PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Risk of cardiovascular events leading to hospitalization after Streptococcus pneumoniae infection: A retrospective cohort LIFE study
AUTHORS	Nishimura, Naoaki; Fukuda, Haruhisa

VERSION 1 – REVIEW

REVIEWER	Romiti, Giulio Francesco Sapienza University of Rome, Department of Translational and Precision Medicine
REVIEW RETURNED	21-Feb-2022

GENERAL COMMENTS	In this manuscript, the authors report about a retrospective, matched analysis on the risk of subsequent cardiovascular events in patients with vs. Without S. Pneumoniae infection in Japan. The authors found that, compared to patients without infection, those infected have a higher risk of developing stroke and arrhythmia. I agree with the authors that the research question is indeed interesting and there is a need for further data on the long-term cardiovascular risk in patients with infections (most of the studies focused on short-term risk). In this view, I think that the authors made a good work in trying to explore this aspects. However, this manuscript has some issues (mainly in the methodology used) which need to be addressed. My comments in detail: 1) One thing that it is unclear is whether the authors have focused on community acquired pneumonia (or, at least, lower tract respiratory infection) or whether all types of infections sustained by S. Pneumoniae were considered (for example, also meningitidis?). This should be specified clearly in the methods section, since the background and the discussion is structured on the comparison between previous evidence related to community acquired pnuemonia, so one would expect that the analysis was restricted on CAP patients. 2) Relatedly, although the authors referred to a previous study for the methods used to identify patients with S. Pneumoniae infection, they should report briefly the methods used to select patients. How were S. Pneumoniae infections identified? Was a microbiological criterion adopted? Please report and specify. 3) As a side note, one may wonder whether the authors restricted their analysis to S. Pneumoniae infected patients - as the authors already acknowledge, community acquired pneumonia is sustained by a broad spectrum of pathogens, so that to improve consistency with previous studies it might have been more suitable to include all patients with a diagnosis of CAP, for example. But this is only a comment.

	4) While I recognize the reasons behind the choice of excluding patients with previous cardiovascular events, one should notice that this approach may have led to selection bias in the population examined. In fact, the real-world population of patients who experience CAP also include patients with previous cardiovascular events (indeed, one may expect that these patients are maybe more prone to develop CAP and other infections due to their inner conditions), so that excluding them actually reduce comparability with a real-world scenario. This may be seen as a limitation. Also, I do not understand why there are 5.4% patients with “congestive heart failure” included for the “HF” analysis; or 9.1% patients with “cerebrovascular disease” in the Stroke analysis. Weren’t these patients expected to be excluded from these analyses? 5) Please reports in table 1 the number (%) of patients with previous arrhythmia. 6) One significant critical aspect of this analysis is the definition of the outcome. The use of ICD codes seem too broad for some of these events. For example, stroke comprises ICD codes from I60 to I69; these includes subarachnoid hemorrhage and other cerebrovascular disease for example, which are only loosely related to “stroke”; similarly, heart failure also comprised I11 code, which is “hypertensive heart disease” (this do not imply heart failure, or at least not in the definition commonly used for that). The most problematic definition is probably that of arrhythmia, which included I47 code (paroxysmal tachycardia) and I49 code (other arrhythmia). These are very different disease, with significant different clinical implications and sequelae. Previous studies mainly focused on atrial fibrillation (for which there is an established relation with CAP). I would suggest the authors to focus on that to improve consistency with previous study and, most important, to avoid referring to a vague definition of “arrhythmia”. This also applies to other outcomes, as stated previously. This is a critical point of this manuscript. 7) Another thing that is important and does not seem to have been taken into account is that death represents here a competitive event in respect to those (cardiovascular events) analyzed in this study. Do the authors have taken into account this aspect? What kind of statistical plan was implemented to take account of this? Please specify. 8) In the results, the atuhors reported that “among the infected patients that could be succesfully matched with non infected patients […]” - this means that some infected patients were actually excluded due to the fact that it was not possible to match them to non-infected controls. This should have been specified in the methods - perhaps one should notice that given the numbers of infected patients (which are overall less than 500 according to incl/exclusion criteria), excluding some others may reduce further the power of the study, and this may be seen as a limitation. 9) Among the limitations, and beyond the ones listed above, I think that also medications and treatments (especially at discharge) are a potential bias; as well as the fact that the events recorded were only those that led to hospitalization (i.e., if a patients developed AF during follow-up but was not hospitalized, this event was not recorded, as per my understanding).

REVIEWER	Mayr, Florian University of Pittsburgh Medical Center
REVIEW RETURNED	04-Mar-2022

GENERAL COMMENTS	Nishimura et al performed a series of case control analyses in a large cohort of residents of 3 municipalities in Japan between 4/2014 and 3/2020 to assess the risk of subsequent different types of cardiovascular events after a diagnosis of pneumococcal pneumonia. In adjusted analyses for age, sex, and pre-existing comorbidities, the risk for stroke and arrhythmias was significantly higher among infected vs. non-infected individuals, whereas it was not for coronary heart disease and heart failure. Please find my comments below: 1. Am I understanding correctly that both inpatient and outpatient claims were used to assess preexisting health status? How long was the look-back period to assess pre-existing health conditions? A sufficiently long look back and use of diverse (e.g., inpatient and outpatient) records is important to adequately assess pre-existing health conditions and reduces the risk of overestimating the outcome of interest. 2. Along these lines, the authors state that patients with any records of cardiovascular events preceding the exposure of interest and during index hospitalization for s pneumonia infection were excluded. I suspect that this was also done for control subjects? How do the authors explain that some patients in table 1 had preexisting myocardial infarction, congestive heart failure, and cerebrovascular disease documented as part of their Charlson Comorbidity index? 3. Please provide more details on the type of claims used. Were pharmacy claims used at all to identify preexisting conditions? 4. I wonder if the matching process as performed may have introduced residual confounding. Am I understanding correctly that controls were never hospitalized? Probably a fairer comparison would be to additionally match on the type of claim used to account for the need for recent hospitalization. I would assume that patients with recent admissions are likely at higher risk for adverse cardiovascular outcomes. Were patients with prior infection-related diagnosis/hospitalizations excluded? How many patients had more than one episode of pneumococcal pneumonia recorded? 5. What is the overall denominator for patients captured in the 3 jurisdictions? This information may be helpful to present in the Results section and it would allow interpreting results in context. How do 698 cases of strep pneumoniae translate into per 100,000? For example, in the US in recent years, the incidence of invasive pneumococcal disease has been 25 per 100K in adults older than 65 years of age (https://www.cdc.gov/pneumococcal/surveillance.html) 6. Multivariate analyses should account for the matching at baseline and likely include a random effect as patients treated in the same hospital may be more similar to each other than others. 7. What was the median follow-up time for each subject? 8. I am a bit concerned that ascertainment of arrhythmias may not be ideal if only based on ICD-10 codes. It would be helpful to understand if these are predominantly episodes of atrial fibrillation or include other types of arrhythmias. Since the Charlson Comorbidity Index, to my knowledge, does not include a separate

	category for arrhythmias, did the authors attempt to exclude subjects with any prior claim indicative of preexisting arrhythmias (i.e., atrial fibrillation 9. What was the rationale to set the index date to the last day of the month during which a Strep pneumoniae infection was recorded instead of using the date of the claim? Could this have introduced lead-time bias? 10. Death definitely is a competing risk to developing the outcome and should likely be incorporated in the proportional hazards models. 11. What was the rationale to only ascertain outcomes that resulted in hospitalization. Could that have resulted in an underestimate of incident cardiovascular disease?

VERSION 1 – AUTHOR RESPONSE

For Reviewer 1
Comment #1
One thing that it is unclear is whether the authors have focused on community acquired pneumonia (or, at least, lower tract respiratory infection) or whether all types of infections sustained by S. Pneumoniae were considered (for example, also meningitidis?). This should be specified clearly in the methods section, since the background and the discussion is structured on the comparison between previous evidence related to community acquired pnuemonia, so one would expect that the analysis was restricted on CAP patients.

Response #1
Thank you for your time and effort in reviewing our manuscript.
Our study analyzed all types of S. pneumonia infections, including invasive pneumococcal diseases such as meningitis and sepsis. There were 22 invasive pneumococcal disease cases (4.5%) and 467 non-invasive pneumococcal disease cases (95.5%) in our study population. Due to this low proportion of invasive cases, we did not conduct separate statistical analyses according to invasiveness. We have clarified the description of our study subjects in the Methods and Results, and agree that this makes the manuscript clearer.

Modification #1
- Methods: Lines 122-123.
- Results: Lines 189-191.

Comment #2
Relatedly, although the authors referred to a previous study for the methods used to identify patients with S. Pneumoniae infection, they should report briefly the methods used to select patients. How were S. Pneumoniae infections identified? Was a microbiological criterion adopted? Please report and specify.

Response #2
Thank you for the question.
S. pneumoniae infections were identified through combinations of ICD-10 codes and/or Japan-specific diagnosis codes as defined by the Ministry of Health, Labour and Welfare. We used the combinations

of codes proposed by Imai et al. (available at https://bmjopen.bmj.com/content/8/3/e018553.long#DC2). Unfortunately, our claims dataset did not include microbiological information to help identify S. pneumoniae infections. As advised, we have added an explanation to the Methods regarding the identification of these patients.

Modification #2

- Methods: Lines 120-122.

Comment #3

As a side note, one may wonder whether the authors restricted their analysis to S. Pneumoniae infected patients - as the authors already acknowledge, community acquired pneumonia is sustained by a broad spectrum of pathogens, so that to improve consistency with previous studies it might have been more suitable to include all patients with a diagnosis of CAP, for example. But this is only a comment.

Response #3

Thank you for the comment.
As you stated, there are other pathogens that can also cause CAP. Nevertheless, pneumonia caused by S. pneumoniae is the best studied with regard to pathogenic mechanisms and cardiovascular events. We had sought to explore the effects of S. pneumoniae-induced pneumonia on subsequent cardiovascular disease based on well-documented pathogenic mechanisms.

Modification #3

- None

Comment #4

While I recognize the reasons behind the choice of excluding patients with previous cardiovascular events, one should notice that this approach may have led to selection bias in the population examined. In fact, the real-world population of patients who experience CAP also include patients with previous cardiovascular events (indeed, one may expect that these patients are maybe more prone to develop CAP and other infections due to their inner conditions), so that excluding them actually reduce comparability with a real-world scenario. This may be seen as a limitation. Also, I do not understand why there are 5.4% patients with “congestive heart failure” included for the “HF” analysis; or 9.1% patients with “cerebrovascular disease” in the Stroke analysis. Weren’t these patients expected to be excluded from these analyses?

Response #4

Thank you for your constructive comments and for pointing this out.
We agree that our approach would overlook patients with previous cardiovascular events, and this could be a study limitation. As advised, we have disclosed this limitation in the Discussion. With regard to the exclusion of patients with previous cardiovascular events, we realize that our explanation was inadequate, and are grateful for the comment. While we used inpatient and outpatient records to identify the comorbidities, we had only excluded patients who had experienced cardiovascular events with in-hospital treatments. Accordingly, the study population included patients who had experienced outpatient treatments for cardiovascular events before the index date. We have added this explanation to the Methods and Fig 2 caption.

Modification #4

- Methods: Lines 125-129.
- Discussion: Lines 319-322.
- Figure 2

Comment #5
Please reports in table 1 the number (%) of patients with previous arrhythmia.

Response #5
Thank you for your suggestion.
We have added that number to Table 1.

Modification #5
- Table 1

Comment #6
One significant critical aspect of this analysis is the definition of the outcome. The use of ICD codes seem too broad for some of these events. For example, stroke comprises ICD codes from I60 to I69; these includes subarachnoid hemorrhage and other cerebrovascular disease for example, which are only loosely related to “stroke”; similarly, heart failure also comprised I11 code, which is “hypertensive heart disease” (this do not imply heart failure, or at least not in the definition commonly used for that). The most problematic definition is probably that of arrhythmia, which included I47 code (paroxysmal tachycardia) and I49 code (other arrhythmia). These are very different disease, with significant different clinical implications and sequelae. Previous studies mainly focused on atrial fibrillation (for which there is an established relation with CAP). I would suggest the authors to focus on that to improve consistency with previous study and, most important, to avoid referring to a vague definition of “arrhythmia”. This also applies to other outcomes, as stated previously. This is a critical point of this manuscript.

Response #6
Thank you for this invaluable advice.
As advised, we have modified the definitions of the outcomes and the use of ICD-10 codes. The ICD-10 codes for stroke, heart failure, and arrythmia have been streamlined for accuracy. The definition of stroke has been narrowed down into intracerebral hemorrhage, cerebral infarction, subarachnoid hemorrhage, and other cerebrovascular disease (ICD-10 codes I61-63, 65-66). The ICD-10 code for heart failure has been limited to only I50. As suggested, we have focused on atrial fibrillation (I48) instead of arrhythmia. The new definitions for these outcomes have been added to the Methods, and arrhythmia has been replaced with atrial fibrillation throughout the manuscript.

Modification #6
- Methods: Lines 154-156.

Comment #7
Another thing that is important and does not seem to have been taken into account is that death represents here a competitive event in respect to those (cardiovascular events) analyzed in this study. Do the authors have taken into account this aspect? What kind of statistical plan was implemented to take account of this? Please specify.

Response #7
Thank you for bringing up this highly pertinent point.
We agree that death is a competing risk in the study, and have added an analysis incorporating death as a competitive event in the proportional hazards models. The results are consistent with the prior analysis that did not take death into account.

Modification #7

- Methods: Lines 173-175.
- Results: Lines 215-218.
- Figure 4

Comment #8

In the results, the atuhors reported that “among the infected patients that could be succesfully matched with non infected patients […]” - this means that some infected patients were actually excluded due to the fact that it was not possible to match them to non-infected controls. This should have been specified in the methods - perhaps one should notice that given the numbers of infected patients (which are overall less than 500 according to incl/exclusion criteria), excluding some others may reduce further the power of the study, and this may be seen as a limitation.

Response #8

Thank you for these invaluable suggestions.
As advised, we have added to the Methods a description of the fact that we excluded infected patients who could not be matched with non-infected controls. We also acknowledged that the exclusion of these non-matched infected cases can affect the power of our statistical analyses as a limitation in the Discussion.

Modification #8

- Methods: Lines 140-141.
- Discussion: Lines 322-325.

Comment #9

Among the limitations, and beyond the ones listed above, I think that also medications and treatments (especially at discharge) are a potential bias; as well as the fact that the events recorded were only those that led to hospitalization (i.e., if a patients developed AF during follow-up but was not hospitalized, this event was not recorded, as per my understanding).

Response #9

Thank you for the comment.
We have added the lack of medications and treatments to the limitations.
As you stated, cardiovascular events that did not lead to hospitalization were not included in the study. However, the validity/accuracy of diagnoses is crucial for database studies, as the imprecise use of codes to identify outcomes can potentially lead to misclassifications. We focused on outcomes that resulted in hospitalization to increase the accuracy and reliability of the cardiovascular event diagnoses. Furthermore, we can ensure a similar level of disease severity among the events by focusing on hospitalization cases only. Nevertheless, it is certainly true that previous outpatient treatments were not included, and this may result in potential bias. We have added this limitation to the Discussion.

Modification #9

- Discussion: Lines 315-316, 328-329.

For Reviewer 2

Comment #1

Am I understanding correctly that both inpatient and outpatient claims were used to assess preexisting health status? How long was the look-back period to assess pre-existing health conditions? A sufficiently long look back and use of diverse (e.g., inpatient and outpatient) records is

important to adequately assess pre-existing health conditions and reduces the risk of overestimating the outcome of interest.

Response #1
Thank you for your time and effort in reviewing our manuscript.
As you mentioned, we used both inpatient and outpatient claims to assess comorbidities. The look-back period to assess the experience of cardiovascular events was at least one year; for these conditions, we looked back to each patient's earliest available records within the observation period. The look-back period to assess the coexisting health conditions was 30 days, which we think is appropriate for assessing coexisting health conditions (as opposed to a history of past conditions). As instructed, we have added to the Methods an explanation of the look-up periods for assessing comorbidities.

Modification #9
- Methods: Lines 113-117,142-143.

Comment #2
Along these lines, the authors state that patients with any records of cardiovascular events preceding the exposure of interest and during index hospitalization for s pneumonia infection were excluded. I suspect that this was also done for control subjects? How do the authors explain that some patients in table 1 had preexisting myocardial infarction, congestive heart failure, and cerebrovascular disease documented as part of their Charlson Comorbidity index?

Response #2
Thank you for pointing this out.
We had indeed excluded non-infected controls who had experienced the exposure of interest before the index date. However, we realize that our explanation on the exclusion of previous cardiovascular events was inadequate. While we used inpatient and outpatient records to identify the comorbidities, we had only excluded infected and non-infected patients who had experienced cardiovascular events with in-hospital treatments. Accordingly, the study population included patients who had experienced outpatient treatments for cardiovascular events before the index date. We have added this explanation to the Methods and Fig 2 caption.

Modification #2
- Methods: Lines 125-129,143-145.
- Figure 2

Comment #3
Please provide more details on the type of claims used. Were pharmacy claims used at all to identify preexisting conditions?

Response #3
Thank you for the comment. The LIFE Study database used in this study largely collects health insurance claims data and provides datasets to researchers for analysis. We used datasets containing records for diagnoses (Japanese diagnosis codes and ICD-10 codes), dates of treatments and admissions, and coexisting conditions (Charlson Comorbidity Index scores).
The Charlson Comorbidity Index scores were generated from the corresponding ICD-10 codes recorded in both inpatient and outpatient claims based on the Quan method (https://pubmed.ncbi.nlm.nih.gov/3558716/, https://pubmed.ncbi.nlm.nih.gov/16224307/). We did not use information on prescribed drugs to identify these conditions. We have clarified the types of claims data used in the Methods.

Modification #3

- Methods: Lines 113-117, 120-122.

Comment #4

Please I wonder if the matching process as performed may have introduced residual confounding. Am I understanding correctly that controls were never hospitalized? Probably a fairer comparison would be to additionally match on the type of claim used to account for the need for recent hospitalization. I would assume that patients with recent admissions are likely at higher risk for adverse cardiovascular outcomes. Were patients with prior infection-related diagnosis/hospitalizations excluded? How many patients had more than one episode of pneumococcal pneumonia recorded?

Response #4

Thank you for your valuable questions and suggestions.
The controls were selected from non-infected patients who had been hospitalized or not hospitalized at the index date. We did not exclude non-infected patients who had been hospitalized. As you pointed out, recent admission would help make a fairer comparison. In accordance with your advice, we have additionally matched the patients on hospitalization at the index date. Regarding patients with prior infection-related diagnoses or hospitalizations, we included only the first infection episode for patients with multiple episodes during the observation period. There were 94 out of 698 S. pneumoniae-infected patients who had experienced more than one infection episode. We have added the above explanation to the Methods.

Modification #4

- Methods: Lines 131-133, 152-153.

Comment #5

What is the overall denominator for patients captured in the 3 jurisdictions? This information may be helpful to present in the Results section and it would allow interpreting results in context. How do 698 cases of strep pneumoniae translate into per 100,000? For example, in the US in recent years, the incidence of invasive pneumococcal disease has been 25 per 100K in adults older than 65 years of age (https://www.cdc.gov/pneumococcal/surveillance.html)

Response #5

Thank you for the valuable suggestions.
We recognize the importance of the overall denominator for patients captured in the three jurisdictions. However, it would not be possible to provide an accurate overall denominator that covers the entire study period because there is a high turnover for National Health Insurance enrollees, with many people changing health insurance types every month (due to changes in employment status or occupation). For reference, there were approximately 180,000 enrollees in the 3 municipalities in 2018, and as many as 20,000 new enrollees annually.
For our study population, there were 868,669 person-years overall (562,238 person-years for older patients aged ≥65 years). Although we analyzed 698 S. pneumoniae-infected patients, these patients were identified after removing multiple episodes and are therefore not appropriate for calculating incidence rates. When including multiple episodes for all ages, we found 914 S. pneumoniae-infected patients (34 cases with invasive pneumococcal disease); these numbers would be more appropriate for calculating incidence rates. The incidence of S. pneumoniae infection was about 105 cases per 100,000 population. The incidence of invasive pneumococcal disease per 100,000 population was 3.9 cases. There were 14 cases (including multiple episodes) of invasive pneumococcal disease for older patients aged ≥65 years. The incidence of invasive pneumococcal disease per 100,000 population was 2.49 cases for older adults aged ≥65 years. For reference, the reported incidences of invasive pneumococcal disease per 100,000 population in Japan between 2014 and 2017 were 1.45–2.53

cases for the total population and 2.96–5.38 for older adults aged ≥65 years (https://www.niid.go.jp/niid/en/basic-science/865-iasr/8187-461te.html).

Modification #5

- None

Comment #6

Multivariate analyses should account for the matching at baseline and likely include a random effect as patients treated in the same hospital may be more similar to each other than others.

Response #6

Thank you for your insightful comments.

We realize that the inclusion of a random effect may be beneficial for our study of residents from three municipalities. However, all the municipalities are located in the same prefecture, with no major differences in characteristics among the municipalities or hospitals. Additionally, pneumonia is one of the most common diseases that hospitals regularly treat, and it is unlikely that there would be a large bias at the hospital level. We have added this limitation to the Discussion.

Modification #6

- Discussion: Lines 331-337.

Comment #7

What was the median follow-up time for each subject?

Response #7

Thank you for the question.

The median observation periods of the matched infected and non-infected patients were 823, 827, 820, and 797 days for CHD, HF, stroke, and AF, respectively.

Modification #7

- Results: Lines 203-205.

Comment #8

I am a bit concerned that ascertainment of arrhythmias may not be ideal if only based on ICD-10 codes. It would be helpful to understand if these are predominantly episodes of atrial fibrillation or include other types of arrhythmias. Since the Charlson Comorbidity Index, to my knowledge, does not include a separate category for arrhythmias, did the authors attempt to exclude subjects with any prior claim indicative of preexisting arrhythmias (i.e., atrial fibrillation

Response #8

Thank you for your constructive comments.

Based on your suggestions, we have modified the definitions of the outcome and the use of ICD-10 codes. The ICD-10 codes for stroke, heart failure, and arrythmia have been streamlined for accuracy. The definition of stroke has been narrowed down into intracerebral hemorrhage, cerebral infarction, subarachnoid hemorrhage, and other cerebrovascular disease (ICD-10 codes I61-63, 65-66). The ICD-10 code for heart failure has been limited to only I50. As suggested, we have focused on atrial fibrillation (I48) instead of arrhythmia.

We realize that our explanation on the exclusion of previous cardiovascular events was inadequate. While we used inpatient and outpatient records to identify the comorbidities, we had only excluded infected and non-infected patients who had experienced cardiovascular events with in-hospital treatments. Therefore, we had excluded patients who had experienced atrial fibrillation with in-hospital treatments, but not patients who had experienced outpatient treatments for atrial fibrillation. As you

stated, the Charlson Comorbidity Index does not include a category for arrhythmia. Accordingly, when examining the occurrence of AF after S. pneumoniae infection, we included the comorbidity of AF as a matching criterion.

Modification #8

- Methods: Lines 125-127, 138-141,154-156.

Comment #9

What was the rationale to set the index date to the last day of the month during which a Strep pneumoniae infection was recorded instead of using the date of the claim? Could this have introduced lead-time bias?

Response #9

Thank you for the question.

Due to the large amount of data on daily medical treatments for many comorbidities, it was impractical to ascertain the non-infected controls' conditions at every claims date for their matched infected patients. The data on the last day of the month were more available and accurate for our database. Nevertheless, we recognize that this could have introduced lead-time bias, and have added this limitation to the Discussion.

Modification #9

- Discussion: Lines 329-331.

Comment #10

Death definitely is a competing risk to developing the outcome and should likely be incorporated in the proportional hazards models.

Response #10

Thank you for bringing up this highly pertinent point.

We agree that death is a competing risk in the study, and have added an analysis incorporating death as a competitive event in the proportional hazards models. The results are consistent with the prior analysis that did not take death into account.

Modification #10

- Methods: Lines 173-175.
- Results: Lines 215-218.
- Figure 4

Comment #11

What was the rationale to only ascertain outcomes that resulted in hospitalization. Could that have resulted in an underestimate of incident cardiovascular disease?

Response #11

Thank you for the question.

As you stated, cardiovascular events that did not lead to hospitalization were not included in the study. However, the validity/accuracy of diagnoses is crucial for database studies, as the imprecise use of

codes to identify outcomes can potentially lead to misclassifications. We focused on outcomes that resulted in hospitalization to increase the accuracy and reliability of the cardiovascular event diagnoses. Furthermore, we can ensure a similar level of disease severity among the events by focusing on hospitalization cases only. Nevertheless, it is certainly true that previous outpatient treatments were not included, and this may result in potential bias. We have added this limitation to the Discussion.

Modification #11

- Discussion: Lines 325-328.

VERSION 2 – REVIEW

REVIEWER	Romiti, Giulio Francesco Sapienza University of Rome, Department of Translational and Precision Medicine
REVIEW RETURNED	30-Jul-2022

GENERAL COMMENTS	The manuscript has improved a lot compared to previous round of revision, and the authors should be commended for this. There are only two minor issues: - please improve legends/representation of Figure 4. In its current form, it is unclear what the red color indicates (there is a "." Label in the legend, which I suppose stands for "none". But it would be better to clarify this). As as side note, wouldn't a line graph a better representation compared to a stacked area plot for cumulative incidence plots? - You have reported in your results results (which were statistically significant) for the Fine-Gray model only for AF and stroke - please report also for the other 2 CV outcomes investigated, even if non-statistically significant. This difference in significance should also be discussed more.

REVIEWER	Mayr, Florian University of Pittsburgh Medical Center
REVIEW RETURNED	15-Sep-2022

GENERAL COMMENTS	I appreciate the opportunity to review the submitted revisions to the original manuscript. The authors have addressed or clarified most of my original comments, however, a few issues remain not addressed which may hamper the validity of the results. 1. I remain confused by the selective use of cardiovascular events for the determination of preexisting cardiovascular disease and outcomes. I recommend consistent use of outpatient and inpatient claims for the determination of chronic comorbidities and outcomes to avoid any inadvertent confounding. If cardiovascular events requiring hospital treatment remains the outcome of choice, the title should reflect this. 2. If the primary outcome remains cardiovascular events requiring hospitalization, the regression models should include a random hospital effect to account for variation in hospital admission practices between hospitals. 3. In follow-up to the responses to reviewer one, I am wondering how many infected patients were excluded due to the inability to match with non-infected patients. Were these patients different

	(demographics, comorbidities, etc.) from infected patients who were able to be matched? 4. Please report pre-post summaries of covariate balance for the matching analysis.

VERSION 2 – AUTHOR RESPONSE

For Reviewer 1

Comment #1

Please improve legends/representation of Figure 4. In its current form, it is unclear what the red color indicates (there is a "." Label in the legend, which I suppose stands for "none". But it would be better to clarify this). As as side note, wouldn't a line graph a better representation compared to a stacked area plot for cumulative incidence plots?

Response #1

Thank you for your time and effort in reviewing our manuscript.

As you mentioned, the "." label in the legend stood for "none". We have clarified the description in the legend as advised. With regard to the type of graph for cumulative incidence, we had elected to use a stacked area plot to clearly present the total cumulative probability of a cardiovascular event and death. As a result, we believe the graphs in their current form are more suitable for our purposes.

Modification #1

- Figure 4

Comment #2

You have reported in your results results (which were statistically significant) for the Fine-Gray model only for AF and stroke - please report also for the other 2 CV outcomes investigated, even if non-statistically significant. This difference in significance should also be discussed more.

Response #2

Thank you for your advice.

As instructed, we have added the results of the Fine-Gray model analysis for CHD and HF to the Results (CHD: adjusted subdistribution HR: 1.19, 95% CI: 0.63–2.26, HF: adjusted subdistribution HR: 1.13, 95% CI: 0.60–2.13). A possible explanation for the difference in significance may be the presence of multiple mechanisms that contribute to cardiovascular events. The impact of these

mechanisms may differ for each type of cardiovascular event. We have added this explanation to the Discussion.

Modification #2

- Results: Lines 199-208
- Discussion: Lines 299-302

For Reviewer 2

Comment #1

I remain confused by the selective use of cardiovascular events for the determination of preexisting cardiovascular disease and outcomes. I recommend consistent use of outpatient and inpatient claims for the determination of chronic comorbidities and outcomes to avoid any inadvertent confounding. If cardiovascular events requiring hospital treatment remains the outcome of choice, the title should reflect this.

Response #1

Thank you for your time and effort in reviewing our manuscript.

We recognize that it would be beneficial to use outpatient and inpatient claims for outcomes to avoid any inadvertent confounding. While the outpatient claims data would include accurate comorbidity-related information, we think that there is an unacceptable level of uncertainty about the accuracy and availability of outcome-related information (e.g., accurate occurrence date, degrees of severity). As suggested, we have modified the title to reflect that the outcomes were focused on hospitalization cases only. The new title is as follows: "**Risk of cardiovascular events leading to hospitalization after *Streptococcus pneumoniae* infection: A retrospective cohort LIFE study**".

Modification #1

- Title

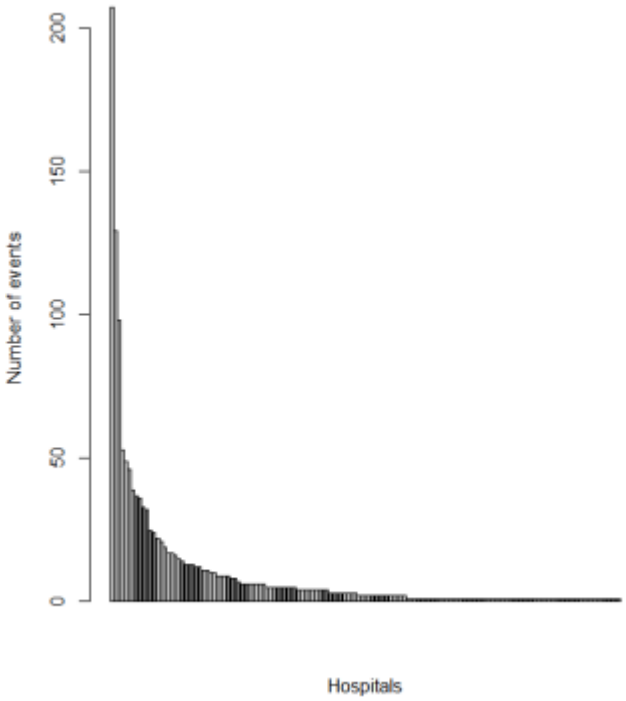
Comment #2

If the primary outcome remains cardiovascular events requiring hospitalization, the regression models should include a random hospital effect to account for variation in hospital admission practices between hospitals.

Response #2

Thank you for pointing this out.

Indeed, a random hospital effect should be considered when hospital admission practices vary between hospitals. However, we do not expect there to be substantial differences in admission practices among hospitals throughout Japan due to the implementation of a universal health insurance system. Thus, we do not think that there is a need to include a random hospital effect in the regression models. As an example, the distribution of event admissions for stroke among hospitals is illustrated in the graph below.



Modification #2

- None

Comment #3

In follow-up to the responses to reviewer one, I am wondering how many infected patients were excluded due to the inability to match with non-infected patients. Were these patients different (demographics, comorbidities, etc.) from infected patients who were able to be matched?

Response #3

Thank you for the valuable questions.

The exact numbers of infected patients who were excluded due to the inability to match with non-infected patients are presented in Figure 2 (CHD: 114, HF: 114, Stroke: 126, and AF: 153).

During the consideration of this point, we discovered that we had made an error in stating that our analyses were conducted using Charlson comorbidity index scores. Instead of scores, matching was actually conducted based on the presence/absence of specific comorbidities that are included in the

Charlson comorbidity index. Therefore, patients were matched according to their exact patterns of comorbidities. Similarly, our Cox proportional hazards regression models included this array of comorbidities (each comorbidity was included as a categorical variable) as covariates. The list of comorbidities is provided in the second paragraph of the “Study subjects” subsection in the Methods, as well as in the covariate balance summaries in the newly added Supplementary Tables 1-4 (as per your Comment #4). We deeply apologize for this error, and have revised all relevant sentences. We have also checked the rest of the manuscript to ensure that there are no similar mistakes.

The infected patients were exactly matched with a cohort of non-infected patients according to age (within 5 years), sex, comorbidities, and hospitalization at the index date. There were many possible patterns in the types of comorbidities, which limited the number of patients who could be matched with the exact same patterns. Moreover, hospitalization narrowed down the non-infected patients that could be matched. With regard to age, each cohort of non-infected patients sufficiently covered a wide range of age groups. Accordingly, the main differences between infected patients that could and could not be matched would be in their patterns of comorbidities. We have clarified the description of exact matching in the Methods.

Modification #3

- Methods: Lines 99-100
- Methods: Lines 115-118
- Methods: Lines 125
- Discussion: Lines 267

Comment #4

Please report pre-post summaries of covariate balance for the matching analysis.

Response #4

Thank you for your suggestion. As instructed, we have added the following covariate balance tables for the matching analysis as Supplementary Tables 1-4.

Supplementary Table 1. Covariate balance before and after matching for coronary heart disease

	Coronary heart disease					
	Unmatched			Matched		
	Mean		Standardized mean differences	Mean		Standardized mean differences
	Infected	Non-infected		Infected	Non-infected	
Age	77.84	68.41	0.48	77.08	77.03	0.0043
Sex	0.48	0.60	0.25	0.49	0.49	0.00
Hospital admission	0.79	0.02	5.58	0.70	0.70	0.00
Myocardial infarction	0.01	0.01	0.02	0.00	0.00	0.00
Congestive heart failure	0.20	0.06	0.60	0.11	0.11	0.00
Peripheral vascular disease	0.08	0.05	0.14	0.03	0.03	0.00
Cerebrovascular disease	0.25	0.12	0.43	0.14	0.14	0.00
Dementia	0.21	0.05	0.68	0.14	0.14	0.00
Chronic pulmonary disease	0.37	0.10	0.89	0.29	0.29	0.00
Rheumatic disease	0.06	0.02	0.32	0.01	0.01	0.00
Peptic ulcer disease	0.13	0.04	0.40	0.07	0.07	0.00
Mild liver disease	0.20	0.12	0.25	0.16	0.16	0.00
Diabetes without chronic complications	0.06	0.01	0.68	0.01	0.01	0.00
Diabetes with chronic complications	0.05	0.03	0.12	0.03	0.03	0.00
Hemiplegia or paraplegia	0.02	0.01	0.19	0.00	0.00	0.00
Renal disease	0.07	0.03	0.26	0.01	0.01	0.00
Malignancy	0.15	0.05	0.42	0.08	0.08	0.00
Moderate or severe liver disease	0.00	0.00	0.05	0.00	0.00	0.00

Metastatic solid tumor	0.03	0.01	0.25	0.02	0.02	0.00
HIV/AIDS	0.00	0.00	0.01	0.00	0.00	0.00

Supplementary Table 2. Covariate balance before and after matching for heart failure

	Heart failure			
	Unmatched			**Match**
	Mean		**Standardized mean differences**	**Mean**
	Infected	**Non-infected**		**Infecte**
Age	77.08	68.42	0.45	75.56
Sex	0.43	0.59	0.34	0.45
Hospital admission	0.78	0.02	5.40	0.66
Myocardial infarction	0.02	0.01	0.13	0.01
Congestive heart failure	0.10	0.05	0.22	0.02
Peripheral vascular disease	0.12	0.05	0.29	0.03
Cerebrovascular disease	0.25	0.12	0.42	0.12
Dementia	0.19	0.05	0.64	0.11
Chronic pulmonary disease	0.39	0.10	0.95	0.32
Rheumatic disease	0.06	0.02	0.31	0.03
Peptic ulcer disease	0.15	0.05	0.51	0.08
Mild liver disease	0.23	0.12	0.33	0.17
Diabetes without chronic complications	0.06	0.01	0.65	0.01
Diabetes with chronic complications	0.06	0.03	0.18	0.04
Hemiplegia or paraplegia	0.02	0.01	0.18	0.00
Renal disease	0.08	0.03	0.33	0.01
Malignancy	0.18	0.05	0.54	0.11
Moderate or severe liver disease	0.01	0.00	0.09	0.00
Metastatic solid tumor	0.02	0.01	0.11	0.02
HIV/AIDS	0.00	0.00	0.01	0.00

Supplementary Table 3. Covariate balance before and after matching for stroke

	Stroke					
	Unmatched			Matched		
	Mean		Standardized mean differences	Mean		Standardized mean differences
	Infected	Non-infected		Infected	Non-infected	
Age	78.20	68.57	0.50	77.66	77.53	0.011
Sex	0.46	0.60	0.29	0.49	0.49	0.00
Hospital admission	0.80	0.02	5.41	0.71	0.71	0.00
Myocardial infarction	0.03	0.01	0.11	0.00	0.00	0.00
Congestive heart failure	0.20	0.07	0.52	0.09	0.09	0.00
Peripheral vascular disease	0.11	0.05	0.24	0.03	0.03	0.00
Cerebrovascular disease	0.20	0.10	0.31	0.10	0.10	0.00
Dementia	0.17	0.05	0.53	0.12	0.12	0.00
Chronic pulmonary disease	0.37	0.11	0.88	0.29	0.29	0.00
Rheumatic disease	0.05	0.02	0.28	0.02	0.02	0.00
Peptic ulcer disease	0.11	0.05	0.31	0.06	0.06	0.00
Mild liver disease	0.19	0.12	0.21	0.15	0.15	0.00
Diabetes without chronic complications	0.04	0.01	0.42	0.01	0.01	0.00
Diabetes with chronic complications	0.05	0.03	0.16	0.03	0.03	0.00
Hemiplegia or paraplegia	0.02	0.01	0.18	0.00	0.00	0.00
Renal disease	0.10	0.03	0.39	0.02	0.02	0.00
Malignancy	0.15	0.06	0.41	0.09	0.09	0.00

Moderate or severe liver disease	0.00	0.00	0.05	0.00	0.00	0.00
Metastatic solid tumor	0.01	0.01	0.07	0.01	0.01	0.00
HIV/AIDS	0.00	0.00	0.01	0.00	0.00	0.00

Supplementary Table 4. Covariate balance before and after matching for atrial fibrillation

	Atrial fibrillation					
	Unmatched			**Matched**		
	Mean		**Standardized mean differences**	**Mean**		**Standardized mean differences**
	Non-infected	**Infected**		**Non-infected**	**Infected**	
Age	78.70	68.92	0.51	77.67	77.52	0.012
Sex	0.45	0.60	0.29	0.48	0.48	0.00
Hospital admission	0.83	0.02	5.42	0.74	0.74	0.00
Myocardial infarction	0.02	0.01	0.09	0.00	0.00	0.00
Congestive heart failure	0.20	0.07	0.52	0.11	0.11	0.00
Peripheral vascular disease	0.10	0.05	0.21	0.03	0.03	0.00
Cerebrovascular disease	0.26	0.12	0.42	0.13	0.13	0.00
Dementia	0.19	0.06	0.55	0.12	0.12	0.00
Chronic pulmonary disease	0.38	0.11	0.90	0.28	0.28	0.00
Rheumatic disease	0.06	0.02	0.32	0.02	0.02	0.00
Peptic ulcer disease	0.12	0.05	0.36	0.05	0.05	0.00
Mild liver disease	0.18	0.12	0.18	0.12	0.12	0.00
Diabetes without chronic complications	0.05	0.01	0.53	0.01	0.01	0.00
Diabetes with chronic complications	0.06	0.03	0.19	0.03	0.03	0.00

Hemiplegia or paraplegia	0.02	0.01	0.16	0.00	0.00	0.00
Renal disease	0.10	0.03	0.41	0.02	0.02	0.00
Malignancy	0.17	0.06	0.50	0.08	0.08	0.00
Moderate or severe liver disease	0.01	0.00	0.05	0.00	0.00	0.00
Metastatic solid tumor	0.02	0.01	0.17	0.02	0.02	0.00
HIV/AIDS	0.00	0.00	0.01	0.00	0.00	0.00
Atrial fibrillation	0.03	0.03	0.02	0.01	0.01	0.00

Modification #4

- Supplementary Tables 1-4

VERSION 3 – REVIEW

REVIEWER	Mayr, Florian University of Pittsburgh Medical Center
REVIEW RETURNED	16-Oct-2022

GENERAL COMMENTS	I appreciate the additional analyses and clarifications. All my concerns have been addressed by additional analyses / as limitations.