nature portfolio

Peer Review File

Evaluating the Safety of XBB.1.5-Containing COVID-19 mRNA Vaccines Using a Self-Controlled Case Series Study

Corresponding Author: Dr Yongqun He

Parts of this Peer Review File have been redacted as indicated to remove third-party material.

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)
The study under review provides a timely and important evaluation of the safety profile of XBB.1.5-containing COVID-19 mRNA vaccines, which is critical given the ongoing vaccination campaign in the United States. By leveraging data from the National COVID Cohort Collaborative (N3C) and employing a self-controlled case series (SCCS) design, the authors examined the risk of 14 adverse events of special interest (AESIs) among over 33.9 million doses administered from September 2023 to March 2024. The study concludes that there is no evidence of an increased risk for the investigated AESIs within the 28-day post-vaccination period, potentially offering valuable reassurance about the vaccine's safety. While this study may provide an essential addition to the safety profile of XBB.1.5-containing COVID-19 mRNA vaccines, I have some concerns regarding certain aspects of the methodology and interpretation of the findings, which I outline below.
Introduction
1. It is important to use the correct names for the XBB.1.5-containing COVID-19 mRNA vaccines to avoid confusion and ensure accuracy. The names Moderna mRNA-1273 and Pfizer–BioNTech BNT162b reference the original formulations targeting the ancestral SARS-CoV-2 strain. It would be more appropriate to refer to these vaccines as the Pfizer XBB.1.5 vaccine and Moderna XBB.1.5 vaccine for clarity.
2. In discussing surveillance systems for COVID-19 vaccine safety, it is essential to highlight the Vaccine Safety Datalink (VSD) as a critical component of the CDC's efforts to monitor vaccine safety. The VSD conducts rapid cycle analyses (RCA) and rigorous epidemiologic studies to assess potential safety signals and provide timely, high-quality safety data. Several significant studies conducted through the VSD have made substantial contributions to understanding the safety profile of COVID-19 vaccines. Key publications include studies with the following PMIDs but not limited to: 34477808, 35471572, 34710075, and 38388239.
Methods
3. It is unclear whether approval was obtained for international use and data sharing. Additionally, clarification is needed on whether Chinese institutions are required to secure IRB approval for accessing and using the data.
4. What is the meaning of "setting" in the subheading "Study Design and Setting"? If it refers to where cases were identified, it is important to note that cases identified in inpatient and ED settings typically have high accuracy for severe and rare events. However, for relatively common events, it may be necessary to include the outpatient setting for case identification. This study does not specify where the events were identified, which warrants clarification.
5. It is recommended to include important time-invariant confounders, such as sex and race/ethnicity, when describing how the SCCS method adjusts for these confounders.
6. It is not appropriate to use a uniform risk interval for all 14 AESIs. Biologically, shorter risk intervals may be more plausible for events such as anaphylaxis, while other events may require longer risk intervals.
7. It is unclear whether day 0 was included in the analyses. Including day 0 can be problematic, as it is challenging to determine whether the vaccination or the adverse event occurred first. Clarification on how day 0 was handled in the analysis is necessary.
8. The start date of the study is inconsistent throughout the manuscript, with both September 11, 2023, and September 13, 2023, being mentioned.
9. In addition, the end date of the study is inconsistent, with both July 1, 2024, and June 1, 2024, being mentioned. Given that very few COVID-19 vaccines were administered after the end of April, it is questionable to include time beyond April in the analysis.

10. It may not be appropriate to combine nonhemorrhagic stroke, transient ischemic attack, and hemorrhagic stroke into a single category, as their etiologies differ significantly. Suggest grouping into two groups: 1) nonhemorrhagic stroke and transient ischemic attack; and 2) hemorrhagic stroke.
11. Suggest changing “Each outcome was analyzed individually” to “Each outcome was analyzed separately”
12. It appears that the authors included only vaccinated individuals who experienced an adverse event in the SCCS analyses. This approach represents a vaccinated-only SCCS, which is a special case of the SCCS method.
13. It is unclear why the authors chose 3-month seasons to adjust for seasonality instead of using a more refined approach, such as adjusting for each individual month. Monthly adjustments could provide a more precise control for seasonal variations, especially if the adverse events of interest exhibit fluctuations within shorter period of time.
RESULTS
14. The IRR for AMI is close to significance, and the selection of the risk interval and sample size could potentially influence the conclusion. Further consideration and justification of these choices are recommended.
15. When describing the number of cases in Table 2, for those with fewer than 20 cases, it would be more informative to provide the actual count instead of using ranges or vague descriptions.
16. For narcolepsy or cataplexy, which are relatively common, clarification is needed on whether diagnoses made in the outpatient setting were included in the analysis.
17. Regarding myocarditis/pericarditis, these are two distinct conditions, and it is possible for one or both to occur in the same patient. Clarification is needed on what the term "myopericarditis" represents. If it refers to either or both conditions, using "myo/pericarditis" would be a clearer and more appropriate term.
DISCUSSION
18. Change “On the other hand, transverse myelitis….” to “On the other hand, transverse myelitis (TM) ….”
19. Regarding ischemic stroke, a recent study (PMID: 38916940) similarly found no significant increase in the risk of ischemic stroke following COVID-19 vaccinations.
20. It is important to discuss the accuracy of capturing these outcomes, including confirmation rates, to assess the reliability of the data.
21. The following sentence conflates two distinct study designs: "However, a large cohort study using the self-controlled case series (SCCS) method found no...". rewording this sentence is needed.
22. In conclusion, it is necessary to address the signal observed among individuals who received the Moderna XBB.1.5 vaccine.

Reviewer #2

(Remarks to the Author)
Abstract

Lines 20-22: This statement is not accurate. In the history of pharmacovigilance, spanning over 80 years, COVID-19 vaccine safety is among the most extensively studied, with comprehensive data derived from both formal active and passive safety surveillance systems.

The abstract lacks specificity. It should detail the adverse events assessed and the effect estimates to enhance informativeness.

Introduction

Lines 46-48: This statement is not accurate. I encourage the authors to review the literature on COVID-19 vaccine safety, including publications and reports from the V-Safe program and CBER Active Surveillance Program.

Lines 48-50: Again, this is not accurate. While there were initial concerns of vaccine safety, there was an unprecedented amount of thorough safety data accumulated and disseminated by the CDC and initial vaccinations rates were very high. More recent declines in vaccination rates are not safety-related.

Lines 50-52: The mention of rarely used vaccines not included in the study is irrelevant to the study's rationale.

Lines 52-55: There are larger, ongoing safety studies capable of detecting rare adverse events. This statement should be revised.

Line 59: "can introduce reporting bias and result" → Missing a space after the period in "methods.These".

Methods

Lines 88-89: Socioeconomic status and health conditions do vary over time.

Lines 89-90: This should be clarified to specify that all patients were a case in one or more of the AE analyses. Clarify how patients were handled during periods when they experienced one adverse event (AE) while serving as controls for other AEs. Specify if case time could overlap across multiple AEs and how control periods were defined (e.g., number of control periods contributed per patient, mean, standard deviation, median, interquartile range, minimum, maximum). Could patients

receiving multiple XBB vaccines (e.g. immunocompromised, booster recommended in those >=65 years) contribute case time more than once?

Figure 1: The figure and associated text are difficult to follow and inconsistent (e.g., 28 vs. 29 days). Specify whether the reference and risk periods are 28 days or 29 days (day 0 + 28 days) and ensure consistency across the manuscript and supplemental materials. If the risk period is actually 29 days (day 0 plus 28 days), this would need to be corrected throughout the manuscript and supplemental materials.

Presumably, the study begins on September 12, 2022, although this is not explicitly stated. It appears that all individuals included in the study must have received a COVID-19 vaccine between September 12, 2022, and September 11, 2023. Following vaccination, there seems to be a 28- or 29-day washout period (the figure and text are unclear on this point), after which control person-days were counted until the XBB vaccination and again after the 29-day risk period. However, it is unclear how individuals who did not receive a COVID-19 vaccine during the 2022-2023 season were handled. Excluding unvaccinated individuals from this timeframe would introduce selection bias and should be addressed explicitly in the methods.

Line 106 : “used to to”.

Line 115: Indicate the exact end date for follow-up. Specify if "younger than 18" applies as of September 12, 2022, or the vaccination date.

Line 116: There is ambiguity in how exclusion criteria 3 was applied. If a 28-day window was used instead of the intended 42 days (28 days for confounding effect elimination plus 14 days for the pre-exposure window), no additional days would remain to account for the pre-exposure window. This requires clarification to ensure the criteria were appropriately applied.

Line 117: Over what timeframe was this assessed, all history back to ___?

Line 119: Figure 2, there is an unexpectedly high percentage (3.8%) of individuals receiving another COVID-19 vaccine within 28 days of the XBB vaccine, as this contradicts vaccine recommendations. This raises concerns about potential inaccuracies in the data or issues with the operational definitions used in the study, especially given the counts of the other exclusions.

Line 128: Written prescriptions should not be used to operationally define vaccine administration or receipt. If written prescriptions were used, the language throughout the manuscript should be revised to specify vaccine administration and prescriptions.

Line 135: What is the rationale for not using the same list and categorizations as the CBER Active Surveillance Program? Also, what is the rationale behind using an outdated adverse event list from February 2021 rather than more recent events (e.g., injection site reactions, rash, headache, menstrual changes).

Lines 142-144: It appears that individuals with any of the 14 adverse events (AEs) during the baseline period were excluded from the study population. If so, these exclusions should be clearly documented in the study population flow chart, such as in Figure 2.

The rationale for excluding individuals with baseline AEs is likely to avoid including those predisposed to these events or already experiencing sequelae or complications. However, this approach conflicts with the inclusion of control periods for these same individuals after they experience an AE during the study period. This practice undermines the rationale for excluding such individuals initially, as it reintroduces them into the analysis despite their prior experience of related conditions, creating inconsistency in the application of criteria.

Excluding individuals based on the specific AE of interest during the baseline period would be a more appropriate strategy to ensure the study focuses on incident (new) cases of that AE. However, the broader exclusion of individuals with other AEs during the baseline period introduces selection bias.

Line 144: Was the baseline period 365 days before the study started, so Sept 12, 2022? Or was the baseline period 365 days before the first vaccination observed between Sept 12, 2022 and Sept 11, 2023. Again, Figure 1 and the associated text are not clear about these different periods.

Line 147: Again, still cannot tell what the baseline period is, as this says something different than other sections.

Lines 154-156: What is the rationale for controlling for season? While vaccine effectiveness can vary by time due to changes in predominant strain, AEs do not vary in any meaningful way over seasons. Due to small sample size, only confounders associated with both exposure and outcome should be controlled for, as controlling for a non-confounder can introduce bias.

Lines 159-160: Citation supporting this threshold?

How were patients developing COVID-19 handled as COVID-19 itself is a risk factor for some of these outcomes? Their follow-up should end if they are diagnosed with or test positive for COVID-19.

Results

Clarify if patients with missing data were excluded (no missing data in Table 1). If so, include this as an exclusion criterion in the methods and flowchart.

Figure 4: The forest plot format is unconventional and confusing. Align IIRs and 95% CIs with the corresponding colored lines, and include events/person-days directly on the figure for clarity.

Incidence rate ratios could not be estimated for encephalitis. As such, encephalitis should be excluded from the tables and figures. The single sentence in the Results section is sufficient to address this.

Discussion

Lines 249-251: Again, this is not accurate, there is substantial recent evidence indicating no association.

Line 327: “can not”.”

Limitations are highly understated and need to be expanded to include study design and data source constraints.

Throughout

Revise terminology: replace "gender" with "sex" where applicable, as per the Reporting Summary.

Pertinent data and analytic details specified in the Reporting Summary were absent from the manuscript.

Reporting Summary

Page 3 of Reporting Summary, Clinical Data should be selected and completed.

Supplemental Materials

Incidence rate ratios could not be estimated for encephalitis. As such, encephalitis should be excluded from the tables and figures. The single sentence in the Results section is sufficient to address this.

For reproducibility, concept IDs of exclusions should be presented.

Unclear how the concept ID code lists were developed. Citations of validation studies of these operational definitions should be provided. If no validation studies exist, the procedures for identifying and selecting these codes should be provided.

Supplemental Tables 2-5: Last column should indicate these are incidence rate ratios and 95% confidence intervals.

Supplemental Figures 1-2: The forest plots utilize an atypical format and are therefore confusing. The IIRs and 95% CI should be next to the colored line of that corresponding strata. This would also allow for the events/person-days to be included in the figure, and then the duplicative tables with IIRs can be removed. All results should be presented similarly (unclear why forest plots only generate for some stratified/sensitivity analyses) and not others.

Reviewer #3

(Remarks to the Author)
Dear Editor,
Thank you for inviting me to review the manuscript by Pan et al., submitted to Nature Communications and titled “Evaluating Safety of XBB.1.5-containing COVID-19 mRNA Vaccines Using a Self-Controlled Case Series Study,” that presents a large-scale analysis utilizing real-world data from the US National COVID-19 Cohort Collaborative (NC3).
The study reports no significant increase in the risk of 14 pre-specified adverse events of special interest following XBB.1.5-containing COVID-19 mRNA vaccination in the general adult population. Although two potential safety signals were noted in the subgroup analysis of the Moderna mRNA-1273 vaccine, these findings warrant cautious interpretation due to wide confidence intervals and the limited number of observed events. The study offers reassurance regarding the overall safety profile of XBB.1.5-containing COVID-19 mRNA vaccines while underscoring the necessity for continued surveillance and focused investigation within specific subgroups. The inherent limitations of EHR-based studies and the SCCS design must also be acknowledged when interpreting these findings. Additional research employing complementary study designs and data sources is needed to confirm the results and assess potential risks within distinct populations.
Strengths:
The study leverages a robust cohort of 259,136 individuals, which increases the probability of detecting uncommon adverse

events. The methodological approach, particularly the self-controlled case series (SCCS) design, effectively account for time-invariant confounders, such as socioeconomic status. Incorporation of seasonal adjustment accounts for temporal confounding as a result of the variable SARS-CoV-2 activity over the year, while the inclusion of a negative control outcome (diverticulitis) addresses vaccine-unrelated factors. Furthermore, the study's reliance on diverse electronic health record (EHR) data sources strengthens the completeness of the safety evaluation.
Limitations:
Several importan limitations must be considered. First, the intrinsic limitations of EHR data may compromise the completeness and accuracy of findings. Although the N3C database provides extensive data, it represents only a subset of the U.S. population, restricting generalizability. Second, the SCCS design, while robust, assumes that adverse events do not influence subsequent exposure—a condition that may not hold if severe events prevent further vaccination. Additionally, the study's focus on first-event occurrences may have overlooked subsequent adverse events during follow-up. Third, the predefined 28-day risk period, while consistent with prior studies, may fail to capture delayed adverse events with longer latency periods. Wide confidence intervals for rare events, such as transverse myelitis and acute myocardial infarction observed in the Moderna mRNA-1273 subgroup, suggest limited precision and necessitate cautious interpretation. Despite adjustments for seasonal variations, residual temporal confounding cannot be entirely excluded. Lastly, the exclusion of individuals without continuous observation or prior vaccination data may introduce selection bias, potentially impacting the representativeness of the sample.
Althoug the authors in the discussion section did acknowledge some of the limitations co,mented above, they missed several critical issues that, in my opinion, should be further discussed:
1. They don't address the potential violation of SCCS assumptions, particularly the event-independence assumption.
2. They don't discuss the rationale or potential limitations of their 28-day risk window choice.
3. While they mention the N3C's partial representation, they don't discuss the implications of their specific exclusion criteria on selection bias.
4. They don't address the limited power for detecting rare events, despite presenting wide confidence intervals for several outcomes.
5. They don't discuss the lack of validation of their outcome definitions or potential misclassification in EHR coding.
6. There's no acknowledgment of the limited subgroup analyses or the potential for differential risks in important subpopulations.
7. The multiple comparison issue is not addressed in their limitations section, though they briefly mention it in their methods.
8. They don't discuss potential limitations in their ascertainment of vaccination status or the quality of vaccination records.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thanks for addressing my concerns, well done. I only have two minor suggestions:
1. Please change "Pfizer–BioNTech's" to "Pfizer–BioNTech" in line 35.
2. In page 4, "While VSD only includes data of member sites." is not a complete sentence.

Reviewer #2

(Remarks to the Author)
Several issues noted during the initial review remain unresolved. The revised manuscript continues to reflect limited understanding of the existing COVID-19 vaccine safety research.

Background

• Lines 42–46: There is a major flaw in the framing of the introduction. Reformulation of vaccines, such as seasonal updates for influenza, is common and not inherently associated with different or novel safety profiles. Updated COVID-19 mRNA vaccines (e.g., bivalent, monovalent XBB.1.5) have similarly shown no major safety concerns attributable to strain changes.

• The generalization that variant updates result in altered safety profiles is not supported by biological plausibility or empirical evidence—especially when the vaccine platform, adjuvants, and delivery mechanisms remain unchanged.

• Regulatory agencies (FDA, CDC, WHO) and the existing published literature have clearly indicated continuity in safety across COVID-19 vaccine reformulations.

• The manuscript still fails to incorporate a comprehensive knowledge on the extensive global surveillance of COVID-19 vaccine safety conducted by agencies such as CDC, FDA, ACIP, and international counterparts.

• Lines 272–273: This statement remains unclear.

• Lines 313–314: Incorrect—analyses of VSD and other data sources, including CDC, FDA, and ACIP reports, are ongoing.

Other Vaccinations

• Lines 88–90: Patients who receive multiple vaccines get them moments apart. This omission is particularly critical since coadministration of vaccines is common and may affect adverse event rates. Other vaccine administrations were not captured by the study or adjusted in the analyses. This is a major limitation that calls the primary findings (on anaphylaxis) into question.

• Lines 198–200: No information is given about other recent vaccinations or how they may have affected the analysis and findings.

Purpose and Existing Literature
• The stated purpose and rationale for the study are not supported by scientific evidence. Multiple assertions are made without appropriate citations or grounding in the field.

• Lines 45–46: The claim that updated vaccines have unknown safety profiles is inaccurate. We have accumulated more safety data for COVID-19 vaccines—including XBB formulations—than for any other vaccine or medication in history.

• Lines 54–55 and 272–273: Numerous large, well-controlled studies have already assessed the safety of COVID-19 vaccines, including XBB. This study does not adequately compare its findings, particularly for anaphylaxis, to the existing literature and CDC/FDA/ACIP reports, nor does it present an appropriate range of rates.

• Lines 314–316: The claim that this study addresses gaps in knowledge is inaccurate.

• Lines 316–319: This overstates the study's contribution, especially given its limited sample size and limitations.

• Lines 320–321: Not accurate. European data are similar to other populations.

• Lines 323–325: Misleading—only one-eighth of the source population was included.

• Lines 323–335: The manuscript overstates its novelty and contribution. The findings confirm the extensive existing research and should be framed as such.

Study Design and Methods

• Key aspects of the study design remain unclear:
The case and control time periods are still not clearly defined. Revisions to Figure 1 do little to improve the clarity of timeline, particularly since there are conflicts with the text in terms of the baseline period.

Wash-out period (Figure 2) is not defined. From when?

Lines 96-97: No rationale for the 14-day pre-exposure window.

Lines 143–144: What happened if experienced again? Post-vaccination reference period should end on recurrence.

Lines 151–152: The baseline period is incorrectly fixed to January 20, 2020. It should be individualized based on each patient's XBB vaccination date. As currently described, outcomes in the reference period are be incorrectly included.

Lines 158–163: Confusing, should simply state controlled for week to account for possible changes in background disease rates over time.

Lines 198–200: Meaning without COVID-19 infection? The method for identifying COVID-19 infection status is not specified (diagnosis, positive lab tests?). Misclassification bias is likely, especially for mild cases that may not be captured in healthcare records, and this is not sufficiently addressed in the limitations.

• The demographic and comorbidity baseline period is defined using a one-year lookback from a uniform date, which does not account for varying vaccination dates.

• The entire results section contains new numbers, but these changes were not clearly marked (e.g., not shown in red).

• Line 84: Shouldn't this be "nature of vaccine-related reactions"?

• Lines 126–127: Shouldn't this be "self-reported vaccination"?

• Figure 1: Still confusing. Inconsistent use of terms for reference, risk, and wash-out periods. These terms must be defined and used consistently across text and figures.

• The forest plot line should be centered at IRR = 1, not 3.

• Lines 173–175: For either reference period or risk period? Use same terminology in figure and text throughout, for naming different periods of time.

Grammar

• Grammatical issues throughout the manuscript and response letter impede clarity and made some responses hard to follow.

• The manuscript should be proofread carefully by all authors.

Limitations

• The revised limitations section is vague and does not adequately respond to concerns raised by reviewers.

• Statements about the study's generalizability and contribution (e.g., Lines 320–335) are misleading. This study confirms what is already known.

Reviewer #3

(Remarks to the Author)
Thank you for your responses. I have read them carefully and all my comments were either answered or incorporated in the manuscript. I don´t have additional questions.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Point-to-point reply

Reviewer #1 :

The study under review provides a timely and important evaluation of the safety profile of XBB.1.5-containing COVID-19 mRNA vaccines, which is critical given the ongoing vaccination campaign in the United States. By leveraging data from the National COVID Cohort Collaborative (N3C) and employing a self-controlled case series (SCCS) design, the authors examined the risk of 14 adverse events of special interest (AESIs) among over 33.9 million doses administered from September 2023 to March 2024. The study concludes that there is no evidence of an increased risk for the investigated AESIs within the 28-day post-vaccination period, potentially offering valuable reassurance about the vaccine's safety. While this study may provide an essential addition to the safety profile of XBB.1.5-containing COVID-19 mRNA vaccines, I have some concerns regarding certain aspects of the methodology and interpretation of the findings, which I outline below.

Introduction

1. It is important to use the correct names for the XBB.1.5-containing COVID-19 mRNA vaccines to avoid confusion and ensure accuracy. The names Moderna mRNA-1273 and Pfizer–BioNTech BNT162b reference the original formulations targeting the ancestral SARS-CoV-2 strain. It would be more appropriate to refer to these vaccines as the Pfizer XBB.1.5 vaccine and Moderna XBB.1.5 vaccine for clarity.

Reply: We agreed with your valuable feedback regarding the naming of the vaccines. In our revised manuscript, we have updated the names of the vaccines to "Pfizer–BioNTech XBB.1.5 vaccine" and "Moderna XBB.1.5 vaccine" throughout the manuscript to ensure clarity and alignment with your suggestion. Please see the changes firstly in Page 3, Line 33.

2. In discussing surveillance systems for COVID-19 vaccine safety, it is essential to highlight the Vaccine Safety Datalink (VSD) as a critical component of the CDC's efforts to monitor vaccine safety. The VSD conducts rapid cycle analyses (RCA) and rigorous epidemiologic studies to assess potential safety signals and provide timely, high-quality

safety data. Several significant studies conducted through the VSD have made substantial contributions to understanding the safety profile of COVID-19 vaccines. Key publications include studies with the following PMIDs but not limited to: 34477808, 35471572, 34710075, and 38388239.

Reply: We agreed that Vaccine Safety Datalink (VSD) is a critical component of the CDC's efforts to monitor vaccine safety, assessing vaccine safety and detecting adverse events from member sites using EHR data. We have added associated text and relevant references about VSD as:

> "The Vaccine Safety Datalink (VSD) has been monitoring COVID-19 vaccine safety by assessing vaccine safety and detecting safety signals of adverse events from member sites using EHR data.[15-17]"

Please see Pages 4, Lines 51-53.

Methods

3. It is unclear whether approval was obtained for international use and data sharing. Additionally, clarification is needed on whether Chinese institutions are required to secure IRB approval for accessing and using the data.

Reply: The N3C only allows data access to researchers from institutions with an executed Data Use Agreement (DUA) with N3C, meaning it is not available for international use and data sharing. The team obtained DUA from the N3C Data Access Committee (DAC). The first author of this manuscript is a visiting student from China at the University of Michigan, with approval from the Data Access Committee (DAC) of N3C and in full compliance with data use policies and obligations. All of our manuscript has been reviewed and approved by the N3C publication committee. More details can be found in N3C webpage: https://covid.cd2h.org/account-instructions/tenant-checklist.jsp

We have clarified it in Page 5 Line 72-75 as:

> "Data usage for this study was authorized by N3C (DUR-36ED2AE) and reviewed and approved by the Medical School Institutional Review Board (IRB) at the University of Michigan (HUM00243962). All data usage was in compliance with N3C's data use policies and obligations."

And Page 24, Line 414 as：

"The N3C Publication committee confirmed that this manuscript MSID: 2137.155 is in accordance with N3C data use and attribution policies."

4. What is the meaning of "setting" in the subheading "Study Design and Setting"? If it refers to where cases were identified, it is important to note that cases identified in inpatient and ED settings typically have high accuracy for severe and rare events. However, for relatively common events, it may be necessary to include the outpatient setting for case identification. This study does not specify where the events were identified, which warrants clarification.

Reply: In the subheading "Study Design and Setting", "setting" refers to the locations or contexts where cases were identified. We agree that the accuracy of case identification can vary by setting. In our study, we included all of the eligible patients in the dataset, including patients from outpatient, inpatient, and ED, which are more reliable for severe and rare events. We have clarified this aspect in the revised manuscript to address your concerns.

"Records of specific outcomes from outpatient, inpatient, and emergency department settings were all retrieved from the dataset."

Please see Page 9, Line 146-147.

5. It is recommended to include important time-invariant confounders, such as sex and race/ethnicity, when describing how the SCCS method adjusts for these confounders.

Reply: We have explicit important time-invariant confounders, such as sex and race/ethnicity, when describing how the SCCS method inherently adjusts for these factors by design.

"The SCCS design is a method commonly utilized in vaccine safety studies, involving within-person comparison of risk and reference periods to eliminate non-time-varying confounders such as sex, race/ethnicity and genetic characteristics."

Please see Page 5, Line 80-81.

6. It is not appropriate to use a uniform risk interval for all 14 AESIs. Biologically, shorter risk intervals may be more plausible for events such as anaphylaxis, while other events may require longer risk intervals.

Reply: We agreed that acute diseases, such as anaphylaxis, require a shorter onset time compared to other events. Based on this, we adjusted the risk window for anaphylaxis to 0, 1, 2 days to more accurately capture the outcome.

We have re-analyzed this event and clarified in manuscript as

> "The exposure was defined as the administration of either brand of XBB.1.5-containing COVID-19 mRNA vaccines. The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases. For anaphylaxis, which requires a much shorter risk window due to its acute onset, we defined 0, 1, and 2-day risk periods to accurately capture relevant outcomes. Day 0 was included in the analysis for the following reasons: anaphylaxis typically occurs within minutes or hours after vaccination,[25,26] so day 0 is the best window to capture events. Additionally, patients who experience anaphylaxis are unlikely to receive further vaccination on the same day, thereby resolving the uncertainty of whether the vaccination or the adverse event occurred first. For other AESIs, the primary risk period extended from day 1 to day 29 after vaccination, this 28-day risk period was determined based on previous studies [23,24]"

Please see Page 6, Line 82-92.

7. It is unclear whether day 0 was included in the analyses. Including day 0 can be problematic, as it is challenging to determine whether the vaccination or the adverse event occurred first. Clarification on how day 0 was handled in the analysis is necessary.

Reply: Day 0 is not included in the analysis except for anaphylaxis. Day 0 for anaphylaxis is included due to its urgent nature and very short risk window. Since a person experiencing anaphylaxis would not be recommended to receive the vaccine on the same day, the issue of whether the vaccination or the adverse event occurred first is effectively resolved. We have clarified this point in the methods section.

> "The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases. For anaphylaxis, which requires a much shorter risk window due to its acute onset, we defined 0, 1, and 2-day risk periods to accurately capture relevant outcomes. Day 0 was included in the analysis for the following reasons: anaphylaxis typically occurs within minutes or hours after vaccination,[25,26] so day 0 is the best window to capture events. Additionally, patients who experience anaphylaxis are unlikely to receive further vaccination on the same day, thereby resolving the uncertainty of whether the vaccination or the adverse event occurred first. For other AESIs, the

primary risk period extended from day 1 to day 29 after vaccination, this 28-day risk period was determined based on previous studies [23,24]"

Please see Page 6, Line 83-92.

8. The start date of the study is inconsistent throughout the manuscript, with both September 11, 2023, and September 13, 2023, being mentioned.

Reply: It's a typo. We have corrected the start date of the study to September 11, 2023, and ensured that it is consistent throughout the manuscript.

"from September 11, 2023, to June 1, 2024."

Please see Page 8, Line 128,129.

9. In addition, the end date of the study is inconsistent, with both July 1, 2024, and June 1, 2024, being mentioned. Given that very few COVID-19 vaccines were administered after the end of April, it is questionable to include time beyond April in the analysis.

Reply: 1. It should be June 1, 2024. We have corrected the end date of the study to June 1, 2024, and ensured that it is consistent throughout the manuscript. Please see Page 8, Line 129.

2. We chose a complete study period including time beyond April in our analysis to capture any possible events and residual effects and to account for variations in vaccination distribution, coverage, and any late vaccination efforts. We believe this broader time frame provides a more comprehensive understanding of vaccine safety, especially in capturing long-term effects.

10. It may not be appropriate to combine nonhemorrhagic stroke, transient ischemic attack, and hemorrhagic stroke into a single category, as their etiologies differ significantly. Suggest grouping into two groups: 1) nonhemorrhagic stroke and transient ischemic attack; and 2) hemorrhagic stroke.

Reply: We appreciate your comments and have revised the grouping as suggested. Bas, separating non-hemorrhagic stroke and transient ischemic attack from hemorrhagic stroke. All results have been re-run and reorganized accordingly.

We have changed it accordingly as:

"hemorrhagic stroke, non-hemorrhagic stroke and transient ischemic attack "

Please group information in Page 9, Line 137.

11. Suggest changing "Each outcome was analyzed individually" to "Each outcome was analyzed separately"

Reply: We have changed it accordingly as:

"Each outcome was analyzed separately."

Please see Page 8, Line148.

12. It appears that the authors included only vaccinated individuals who experienced an adverse event in the SCCS analyses. This approach represents a vaccinated-only SCCS, which is a special case of the SCCS method.

Reply: Yes, this paper only included vaccinated patients who experienced the adverse event in the SCCS, which is consistent with the use of SCCS in previous vaccination studies [1-2]. The SCCS method is initially designed for vaccine safety, aiming to estimate a relative incidence. Only those who have experienced an event (cases) can contribute any information on when the event occurred, so only data on these individuals need to be collected.. This method has been used in previous research, and it's a vaccination-specific case.

Refs:

1. Andersson NW, Thiesson EM, Hansen JV, Hviid A. Safety of BA.4-5 or BA.1 bivalent mRNA booster vaccines: nationwide cohort study. *BMJ*. 2023;382:e075015. Published 2023 Jul 25. doi:10.1136/bmj-2023-075015
2. Lu Y, Matuska K, Nadimpalli G, et al. Stroke Risk After COVID-19 Bivalent Vaccination Among US Older Adults. *JAMA*. 2024;331(11):938-950. doi:10.1001/jama.2024.1059

13. It is unclear why the authors chose 3-month seasons to adjust for seasonality instead of using a more refined approach, such as adjusting for each individual month. Monthly adjustments could provide a more precise control for seasonal variations, especially if the adverse events of interest exhibit fluctuations within shorter period of time.

Reply: Thank you for raising this important point. We agree that monthly adjustments could provide more precise control for seasonal variations, particularly if the adverse events of interest show significant intra-seasonal variability. To address this, we reviewed the literature and

identified a study using weekly adjustments[1]. Following this approach, we have reanalyzed our data accordingly.

"Background rates of disease temporal changes were accounted for by dividing the observation period into weeks and including them as covariates in the analysis."

1. Hippisley-Cox J, Patone M, Mei X W, Saatci D, Dixon S, Khunti K et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study BMJ 2021; 374 :n1931 doi:10.1136/bmj.n1931

Please see Page 10, Line 162.

RESULTS

14. The IRR for AMI is close to significance, and the selection of the risk interval and sample size could potentially influence the conclusion. Further consideration and justification of these choices are recommended.

Reply: The primary risk window we defined as 28 days is appropriate for the disease nature (0-28 days). Additionally, we performed a sensitivity analysis using risk windows of 14 and 70 days to test the robustness of the results. After re-conducting the analysis and adding new adjustments, such as COVID infection and weekly time, the IRR for AMI is now 0.94 (0.78-1.14).
Please see Page 14, Line 212.

15. When describing the number of cases in Table 2, for those with fewer than 20 cases, it would be more informative to provide the actual count instead of using ranges or vague descriptions.

Reply: In accordance with N3C data policy, any cell size with fewer than 20 cases must be redacted and replaced with '<20' for privacy and confidentiality reasons. We are sorry about not providing exact numbers.

16. For narcolepsy or cataplexy, which are relatively common, clarification is needed on whether diagnoses made in the outpatient setting were included in the analysis.

Reply: We include all of the eligible patients in the dataset, including outpatients.
We have clarified this aspect in the revised manuscript.

"Records of specific outcomes from outpatient, inpatient, and emergency department settings were all retrieved from the dataset."

Please see Page 9, Line 146-147.

17. Regarding myocarditis/pericarditis, these are two distinct conditions, and it is possible for one or both to occur in the same patient. Clarification is needed on what the term "myopericarditis" represents. If it refers to either or both conditions, using "myo/pericarditis" would be a clearer and more appropriate term.

Reply: 'Myopericarditis' means either or both conditions in this study. We agree that 'myo/pericarditis' is a clearer and more appropriate term. Thanks for your suggestion. We have changed 'Myopericarditis' to 'myo/pericarditis' throughout the manuscript.

Please see in Page 9, Line 139.

DISCUSSION

18. Change "On the other hand, transverse myelitis…." to "On the other hand, transverse myelitis (TM) …."

Reply: We have changed the text because of new findings.

"Similarly, our primary analysis of XBB.1.5-containing mRNA vaccines showed no increased risk of Bell's palsy, encephalitis, Guillain-Barré syndrome, and transverse myelitis."

Please see Page 19, Line 302.

19. Regarding ischemic stroke, a recent study (PMID: 38916940) similarly found no significant increase in the risk of ischemic stroke following COVID-19 vaccinations.

Reply: Thank you for your suggestion. We have added this reference to enrich our references.

Please see in Page 20, Line311.

20. It is important to discuss the accuracy of capturing these outcomes, including confirmation rates, to assess the reliability of the data.

Reply: We agree that limitation remains while we have tried several ways to include all outcomes as much as possible.

We have discussed it in the Discussion section as follow:

> "Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, or incomplete records. Vaccination history and adverse events may not be fully captured."

Please see Page 21, Line 336-338.

21. The following sentence conflates two distinct study designs: "However, a large cohort study using the self-controlled case series (SCCS) method found no...". rewording this sentence is needed.

Reply: We have checked this publication and reworded this sentence as:

> "A prior study found no significant association between COVID-19 vaccines (including ChAdOx1 nCoV-19, BNT162b2, mRNA-1273, and Ad.26.COV2.S across first and second doses) and immune-mediated neurological disorders"

Please see Page 19, Line 300 -302.

22. In conclusion, it is necessary to address the signal observed among individuals who received the Moderna XBB.1.5 vaccine.

Reply: Reanalysis was conducted after incorporating the feedback of several reviewers. The conclusion has changed as:

> "In conclusion, we found that anaphylaxis was strongly associated with XBB.1.5-containing COVID-19 mRNA vaccines in day 0-2 risk window, while no significant association was observed for other AESIs within the 28-day risk period following vaccination."

Please see Page 21 Line 353-355.

Reviewer #2 (Remarks to the Author):

Abstract

1.Lines 20-22: This statement is not accurate. In the history of pharmacovigilance, spanning over 80 years, COVID-19 vaccine safety is among the most extensively studied, with comprehensive data derived from both formal active and passive safety surveillance systems.

Reply: We agreed with your comment. We have reworded the sentences as:

'While extensive studies have been conducted on earlier vaccine formulations, the safety of XBB.1.5-containing COVID-19 mRNA vaccines warrants further investigation.'

Please see Page 3, Line 42 - 46.

2. The abstract lacks specificity. It should detail the adverse events assessed and the effect estimates to enhance informativeness.

Reply: We have reworded the abstract as below accordingly:

"The safety of XBB.1.5-containing COVID-19 mRNA vaccines warrants investigation. This study assessed the relative risk of 15 adverse events following the XBB.1.5 vaccination using a self-controlled case series study design with data from the National COVID Cohort Collaborative from September 11, 2023, to June 1, 2024. Investigated adverse events included Guillain-Barré syndrome, seizure, nonhemorrhagic stroke and transient ischemic attack, hemorrhagic stroke, narcolepsy or cataplexy, anaphylaxis, acute myocardial infarction, myo/pericarditis, coagulopathy, multisystem inflammatory syndrome, Bell's palsy, transverse myelitis, appendicitis, pulmonary embolism, and encephalitis. We found that anaphylaxis (day 0 IRR: 34.47 [95% CI, 18.51-59.67]) was highly associated with vaccination. Other outcomes were not observed with significant risk following vaccination. This study is the first to utilize N3C big data to evaluate the safety of XBB.1.5-containing COVID-19 mRNA vaccines, contributing to the safety profile of COVID-19 vaccines."

Please see Page 2, Line 16-27.

Introduction

3.Lines 46-48: This statement is not accurate. I encourage the authors to review the literature on COVID-19 vaccine safety, including publications and reports from the V-Safe program and CBER Active Surveillance Program.

Reply: Thank you for your advice. The author had reviewed related literature and reworded the sentences. We also added content about Vaccine Safety Datalink (VSD). We have reworded the sentences as:

> "While extensive studies have been conducted on earlier vaccine formulations, the safety of XBB.1.5-containing COVID-19 mRNA vaccines warrants further investigation. Targeting new variants of the virus, changes in the vaccine formulation may introduce differences in safety profiles. Continuous surveillance of COVID-19 vaccine safety is crucial to promote public health.[9] Pharmacovigilance platforms, such as the Vaccine Adverse Event Reporting System (VAERS)[10] in the USA and EudraVigilance[11] in Europe, are examples of passive surveillance methods. These systems rely on self-reports from patients, which can introduce reporting bias and result in incomplete data representation across the vaccinated population.[12] In contrast, utilizing electronic health record (EHR) data for investigating vaccine-related adverse events has proven to be an effective active surveillance method.[13,14] The Vaccine Safety Datalink (VSD) has been monitoring COVID-19 vaccine safety by assessing vaccine safety and detecting safety signals of adverse events from member sites using EHR data.[15-17] While VSD only includes data of member sites. Hence, more studies into vaccine safety using extensive EHR data are needed to provide robust insights into XBB.1.5-containing COVID-19 mRNA vaccine safety."

Please see Page 3-4, Line 42-55.

3.Lines 48-50: Again, this is not accurate. While there were initial concerns of vaccine safety, there was an unprecedented amount of thorough safety data accumulated and disseminated by the CDC and initial vaccinations rates were very high. More recent declines in vaccination rates are not safety-related.

Reply: We have reworded the sentence to:

> "Continuous surveillance of COVID-19 vaccine safety is crucial to promote public health."

Please see Page 3, Line 45.

4.Lines 50-52: The mention of rarely used vaccines not included in the study is irrelevant to the study's rationale.

Reply: We have deleted the irrelevant content of rarely used vaccines.
Please see Page 2, Line 42-50.

5.Lines 52-55: There are larger, ongoing safety studies capable of detecting rare adverse events. This statement should be revised.

Reply: We have reworded these sentences to focus on passive and active surveillance methods.
Please see Page 2, Line 42-50.

6.Line 59: "can introduce reporting bias and result" → Missing a space after the period in "methods.These".

Reply: Thank you for your comments. We have added the space.
Please see Page 3, Line 48.

Methods

7. Lines 88-89: Socioeconomic status and health conditions do vary over time.

Reply: We have explicit important time-invariant confounders, such as sex and race/ethnicity, when describing how the SCCS method inherently adjusts for these factors by design.

> "The SCCS design is a method commonly utilized in vaccine safety studies, involving within-person comparison of risk and reference periods to eliminate non-time-varying confounders such as sex, race/ethnicity and genetic characteristics."

Please see Page 5, Line 80-81.

8. Lines 89-90:

1)This should be clarified to specify that all patients were a case in one or more of the AE analyses.

2)Clarify how patients were handled during periods when they experienced one adverse event (AE) while serving as controls for other AEs.

3)Specify if case time could overlap across multiple AEs and how control periods were defined (e.g., number of control periods contributed per patient, mean, standard deviation, median, interquartile range, minimum, maximum).

4)Could patients receiving multiple XBB vaccines (e.g. immunocompromised, booster recommended in those >=65 years) contribute case time more than once?

Reply:

1) Each outcome was analyzed separately, meaning that patients contributed person-time only one time to one specific AE analyse. Even patients may be involved in more than one AE analyses, but each analyse was independent without cross effect. This will be explicitly clarified in the revised manuscript.

2) Patients would not serve as a case for one AE and control for other AEs in one AE analysis. The patients would only be included in one AE analyse because each AE was analyzed independently. Furthermore, self-controlled study design was employed, one patient would serve as both case and control for in-person comparison.

3) Case time won't be overlapped across multiple AEs because each AE was carried out separately from patient selection to statistical analysis. Control period was defined as observation time without any exposure, consisting of both pre- and post-vaccination intervals.

We have clarified this as:

> "Each outcome was analyzed separately, and patients would contribute as a single case in the analysis of each AESI."
>
> "The reference period consisted of both pre- and post-vaccination intervals."

Please see Page 9, Line 147.

4) No, patients only contributed once in this study. First, the XBB.1.5 vaccine is designed as one dose. And we didn't include any immunocompromised booster. We assessed the first recorded XBB vaccine record in the dataset to make sure it's consistent with the vaccine policy.

We have clarified as

> "Only the first dose was included for each patient because all brands of XBB.1.5 vaccine was one dose formulation."

Please see Page 8, Line 132.

9. Figure 1: The figure and associated text are difficult to follow and inconsistent (e.g., 28 vs. 29 days). Specify whether the reference and risk periods are 28 days or 29 days (day 0 + 28 days) and ensure consistency across the manuscript and supplemental materials. If the risk period is actually 29 days (day 0 plus 28 days), this would need to be corrected throughout the manuscript and supplemental materials.

Reply: The risk periods are 28 days (day 1 to day 29), day 0 was excluded.

We have clarified as

> "The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases……. the primary risk period extended from day 1 to day 29 after vaccination."

Please see Page 6, Line 83-91.

We have changed Figure 1 accordingly. Please see Page 6 Line 101.

10. Presumably, the study begins on September 12, 2022, although this is not explicitly stated. It appears that all individuals included in the study must have received a COVID-19 vaccine between September 12, 2022, and September 11, 2023. Following vaccination, there seems to be a 28- or 29-day washout period (the figure and text are unclear on this point), after which control person-days were counted until the XBB vaccination and again after the 29-day risk period. However, it is unclear how individuals who did not receive a COVID-19 vaccine during the 2022-2023 season were handled. Excluding unvaccinated individuals from this timeframe would introduce selection bias and should be addressed explicitly in the methods.

Reply: We did require the individual to have a prior COVID-19 record before their first XBB, so the very first date of prior vaccination could date back to Dec 2020, the date of first emergency use approval of COVID-19 vaccines. Then the washout period was 28 days, after which control person-days were counted until the XBB vaccination and again after the 28-day risk period.

We agreed that our figure caused confusion. To address this, we have generated a new figure for improved clarity.

We consider patients who had not recorded vaccination before as a potential indicator of incomplete vaccine records or non-continuous observation. We have calculated that patients without any vaccination before were less than 5%.

"The exposure was defined as the administration of either brand of XBB.1.5-containing COVID-19 mRNA vaccines. The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases. For anaphylaxis, which requires a much shorter risk window due to its acute onset, we defined 0, 1, and 2-day risk periods to accurately capture relevant outcomes. Day 0 was included in the analysis for the following reasons: anaphylaxis typically occurs within minutes or hours after vaccination,[25,26] so day 0 is the best window to capture events. Additionally, patients who experience anaphylaxis are unlikely to receive further vaccination on the same day, thereby resolving the uncertainty of whether the vaccination or the adverse event occurred first. For other AESIs, the primary risk period extended from day 1 to day 29 after vaccination, this 28-day risk period was determined based on previous studies[23,24](see Figure 1).

The reference period consisted of both pre- and post-vaccination intervals. The pre-vaccination period extended from 28 days after prior non-XBB.1.5 COVID-19 vaccinations to 14 days before the current vaccination, with the 28-day period implemented to eliminate potential confounding effects from prior vaccines. A 14-day pre-exposure window was excluded to control for event-dependent exposure. The post-vaccination reference period began 30 days after XBB.1.5 vaccination and continued until the earliest of either a discontinuous observation in OMOP CDM, death, or the study's end. Each patient contributed data to both the risk and reference periods (see Figure 1)."

Please see Page 6, Line 82-100.

11. Line 106 : "used to to".

Reply: Thank you for pointing out this typo. We have corrected the wrong sentence.

12. Line 115: Indicate the exact end date for follow-up. Specify if "younger than 18" applies as of September 12, 2022, or the vaccination date.

Reply: The exact end date for follow-up was 'June 1, 2024'. The criteria 3 was 'younger than 18 as of the XBB.1.5 COVID-19 mRNA vaccination date.'
We have clarified it in the method section as:

"were younger than 18 years old as of the XBB.1.5 COVID-19 mRNA vaccination date"
Please see Page 7 Line 114.

13. Line 116: There is ambiguity in how exclusion criteria 3 was applied. If a 28-day window was used instead of the intended 42 days (28 days for confounding effect elimination plus 14 days for the pre-exposure window), no additional days would remain to account for the pre-exposure window. This requires clarification to ensure the criteria were appropriately applied.

Reply: The 14-day pre-exposure window accounted for event-dependent exposure, and we excluded any events that occurred within this window. Therefore, this period was not part of the inclusion criteria. If a patient had only 28 days between two vaccinations, although this condition is rare, any events occurring in that period would not be observed either. We agree that our original figure caused confusion, and we have generated a new one to clarify this point and avoid further misunderstanding.
We have changed our Figure 1, Please see in Page 6 Line 101.

14. Line 117: Over what timeframe was this assessed, all history back to ___?

Reply: With history back to "January 20, 2020", We have it in the method section. Please see Page 9, Line 152.

15. Line 119: Figure 2, there is an unexpectedly high percentage (3.8%) of individuals receiving another COVID-19 vaccine within 28 days of the XBB vaccine, as this contradicts vaccine recommendations. This raises concerns about potential inaccuracies in the data or issues with the operational definitions used in the study, especially given the counts of the other exclusions.

Reply: Thank you for your comment. The actual number of individuals who received another COVID-19 vaccine within 28 days was 34. We have regenerated the figure to eliminate any confusion. Please refer to Page 8, Line 120.

16. Line 128: Written prescriptions should not be used to operationally define vaccine administration or receipt. If written prescriptions were used, the language throughout the manuscript should be revised to specify vaccine administration and prescriptions.

Reply: We have re-checked data and are sure that written prescriptions were not included. We have reworded the text as:

> "The sources of N3C vaccination records included EHR, claims data, and self-reported medication information. "

Please see Page 8, Line 126-127.

17. Line 135: What is the rationale for not using the same list and categorizations as the CBER Active Surveillance Program? Also, what is the rationale behind using an outdated adverse event list from February 2021 rather than more recent events (e.g., injection site reactions, rash, headache, menstrual changes).

Reply: We appreciate your introduction of CBER Active Surveillance Program. After careful comparison, we have found all of our AESI were included in this list. We also updated our list based on the CBER Active Surveillance Program and cited it as reference.
Please Page 9, Line 136.
This study was more focused on rare events (incidence rate less than 1 per 10000 people). Recent events (e.g., injection site reactions, rash, headache, menstrual changes), Firstly, these conditions were quite common with much more cause, and these conditions may not be very well recorded in EHR.

18. Lines 142-144: It appears that individuals with any of the 14 adverse events (AEs) during the baseLine period were excluded from the study population. If so, these exclusions should be clearly documented in the study population flow chart, such as in Figure 2.

Reply: We have changed Figure 2 to illustrate the number excluded in each adverse event.
Please see Figure 2 in Page 8, Line 120.

19. The rationale for excluding individuals with baseLine AEs is likely to avoid including those predisposed to these events or already experiencing sequelae or complications.

However, this approach conflicts with the inclusion of control periods for these same individuals after they experience an AE during the study period. This practice undermines the rationale for excluding such individuals initially, as it reintroduces them into the analysis despite their prior experience of related conditions, creating inconsistency in the application of criteria.

Reply: There wouldn't not be conflicts of inclusion of control periods for these same individuals after they experience an AE during the study period. Self-controlled case series study design, only made within-person comparison, both reference and risk period were taken from the same patients to calculate the relative risk of exposure. We only included the first event in the observation period (either reference period and risk period), So if the event was observed in the risk period, it would be never observed in the reference period. We carefully followed the design of SCCS.

20. Excluding individuals based on the specific AE of interest during the baseline period would be a more appropriate strategy to ensure the study focuses on incident (new) cases of that AE. However, the broader exclusion of individuals with other AEs during the baseline period introduces selection bias.

Reply: Each AE was analyzed separately, so excluding individuals was actually based on the specific AE during the baseline, which aligned with your concern. For example, when we studied seizure, we just excluded patients with a history of seizure. We have clarified this as:

> "Patients who experienced specific AESI within 365 days (30 days for anaphylaxis) before the study period started were excluded in each analysis."

Please see Page 9, Line 144-145.

21. Line 144: Was the baseline period 365 days before the study started, so Sept 12, 2022? Or was the baseline period 365 days before the first vaccination observed between Sept 12, 2022 and Sept 11, 2023. Again, Figure 1 and the associated text are not clear about these different periods.

Reply: The baseline period was defined as the 365 days preceding the date of prior COVID vaccination plus 28 days after vaccination. The earliest record in N3C was December 22, 2020,

so the baseline period would start from January 20, 2020. Additionally, we have updated the figure diagram and text to better reflect our study design and improve clarity as:

> "We evaluated the demographic characteristics and comorbidity status of the study population during the one-year period before the study's start date (January 20, 2020)."

Please see Page 9, Line 151.

22. Line 147: Again, still cannot tell what the baseline period is, as this says something different than other sections.

Reply: We appreciate the reviewer's feedback regarding the clarity of the baseline period. To address this, we have made substantial edits to the Methods section to ensure consistency in the description of the baseline period. Additionally, we have updated the figure diagram to better reflect our study design and improve clarity.

23. Lines 154-156: What is the rationale for controlling for season? While vaccine effectiveness can vary by time due to changes in predominant strain, AEs do not vary in any meaningful way over seasons. Due to small sample size, only confounders associated with both exposure and outcome should be controlled for, as controlling for a non-confounder can introduce bias.

Reply: Thank you for raising this important point. To address this, we reviewed the literature and identified a study using weekly adjustments to account for the background rates of AEs in model[1]. Following this approach, we have reanalyzed our data accordingly. We have added the adjustment as:

> "Background rates of disease temporal changes were accounted for by dividing the observation period into weeks and including them as covariates in the analysis."

1. Hippisley-Cox J, Patone M, Mei X W, Saatci D, Dixon S, Khunti K et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study BMJ 2021; 374 :n1931 doi:10.1136/bmj.n1931

Please see Page 10, Line 162.

24. Lines 159-160: Citation supporting this threshold?

Reply: Multiple Comparisons Issue may be introduced by making many statistical tests, the probability of obtaining at least one false positive increases. Adjusting the significance threshold helps control for this, especially in studies with many variables or comparisons. The Bonferroni correction is one commonly used method to control for this issue, which involves adjusting the p-value threshold based on the number of tests.

- Reference:
  *Bonferroni, C. (1936). "Teoria statistica delle classi e calcolo delle probabilità." *Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze*.

25. How were patients developing COVID-19 handled as COVID-19 itself is a risk factor for some of these outcomes? Their follow-up should end if they are diagnosed with or test positive for COVID-19.

Reply: We appreciate the reviewer's concern regarding the impact of COVID-19 infection on the study outcomes. To account for this, firstly, we have adjusted for COVID-19 infections as confounders using time-dependent covariates. Specifically, we calculated the total number of overlapping days between infections and each risk/reference period and incorporated this into the analysis to mitigate potential bias. Secondly, we conducted a sensitivity analysis by excluding any patients who had a COVID-19 infection in the 90 days prior to the start of the observation period and continuing through the end of the observation period.
We have added the infection adjustment as:

> "The model was adjusted for COVID-19 infection and background rates of disease temporal changes. COVID-19 infection was treated as a confounder using a time-dependent covariate. Specifically, we calculated the total number of overlapping days between the 90-day post-infection period and each risk or reference period and incorporated this into the model. Background rates of disease temporal changes were accounted for by dividing the observation period into weeks and including them as covariates in the analysis."

Please see Page 10 Line 158-163.

> "To better account for the potential impact of COVID-19 infection on AESIs, we conducted a sensitivity analysis by excluding any patients who had a COVID-19 infection in the 90 days prior to the start of the observation period and continuing through the end of the observation period."

And Page 10 Line 172-175.

Results

26. Clarify if patients with missing data were excluded (no missing data in Table 1). If so, include this as an exclusion criterion in the methods and flowchart.

Reply: We appreciate the reviewer's comment regarding missing data. We do require patients without missing data. We have updated the exclusion criterion of missing data and listed the exact number of missing data excluded in Figure 1.
Please see Page 7 Line 118 and Figure 1 at Page 8 Line 120.

27. Figure 4: The forest plot format is unconventional and confusing. Align IIRs and 95% CIs with the corresponding colored Lines, and include events/person-days directly on the figure for clarity. Incidence rate ratios could not be estimated for encephalitis. As such, encephalitis should be excluded from the tables and figures. The single sentence in the Results section is sufficient to address this.

Reply: Thank you for your comments. We have revised Figure 4 as comment for better clarity and readability.
Please see Page 15 Line 234.

Discussion

28. Lines 249-251: Again, this is not accurate, there is substantial recent evidence indicating no association.

Reply: We have rewording this sentence to

> 'Conflicting conclusions had been made by previous studies regarding risks of coagulative disorders, such as thrombocytopenia, following mRNA vaccines targeting earlier COVID-19 strains.[25,26]'

Please see Page 19 Line 296-298.

29. Line 327: "can not"."

Reply: Thank you for pointing out this typo. We have changed this sentence.

30. Limitations are highly understated and need to be expanded to include study design and data source constraints.

Reply: We appreciate the reviewer's feedback. We have expanded the limitations section to further discuss the constraints of a retrospective study using EHR data, including potential biases, missing data, and the challenges in capturing complete vaccination and outcome records as:

> "Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, misclassifications in EHR coding, or incomplete records. These issues could impact the reliability of vaccination and adverse event records. Specifically, vaccination records and adverse events may not be fully captured or may contain erroneous information. Despite the extensive data available in N3C, it represents only a portion of the country rather than its entirety. We excluded patients based on certain criteria, which may have resulted in the loss of potentially eligible participants. The fixed 28-day risk window may not be applicable to all populations or all types of adverse reactions. By focusing on only the first event, there is a possibility of missing subsequent events during the follow-up. Causal relationships between outcomes and exposures cannot be established given to retrospective and observational study design. The multiple comparisons conducted in our analysis may lead to an increased risk of Type 1 error, which refers to falsely rejecting the null hypothesis when it is actually true. Subgroup analyses were not conducted throughout the study due to sample size limitations. Further investigation into specific subpopulations is warranted to explore potential differential risks and to better understand how these factors may influence the outcomes. Given these limitations, our findings need to be interpreted with caution."

Please see Page 21 Line 337-352.

Throughout

31. Revise terminology: replace "gender" with "sex" where applicable, as per the Reporting Summary.

Reply: We have replaced "gender" with "sex" where applicable in main text, supplementary materials and Reporting Summary.

32. Pertinent data and analytic details specified in the Reporting Summary were absent from the manuscript.

Reply: We have updated the manuscript to ensure that all pertinent data and analytical details specified in the Reporting Summary are clearly presented**.**

Reporting Summary

33. Page 3 of Reporting Summary, Clinical Data should be selected and completed.

Reply: We only used diagnosis codes and did not incorporate other clinical data. The 'Clinical Data' section in the Reporting Summary is not applicable to our study.

Supplemental Materials

34. Incidence rate ratios could not be estimated for encephalitis. As such, encephalitis should be excluded from the tables and figures. The single sentence in the Results section is sufficient to address this.

Reply: We have updated all fo our figures as suggested.

35. For reproducibility, concept IDs of exclusions should be presented.

Reply: We acknowledge the reviewer's suggestion. The concept IDs were selected based on existing knowledge and concept sets from N3C and OHDSI, and they have been rechecked by domain experts. Reproducibility can be achieved by using the concept IDs provided in the Supplementary Materials.
In general, the exclusion criteria include the following:

1. Inactive Concepts: These are concepts that are no longer in use or are considered outdated.
2. Non-standard Concepts: In many analyses, only standard concepts are of interest.

3. Specific Domain Exclusions: We only work with possible vaccine related drug exposure and outcome, any concepts with sure vaccine irrelevant should be excluded.

36. Unclear how the concept ID code lists were developed. Citations of validation studies of these operational definitions should be provided. If no validation studies exist, the procedures for identifying and selecting these codes should be provided.

Reply: We acknowledge the reviewer's suggestion. The concept IDs were selected based on existing studies[1,2] and concept sets from N3C and OHDSI, and they have been rechecked by domain experts.

1. Andersson NW, Thiesson EM, Hansen JV, Hviid A. Safety of BA.4-5 or BA.1 bivalent mRNA booster vaccines: nationwide cohort study. BMJ. 2023 Jul 25;382:e075015. doi: 10.1136/bmj-2023-075015. PMID: 37491031; PMCID: PMC10364193.
2. Andersson NW, Thiesson EM, Hviid A. Adverse Events After XBB.1.5-Containing COVID-19 mRNA Vaccines. JAMA. 2024;331(12):1057–1059. doi:10.1001/jama.2024.1036

37. Supplemental Tables 2-5: Last column should indicate these are incidence rate ratios and 95% confidence intervals.

Reply: We have updated our figures as comments.

38. Supplemental Figures 1-2: The forest plots utilize an atypical format and are therefore confusing. The IIRs and 95% CI should be next to the colored Line of that corresponding strata. This would also allow for the events/person-days to be included in the figure, and then the duplicative tables with IIRs can be removed. All results should be presented similarly (unclear why forest plots only generate for some stratified/sensitivity analyses) and not others.

Reply: We have updated our figures as comments.

Reviewer #3 (Remarks to the Author):

Dear Editor,

Thank you for inviting me to review the manuscript by Pan et al., submitted to Nature Communications and titled "Evaluating Safety of XBB.1.5-containing COVID-19 mRNA Vaccines Using a Self-Controlled Case Series Study," that presents a large-scale analysis utilizing real-world data from the US National COVID-19 Cohort Collaborative (NC3).

The study reports no significant increase in the risk of 14 pre-specified adverse events of special interest following XBB.1.5-containing COVID-19 mRNA vaccination in the general adult population. Although two potential safety signals were noted in the subgroup analysis of the Moderna mRNA-1273 vaccine, these findings warrant cautious interpretation due to wide confidence intervals and the limited number of observed events. The study offers reassurance regarding the overall safety profile of XBB.1.5-containing COVID-19 mRNA vaccines while underscoring the necessity for continued surveillance and focused investigation within specific subgroups. The inherent limitations of EHR-based studies and the SCCS design must also be acknowledged when interpreting these findings. Additional research employing complementary study designs and data sources is needed to confirm the results and assess potential risks within distinct populations.

Strengths:

The study leverages a robust cohort of 259,136 individuals, which increases the probability of detecting uncommon adverse events. The methodological approach, particularly the self-controlled case series (SCCS) design, effectively account for time-invariant confounders, such as socioeconomic status. Incorporation of seasonal adjustment accounts for temporal confounding as a result of the variable SARS-CoV-2 activity over the year, while the inclusion of a negative control outcome (diverticulitis) addresses vaccine-unrelated factors. Furthermore, the study's reliance on diverse electronic health record (EHR) data sources strengthens the completeness of the safety evaluation.

Limitations:

Several important limitations must be considered. First, the intrinsic limitations of EHR data may compromise the completeness and accuracy of findings. Although the N3C database provides extensive data, it represents only a subset of the U.S. population,

restricting generalizability. Second, the SCCS design, while robust, assumes that adverse events do not influence subsequent exposure—a condition that may not hold if severe events prevent further vaccination. Additionally, the study's focus on first-event occurrences may have overlooked subsequent adverse events during follow-up. Third, the predefined 28-day risk period, while consistent with prior studies, may fail to capture delayed adverse events with longer latency periods. Wide confidence intervals for rare events, such as transverse myelitis and acute myocardial infarction observed in the Moderna mRNA-1273 subgroup, suggest limited precision and necessitate cautious interpretation. Despite adjustments for seasonal variations, residual temporal confounding cannot be entirely excluded. Lastly, the exclusion of individuals without continuous observation or prior vaccination data may introduce selection bias, potentially impacting the representativeness of the sample.
Although the authors in the discussion section did acknowledge some of the limitations commented above, they missed several critical issues that, in my opinion, should be further discussed:

1. They don't address the potential violation of SCCS assumptions, particularly the event-independence assumption.

Reply: Regarding the event-independence assumption, we clarify that our analysis focuses on individual adverse events separately, and we only included the first event of the whole study period of each patient to maintain the assumption of event independence. This approach aligns with standard SCCS methodology and minimizes potential biases from recurrent events.
We have clarified this as

> "We included only the first event of each outcome during the whole observation to maintain the SCCS event-independence assumption."

Please see Page 9 Line 143-144.

2. They don't discuss the rationale or potential limitations of their 28-day risk window choice.

Reply: 1. Rationale for Choosing the 28-Day Risk Window: We selected a 28-day risk window based on previous studies and established vaccine adverse event surveillance protocols. This time frame has been commonly used in similar research to capture acute reactions while

balancing the risk of including events that may not be causally related to the exposure. Based on comments, we selected a 0-2 day risk window for anaphylaxis.

We have noted this discuss the rationale in the method as:

> "The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases. For anaphylaxis, which requires a much shorter risk window due to its acute onset, we defined 0, 1, and 2-day risk periods to accurately capture relevant outcomes. Day 0 was included in the analysis for the following reasons: anaphylaxis typically occurs within minutes or hours after vaccination,[25,26] so day 0 is the best window to capture events. Additionally, patients who experience anaphylaxis are unlikely to receive further vaccination on the same day, thereby resolving the uncertainty of whether the vaccination or the adverse event occurred first. For other AESIs, the primary risk period extended from day 1 to day 29 after vaccination, this 28-day risk period was determined based on previous studies."

Please see Page 6,Line 83-92.

2. Addressing Potential Limitations: To address potential limitations related to the choice of the 28-day window, we took several steps:

a. We modified the risk window for anaphylaxis to a more immediate 0, 1, and 2-day window, as anaphylaxis is an acute reaction that typically occurs shortly after exposure.
b. We conducted secondary analyses using 14-day and 70-day risk windows to test the sensitivity of our findings to the length of the risk window. These analyses allowed us to assess whether the choice of window influenced the results.

We have noted this as a limitation in the discussion as

> " The fixed 28-day risk window may not be applicable to all populations or all types of adverse reactions."

Please see Page 21,Line 342-344.

3. While they mention the N3C's partial representation, they don't discuss the implications of their specific exclusion criteria on selection bias.

Reply: We have noted this as a limitation in the discussion as:

> "We excluded patients based on certain criteria, which may have resulted in the loss of potentially eligible participants."

Please see Page 21,Line 341.

4. They don't address the limited power for detecting rare events, despite presenting wide confidence intervals for several outcomes.

Reply: We appreciate the reviewer's concern regarding statistical power for detecting rare events. While we utilized a large EHR dataset, we acknowledge that some outcomes had low event counts, leading to wider confidence intervals. We have noted this as a limitation in the discussion and recognize that future studies with even larger case numbers may be needed to improve the precision of risk estimates for rare events.
We have noted this as a limitation in the discussion as:

> " Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, misclassifications in EHR coding, or incomplete records. These issues could impact the reliability of vaccination and adverse event records. Specifically, vaccination records and adverse events may not be fully captured or may contain erroneous information."

Please see Page 21,Line 336-340.

5. They don't discuss the lack of validation of their outcome definitions or potential misclassification in EHR coding.

Reply: We have noted this as a limitation in the discussion as:

> " Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, misclassifications in EHR coding, or incomplete records.

Please see Page 21,Line 336-338.

6. There's no acknowledgment of the limited subgroup analyses or the potential for differential risks in important subpopulations.

Reply: We have conducted several subgroup analyses, including sex and vaccine brand. We appreciate the reviewer's suggestion regarding additional subgroup analyses, as these could provide further insights into potential differential risks in specific populations. We have

acknowledged this as a future research direction and added it to the end of the discussion section.

We have noted this as a limitation in the discussion as

> "Subgroup analyses were not conducted throughout the study due to sample size limitations. Further investigation into specific subpopulations is warranted to explore potential differential risks and to better understand how these factors may influence the outcomes."

Please see Page 21,Line 348-351.

7. The multiple comparison issue is not addressed in their limitations section, though they briefly mention it in their methods.

Reply: We appreciate the reviewer's comment regarding multiple comparisons. To address this, we used a more stringent significance threshold ($p < 0.01$/$p<-.001$, Yun suggested picking one to discuss in the discussion, then confirming with Dr.Zhao) to reduce the likelihood of false-positive findings. Additionally, we carefully chose outcomes that are proved to be possible adverse events of COVID-19 vaccines by existing evidence to avoid false positive errors. Finally, we have now explicitly mentioned this in the limitations section to acknowledge the potential impact of multiple comparisons on our results as

> "The multiple comparisons conducted in our analysis may lead to an increased risk of Type 1 error, which refers to falsely rejecting the null hypothesis when it is actually true."

Please see Page 21,Line 346-348.

8. They don't discuss potential limitations in their ascertainment of vaccination status or the quality of vaccination records.

Reply: We appreciate the reviewer's comment regarding ascertainment of vaccination data. We have added related content in the Discussion as:

> "Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, misclassifications in EHR coding, or incomplete records. These issues could impact the reliability of vaccination and adverse event records. Specifically, vaccination records and adverse events may not be fully captured or may contain erroneous information. "

Please see Page 21,Line 336-340.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We appreciate the reviewer's comments and efforts in improving this manuscript.

Point-by-point responses to reviewers' comments:

Reviewer #1 (Remarks to the Author):

Thanks for addressing my concerns, well done. I only have two minor suggestions:

Reply: We appreciate the reviewer's positive comment. We have incorporated the two minor suggestions as described below.

1. Please change "Pfizer–BioNTech's" to "Pfizer–BioNTech" in line 35.

Reply: We have made the suggested changes(Page 3 Line 34).

2. In page 4, "While VSD only includes data of member sites." is not a complete sentence.

Reply: We have completed the sentence.

"The Vaccine Safety Datalink (VSD) project has been monitoring COVID-19 vaccine safety in large populations using member sites data." (Page 3 Line 48-50)

Reviewer #2 (Remarks to the Author):

Several issues noted during the initial review remain unresolved. The revised manuscript continues to reflect limited understanding of the existing COVID-19 vaccine safety research.

Background

Lines 42–46: There is a major flaw in the framing of the introduction. Reformulation of vaccines, such as seasonal updates for influenza, is common and not inherently associated with different or novel safety profiles. Updated COVID-19 mRNA vaccines (e.g., bivalent, monovalent XBB.1.5) have similarly shown no major safety concerns attributable to strain changes.

• The generalization that variant updates result in altered safety profiles is not supported by biological plausibility or empirical evidence—especially when the vaccine platform, adjuvants, and delivery mechanisms remain unchanged.

• Regulatory agencies (FDA, CDC, WHO) and the existing published literature have clearly indicated continuity in safety across COVID-19 vaccine reformulations.

• The manuscript still fails to incorporate a comprehensive knowledge on the extensive global surveillance of COVID-19 vaccine safety conducted by agencies such as CDC, FDA, ACIP, and international counterparts.

Reply: We believe that our work makes a meaningful contribution to the understanding of COVID-19 vaccine safety profiles. While COVID-19 vaccines have consistently demonstrated favorable safety records, our study further supports this evidence by evaluating their safety in the context of reformulated vaccines.

We have revised the introduction to more clearly emphasize the value of incorporating N3C EHR data in the broader landscape of vaccine safety research, underscoring its contribution to the ongoing monitoring of vaccine reformulations.

We have changed the main text as:

> "Extensive studies have been conducted on earlier vaccine formulations. Pharmacovigilance platforms, such as the Vaccine Adverse Event Reporting System (VAERS)[10] in the USA and EudraVigilance[11] in Europe, are examples of passive surveillance methods. These systems rely on self-reports from patients, which may introduce reporting bias and result in incomplete data representation across the vaccinated population.[12] In contrast, utilizing electronic health record(EHR) data for investigating vaccine-related adverse events has proven to be an effective active surveillance method.[13,14] The Vaccine Safety Datalink (VSD) has been monitoring COVID-19 vaccine safety by assessing vaccine safety and detecting safety signals of adverse events from member sites using EHR data.[15-17]. Overall, the COVID-19 related vaccines showed good safety in previous studies.
>
> However, continuous safety monitoring remains essential to support public health efforts, particularly some rare but serious types of adverse events.[21] The National COVID Cohort Collaborative (N3C)[22] is one of the largest centralized and harmonized collections of EHR data in the United States. Leveraging N3C enables comprehensive, real-world analyses that can provide robust insights into the safety of XBB.1.5-containing COVID-19 mRNA vaccines." (Page 3-4, Line 42-55.)

Lines 272–273: This statement remains unclear

Reply: We have changed the statement to:

> " Our findings support CDC surveillance data indicating that anaphylaxis remains a rare but serious adverse event following COVID-19 vaccination, with a reported incidence of approximately 5 cases per million doses administered." (Page 18, Line 272-275.)

• Lines 313–314: Incorrect—analyses of VSD and other data sources, including CDC, FDA, and ACIP reports, are ongoing.

Reply: We have changed the statement

" Real-world data is a robust enhancement for a comprehensive and continuous safety assessment of XBB.1.5-containing COVID-19 mRNA vaccine safety. Consequently, our findings provide post-marketing evidence that complements data from clinical trials." (Page 19 Line 316-319)

Other Vaccinations

• Lines 88–90: Patients who receive multiple vaccines get them moments apart. This omission is particularly critical since coadministration of vaccines is common and may affect adverse event rates. Other vaccine administrations were not captured by the study or adjusted in the analyses. This is a major limitation that calls the primary findings (on anaphylaxis) into question.

• Lines 198–200: No information is given about other recent vaccinations or how they may have affected the analysis and findings.

Reply: We examined the vaccines administered on the same day as COVID-19 vaccinations and found that the vast majority were flu vaccines, consistent with established vaccination practices. In our cohort, co-administration with flu vaccines accounted for only a small proportion (19,563 out of 244,494 in the overall cohort; 35 out of 531 in the anaphylaxis cohort). We also performed a post hoc analysis of anaphylaxis. While the model could not be fitted when including flu vaccine co-administration as an interaction term, incorporating it as a covariate yielded results consistent with our original findings.

Combo vaccine fact		
	No. of patients	No.of combo vaccine
Overall cohort	244,494	19,563
Anaphylaxis cohort	531	35

	before	With combo as intreation	With combo as covariate
IRR	17.35	NA	17.35

We have incorporated the above information to the main text:

" We also examined co-administration with influenza vaccines on the same day, which accounted for only a small proportion of cases (19,563 out of 244,494 in the overall cohort; 35 out of 531 in the anaphylaxis cohort)." (Page 16 Line 260-262)

Purpose and Existing Literature

• The stated purpose and rationale for the study are not supported by scientific evidence. Multiple assertions are made without appropriate citations or grounding in the field.

Reply: We have rewritten this section and added relevant references to support our study design.

> "Extensive studies have been conducted on earlier vaccine formulations. Pharmacovigilance platforms, such as the Vaccine Adverse Event Reporting System (VAERS)[9] in the USA and EudraVigilance[10] in Europe, are examples of passive surveillance methods. These systems rely on self-reports from patients, which may introduce reporting bias and result in incomplete data representation across the vaccinated population.[11] In contrast, utilizing electronic health record (EHR) data for investigating vaccine-related adverse events has proven to be an effective active surveillance method.[12] The Vaccine Safety Datalink (VSD) project has been monitoring COVID-19 vaccine safety in large populations using member sites data.[13,14] Overall, the COVID-19 related vaccines showed good safety in previous studies.[2,15-20] " (Page 3, Line 42-50)

• Lines 45–46: The claim that updated vaccines have unknown safety profiles is inaccurate. We have accumulated more safety data for COVID-19 vaccines—including XBB formulations—than for any other vaccine or medication in history.

Reply: We have updated the sentence to:

> "Extensive studies have been conducted on earlier vaccine formulations." and "Overall, the COVID-19 related vaccines showed good safety in previous studies." (Page 3, Line 42 and 49)

• Lines 54–55 and 272–273: Numerous large, well-controlled studies have already assessed the safety of COVID-19 vaccines, including XBB. This study does not adequately compare its findings, particularly for anaphylaxis, to the existing literature and CDC/FDA/ACIP reports, nor does it present an appropriate range of rates.

Reply: Lines 54–55 has been updated in the revised manuscript to:

> "Overall, the COVID-19 related vaccines showed good safety in previous studies.[2,15-20] However, continuous safety monitoring remains essential to support public health efforts, particularly some rare but serious types of adverse events.[21] " (Page 3, Line 49-52)

We have added more comparisons than before of our anaphylaxis findings with CDC existing documentation of anaphylaxis.

Lines 272–273 has been updated to:

> "Our findings support CDC surveillance data indicating that anaphylaxis remains a rare but serious adverse event following COVID-19 vaccination, with a reported incidence of approximately 5 cases per million doses administered.[21]" (Page 17, Line 272-275)

• Lines 314–316: The claim that this study addresses gaps in knowledge is inaccurate.
• Lines 316–319: This overstates the study's contribution, especially given its limited sample size and limitations.
• Lines 320–321: Not accurate. European data are similar to other populations.

Reply: We have updated the text to avoid any confusion as below:

> "While previous randomized clinical trials have concluded that the vaccines are generally safe,[51-53] their limited sample sizes and follow-up durations may reduce the likelihood of detecting rare adverse AEs. Real-world data is a robust enhancement for a comprehensive and continuous safety assessment of XBB.1.5-containing COVID-19 mRNA vaccines safety. Consequently, our findings provide post-marketing evidence that complements data from clinical trials. A prior research found no increased risk of 28 AEs following vaccination with a monovalent XBB.1.5-containing vaccine, it was based solely on EHR data of elder population in Denmark and lacked sensitivity analyses due to the brief nature of the research letter.[30] Our study expands on these findings by analyzing U.S. data with a more robust study design." (Page 19, Line 312-320.)

• Lines 323–325: Misleading—only one-eighth of the source population was included.
• Lines 323–335: The manuscript overstates its novelty and contribution. The findings confirm the extensive existing research and should be framed as such.

Reply: Even though only one-eighth of the source population was included, our cohort was extracted from 2 million patients. Our study confirmed what was already known. But continuous safety monitoring remains essential to support public health.

We have refined this in our revision as:

> "Furthermore, our study leveraged the N3C database, which includes data from over 2 million patients across more than 70 institutions nationwide, to construct a cohort of over 240,000 patients—ensuring a sufficient sample size to detect rare vaccine-associated adverse events. This expansive, nationally representative cohort enhances the generalizability of our findings. The N3C data undergo rigorous quality control measures, standardized harmonization protocols of OMOP CDM and institutional review board approvals at all participating sites, ensuring high reliability and reproducibility for safety surveillance analyses.[22,54]" (Page 19, Line 321-327.)

• Key aspects of the study design remain unclear:
The case and control time periods are still not clearly defined. Revisions to Figure 1 do little to improve the clarity of timeline, particularly since there are conflicts with the text in terms of the baseline period.

Reply: We have clearly defined the case periods as:

> "The day of vaccination was considered day 0, with different risk periods determined based on the nature of the diseases. For anaphylaxis, which requires a much shorter risk window due to its acute onset, we defined 0, 1, and 2-day risk periods to accurately capture relevant outcomes." (Page 5, Line 83-86.)
>
> "For other AESIs, the primary risk period extended from day 1 to day 29 after vaccination, this 28-day risk period was determined based on previous studies." (Page 5, Line 90-91.)

Regarding the control time periods:

> "The reference period consisted of both pre- and post-vaccination intervals. The pre-vaccination period extended from 28 days after prior non-XBB.1.5 COVID-19 vaccinations to 14 days before the current vaccination."
> which is pretty clear. But we can make more edits if accurate comments are provided.

wash-out period (Figure 2) is not defined. From when?

Reply: The wash-out period was indeed defined in the early version.

In the main text: "Patients who experienced specific AESI within 365 days (30 days for anaphylaxis) before the study period started were excluded in each analysis." (Page 9, Line 144-146.)

In the note of Figure 2: "The washout period for anaphylaxis was 30 days, for other outcomes was 365 days." (Page 7, Line 123.)

Lines 96-97: No rationale for the 14-day pre-exposure window.

Reply: The 14-day pre-exposure window exclusion was implemented to account for potential event-dependent exposure bias, following established methodology in recent high-quality vaccine safety studies.

The method of using the 14-day pre-exposure window has been commonly used, for example, this approach has been used in the nationwide cohort study by Andersson et al. (2023) [PMID: 37491031] in BMJ that similarly excluded 14 days before booster vaccination from the reference period to address the same bias.

We have cited the above and other references in the revised manuscript.

Lines 143–144: What happened if experienced again? Post-vaccination reference period should end on recurrence.

Reply: We only included the first event (the earliest event) of every patient to avoid recurrence of adverse events. This design followed previous published studies (PMID: 35296468 and PMID: 34446426).

Lines 151–152: The baseline period is incorrectly fixed to January 20, 2020. It should be individualized based on each patient's XBB vaccination date. As currently described, outcomes in the reference period are be incorrectly included.

Reply: The baseline period is not fixed to January 20, 2020, this is just the earliest date of the baseline period.

Lines 158–163: Confusing, should simply state controlled for week to account for possible changes in background disease rates over time.

Reply: We appreciate the reviewer's comment and suggestion. As suggested , we have modified the sentence as:

> " Temporal changes in background disease rates were adjusted on a weekly basis in the model. " (Page 9, Line 162-163.)

Lines 198–200: Meaning without COVID-19 infection? The method for identifying COVID-19 infection status is not specified (diagnosis, positive lab tests?). Misclassification bias is likely, especially for mild cases that may not be captured in healthcare records, and this is not sufficiently addressed in the limitations.

Reply: We used both COVID-19 diagnosis records and laboratory-confirmed positive test results to define infections. This methodological detail was not emphasized in the manuscript as it was not the primary focus of our study.

• The demographic and comorbidity baseline period is defined using a one-year lookback from a uniform date, which does not account for varying vaccination dates.

Reply: This is not true. January 20, 2020 is the earliest day not the uniform date.

• The entire results section contains new numbers, but these changes were not clearly marked (e.g., not shown in red).

Reply: We have checked and make sure all changes were showed in red.

• Line 84: Shouldn't this be "nature of vaccine-related reactions"?

Reply: Risk period based on nature of the diseases, also "nature of vaccine-related reactions"

• Lines 126–127: Shouldn't this be "self-reported vaccination"?

Reply: Did the reviewer mean "self-reported medication" should be "self-reported vaccination"? The vaccination history is from the "Drug exposure" table of data model, so we used "self-reported medication information".

• Figure 1: Still confusing. Inconsistent use of terms for reference, risk, and wash-out periods. These terms must be defined and used consistently across text and figures.

Reply: We believe our figure is quite clear to show a SCCS design. We followed the published as our reference, for example:

[REDACTED]

From: Andersson NW, Thiesson EM, Hansen JV, Hviid A. Safety of BA.4-5 or BA.1 bivalent mRNA booster vaccines: nationwide cohort study. BMJ. 2023 Jul 25;382:e075015. doi: 10.1136/bmj-2023-075015. PMID: 37491031; PMCID: PMC10364193. https://pmc.ncbi.nlm.nih.gov/articles/PMC10364193/

We have double checked the text and make sure that we are consistent across text and figures in using terms for reference, risk, and wash-out periods. All specially defined terms such as "wash-out period" have been defined and used consistently.

• The forest plot line should be centered at IRR = 1, not 3.

Reply: In our plot, IRR = 1 is indeed the reference line indicating no effect. We did not define IRR = 3 as the center; rather, it appears due to the automatic scaling of the axis. For a better plot, We have used log scale and use 1 as center. We have updated every forestplot.

• Lines 173–175: For either reference period or risk period? Use same terminology in figure and text throughout, for naming different periods of time.

Reply: We believe there are misunderstandings here. The terms *reference period* and *risk period* are used consistently throughout the text. However, the terms *follow-up period* and *observation period* mentioned in Lines 173–175 have different meanings. Specifically, the *follow-up period* refers to the combined duration of the *reference period* and the *risk period*. In contrast, the *observation period* encompasses the entire duration, including the *washout period*, the *reference period*, and the *risk period.*

Grammar

• Grammatical issues throughout the manuscript and response letter impede clarity and made some responses hard to follow.

• The manuscript should be proofread carefully by all authors.

Reply: Thank you for your valuable comment. We sincerely apologize for the grammatical issues and lack of clarity in the manuscript and the response letter. All authors have now carefully reviewed and revised the manuscript to improve the language, grammar, and overall clarity.

Limitations

• The revised limitations section is vague and does not adequately respond to concerns raised by reviewers.

Reply: We believe the content of limitation is clear and enough. We can also add some content about the weakness of infection records.

> “Due to the inherent limitations of EHR data, the N3C database may contain inaccuracies, missing values, misclassifications in EHR coding, or incomplete records. These issues could impact the reliability of vaccination and adverse event records. Specifically, vaccination records and adverse events may not be fully captured or may contain erroneous information. Despite the extensive data available in N3C, it represents only a portion of the country rather than its entirety. We excluded patients based on certain criteria, which may have resulted in the loss of potentially eligible participants. The fixed 28-day risk window may not be applicable to all populations or all types of adverse reactions. By focusing on only the first event, there is a possibility of missing subsequent events during the follow-up. Causal relationships between outcomes and exposures cannot be established given to retrospective and observational study design. The multiple comparisons conducted in our analysis may lead to an increased risk of Type 1 error, which refers to falsely rejecting the null hypothesis when it is actually true. Subgroup analyses were not conducted throughout the study due to sample size

limitations. Further investigation into specific subpopulations is warranted to explore potential differential risks and to better understand how these factors may influence the outcomes. The infection records used to adjust in the model may be incomplete. Given these limitations, our findings need to be interpreted with caution." (Page 20, Line 339-354.)

• Statements about the study's generalizability and contribution (e.g., Lines 320–335) are misleading. This study confirms what is already known.

Reply: We believe even though our study confirms what is already known, continuous safety monitoring remains essential to support public health efforts, particularly some rare but serious types of adverse events.

We have made changes as:
"While previous randomized clinical trials have concluded that the vaccines are generally safe,[51-53] their limited sample sizes and follow-up durations may reduce the likelihood of detecting rare adverse AEs. Real-world data is a robust enhancement for a comprehensive and continuous safety assessment of XBB.1.5-containing COVID-19 mRNA vaccine safety. Consequently, our findings provide post-marketing evidence that complements data from clinical trials. A prior research found no increased risk of 28 AEs following vaccination with a monovalent XBB.1.5-containing vaccine, it was based solely on EHR data of elder population in Denmark and lacked sensitivity analyses due to the brief nature of the research letter.[30] Our study expands on these findings by analyzing U.S. data with a more robust study design." (Page 19, Line 312-320.)

Reviewer #3 (Remarks to the Author):

Thank you for your responses. I have read them carefully and all my comments were either answered or incorporated in the manuscript. I don´t have additional questions.

Reply: We appreciate the reviewer's positive comments.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the *Nature Communications* initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We appreciate the reviewer's co-reviewing this manuscript with one of the reviewers.