August 14, 2022

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Dear Dr. Islam,

Thank you for the review of our manuscript. Based on the comments from editors and reviewers, we have revised the manuscript and provided responses to each specific comment (below). The revision process has helped us to improve the paper; thank you. The “R1” version of the manuscript is now submitted in conjunction with this letter. We are excited about the potential of publishing this work in The BMJ.

Sincerely,

Katherine Adams, US Centers for Disease Control and Prevention
Wesley Self, Vanderbilt University Medical Center
For The IVY Network
Comments from the editors and reviewers

Manuscript Committee Comments

1. The VE of the third dose has been widely reported, including that on Omicron variants. Therefore, the manuscript in its current form was not evaluated as a priority by the Editorial Board. However, as we discussed, data on Omicron sub-variants may be of interest, and we look forward to the revised version with data on the sub-variants (and possibly the 4th dose) before any formal decision is made.

Response: Thank you for your comments. We have revised the manuscript to include VE estimates based on Omicron sublineage as well as on 4th dose recipients. We agree this substantially improves the work; thank you for the opportunity to includes these new data.

2. With the updated data, it is also expected that additional analysis could be conducted on immunocompromised patients.

Response: Thank you for your comment. We have updated the manuscript to include detailed results for immunocompromised patients. Updating our dataset with enrollments through 30 June 2022 enabled us to have adequate sample size to provide more complete results on the immunocompromised population. We agree this is an important addition to the work.

3. Cases and controls were not matched, and the comparison was adjusted only for admission date (biweekly), age (with broad categories), sex, race/ethnicity, and region of the admitting hospital. Could you please comment on the adequacy of this fairly minimal adjustment. Some Editors mentioned other potentially important covariates such as comorbidities, SES, residential status, etc.

Response: Thank you for your comments; they are important to consider. Patients were not matched on individual patient characteristics. After careful consideration during the design phase of our platform, we decided not to match cases and controls based on patient characteristics for several reasons, including: to avoid loss of flexibility in enrollment, to maximize sample size, and to avoid potential introduction of selection bias.

Covariates for adjustment have been selected using recommended methods for identification of potential confounders (https://doi.org/10.1093/oxfordjournals.aje.a116813), using a prespecified cutoff for inclusion in the final model of absolute change of >5% in the odds ratio of vaccination.

As requested, we explored the potential of using additional co-variables, including comorbidities, residential status, and socioeconomic status (highest education level completed is the best variable in our dataset to approximate socioeconomic status). These sensitivity analyses have been added to the manuscript (Table S5) and are summarized here:

- We analyzed comorbidity burden using an ordinal variable consisting of the count of categories of chronic medical conditions. When this covariable was added to our current primary series model, the point estimate for the adjusted odds ratio changed from 31% (current model) to 30% (current model plus number of categories of chronic conditions co-variable).
- We classified patient’s home living status using a binary variable of community living vs residential living (which included nursing homes, assisted living facilities, rehab hospitals and other setting with profession care). When this covariable was added to our current primary...
series model, the point estimate for the adjusted odds ratio changed from 31% (current model) to 29% (current model plus residential status co-variable).

- We used highest education level completed as a marker for socioeconomic status (we recognize that education level is not a perfect measure for socioeconomic status). Highest education completed was classified using a binary variable: college or less vs more than college. When this covariable was added to our current primary series model, the point estimate for the adjusted odds ratio changed from 31% (current model) to 33% (current model plus educational status co-variable).

The results of these sensitivity analyses (Table S5) are consistent with analyses we have conducted over the past two years, in which the adding variables for chronic conditions, living status, and socioeconomic variables did not substantively change vaccine effectiveness results (+/- >5%) compared to our base/primary model (https://doi.org/10.1093/cid/ciab687, https://doi.org/10.1001/jama.2021.19499, https://doi.org/10.1136/bmj-2021-069761).

4. A recent publication recommended against using Omicron hospitalization as an indicator for severe Covid-19 diseases and indicated some approaches that better characterize vaccine protection against severe Covid-19 disease caused by Omicron, e.g., indicators of respiratory distress. (https://doi.org/10.1016/j.vaccine.2022.04.069) Could you please address the issues raised by them.
Response: Thank you for your comment. To address the question of COVID-19-associated hospitalization (versus hospitalization with incidental COVID-19), our case definition in IVY specifies that patients must have tested positive for SARS-CoV-2 within 14 days of onset of at least one of the following signs or symptoms believed to be due to an acute viral infection: fever; cough; shortness of breath; loss of taste; loss of smell; use of respiratory support (high flow oxygen by nasal cannula, non-invasive ventilation or invasive ventilation) for the acute illness; new pulmonary findings on chest imaging consistent with pneumonia. Thus, all enrolled COVID-19 cases have laboratory confirmed and symptomatic COVID-19; that is, patients hospitalized for another non-COVID reason and without clinical signs/symptoms of COVID-19 were not enrolled in this study because of an isolated positive SARS-CoV-2 test. Furthermore, we have added a subgroup analysis to evaluate vaccine effectiveness among immunocompetent COVID-19 patients admitted with hypoxemia, defined as patients who received supplemental oxygen or had an oxygen saturation (SpO2) <92% within 24 hours of admission. This sensitivity analysis closely follows the recommendations outlined in the Feikin et al reference in Vaccine referenced in the editors’ comment. The subgroup analysis defining cases as patients hospitalized with COVID-associated hypoxemia demonstrated similar trends across vaccination groups, with a lower VE for immunocompetent patients receiving a primary series alone (48%, 95% CI: 36–57%) than for a primary series plus one booster dose (74%, 95% CI: 67–79%) or a primary series plus two booster doses (63%, 95% CI: 29–81%).

Reviewer 1 Comments

1. Of 1,572 COVID-19 patients only in 648 (41 %) whole-genome sequencing was performed and in 78 % a variant could be identified. Among identified variants, 13 % were Delta and were excluded from further analysis. I see no point for this exclusion. Very likely the same percentage of not-Omicron variants was the infecting agent in those not submitted to WGS. But they remain in the analysis. WGS should only be used to substantiate that indeed the predominant agent in the enrolled subjects was
the Omicron variant of SARS-CoV-2. I suggest to change the title to “Vaccine Effectiveness of Primary Series and Booster Doses During a Period of Omicron Variant Dominance against COVID-19-Associated Hospitalization in the United States”

Response: Thank you for your comment. We have included additional sequencing information in the revised manuscript and the percentage of COVID-19 cases with a sequenced lineage available is now 41%. We agree it is important to note that not all cases had the infecting virus successfully sequenced and that cases without a sequenced variant be included in the analysis. In this analysis, which focuses on the Omicron variant, we think it is most rational to exclude cases with a known variant other than Omicron. Exclusion of sequenced samples with a lineage identified as discordant with the time period of variant predominance is consistent with our previous analyses published in BMJ (https://doi.org/10.1136/bmj-2021-069761), with the rationale that while sequencing was not able to be performed for every case-patient, known cases of other variants should be excluded for more precise estimates of VE for the Omicron variant. The same approach is taken in our subgroup analysis of VE by Omicron sublineage (BA.1, BA.2, BA.4/5).

We have revised the title of the manuscript to “Vaccine Effectiveness of Primary Series and Booster Doses against COVID-19-Associated Hospitalization During a Period of Omicron Variant Dominance in the United States”.

2. There are some important differences between unvaccinated and vaccinated as well as between cases and controls as reported in Tables 1 and 2. Unvaccinated had less often a prior hospital visit that may point to differences in health-care seeking behavior. Another important difference is the higher rate of self-reported prior SARS-CoV-2 infection in controls. The model was not adjusted for prior infection. It seems that this information was not available for all participants (which is strange in my view and could be due to a wrong assignment of missing values for those that responded they cannot remember). I strongly recommend inclusion of prior infection at least within a sensitivity analysis that may be reported in the supplement.

Response: Thank you for your comments. To examine the role of prior hospitalization as a potential confounder, we have repeated our regression analysis with prior hospitalization as a covariate. Prior hospitalization was omitted from the final model due to not meeting the prespecified absolute change of 5% in the odds ratio of vaccination (Table S5). This has been added to the methods section.

Information on known prior infection was obtained during the patient interview upon enrollment. However, definitive prior infection history is not available for some of these hospitalized patients for many reasons, including patient refusal to answer, patient being unable to communicate due to illness, and the source of the information being a proxy (i.e., surrogate). For the current patient cohort, the proportion of patients with self-reported prior infection is well below anticipated levels of US population with SARS-CoV-2 antibodies based on national seroprevalence data (https://doi.org/10.15585/mmwr.mm7117e3), suggesting under-capturing. Given the heterogeneity of self-reporting of prior infection status as well as date of prior infection, lack of antibody testing for all patients (which itself may underestimate prior infection due to declining antibody titers as previously examined by our group (https://doi.org/10.15585/mmwr.mm6947a2) and others), and the milder and potentially subclinical disease presentation of Omicron leading to greater uncertainty around prior infection status, we believe the best course of action is to include a sensitivity analysis that excludes patients with self-report prior SARS-CoV-2 infection and retain all participants in the primary analysis. In
the supplement, we have now included results for this sensitivity analysis (Table S5) that excludes patients with self-report of a prior SARS-CoV-2 infection within the past 120 days. Results of this sensitivity analysis show that the point estimate for the adjusted odds ratio did not change (31% with current model, 31% with those with prior SARS-CoV-2 infections within 120 days removed).

3. Table 1 would benefit from columns with p-values comparing vaccinated/unvaccinated and cases/controls. Table 2 has a p-value comparing the three groups simultaneously. I’d rather like to see a comparison vaccinated/unvaccinated and primary/booster vaccinated. These comparisons are orthogonal and no adjustment is needed.

Response: Thank you for your comment. We note that different journals, editors, and reviewers have different policies and views regarding the inclusion of p-values in tables of baseline characteristics. (For example, reviewer #3 suggested that we remove all p-values for baseline comparisons). We are happy to include the p-values or omit them based on directions from The BMJ. In the revised manuscript, we have added p-values to Table 1 for completeness; these can be easily removed if we are requested to do so. For Table 2, we have a p-value comparing unvaccinated versus vaccinated (any group).

4. Although a waiver of informed consent was granted for the public health surveillance, this does not hold for the investigation of the antibody response. Nothing is specified how the patients for this study were recruited and whether or not informed consent was obtained.

Response: The project evaluating antibody responses was determined to be public health surveillance with waiver of informed consent by IRBs at the US Centers for Disease Control and Prevention and three of the participating sites. At one participating site, the antibody response project was determined to be a research study and participants at that site provided written informed consent for participation in this component of the program. We would be happy to add this information to the manuscript if The BMJ would like this information included.

5. Concerning antibody responses it should be mentioned that the BAUs are with respect to a standard pool from 2020 with antibodies against the original virus (predominantly D614G) and that values against Omicron are likely a factor of about 100 lower.

Response: Thank you for noting this. In the revised manuscript we added the following language to the methods section:

“An international reference standard (WHO NIBSC 20/136) consisting of pooled sera from patients infected during 2020 with ancestral strain viruses was used to establish the BAU / ml scale. Notably, as Omicron variants have more than 30 mutations in spike and 15 or more in the receptor binding domain, titers against Omicron spikes are expected to be lower for Omicron than ancestral strains.”

Reviewer 2 Comments

1. The use of hospitalization together with a positive test is, as the authors themselves note in the Introduction, problematic in the Omicron period of dominance. It can be difficult to disentangle the “because of covid-19” hospitalizations from the “with covid-19” hospitalizations. The proportion of “with covid-19” hospitalizations becomes significant when infections are common in the population. As I understand it, the authors use signs and symptoms of covid-19 together with a PCR positive test
to identify cases. But this does not ensure that the hospitalization “was because of covid-19”, i.e. you can still be admitted due to a non-covid related issue and have incidental symptomatic infection? The authors write “admitted to the hospital with COVID-19” lines 255-56. It should be clarified what this means.

Response: These are good and important comments. As detailed in our response to Manuscript Committee comment #4, we approached the issue of incidental omicron infection among hospitalized patients in two ways. First, we only enrolled cases who were symptomatic with signs and symptoms of COVID-19; thus, unlike some studies that analyze administrative datasets consisting of hospitalized patients with a positive SARS-CoV-2 test, we only enrolled cases confirmed to have symptomatic COVID-19. As noted by the reviewer, even with symptomatic laboratory-confirmed COVID-19, it is possible that a concurrent condition contributed to the decision to hospitalize a patient and without the concurrent condition the symptomatic COVID-19 would have been managed as an outpatient. Thus, in the revised manuscript, we added a subgroup analysis limiting COVID-19 cases to those who were hypoxemic (defined as SpO2 <92% or treated with supplemental oxygen); these patients had a very high likelihood of COVID-19 directly causing illness severity to warrant in-hospital treatment.

2. Have the authors evaluated the admission/discharge diagnoses associated with the hospitalisations?

Response: Surveillance program personnel enrolled all participants prospectively with real-time collection of symptoms, signs, viral testing results, and vaccination status. All participants (including cases and controls) were hospitalized with signs and symptoms of an acute respiratory illness. We did not collect diagnostic codes (such as ICD-10 codes). We have collected diagnostic codes in prior iterations of the surveillance program but did not find them particularly useful as adjuncts to the information we collected from participants and medical records in real-time; thus, we dropped the collection of diagnostic codes for this iteration of the program.

3. It is a weakness that no information on prior infection is included. Can previously infected individuals be excluded from the study? In table 1, self-reported prior infection is mentioned. Please provide sensitivity analyses, where patients with self-reported infection are excluded.

Response: Thank you for your comment. Please see Reviewer 1 response #2. We have added a sensitivity analysis in which patients who self-reported a prior episode of COVID-19 were excluded.

4. The study estimates VE with unvaccinated as a reference group. I do not think this is the most relevant comparison at this point, neither from the individual level (will I benefit from a booster) or a public health perspective (should we roll-out a booster). The most relevant comparison is booster vs primary course. I would refocus the paper with this in mind.

Response: Thank you for bringing attention to the important concepts of absolute VE (vaccinated vs. unvaccinated) and relative VE (vaccinated group 2 vs. vaccinated group 1). While relative VE (rVE) is of interest for decision-making, rVE relies on these critical absolute VE estimates we report here. For example, an rVE estimate for 3 doses vs 2 doses translates to the proportion of residual disease remaining after full implementation of the 2-dose primary series program that implementation of a 3-dose program would prevent; however, it does not truly compare how effective (in terms of true VE) one regimen is compared to the other. We believe that absolute VE calculations are preferable to rVE when an unvaccinated comparison group is available for several reasons. First, reporting absolute VE
allows for the number of events averted (such as COVID-19 hospitalizations) due to vaccination to be estimated, showing the effect of booster implementation on a population level. Second, reporting absolute VE provides important reference values for other studies of booster effectiveness during the Omicron-predominant era. Since other surveillance networks that lack unvaccinated participants may not be able to report absolute VE, estimates of absolute VE from other studies (such as the current study, or earlier studies from the same locale) are necessary to provide context for what the absolute VE of the primary vaccine series was likely to have been.

5. A case could be made for excluding the antibody measurements part from the manuscript. The volunteers for this part are not included in the VE part on hospitalized patients and the study periods do not align, i.e. it is essentially another mini study. The results are also not discussed in any detail, nor are they novel. The presentation of the manuscript will be improved by dropping this part.
Response: The antibody measurement results are presented as supporting data for the clinical vaccine effectiveness results. We believe they add to the report by internally providing data that support the biological plausibility for the clinical findings. We would prefer to retain this component of the manuscript but are happy to defer to the preferences of The BMJ.

6. Please discuss the potential weaknesses of the test-negative design in this scenario in more detail – see e.g., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6888869/#BX2.
Response: We agree that the test negative design, like all study designs, has potential limitations that deserve careful consideration. Leading up to the release of COVID-19 vaccines, the World Health Organization convened a panel of experts to provide guidance on optimal approaches for observational COVID-19 vaccine effectiveness studies (PMID: 34119350). The group recommended the use of a test negative design and several methodological details. Our study design for the current study is consistent with these WHO recommendations. As noted by the Chua et al reference highlighted by the reviewer, approximately 90% of vaccine effectiveness studies published between 2011 and 2020 used a test negative design. The Chua et al review notes several potential limitations of the test-negative design and recommendations for minimizing these limitations (largely outlined on pages 52-54 of the article). The current study fulfills nearly all the recommendations by Chua et al. In the table below, we list key limitations and recommendations from Chua et al and the approach we took in the current study related to these limitations and recommendations.

<table>
<thead>
<tr>
<th>Potential limitation of the test negative design noted by Chua et al (PMID: 34119350)</th>
<th>Approach to taken in the current study</th>
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<tbody>
<tr>
<td>Bias estimates may arise if the study fails to adjust for calendar time (page 52)</td>
<td>We adjusted for calendar time using admission date in biweekly intervals.</td>
</tr>
<tr>
<td>Bias estimates may arise if vaccination affects the probability of healthcare seeking behavior between cases and controls (page 52)</td>
<td>We designed the study so that both the case and control group consisted of adults hospitalized with acute signs and symptoms of an acute respiratory infection. This approach to a control group tends to lessen the concern of controls having different healthcare seeking behavior than cases because they have similar clinical symptoms. We have extensively evaluated alternative control groups in 2021 and found no difference in COVID-19 vaccine effectiveness estimates using alternative control</td>
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<tr>
<td>Bias estimates may arise from misclassification of case vs control status or vaccinated vs unvaccinated status. (page 52)</td>
<td>We worked hard to minimize misclassification of case-control status and vaccination status. To classify case-control status we used both clinical SARS-CoV-2 test results obtained clinically at the local hospital and systematically tested nasal swabs from participants at a central research laboratory using standardized RT-PCR methods. Controls had negative SARS-CoV-2 tests both locally and at the central laboratory. To minimize the risk of vaccination status misclassification, vaccination status was ascertained via multiple parallel methods including patient report and systematic searches of CDC vaccination cards, state vaccine registries, and hospital electronic records.</td>
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<td>“Prospective test-negative design studies (as opposed to retrospective studies using administrative data) may be the most robust” (page 52).</td>
<td>We conducted a prospective study with real-time enrollment of all participants with confirmation of COVID-like illness at the bedside.</td>
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<tr>
<td>“Biases could persist under the test-negative design if broad variation in disease manifestation gives rise to differential healthcare-seeking behavior in terms of vaccination and care seeking for symptoms and affect the probability of being tested” (page 52).</td>
<td>Essentially all hospitalized patients during this time period were tested for SARS-CoV-2 based on usual care hospital practices. However, our study included only hospitalized patients who met a prespecified case definition including signs and symptoms of an acute respiratory illness compatible with COVID19. Furthermore, we did not enroll patients with only gastrointestinal manifestations of COVID-19 illness to minimize the concern for differential healthcare seeking behavior that could occur with a broader case definition.</td>
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<tr>
<td>“A more stringent clinical case definition could be used to reduce this source of bias, for example by recruiting severely ill inpatients as in the case of pneumococcus” (page 52).</td>
<td>We used a stringent and prespecified case definition which included a positive laboratory test for SARS-CoV-2, hospitalization, and acute COVID-like illness symptoms and signs, defined as fever, cough, shortness of breath, loss of taste, loss of smell, use of respiratory support (high-flow oxygen by nasal cannula, non-invasive ventilation, or invasive mechanical ventilation), or new pulmonary findings on chest imaging indicating pneumonia. In a subgroup analysis, we applied a more stringent case definition to focus on the subset of patients with even more severe disease (i.e., with documented hypoxemia).</td>
</tr>
<tr>
<td>“in general it may not be appropriate to pool data from inpatients and outpatients when estimating VE” (page 52).</td>
<td>We only report on in-patients.</td>
</tr>
<tr>
<td>“Nevertheless, the choice of clinical symptoms for enrolled patients should ensure similarity between cases and controls” (page 52)</td>
<td>We used identical clinical eligibility criteria for cases and controls.</td>
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<td>“An important consideration, therefore, in the application of the test-negative design is whether the vaccine in question causes there to be a difference in the source populations from which the cases and controls are derived. For example, in the</td>
<td>The current study was conducted in US hospitals during a period when testing for SARS-CoV-2 was virtually universal and unrelated to disease severity. Our study only enrolled hospitalized patients who met a prespecified clinical definition. Furthermore, to address</td>
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case of rotavirus vaccine, where vaccination can mitigate the severity of disease, the propensity to seek care differs between cases and controls leading to a form of selection (collider) bias that can harm the validity of VE estimates” (page 52).

“We would be particularly cautious over the choice of nonvaccine-type patients as controls for vaccine-type cases, as observed in most of the pneumococcal studies. (page 53)

“We would be particularly cautious over the choice of nonvaccine-type patients as controls for vaccine-type cases, as observed in most of the pneumococcal studies. (page 53)

Concerns about selection issues, our study includes a subgroup analysis, which applied a more stringent case definition to focus on the subset of patients with even more severe disease (i.e., with documented hypoxemia) which should further mitigate concerns about differential healthcare seeking between cases and controls.

“Our controls tested negative for SARS-CoV-2 (controls were not infected with a particular variant of SARS-CoV-2).

“Almost all VE studies should control for age.” (page 53)

“We adjusted for age.

“Controlling for calendar time is important in situations when vaccination uptake and risk of disease vary over time.” (page 53)

“We adjusted for calendar time using admission date in biweekly intervals.

Recommendation 1: “Select cases and controls that manifest similar degree of severity” (page 54)

All cases and controls were hospitalized with the same acute signs and symptoms of an acute respiratory illness.

Recommendation 2: “Select controls that are unaffected by vaccination” (page 54)

Our controls were hospitalized with an acute respiratory illness and tested negative for SARS-CoV-2 by two tests. Disease that is not from SARS-CoV-2 infection should not be affected by COVID-19 vaccination.

Recommendation 3: “Provide clearly defined case criteria” (page 54)

A single, clear and prespecified case definition was prospectively used by all enrolling sites through the entire enrollment period.

Recommendation 4: “Make appropriate adjustments for confounding and report VE estimates that reflect the causal effect of vaccination in reducing the risk of disease In a VE test-negative design study, unbiased VE estimates can be obtained under the following assumptions:

1. Vaccination does not affect the probability of becoming a control.
2. Vaccination does not affect the probability of seeking medical care.
3. Absence of misclassification of exposure and outcome status.” (page 54)

As detailed above, we adjusted for age and calendar time and have evaluated the effect of many other variables on vaccine effectiveness estimates (none of which substantively change the estimates). Additionally, we have carefully designed the study to minimize the risks of vaccination affecting the probability of a patient being a control (all controls were confirmed not to have SARS-CoV-2 by two tests), of vaccination affecting medical care (our VE estimates are limited to severe disease resulting in hospitalization, which should minimize variation in healthcare seeking behavior and a subgroup analysis was further limited to the subset of patients hospitalized with severe disease [i.e., documented hypoxemia]), and of misclassification (multiple parallel methods were used to maximize the accuracy of case-control classification and vaccination status classification).

In the limitations section of the revised manuscript, we more clearly note the test negative design has potential limitations and include reference to the Chua et al article. The limitations section now includes this language:

“While the test-negative design is a preferred method for evaluating vaccine effectiveness with observational data and is the method recommended by the World Health Organization for COVID-19 vaccine effectiveness studies, potential limitations of the test-negative design should be considered. These limitations include collider bias, which can occur when attenuation of disease severity from
vaccination results in different healthcare seeking behavior, leading to differences in testing or hospitalization, between cases and controls. However, the impact of collider bias is likely lower in this study than outpatient vaccine effectiveness studies due to its focus on severe COVID-19 in the hospital, where a high proportion of COVID-19 cases should be detected due to near universal testing for SARS-CoV-2 (regardless of disease severity) among hospitalized adults in the US during the time of period of this study.”

7. Generalizability: these are older hospitalized patients (maybe add “older adults” to the title). Can these results be used to support 18+ recommendations in the general non-hospitalized population?
Response: All adults at least 18 years old were eligible for enrollment. We believe the enrolled population is a good representation of adult patients who were hospitalized in the US during the study period. The median age was 64 years. We provided vaccine effectiveness results stratified by age group (18-49 years old; ≥50 years old). We believe the relatively low number of very young adults (e.g., <40 years old) reflects a low volume of younger patients getting severe disease requiring hospitalization from Omicron infection.

Reviewer 3 Comments

1. The authors used the so-called test-negative design in which cases status is defined by a positive test, here against SARS-CoV-2. The test-negative group has similar symptoms (i.e., respiratory infections) cause by another virus, e.g., influenza. The underlying conception for this design assumes that if two (or more) viruses do not interact and circulate independently, then the frequency of co-detection estimated from cross-sectional data should be approximately equal to the product of each virus’s detection frequency. Conversely, any significant deviation from equality should then indicate interaction. While this study design has been uses in other studies, the fundamental validity of this design has hardly been tested. In a recent paper by Domenech de Cellès and colleagues (https://doi.org/10.1098/rspb.2021.2358), the fundamental principles of the test-negative design have been challenged. The authors of that paper show that by focusing on the pair influenza –SARS-CoV-2, the prevalence ratio systematically underestimates the strength of interaction, and can even misclassify antagonistic or synergistic interactions that persist after clearance of infection. Overall, this paper suggests that ecological or epidemiological studies based on co-detection prevalence data provide a poor guide to assess interactions among respiratory viruses. Consequently, inferences about vaccination effects can be equally poorly estimated from such studies.

Response: We agree that the test negative design has limitations, but it has been used for the vast majority of observational vaccine effectiveness studies during the past decade (~90% as estimated by Chua et al, PMID: 31609860) and is the recommended design by the WHO for COVID-19 vaccine effectiveness studies (Patel et al, PMID: 34119350).

The article by Domenech de Celles et al referenced by the reviewer notes that prevalence studies that examine the co-detection of SARS-CoV-2 and influenza may underestimate the biological interaction of these two viruses. Our understanding of this paper is that the authors are challenging the use of cross-sectional prevalence data to assess biological interactions between respiratory viruses. The test negative design is mentioned on page 2 of the article:
“In particular, recent studies of SARS-CoV-2 interactions used a test-negative design to compare the risk of SARS-CoV-2 infection among those infected with another respiratory virus (e.g., influenza) to that among those uninfected. The underlying idea is conceptually simple: if two (or more) viruses do not interact and circulate independently, then the frequency of co-detection estimated from cross-sectional data should be approximately equal to the product of each virus’s detection frequency—conversely, any significant deviation from equality should indicate interaction. However, earlier epidemiological and ecological modelling studies have cautioned against seemingly intuitive statistics of interaction [14,19,20]. In fact, to our knowledge the validity of this study design has not yet been systematically tested for emerging or seasonal respiratory viruses.”

Our understanding is that the authors are not challenging the use of the test negative design to evaluate COVID-19 vaccine effectiveness but rather are challenging the notion of comparing the prevalence of influenza detection among SARS-CoV-2 positive cases and SARS-CoV-2 negative controls as a reliable method of evaluating the biological interaction between SARS-CoV-2 and influenza. From the perspective of designing a COVID-19 vaccine effectiveness study using a test negative design, we think the most important take away message is that patients with influenza should not be used in the control group. The current study does not include patients with influenza in the control group. Patients in the control group were negative for SARS-CoV-2, influenza, and RSV.

2. As study participants all have been hospitalized, there is an inherent selection bias. As there are other reasons for hospitalizations that may not have been recorded, Selection of participants represent a collider-stratification bias. More details on the selection bias in test-negative studies can be found in a commentary by Westreich and Hudgens: https://doi.org/10.1093/aje/kww063 and in the study by Sullivan and colleagues (https://doi.org/10.1093/aje/kww064). From the analysis approach of the paper by Adams and colleagues, I do not see an attempt to correct for this bias, if that is possible at all.

Response: The reviewer raises an important question about the potential of collider bias. In our study, the outcome is hospitalization for COVID19, and the main exposure of interest is SARS-CoV-2 vaccination. In our study, cases and controls were patients hospitalized who met a prespecified clinical definition compatible with COVID19. Furthermore, in a subgroup analysis, we further limited the population to the subset of more severe hospitalized patients with documented hypoxemia.

We acknowledge that our study design conditions on testing, which has been identified as a source of collider bias in other study designs. Traditional test-negative design studies focus on infection as the outcome (e.g., usually identified in the outpatient setting) and considers that healthcare seeking behaviors may influence vaccination and also lead to more testing. Since the infection outcome itself would also lead to testing, conditioning on testing represents a collider issue in those designs (as noted by the references provided by the reviewer, including Sullivan et al AJE 2016 and Westreich et al AJE 2016). This concern has been extensively studied in preparation for COVID-19 vaccine effectiveness studies, and formal recommendations have been made by a technical group of methodology advisors convened by the World Health Organization (WHO) to guide the design of vaccine effectiveness studies (Patel MK et al. Vaccine 2021). In line with those recommendations to mitigate concerns about collider bias (Please see Table 3 of the WHO report https://doi.org/10.1016/j.vaccine.2021.05.099), our study
focuses on patients meeting a clear, prespecified clinical syndrome definition and we have also built in an analysis that focuses on a subset of participants experiencing severe disease (i.e., documented hypoxemia), which is less influenced by healthcare seeking behaviors.

Importantly, several lines of evidence suggest that the impact of this potential bias may be low in the current study. First, the findings of the vast majority of COVID-19 vaccine effectiveness studies using the test-negative design, as well as other designs, have been consistent with the vaccine efficacy demonstrated by randomized controlled trials. To address concerns similar to those raised by conditioning on testing as a source of collider bias, our group extensively examined two distinct control groups (test negative and syndrome negative) for test-negative design studies of COVID-19 vaccine effectiveness throughout 2021 and found virtually identical vaccine effectiveness estimates using both traditional test-negative controls (patients hospitalized with an acute respiratory illness and test-negative for SARS-CoV-2) and “syndrome-negative” controls (patients hospitalized without an acute respiratory illness); these findings are detailed in: CID 2021, PMID: 34358310 and JAMA 2021, PMID: 34734975. Finally, unlike with other vaccine preventable diseases such as influenza, testing for SARS-CoV-2 has been virtually universal and unrelated to disease severity in the hospital setting, thus likely reducing concerns about selective testing, and the conditioning on testing as a collider.

We agree that acknowledging potential limitations of the test-negative design is important. Thus, we have added language to the limitations section of the manuscript and referenced literature noted by the reviewers that highlight potential limitations of the test negative design. The updated limitations section now includes:

“While the test-negative design is a preferred method for evaluating vaccine effectiveness with observational data and is the method recommended by the World Health Organization for COVID-19 vaccine effectiveness studies, potential limitations of the test-negative design should be considered. These limitations include collider bias, which can occur when attenuation of disease severity from vaccination results in different healthcare seeking behavior, leading to differences in testing or hospitalization, between cases and controls. However, the impact of collider bias is likely lower in this study than outpatient vaccine effectiveness studies due to its focus on severe COVID-19 in the hospital, where a high proportion of COVID-19 cases should be detected due to near universal testing for SARS-CoV-2 (regardless of disease severity) among hospitalized adults in the US during the time of period of this study.”

3. (Minor) I suggest that the authors do not conduct statistical null hypothesis tests to compared baseline differences of the participants.
Response: Please see our response to Reviewer 1, Comment 3. Reviewer 1 and Reviewer 3 seem to have given contradictory recommendations regarding this topic. We are happy to proceed as directed by The BMJ.