

Correlates of Risk of Respiratory Syncytial Virus Disease: A Prospective Cohort Study

Corresponding Author: Ms Collrane Frivold

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

With interest I read the manuscript entitled: "Correlates of Protection Against Respiratory Syncytial Virus Disease: A Prospective Cohort Study" in which they prospectively evaluate the association between baseline RSV antibody titers and risk of RSV illness among household members in a community setting to determine correlates of protection (CoP) against RSV disease. These CoP are important in the light of (future) vaccination against RSV in various age groups. I've a few comments reflecting the methodology:

Major:

- Lines 146-147; "41.9% had another respiratory pathogen co-detected (n=177/422)". Were these co-detections also in cases with symptomatic RSV? And was the load of RSV in these cases higher as compared to the other pathogen detected? My question; was RSV indeed judged to be the causal pathogen in this symptomatic disease "RSV positive cases", because if that is unsure, this could potentially interfere with the analyses for CoP.
- Lines 293-300; the study by Öner and colleagues (PMID 39493753) demonstrated that higher levels of RSV-pre-F binding IgG antibodies pre-season are associated with protection from RSV infection regardless of symptoms in older adults (>60y). The number of RSV cases in this study was smaller (n=36) as compared to the current study in the group aged 18-50 (n=99) and severe disease (hospitalization or pneumonia) was not observed, but still the effect was statistically significant. The log transformed RSV pre-F binding IgG levels are also substantially lower in the current cohort (18-50; median 5.27) as compared to those in this study (>60y, median 7.92-8.55), is this a population difference, post- versus pre-covid difference or perhaps a difference in diagnostic methods? Can this be reflected upon in the discussion?
- Can you reflect on the sensitivity of the Mid-turbinate nasal swabs (RHINOstic™) as compared to nasopharyngeal collected flocculated swabs? Could RSV cases be missed using this collection method leading them to be falsely classified as RSV negative and placed in the control group which can influence the validity of the CoP analyses?

Minor:

- Initially samples were only tested for RSV A (in case no symptoms were recorded, supplemental file 292-298), Does the incidence; "0.43% (n=345 positive; n=4 inconclusive) and 0.09% (n=72 positive; n=1 inconclusive) tested 146 positive/inconclusive for RSV subtypes A and B, respectively" reflect underdiagnosis or was RSV B circulation lower during the study period?

Reviewer #2

(Remarks to the Author)

This was a prospective, community-based RSV surveillance study among individuals 6 months to 50 years of age, which was conducted in Oregon and Washington state from June 2022-May 2023. Participants answered a baseline questionnaire and had blood collection for RSV serology (pre-F binding antibodies [all] and RSV-A neutralizing antibodies [children]). They self-collected mid-turbinate swabs weekly for RSV RT-qPCR and performed weekly symptom surveys. Of 3,237 participants, 305 RSV illnesses were identified. The highest incidence was in children 6 months to 1 year of age. Using proportional hazards regression, the investigators found that higher binding and neutralizing antibody titers were associated with significant reduction in hazard of RSV illness. This is a timely analysis and a well written manuscript.

Major Comments:

- It would be helpful for clinicians and scientists to know what antibody titer (whether binding or neutralizing) correlates with protection. There is a lot of great data in this manuscript, but the CoP seems to be missing. Can you provide the titer? Or could you show the probability of infection for a given discretized titer?
- Was any analysis done for the asymptomatic RSV infections? Can anything be concluded from their data?
- Why do you think antibodies do not correlate as well with protection among the infants 6-12 months, adolescents, and the adults 18-50 years?

Minor Comments:

- Consider noting in the text that the study was conducted prior to the approval of RSV prevention products (older adult vaccines, maternal vaccine, nirsevimab), which are likely to impact epidemiology in these other age groups
- Lines 118-119: Consider specifying "... prior to full-scale implementation of RSV prevention products in these age groups."
- Line 121: Suggest modifying to: "... (CoPs) against RSV are incompletely defined."
- Line 141: Did study begin in June 2022 or August 2022?
- Line 142: What was the median duration of time in the study?
- Line 147: Suggest noting the frequency of other pathogen detections in the RSV-negative samples, and specifically rhinovirus.
- Line 154: Somewhat awkward to call myalgia a respiratory symptom
- Line 320: Could you comment on the relevance of antibody waning during the course of the study?
- Limitations paragraph: Could you comment on the impact of coinfections on the analysis?
- Line 339: Suggest including young children 2-4 years in this sentence.
- Methods: CASCADIA: Could you define abbreviation and include this earlier in the manuscript?
- Methods: Could you comment on the impact of self- and home-collection of mid-turbinate swabs and the dry specimen shipping on viral detection by the RT-qPCR assay?
- Supplement: The organization is somewhat awkward with the eResults in the middle of Tables/Figures
- Table 3: Suggest making p-values <0.001 rather than 0.000
- Supplemental Table 3: Suggest noting that 5 people had RSV A/B coinfections
- Supplemental Table 6: Suggest noting that this includes people with asymptomatic infection
- Supplemental Figure 7: Suggest noting why only 278 are included in this figure
- I think some of your supplemental figures are more interesting than the current figure 2.

Reviewer #3

(Remarks to the Author)

The study presented in this manuscript aimed to examine the correlation of protection (CoP) of RSV antibodies (both binding and neutralizing) with the incidence of RSV across different age groups. Additionally, it characterized the clinical features of RSV across these age groups. This is a well-written manuscript with a large sample size from a community-based cohort and an intensive RSV detection follow-up.

However, some aspects require further clarification:

1. In the abstract, the study duration is stated as August 2022 to May 2023 (line 90), whereas in the results section (line 141), it is given as June 2022 to May 2023. Please clarify the study duration, the participant enrollment period, and the follow-up duration for detecting RSV infections for each participant.
2. The reference numbering is not aligned with the order of appearance. At line 107, references 1–6 are cited, but the next citation jumps to reference 10.
3. References 11–12 were cited for the recently approved RSV prevention products; however, these references do not cover the maternal RSV vaccine. Please include an appropriate reference for the RSV maternal vaccine.
4. In Supplementary Figure 4, several samples have Ct values below 10, with two cases even below 5. Could these values be accurate, or might they be artifacts due to primer-dimers or high background noise, given that these samples were clinical nasal swabs rather than viral cultures? Additionally, the sensitivity and specificity of the RSV test used in this study are unclear and should be briefly mentioned in the Methods section.
5. Lines 178–186: How were pediatric patients able to report all symptoms listed in Table 2 and Supplementary Tables 3–6, particularly headache and myalgia?
6. Lines 189–191: It is unclear whether this sentence refers to adult or pediatric participants.
7. The statement on lines 195–196, "However, the Crt distribution was similar between children and adults (Supplemental Figure 4)" requires further explanation. Why is this finding relevant?
8. The statement on lines 197–198, "measurable Crt values on multiple swabs after the first positive swab were more common among children (Supplemental Figure 5)." should be revised to emphasize the persistence of viral load, which was more frequently observed in pediatric participants.
9. Data presentation: The manuscript should maintain consistency by using either one or two decimal places throughout.

Reviewer #4

(Remarks to the Author)

This prospective cohort study examined correlates of protection against respiratory syncytial virus (RSV) in individuals aged 6 months to 50 years in Oregon and Washington. Researchers assessed RSV antibody titers and their association with RSV illness using weekly symptom surveys and swab testing. Higher baseline RSV-specific binding and neutralizing antibodies were linked to reduced RSV risk, particularly in children. The study highlights the importance of pre-existing immunity in shaping RSV susceptibility and provides critical data for developing targeted RSV vaccines and prevention strategies.

across different age groups. I found the data on RSV epidemiology to be important, but was not convinced about the framing of the CoP analysis.

Major comments

A “correlate of protection” is often determined for vaccines because it can then be used as a proxy measure of vaccine performance in immunogenicity studies. It is important to recognise that for a given pathogen, vaccine A might have different CoP compared to vaccine B. For example for the live attenuated influenza vaccine it seems like HA titers are at most weakly correlated with protection, whereas for inactivated vaccines the HA titer is a well-established CoP. For RSV vaccines it is important to specify which assay is a CoP for which vaccine, and not simply imply that neutralizing antibodies are a CoP for all RSV vaccines. In the present analysis, authors examine antibody titers that are a result of natural infections (I think) and therefore strictly speaking it is not a CoP analysis because there is no vaccination in the data or analysis. A CoP analysis is a specific type of analysis where we look at post-vaccination antibody levels and risk of subsequent infection. Authors could frame this as an examination of the correlation of RSV antibody levels with risk of infection and it would be fine. But it should not be called a CoP analysis because the P in CoP analysis refers explicitly to protection from vaccination (in my opinion). Given that RSV infections stimulate antibodies, and the risk of RSV infection is lower in those with a prior history of RSV, it is not too surprising that there is a correlation between antibody levels and risk of subsequent infection, nevertheless the quantitative findings are important and this is a unique cohort.

Major comment

Lines 304 onwards - I am wondering whether suggesting alternative CoPs is not something you can do without post-vaccination sera. Its plausible that other post-vaccination markers would be better CoPs for existing RSV vaccines but I don't think your study can answer that question.

Minor comments

Compare Line 121 - “correlates of protection against RSV are undefined” versus line 284-5 “RSV neutralising antibody is an established CoP ...” only one of these statements is correct? And please specify which vaccine platform(s) you are referring to in line 284-5.

In line 291-3 you mention that increasing RSV titers through vaccination may be protective but hasn't it already been shown that vaccination reduces RSV illness at least for the approved vaccines? Whether or not RSV antibody titers are also boosted by vaccination, and contribute to protection, is surely of interest but cannot be answered by the present study of “natural” immunity. And for vaccine policy as long as the vaccine works we are happy. The reason we would like CoPs for vaccines is so we can more easily monitor their performance in future years, and more quickly approve vaccines of the same platform from other manufacturers (competition is good, e.g. to keep prices down and hedge against shortages from one manufacturer).

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I am happy with the revised manuscript and have no further comments

Reviewer #2

(Remarks to the Author)

Thank you, the authors have well addressed the considerations raised in the peer review.

Reviewer #3

(Remarks to the Author)

The revision has been substantially improved the clarity of the manuscript.

I only have some minor comments below:

- Main manuscript:

*line 428, define SD (in sample SD)

*line 449, define PH (in PH regression)

*line 453: define HRs

- Supp.Fig2 Legends for “0” and “1” and “NA” values.

- Data presentation: The manuscript and Supp.files should maintain consistency by using either one or two decimal places throughout. One decimal should be sufficient for the precision.

Reviewer #4

(Remarks to the Author)

No further comments

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Response to reviewers' comments:

Reviewer comment	Authors' response
Reviewer 1	
With interest I read the manuscript entitled: "Correlates of Protection Against Respiratory Syncytial Virus Disease: A Prospective Cohort Study" in which they prospectively evaluate the association between baseline RSV antibody titers and risk of RSV illness among household members in a community setting to determine correlates of protection (CoP) against RSV disease. These CoP are important in the light of (future) vaccination against RSV in various age groups. I've a few comments reflecting the methodology:	Thank you for your review of our manuscript and your comments, which have helped improve our manuscript. We provide responses below on how we have addressed your comments in the manuscript. Please note that the line numbers mentioned in the responses below refer to the line numbers in the clean/simple markup versions of the revised manuscript.
<i>Major comments</i>	
- Lines 146-147; "41.9% had another respiratory pathogen co-detected (n=177/422)". Were these co-detections also in cases with symptomatic RSV? And was the load of RSV in these cases higher as compared to the other pathogen detected? My question; was RSV indeed judged to be the causal pathogen in this symptomatic disease "RSV positive cases", because if that is unsure, this could potentially interfere with the analyses for CoP.	Thanks for your comment. The text referred to unique swabs and not overall illness episodes. We have added the following text to the supplemental information (lines 32-37): "Of 305 RSV symptomatic infections, 29.8% had another virus detected. Of the RSV illness swabs with another pathogen co-detected (n=91), the following viruses were detected with some swabs testing positive for two or more viruses: rhinovirus (n=64); adenovirus (n=15); influenza (n=11); human coronavirus (n=10); SARS-CoV-2 (n=8); pan-enterovirus (n=4 including n=3 EVD-68); human metapneumovirus (n=1); Parainfluenza virus subtype 1-2 (n=1); Parainfluenza virus subtype 3-4 (n=5). See Supplementary Figure 5 for the distribution of Crt values stratified by co-detection status (Supplementary Figure 5)."

Reviewer comment	Authors' response
	We have also added details on the most common co-detected pathogens to the main text (lines 153-156): “During RSV illness episodes, 29.84% of participants had RSV co-detected with one or more other pathogens (n=91/305). RSV A/B was most frequently co-detected with rhinovirus (70.33%; n=64/91); adenovirus (16.48%; n=15/91); and influenza (12.09%; n=11/91).” The literature supports that RSV is often the driver of the illness, even when co-detected with other pathogens (Martin et al. 2013; Mazur et al. 2017). In our analysis, rhinovirus was the most commonly co-detected (70.33%), which is also less likely to be the driver of symptoms. For pathogens like SARS-CoV-2 and influenza, it is more challenging to identify the causal pathogen. However, these pathogens were only co-detected for 8.8% (SARS-CoV-2) and 12.09% (influenza) of the RSV illness swabs where co-detection was present (n=91). Therefore, we expect these co-detections to have a minimal impact on our key findings. However, we have added this limitation to the discussion section (lines 361-365): “Furthermore, although RSV is often the driver of the illness when co-detected with other pathogens, co-detections may have impacted our observed association between RSV antibodies and RSV illness. However, we expect the impact of co-detections to be minimal since RSV was most frequently co-detected with rhinovirus (70.33%; n=64/91) and Crt values were similar by co-detection status.”

Reviewer comment	Authors' response
	We have also added a new figure to illustrate that the distribution of Crt values for a participant's first RSV-positive swab was similar between participants who did and did not have another pathogen co-detected with RSV (Supplemental Figure 5).
- Lines 293-300; the study by Öner and colleagues (PMID 39493753) demonstrated that higher levels of RSV-pre-F binding IgG antibodies pre-season are associated with protection from RSV infection regardless of symptoms in older adults (>60y). The number of RSV cases in this study was smaller (n=36) as compared to the current study in the group aged 18-50 (n=99) and severe disease (hospitalization or pneumonia) was not observed, but still the effect was statistically significant. The log transformed RSV pre-F binding IgG levels are also substantially lower in the current cohort (18-50; median 5.27) as compared to those in this study (>60y, median 7.92-8.55), is this a population difference, post- versus pre-covid difference or perhaps a difference in diagnostic methods? Can this be reflected upon in the discussion?	Thank you for your comment. The ELISA RSV binding antibody assay described by Öner and colleagues is a different assay than the Mesoscale Diagnostics electrochemiluminescence immunoassay used in this analysis. The results presented by Öner and colleagues are also in different units (EU/L), which makes it challenging to directly compare the magnitude of the RSV binding antibody titers across studies. To address this limitation, we also present our results using standardized antibody titer variables based on standard deviation-standardized antibody titers as well as categorical antibody titer variables. Moreover, our variable importance analysis directly quantifies the predictive ability of binding and neutralizing antibodies to account for differences in their measurement scales/units. Challenges with directly comparing different diagnostic methods has been added to the discussion in lines 345-347: "Researchers should also consider incorporating variable importance analyses into future studies to facilitate comparing RSV antibodies reported in different scales/units across studies."
Can you reflect on the sensitivity of the Mid-turbinate nasal swabs (RHINOstic™) as compared to nasopharyngeal collected flocked swabs? Could RSV cases be missed using this collection method leading them to be falsely classified as RSV	A comparison of unsupervised, home-collection of midnasal swabs compared to clinician-collected nasopharyngeal swabs was previously published for SARS-CoV-2 (McCulloch et al. JAMA Netw Open. 2020). This study found that sensitivity and specificity of midnasal swabs for SARS-CoV-2 were 80.0% (95%

Reviewer comment	Authors' response
negative and placed in the control group which can influence the validity of the CoP analyses?	CI, 63%-91%) and 97.9% (95% CI, 94%-99.5%), respectively. We expect similar trends for detection of RSV by self-/caregiver-collection using midnasal compared to clinician-collected nasopharyngeal swabs. Home-based collection of midnasal swabs was also reported by Kim et al. (J Clin Microbiol. 2021), which found that self-collection was a safe and feasible method for community surveillance of respiratory pathogens. As part of this analysis, we also performed further validation of the RSV assay based on midnasal swab collection. Analytical specificity was evaluated by concordance studies on matched specimens, which compared each method with a reference standard assay, the Xpert Xpress CoV-2/Flu/RSV plus (Cepheid, Sunnyvale, CA), with further RSV subtyping performed by UW Virology. There was 100% result concordance compared to negative specimens for both RSV A and RSV B. Additional information describing midnasal versus nasopharyngeal swabs and the assay validation process have been added to the supplemental appendix. Moreover, among our study population, most nasal swabs (>99%) had adequate mucosal sampling as measured by sufficient Ribonuclease P (RNase P) detection, which suggests that there was sufficient sample to detect RSV. However, we agree with you that misclassification is a potential limitation, which we have added to the discussion (lines 359-360): “There is also a potential risk for misclassification of RSV illness status if a false-negative RT-qPCR result was reported and/or due to missing swabs.”
<i>Minor comments</i>	

Reviewer comment	Authors' response
- Initially samples were only tested for RSV A (in case no symptoms were recorded, supplemental file 292-298), Does the incidence; “0.43% (n=345 positive; n=4 inconclusive) and 0.09% (n=72 positive; n=1 inconclusive) tested 146 positive/inconclusive for RSV subtypes A and B, respectively” reflect underdiagnosis or was RSV B circulation lower during the study period?	RSV B circulation was lower in the 2022-2023 season, which has also been observed in other studies (Rios-Guzman et al. Nat Commun. 2024). For our primary analysis, we also focus on symptomatic RSV A/B infections to account for the different testing criteria for RSV A and B.
Reviewer 2	
This was a prospective, community-based RSV surveillance study among individuals 6 months to 50 years of age, which was conducted in Oregon and Washington state from June 2022-May 2023. Participants answered a baseline questionnaire and had blood collection for RSV serology (pre-F binding antibodies [all] and RSV-A neutralizing antibodies [children]). They self-collected mid-turbinate swabs weekly for RSV RT-qPCR and performed weekly symptom surveys. Of 3,237 participants, 305 RSV illnesses were identified. The highest incidence was in children 6 months to 1 year of age. Using proportional hazards regression, the investigators found that higher binding and neutralizing antibody titers were associated with significant reduction in hazard of RSV illness. This is a timely analysis and a well written manuscript.	Thank you for the thoughtful summary and positive comments. We directly address questions and suggestions below and have made revisions throughout the manuscript to improve clarity. Please note that the line numbers mentioned in the responses below refer to the line numbers in the clean/simple markup versions of the revised manuscript.
<i>Major comments</i>	
- It would be helpful for clinicians and scientists to know what antibody titer (whether binding or neutralizing) correlates with protection. There is a lot of great data in this manuscript, but the CoP seems to be missing. Can you provide the titer? Or could you show the probability of infection for a given discretized titer?	Thank you for this comment. In addition to the hazard ratios of the association between the categorical antibody titer variables and RSV illness (Figure 3), we have added a new figure illustrating the cumulative incidence of RSV illness over time by antibody titer category (Figure 4). The cumulative incidence results are summarized in lines 244-247:

Reviewer comment	Authors' response
	“. At 180 days since baseline blood collection, the cumulative incidence in the low antibody category was estimated to be 21.70% and 19.77% for binding and neutralizing antibodies, respectively. For the very high antibody category, the cumulative incidence was estimated to be 3.50% and 3.95% for binding and neutralizing antibodies, respectively.”
- Was any analysis done for the asymptomatic RSV infections? Can anything be concluded from their data?	Sensitivity analyses reporting results regardless of symptom status are shown in Supplemental Tables 6, 10, and 11. However, since most participants reported symptoms in our study population, only 7% (n=23/328) of participants with an RSV infection did not meet our RSV illness case definition. Due to the small sample size of participants with an asymptomatic RSV infection, we did not report results for this subpopulation. We have clarified the number of overall RSV infections versus symptomatic infections in the results (lines 161-164): “Overall, 328 participants experienced an RSV infection, regardless of symptom status, during the study period. Of these infections, 92.99% (n=305/328) were symptomatic defined as ≥ 1 respiratory illness symptom (e.g., fever, cough, sore throat, shortness of breath, myalgia, rhinorrhea).” Our ability to capture asymptomatic disease was also partially limited by our protocol where all swabs were tested for RSV A, but only one subset was tested for RSV B, particularly for symptomatic participants, which we have highlighted in the discussion (lines 357-359): “Moreover, our ability to capture asymptomatic disease was partially limited by our protocol where all swabs were tested for

Reviewer comment	Authors' response
- Why do you think antibodies do not correlate as well with protection among the infants 6-12 months, adolescents, and the adults 18-50 years?	RSV A, but only a subset were tested for RSV B, particularly for symptomatic participants.” Due to the small sample sizes of some of the age groups, this analysis may be underpowered to detect statistically significant differences by age group. Moreover, when evaluating whether the association of interest was modified by pediatric versus adult age group, we failed to reject the null hypothesis that the estimated difference in the hazard of symptomatic RSV infection for two groups differing by 1-unit in $\log_{10}$- antibody titers is equal for adults and children (p-value=0.80), which suggests that the association of interest may not be modified by age (Table 3; footnote 1). As such, we focus on the overall HRs in the main text when interpreting our findings. There may also be different relationships between RSV antibodies and RSV illness by age group, which we comment on in the discussion for adults (lines 320-327): “when further stratifying by adult versus pediatric participants, this association was not statistically significant among adults; this may be because our adult participants had baseline binding antibody titers in a narrow range of high values, consistent with adults being exposed to RSV many times and building up higher antibody titer levels on average compared to young children. Moreover, our adult participants only experienced mild illnesses. As such, we may have observed a stronger protective association of binding antibody titers in a hospital- or outpatient-setting compared to our community-based cohort or if our study had included higher-risk adults.”

Reviewer comment	Authors' response
	Another important consideration is that this study only assessed how levels of IgG detected in serum contribute to risk of RSV and we did not measure mucosal immunity or T-cells, which may also contribute to protection.
<i>Minor comments</i>	
- Consider noting in the text that the study was conducted prior to the approval of RSV prevention	Thank you for the suggestion. We have clarified this context in the introduction (lines 134-137): "We aimed to prospectively evaluate the association between baseline RSV antibody titers and risk of RSV illness among household members in a community setting after COVID-19 mitigation measures were lifted and prior to the approval of RSV prevention products to protect high-risk infants and older adults." In the methods, we have also added the following text (lines 416-419): "In the 2022-2023 respiratory season prior to the introduction of novel RSV vaccines and monoclonal antibodies, we included participants who completed an enrollment survey, attended a baseline blood draw appointment (regardless of blood draw success), and provided at least one nasal swab by May 31, 2023."
- Lines 118-119: Consider specifying "... prior to full-scale implementation of RSV prevention products in these age groups."	Added. Thanks for the suggestion.
- Line 121: Suggest modifying to: "... (CoPs) against RSV are incompletely defined."	Added. Thanks for the suggestion.
- Line 141: Did study begin in June 2022 or August 2022?	Participant enrollment began in June 2022 on a rolling basis and individuals attended a baseline blood draw appointment began swabbing weekly upon enrollment. Since we detected our first RSV-positive swab in September, we presented monthly RSV

Reviewer comment	Authors' response
	incidence estimates starting in August 2022. We have revised these inconsistencies between June/August throughout the manuscript.
- Line 142: What was the median duration of time in the study?	The median duration of follow up time was 189 days from the baseline blood draw to when a participant subsequently tested positive for RSV, withdrew from the study, or the end of the analytic period (May 31, 2023). This information has been added to lines 144-146 of the main text: “The median follow-up duration from the baseline blood draw to when a participant subsequently tested positive for RSV, withdrew from the study, or the end of the analytic period (May 31, 2023) was 189 days.”
- Line 147: Suggest noting the frequency of other pathogen detections in the RSV-negative samples, and specifically rhinovirus.	Thanks for the suggestion. These results have been added to the supplementary information (lines 39-42): “Among the swabs not associated with an RSV illness episode (n=79469), the most frequently detected pathogens were as follows: rhinovirus (n= 2453; 22%); human coronavirus (n=1154; 10.3%); adenovirus (n=370; 3.3%); enterovirus (n=336; 3.0%); human metapneumovirus (n=298; 2.7%); SARS-CoV-2 (n=1878; 2.4%); Parainfluenza virus subtype 3-4 (n=258; 2.3%); Parainfluenza virus subtype 1-2 (n=119; 1.1%); influenza (n=477; 0.6%).”
- Line 154: Somewhat awkward to call myalgia a respiratory symptom	Although myalgia is a systemic symptom and could have other causes, we included it in our RSV illness definition since it is a systemic symptom of a respiratory viral infection similar to fever. We have revised lines 162-164 to clarify that we are referring to symptoms of respiratory illness instead of only symptoms associated with the respiratory tract:

Reviewer comment	Authors' response
	"Of these infections, 92.99% (n=305/328) were symptomatic defined as ≥ 1 respiratory illness symptom (e.g., fever, cough, sore throat, shortness of breath, myalgia, rhinorrhea)."
- Line 320: Could you comment on the relevance of antibody waning during the course of the study?	While the literature suggests that RSV antibody titers are relatively stable among uninfected adults, if a participant was recently infected prior to study enrollment and collection of their baseline blood draw then their antibody titers measured at baseline may decline to pre-infection levels rapidly within a few months (Blunck et al. 2021; Sande et al 2013). We accounted for potential changes in antibody levels over time by conducting a survival analysis where the primary outcome of interest was time from the baseline blood draw to RSV illness. The longitudinal kinetics of RSV binding antibody titers pre- and post-RSV infection over two seasons will also be addressed in a separate manuscript.
- Limitations paragraph: Could you comment on the impact of coinfections on the analysis?	The literature supports that RSV is often the driver of the illness, even when co-detected with other pathogens (Martin et al. 2013; Mazur et al. 2017). In our analysis, of 305 RSV symptomatic infections, 29.84% had another virus detected. Rhinovirus was the most commonly co-detected (70.33%), which is also less likely to be the driver of symptoms. These details have been added to lines 153-156: "During RSV illness episodes, 29.84% of participants had RSV co-detected with one or more other pathogens (n=91/305). RSV A/B was most frequently co-detected with rhinovirus (70.33%; n=64/91); adenovirus (16.48%; n=15/91); and influenza (12.09%; n=11/91)." For pathogens like SARS-CoV-2 and influenza, it is more challenging to identify the causal pathogen. However, these

Reviewer comment	Authors' response
	pathogens were only co-detected with RSV in 6.23% of illness episodes overall (n=19/205). Therefore, we expect these co-detections to have a minimal impact on our key findings. However, we have added this limitation to the discussion section (lines 361-365): “Furthermore, although RSV is often the driver of the illness when co-detected with other pathogens, co-detections may have impacted our observed association between RSV antibodies and RSV illness. However, we expect the impact of co-detections to be minimal since RSV was most frequently co-detected with rhinovirus (70.33%; n=64/91) and Crt values were similar by co-detection status.” We have also added a new figure to illustrate that the distribution of Crt values for a participant's first RSV-positive swab was similar between participants who did and did not have another pathogen co-detected with RSV (Supplementary Figure 5).
- Line 339: Suggest including young children 2-4 years in this sentence.	Added.
- Methods: CASCADIA: Could you define abbreviation and include this earlier in the manuscript?	CASCADIA is a pseudo-acronym and does not stand for anything. We have added a sentence to the end of the Introduction Section to introduce the study name earlier in the manuscript (line 134).
Methods: Could you comment on the impact of self- and home-collection of mid-turbinate swabs and the dry specimen shipping on viral detection by the RT-qPCR assay?	A comparison of unsupervised, home-collection of midnasal swabs compared to clinician-collected nasopharyngeal swabs was previously published for SARS-CoV-2 (McCulloch et al. JAMA Netw Open. 2020). Home-based collection of midnasal swabs was also reported by Kim et al. (J Clin Microbiol. 2021), which found that self-collection was a safe and feasible method for

Reviewer comment	Authors' response
	community surveillance of respiratory pathogens. Among our study population, most nasal swabs (>99%) had adequate mucosal sampling as measured by sufficient Ribonuclease P (RNase P) detection, which suggests that there was sufficient sample to detect RSV. Regarding dry specimen shipping, dry transport with no refrigeration for 72 hours had negligible differences on cycle threshold. We have added the following to the supplementary methods: “Specimen stability was confirmed by IATA-recommended shipping studies that mimic winter conditions for 75 hours. High (Ct<30) and low (Ct>30) concentration samples were tested for the RSV-A pathogen. Ct values shifted by -0.22 for high-concentration samples and -0.2 for low concentration samples, indicating negligible differences in Ct when cycling dry-swabs through varied winter shipping temperatures.”
Supplement: The organization is somewhat awkward with the eResults in the middle of Tables/Figures	Thanks for your feedback. We have moved all the tables and figures to the end of the supplement instead of arranging by eResults followed by eMethods with embedded tables/figures.
Table 3: Suggest making p-values <0.001 rather than 0.000	Thanks for the suggestion. The p-values <0.001 have been updated as you recommended in both the main text and supplement.
Supplemental Table 3: Suggest noting that 5 people had RSV A/B coinfections	We have added a footnote to clarify that five participants had unique RSV A and B illness episodes during the follow-up period. However, both subtypes were not co-detected at the same time. Note that Table 3 only presents incident RSV illness episodes so the participants with unique RSV A and B illnesses are only included once for their incident infection with the first subtype.

Reviewer comment	Authors' response
Supplemental Table 6: Suggest noting that this includes people with asymptomatic infection	The following footnote has been added to the definition of any RSV infection (regardless of symptom status): "Includes both symptomatic and asymptomatic infections."
Supplemental Figure 7: Suggest noting why only 278 are included in this figure	We have expanded the footnote as follows: "Open Array relative cycle threshold (Crt) values are ~10 cycles lower than conventional RT-qPCR CT values (Crt of 5 would be equivalent to a CT ~15). For comparability, Crt values are reported for swabs tested on the multipathogen ("open array") assay. As such, results are only shown for n=278 participants instead of the n=305 participants who experienced an RSV illness."
I think some of your supplemental figures are more interesting than the current figure 2.	Thanks for flagging this. We have moved the following supplemental figures to the main text: -Hazard ratios of the association between categorical baseline antibody titers and RSV A/B illness by antibody type (now Figure 3) - Area under the receiver operating curve variable importance for baseline antibody titers relative to covariates (now Figure 5)
Reviewer 3	
The study presented in this manuscript aimed to examine the correlation of protection (CoP) of RSV antibodies (both binding and neutralizing) with the incidence of RSV across different age groups. Additionally, it characterized the clinical features of RSV across these age groups. This is a well-written manuscript with a large sample size from a community-based cohort and an intensive RSV detection follow-up. However, some aspects require further clarification:	Thank you for the thoughtful summary and positive comments about the strengths of this analysis. We provide responses below on how we have addressed your comments in the manuscript. Please note that the line numbers mentioned in the responses below refer to the line numbers in the clean/simple markup versions of the revised manuscript.
1. In the abstract, the study duration is stated as August 2022 to May 2023 (line 90), whereas in the results section (line 141), it is given as June 2022 to May 2023. Please clarify the study	Thanks for highlighting this inconsistency. Participant enrollment began in June 2022 on a rolling basis and individuals began swabbing upon enrollment. Since we detected our first RSV-

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duration, the participant enrollment period, and the follow-up duration for detecting RSV infections for each participant.	positive swab in September, we presented monthly RSV incidence estimates starting in August 2022. We have revised the abstract to state that the study period began in June 2022 when enrollment opened. For the monthly incidence estimates, a participant's follow-up time started on the date of their first nasal swab collection. For the proportional hazards analysis, time at risk was calculated as the time elapsed between baseline blood draw (beginning in June) and when a participant subsequently tested positive for RSV, withdrew from the study, or the end of the analytic period (May 31, 2023). The follow-up duration for each individual participant is shown in the participant timelines (Supplemental Figure 2). To highlight the participant timelines in the main text, we added the following sentence (lines 146-147): "See Supplementary Figure 2 for individual participant timelines illustrating when participants enrolled and collected swabs throughout the analytic period among participants with RSV A and B illnesses, respectively." These details on follow-up duration are described in the supplemental methods and we have also added them to the methods in the main text for clarity: Lines 443-445 for the incidence rate estimates: "Participants contributed person-time at risk during the weeks they self-collected a nasal swab beginning on the date of their first nasal swab collection." Lines 450-452 for the proportional hazards analysis: "Time at risk was calculated as the time elapsed between baseline blood

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	draw and when a participant subsequently tested positive for RSV, withdrew from the study, or the end of the analytic period (May 31, 2023)."
2. The reference numbering is not aligned with the order of appearance. At line 107, references 1–6 are cited, but the next citation jumps to reference 10.	Thank you for flagging this error. The reference numbering and field codes have been updated.
3. References 11–12 were cited for the recently approved RSV prevention products; however, these references do not cover the maternal RSV vaccine. Please include an appropriate reference for the RSV maternal vaccine.	Thank you for bringing this to our attention. The following references have been added: -Kampmann B, Madhi SA, Munjal I, et al. Bivalent Prefusion F Vaccine in Pregnancy to Prevent RSV Illness in Infants. <i>N Engl J Med.</i> 2023;388(16):1451-1464. doi:10.1056/NEJMoa2216480 -Fleming-Dutra KE, Jones JM, Roper LE, et al. Use of the Pfizer Respiratory Syncytial Virus Vaccine During Pregnancy for the Prevention of Respiratory Syncytial Virus-Associated Lower Respiratory Tract Disease in Infants: Recommendations of the Advisory Committee on Immunization Practices - United States, 2023. <i>MMWR Morb Mortal Wkly Rep.</i> 2023;72(41):1115-1122. doi:10.15585/mmwr.mm7241e1
4. In Supplementary Figure 4, several samples have Ct values below 10, with two cases even below NPOs	We report Crt values from our arrayed RT-qPCR assay (Open Array). The Open Array performs 14 cycles of pre-amplification and dilution before the detection qPCR. Thus, Open Array Crts are ~10 cycles lower than conventional RT-qPCR CT values (Crts of 5 would be equivalent to a Ct ~15). We have added these details to the lab methods section in the supplementary methods (lines 212-214): "We report Crt values from our arrayed RT-qPCR assay (Open Array). The Open Array performs 14 cycles of pre-amplification and dilution before the detection qPCR. Thus, Open Array Crt

Reviewer comment	Authors' response
	values are ~10 cycles lower than conventional RT-qPCR CT values (Crt of 5 would be equivalent to a Ct ~15)." We have also added the following footnote to figures including Crt values for clarity: "Open Array relative cycle threshold (Crt) values are ~10 cycles lower than conventional RT-qPCR CT values (Crt of 5 would be equivalent to a CT ~15). For comparability, Crt values are reported for swabs tested on the multipathogen ("open array") assay. As such, results are only shown for n=278 participants instead of the n=305 participants who experienced an RSV illness."
5. Could these values be accurate, or might they be artifacts due to primer-dimers or high background noise, given that these samples were clinical nasal swabs rather than viral cultures? Additionally, the sensitivity and specificity of the RSV test used in this study are unclear and should be briefly mentioned in the Methods section.	Thank you for your comment. We have added a section on assay validation to the supplemental methods, which describes the limit of detection for the RT-qPCR assays as well as the specificity. The following text has been added to the supplementary methods: "To evaluate the RT-qPCR assays developed by UW Brotman Baty Laboratory, we performed a minimum of 24 replicate reactions for each RT-qPCR target and determined limit of detection (LoD). LoD is defined by the minimum number of molecules that can be detected in greater than 95% of replicate RT-qPCR reactions. For RSV A, the LoD for the CRI, extraction-free assay was 625 molecules in a 5 ul reaction. The LoD for the extracted assay was 75 molecules in a 5 ul reaction for RSV A. For RSV B, the LoD for extraction-free was 83.3 molecules in a 5 ul reaction; for assay with extraction it was 30 molecules/5 ul rection. Analytical specificity was evaluated by concordance

Reviewer comment	Authors' response
	studies on matched specimens, which compared each method with a reference standard assay, the Xpert Xpress CoV-2/Flu/RSV plus (Cepheid, Sunnyvale, CA), with further RSV subtyping performed by UW Virology. There was 100% result concordance compared to negative specimens for both RSV A and RSV B. Additionally, included in each validation plate is a control that has no template, called a “no template controls” (NTC). All NTC specimens failed to show any amplification of viral targets or RNase P in either assay.”
5. Lines 178–186: How were pediatric patients able to report all symptoms listed in Table 2 and Supplementary Tables 3–6, particularly headache and myalgia?	For all pediatric participants <13 years of age, symptoms were reported by their caregiver. This is specified in the following footnote included in the symptom tables: “Symptoms were self- and/or caregiver-reported -7 days to +14 days from Day 0; for participants <13 years, surveys were sent to the caregiver instead of the participant; symptoms were not evaluated by a clinician.” We have also added these details to the methods so that the data collection procedures are clearer to the reader (lines 395-397): “For participants <13 years of age, symptoms were reported by the participant’s parent/guardian; symptoms were not evaluated by a clinician. All data were collected using REDCap.”
6. Lines 189–191: It is unclear whether this sentence refers to adult or pediatric participants.	The sentence has been revised to clarify that all participants (both adult and pediatric) sought care at a doctor’s office or urgent care facility most frequently followed by telehealth services.
7. The statement on lines 195–196, " However, the Crt distribution was similar between children and adults	Since we observed that adult participants tended to experience milder illness, we were also interested in comparing Crt values between pediatric and adult participants. In particular, fever,

Reviewer comment	Authors' response
(Supplemental Figure 4)" requires further explanation. Why is this finding relevant?	shortness of breath, care-seeking, and absenteeism were less frequently reported among symptomatic adults than pediatric participants. We have clarified the rationale for this comparison as follows (lines 207-208): "Although adult participants tended to experience more mild illness, the C_{rt} distribution was similar between children and adults"
8. The statement on lines 197–198, "measurable C _{rt} values on multiple swabs after the first positive swab were more common among children (Supplemental Figure 5)." should be revised to emphasize the persistence of viral load, which was more frequently observed in pediatric participants.	Thank you for your suggestion. We revised the sentence as follows (lines 211-212): "measurable C_{rt} values on multiple swabs after the first positive swab, suggesting longer time to viral clearance, was more common among children."
9. Data presentation: The manuscript should maintain consistency by using either one or two decimal places throughout.	Thank you for flagging this inconsistency. We have harmonized throughout the manuscript using two decimal places in the main text.
Reviewer 4	
This prospective cohort study examined correlates of protection against respiratory syncytial virus (RSV) in individuals aged 6 months to 50 years in Oregon and Washington. Researchers assessed RSV antibody titers and their association with RSV illness using weekly symptom surveys and swab testing. Higher baseline RSV-specific binding and neutralizing antibodies were linked to reduced RSV risk, particularly in children. The study highlights the importance of pre-existing immunity in shaping RSV susceptibility and provides critical data for developing targeted RSV vaccines and prevention strategies across different age groups. I found the	Thank you for your review of our manuscript and your comments which have helped improve our manuscript. We provide responses below on how we have addressed your comments in the manuscript including reframing the analysis as an assessment of the association between pre-existing RSV antibodies and RSV illness instead of a CoP analysis. Please note that the line numbers mentioned in the responses below refer to the line numbers in the clean/simple markup versions of the revised manuscript.

Reviewer comment	Authors' response
data on RSV epidemiology to be important, but was not convinced about the framing of the CoP analysis.	
<i>Major comment</i>	
A “correlate of protection” is often determined for vaccines because it can then be used as a proxy measure of vaccine performance in immunogenicity studies. It is important to recognise that for a given pathogen, vaccine A might have different CoP compared to vaccine B. For example for the live attenuated influenza vaccine it seems like HA titers are at most weakly correlated with protection, whereas for inactivated vaccines the HA titer is a well-established CoP. For RSV vaccines it is important to specify which assay is a CoP for which vaccine, and not simply imply that neutralizing antibodies are a CoP for all RSV vaccines. In the present analysis, authors examine antibody titers that are a result of natural infections (I think) and therefore strictly speaking it is not a CoP analysis because there is no vaccination in the data or analysis. A CoP analysis is a specific type of analysis where we look at post-vaccination antibody levels and risk of subsequent infection. Authors could frame this as an examination of the correlation of RSV antibody levels with risk of infection and it would be fine. But it should not be called a CoP analysis because the P in CoP analysis refers explicitly to protection from vaccination (in my opinion). Given that RSV infections stimulate antibodies, and the risk of RSV infection is lower in those with a prior history of RSV, it is not too surprising that there is a correlation between antibody levels and risk of subsequent infection, nevertheless the quantitative findings are important and this is a unique cohort.	Thank you for your critical review of our manuscript and comments regarding the terminology used throughout the manuscript. We have revised throughout the manuscript to emphasize that this analysis evaluated the association between baseline RSV antibody titers and risk of RSV illness in a vaccine-naïve population (i.e., correlate of risk) and how our results can inform future CoP analyses as part of vaccine clinical trials. Moreover, as part of the variable importance analysis among pediatric participants, we observed that binding and neutralizing antibody titers improved illness prediction beyond baseline covariates (e.g., age; school and daycare attendance; immunocompromised status) alone.

Reviewer comment	Authors' response
Lines 304 onwards - I am wondering whether suggesting alternative CoPs is not something you can do without post-vaccination sera. Its plausible that other post-vaccination markers would be better CoPs for existing RSV vaccines but I don't think your study can answer that question.	Thank you for your feedback. We have modified the discussion to highlight that binding antibody could be an RSV CoP, however, binding antibody as a CoP should be validated using post-vaccination sera for specific vaccine constructs. As a next step, our results can be leveraged to inform the design of future RSV vaccine clinical trials for next-generation prevention products. See lines 331-345 for details: "While these findings should be validated using post-vaccination sera as part of upcoming RSV vaccine clinical trials, our results suggest that binding antibody titers could be a viable RSV correlate of protection and alternative to neutralizing antibody, which is considerably more time- and resource-intensive to measure. The relevance of binding antibody as a potential RSV correlate of protection was further supported by the variable importance analysis, which suggested that binding and neutralizing antibody titers have a similar ability to predict RSV illness beyond baseline covariates when the predictive ability of these antibody titers was directly quantified to account for differences in their measurement scales/units. Moreover, since using binding and neutralizing antibody titers together resulted in the highest estimated AUC, our findings suggest that these biomarkers may partly provide complementary information for predicting RSV illness. Future studies should further explore binding antibodies as a correlate of risk against RSV illness, the role of binding versus neutralizing antibodies in protection against different RSV disease outcomes, as well as a composite biomarker leveraging both binding and neutralizing antibodies."
<i>Minor comments</i>	

Reviewer comment	Authors' response
Compare Line 121 - "correlates of protection against RSV are undefined" versus line 284-5 "RSV neutralising antibody is an established CoP ..." only one of these statements is correct? And please specify which vaccine platform(s) you are referring to in line 284-5.	Thanks for highlighting this inconsistency. We have modified lines 120-121 as follows: "Moreover, the impact of pre-existing RSV antibodies on RSV susceptibility is incompletely defined." We have also clarified that we are referring to pre-F subunit RSV vaccines administered to maternal and older adult populations and monoclonal antibody prophylaxis administered to infants (lines 307-310): "Higher levels of RSV neutralizing antibody have been shown to protect against disease in both observational studies and clinical trials of pre-F subunit RSV vaccines administered to maternal and older adult populations and monoclonal antibody prophylaxis administered to infants."
In line 291-3 you mention that increasing RSV titers through vaccination may be protective but hasn't it already been shown that vaccination reduces RSV illness at least for the approved vaccines? Whether or not RSV antibody titers are also boosted by vaccination, and contribute to protection, is surely of interest but cannot be answered by the present study of "natural" immunity. And for vaccine policy as long as the vaccine works we are happy. The reason we would like CoPs for vaccines is so we can more easily monitor their performance in future years, and more quickly approve vaccines of the same platform from other manufacturers (competition is good, e.g. to keep prices down and hedge against shortages from one manufacturer).	Thanks for your comment. While previous studies have reported that RSV-specific antibodies protect against symptomatic and lower respiratory tract disease in infants and older adults, this relationship has not been as well-documented among other age groups such as older children, who are the next target population for RSV vaccine development. As such, we were interested in evaluating the association between pre-existing RSV antibody and RSV illness among children >6 months of age and younger adults who were not included in the recent clinical trials (i.e., individuals not eligible for the currently approved RSV prevention interventions). We hypothesized that vaccine responses in children aged 5-12 years who have likely been exposed to RSV multiple times may differ from RSV-naïve infants entering their first season as well as older adults due to immunosenescence. Therefore, when evaluating next-generation RSV prevention products in children <6 months as

Reviewer comment	Authors' response
	part of upcoming clinical trials, different trends may be observed. This rationale is highlighted in the introduction (lines 129-133): “As such, antibody data for these age groups are needed to inform the development of next-generation prevention products. In particular, vaccine responses in children aged 5-12 years who have likely been exposed to RSV multiple times may differ from RSV-naïve infants entering their first season as well as older adults due to immunosenescence.” We have also re-emphasized these details in the discussion to clarify why understanding the association between RSV-specific antibodies and RSV illness among an unvaccinated population has relevance to upcoming clinical trials among children >6 months of age (lines 314-318): “Since previous studies have reported that RSV-specific antibodies induced via both natural infection and vaccination protected against RSV disease in infants, our results may also translate into a protective effect of vaccine-induced RSV antibodies against RSV illness in future clinical trials conducted among children >6 months of age.”

Response to reviewers' comments

Reviewer comment	Response
Reviewer #1 (Remarks to the Author): I am happy with the revised manuscript and have no further comments	Thank you for taking the time to review our revised manuscript. We're glad that our revisions addressed your previous comments.
Reviewer #2 (Remarks to the Author): Thank you, the authors have well addressed the considerations raised in the peer review.	Thank you for taking the time to review our revised manuscript. We're glad that our revisions addressed your previous comments.
Reviewer #3 (Remarks to the Author): The revision has been substantially improved the clarity of the manuscript. I only have some minor comments below: - Main manuscript: *line 428, define SD (in sample SD) *line 449, define PH (in PH regression) *line 453: define HRs	Standard deviation (SD) is defined upon first usage in line 175; proportional hazards (PH) is defined upon first usage in line 206; hazard ratio (HR) is defined upon first usage in line 182. All line numbers correspond to the main text file viewed in simple markup.
- Supp.Fig2 Legends for "0" and "1" and "NA" values. - Data presentation: The manuscript and Supp.files should maintain consistency by using either one or two decimal places throughout. One decimal should be sufficient for the precision.	The legend legends have been updated to define "0", "1", and "NA". The decimal places in the supplementary data file have been updated to align with the main text as well as the editorial feedback. For Figure 5, we have retained three significant figures due to the small differences between the AUC differences.
Reviewer #4 (Remarks to the Author): No further comments	Thank you for taking the time to review our revised manuscript. We're glad that our revisions addressed your previous comments.