIBUTABLE HOSPITAL ADMISSION

“The term RSV-attributable hospital admission or disease is also a misnomer in this study because of the presence of bacterial and viral co-infections in a number of their samples. The analysis of comparators should have been chosen amongst those without any other identifiable virus/pathogen to attribute disease burden solely to RSV.”

We appreciate both comments. Table 2 separates “all-cause hospital admission”, which includes patients admitted for other reasons (e.g chemotherapy) than a respiratory condition from those admitted for respiratory symptoms with a concomitant documentation of RSV in NP swabs, defined as ‘’RSV-attributable hospital admission.’ To avoid any further confusion and provide consistency with point 3) we replaced ‘’RSV-attributable hospital admission” with “acute-respiratory-tract infection (ARTI)-attributable hospital admission” throughout the manuscript and the tables. We agree that having a comparator group of patients without any identifiable virus or bacteria would have clarified the attribute disease solely to RSV. Unfortunately, the retrospective nature of our study did not enable the use of this comparator group. We have stated this comment as a limitation

p.12, ll. 16-18

5) ANALYSES

“There should be adjustment in the analysis for other confounders like comorbidities”; AND Page 9, lines 45-56 “Would other underlying medical conditions (e.g., diabetes, obesity, etc) also be factors for the severe disease in adult patients (aged 42-64 y/o) compared to children?”

None of the included children presented associated co-morbidities as children with congenital heart disease were excluded, by definition, of our study population because of their eligibility to receive palivizumab. No information regarding additional co-morbidities such as diabetes obesity was collected from our adult patient-population. This information has been added in the revised manuscript p. 10, ll.15-17.

“The authors should also make it clear why their analysis in Table 3 and 4 were only done for 215 patients compared to the 239 overall positive samples? Additionally, Table 1 and 2 are showing N=175 for adults but about 5 have missing values and were not included in calculations which makes the data presentation confusing to follow. Would weeding out the 5 samples from total N have a significant impact on the overall outcome/conclusions?.

We thank the reviewer for their very valuable comment. We favored manual exclusion of cases with missing data over automatic procedures and proceeded with “complete cases analyses” with the hypothesis that data were missing completely at random (MCAR), as we wanted to ensure that the same patients with missing data would be excluded from univariable and multivariable models. From the 239 patients (175 adults and 63 children) we excluded a total of 24 patients (23 adults and 1 child) with missing data on nALC. As such, from the 239 patients included, we analyzed data from the same total of 215 patients (151 adults and 63 children) in univariable and multivariable analyses. We believe that the exclusion of patients with missing data, including those 5 samples mentioned above from the total number did not affect our risk estimates.

- MINOR COMMENTS

1) “Title should be re-written as "Burden of severe RSV disease among immunocompromised children and adults: a 10-year retrospective study"

We have followed this suggestion in the revised manuscript

2) “Page 3, lines 43-48. Consider revising this statement. It is unclear as it is written.”

P3; ll.18-19 the following sentence has been suppressed. “As such, RSV infection could result in CLAD although further studies should better delineate the contribution of RSV in the pathogenesis of CLAD.”

3) “Page 8, lines 14-16. Requires revision for grammatical error.”

The sentence has been corrected.

Reviewer 2

“To not only focus on adults within the introduction and discussion section, I suggest to incorporate the following papers: Paediatric Respiratory Reviews 10 (2009) 148-153; N Engl J Med. 1986 Jul 10;315(2):77-81; Influenza Other Respir Viruses 2016 May;10(3):205-10; J Immunol Res. 2014;2014:850831.”

As suggested, we have incorporated the suggested papers within the introduction (p.3, ll 3-5) and discussion (p. 11, ll. 6)

Reviewer 3

- MAJOR COMMENTS

Expand, re-write the methods section

1) METHODS

"What was the primary selection criteria for study participation? Immunocompromised patients who were RSV positive or RSV positive patients that had an underlying immunocompromised condition? "

The primary selection criteria for study participation was RSV-positive patients that had an underlying immunocompromised condition. We have provided this information in the revised manuscript, methods section (p. 4; ll. 7-9)

"What was the initial study size regarding just immunocompromised patients? "

Given the lack of an immunocompromised cohort, with the exception of the Swiss- National Transplant cohort, we cannot provide the initial study size. Regarding solid-organ transplant recipients, it is difficult to estimate the denominator of all patients followed at both centers. Overall, during the study period there were more than 1300 SOT recipients performed between Lausanne and Geneva. Patient mortality is approximately 25% at 10 years, all organs confounded. Some of these patients are currently followed at other centers. As a result, we cannot provide data on RSV infections from all patients with SOT.

"What was the initial study size regarding RSV positive patients?"

From January 2005 to December 2014, a total of 17'208 respiratory samples (NP swabs, BAL) were submitted for RSV detection. From those, 4'605 samples collected from 3'223 patients (initial study size regarding RSV-positive patients) were detected positive for RSV of whom 239 (7.4%) presented with an underlying immunosuppressive condition.

"How many study eligible patients were RSV negative? and why? lack of laboratory viral testing results?" "Need to add information regarding this group. Possibly add a study inclusion flow chart."

From our Laboratory data, 12'603 respiratory samples were tested RSV-negative by any diagnostic method (antigen assays and PCR). Unfortunately, we were not able to provide information on the number of RSV-negative eligible patients given the retrospective nature of

our study, the number of different immunosuppressive conditions included and the inclusion of outpatients.

Please refer to the flow diagram inserted included in our supplementary data (figure 1).

VIROLOGY

"Virology section is well described - assuming that RSV testing was performed on all immunocompromised patients presenting or developing respiratory symptoms"

Screening for respiratory viruses, including RSV, is recommended for all immunocompromised patients presenting with respiratory symptoms at both university centers of Lausanne and Geneva. However, the retrospective design of our study, which relied on Laboratory-based data rather than prospectively collected data, possibly affected the quality of the data collected as the motivation for respiratory viral screening was not clearly stated. However, we believe that the use of standardized guidelines for respiratory viral testing at both sites ensured that most of immunocompromised patients with respiratory symptoms were screened for respiratory viruses.

3) STATISTICAL ANALYSES

"Statistical analyses section needs significant expansion in describing statistical methods used in this study - especially all regression analyses. This includes the multi-regression method used, the level of significance for multiple regression, define (in clear terms) the dependent and independent variables used in the multi-regression analyses (include the univariate results showing which independent variables to include), include sample size numbers for the initiation of multi-regression and the final sample size included in the final model."

We thank the reviewers for their comments. We have specified all requested points relevant to statistical analyses (p.6; ll.13-21). We used logistic univariable and multivariable analyses with a level of significance for multiple regression of $P < 0.05$. Dependent variables included ARTI-attributed hospital admission as our primary outcome of interest and presence or absence of LRTI or pneumonia as our secondary outcomes of interest. Independent variables included in our

univariable models were: Age, bacterial co-infection, ALC and immunosuppressive categories for “all patients” and the adult subgroup, whereas only bacterial co-infection and ALC were included in the children subset as a result of limited observations.

We selected independent variables a priori based on their clinical relevance. Variables showing statistical significance in univariable models were subsequently included in multivariable models. Tables 3 and 4 summarize risk estimates for univariable as multivariable analyses. As specified above, the same sample size for univariable and multivariable models was determined by the number of complete cases observations (215 from the total number of 239 patients included) as 24 cases (23 adults and 1 child) had missing nALC values.

“It is unclear why patients with missing data were excluded from statistical analysis when regression methods automatically exclude data records with missing data points. This will affect the level of significance used to properly identify significant covariates.”

Please refer to REVIEWER 1, 5) ANALYSES.

4) RESULTS

After clarifying the statistical methods used, we believe that the result section, as originally written should reflect our findings.

5) DISCUSSION

“Discuss the overall burden of RSV among health-seeking populations”

A ten year retrospective study in which only 239 subjects were study eligible - this suggests that among a general population, RSV has only a slight burden and only among a small sub-set of immunocompromised patient categories. “

We thank the reviewer for this excellent comment. Given the lack of an active surveillance system of RSV in our country, the burden of RSV-attributable cannot be precisely appreciated. Therefore, the number of 239 RSV-positive immunocompromised adults collected over 10 years might only suggest burden among a small subset of immunocompromised patients but could also result from an inadequate active surveillance system. Please refer to p. 9-10, ll. 21-25; ll.1-5 and p.11-12, ll. 25-27; ll.1-3.

6) CONCLUSION

"RSV infection lends itself to significant morbidity and mortality among immunocompromised patients, both adult and pediatric. However, the numbers and analyses reported in this study appear to support that premise in a very small sub-set of patients. "

We thank the reviewer for this comment and have modified our conclusion accordingly. (p.11, ll. 25-27 and p.12 ll.1-3)

REVIEWER 4

1. ABSTRACT

"As an editorial point, I am not sure why the abstract is written as sentence fragments rather than full sentences. Please revise. "

We thank the reviewer and have modified the abstract accordingly. (p. 2, ll 6-8; 10 and 22-23)

2. INTRODUCTION

We have modified the sentence as suggested by the reviewer. (p.3, ll.4-5)

We have deleted the sentence (p. 3, ll. 18-19)

3. METHODS

“Please provide more information on how persons were determined to have included as having the different conditions listed.”

Extensive chart review from health records provided information on the different immunosuppressive conditions listed.

“Please list the relevant baseline characteristics described on page 5, line 3.”

We listed relevant baseline characteristics (p. 5; ll 5-7)

“It is difficult to determine the appropriateness of the multivariate logistic model, because it is not described. Are all the covariates used listed in the tables? I also did not fully understand how age in 10 year increments was incorporated in the model, but in the table it is divided as child/adult.”

Please refer to Reviewer 3, statistical analyses.

We have addressed this suggestion in the revised manuscript (p 6, ll 13-21)

Our Tables provide information on all patients with subsequent stratified analyses for children and adults. Risk estimates for variables included in univariable and multivariable models were reported in Tables 3 and 4. Dashes (-) referred to the non-inclusion of the variable in multivariable models for the children subgroup as a result of a limited sample size. Age in 10 years was reported as a continuous variable. We chose the scale of 10 years range for commodity and clarity reasons.

4. DISCUSSION

“Do you expect that varying levels of error rates with rapid testing/PCR impacted your outcomes? Why or why not?”

PCR diagnostic assays are known to be up to five times more sensitive than antigen assays although good sensitivity of antigen assays has been reported from pediatric populations. Given the different diagnostic procedures used, we performed post-hoc sensitivity analyses excluding children detected by rapid antigen assays, which did not affect our risk estimates. Therefore, we believe that our findings were still suggestive of worse clinical outcomes among our RSV-infected adult cohort.

Please refer to p. 12, ll 3-10.

Please refer to our supplementary addendum tables 6 and 7 for detailed information.