PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Post-marketing observational study on the safety of 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy: TheShinISS-Vax|Flu study protocol
AUTHORS	Spila Alegiani, Stefania; Morciano, Cristina; Menniti-Ippolito, Francesca; Da Cas, Roberto; Felicetti, Patrizia; Marchione, Pasquale; Petronzelli, Fiorella; Marra, Anna Rosa; Massari, Marco

VERSION 1 – REVIEW

REVIEWER	Pérez Rivas, Francisco Javier Universidad Complutense de Madrid, Enfermería
REVIEW RETURNED	31-May-2023

GENERAL COMMENTS	The study protocol sets out an interesting research project to identify adverse events after administration of the influenza vaccine, which have required emergency care or hospital admission. Although the approach of the project is quite correct, there are aspects that need to be reviewed/clarified before publication. I have added my contributions in the form of comments in the attached document – contact publisher to view this file. Professor Nuria Alcolea Ruiz has also participated in the revision of the article.

REVIEWER	Wilhelm, Marcel Philipps-Universität Marburg
REVIEW RETURNED	05-Jun-2023

GENERAL COMMENTS	The submitted study protocol refers to a study that examines the adverse events of influenza vaccinations using real-world data from a large nationwide dataset. I suspect that this study could provide important information on vaccine side effects, particularly with regard to severe adverse events. However, the protocol should include much more relevant information to understand the rationale for the study as well as the methodology. Introduction: The text starts with “The Italian Ministry of Health..” I think the introduction would improve if the first paragraph is dedicated to catch the readers attention. What is the problem that this study addresses? Why should I continue reading? Why is this study relevant for people living outside of Italy? The study name is really long “TheShinISS-Vax|Flu” and the first part “TheShinISS” is introduced too late to understand why this name was chosen. The introduction lacks theory and previous findings: Why is it important to access AESI of influenza? What are common AEs

	following immunization with influenza vaccines (see: https://doi.org/10.1016/j.vaccine.2022.11.033 for more information)? What are the most common AESIs (to ground your choice later in Table 1)? What are the problems in recording AESIs/AEFIs, which make study designs like SCCS necessary? You should make a convincing point on why you think this study will help to understand adverse events. Methods: p. 6: Why are the diagnoses coded in ICD-9? ICD-10 is used since the 90s. If this is due to the predefined database structure, please discuss as a limitation Describing the SCCS study design and TheShinISS takes a lot of space without specifying how they work. How is the SCCS design employed in this study? How was the SCCS modified to handle event dependent exposures? When you write "TheShinISS allows to carry out distributed analyses in multi-database pharmacoepidemiological studies according to a Common Data Model strategy which is study tailored" --> How was the strategy tailored in this study? Figure 1 is not self-explanatory

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1.1

Line 10 page 3: The purpose of TheShinISS-Vax|Flu study is to examine the association between
influenza vaccines and adverse events during the influenza vaccination campaigns 2021/2022 and 2022/2023 in Italy.
Comment: serious adverse events requiring hospital admission or emergency care

We added the sentence in the abstract.

1.2

Line 36 page 4: The main limitations are: no validation of the outcomes of interest through review of
clinical records and absence of information on obesity, smoking and other life style factors.
Comment: It could be added as a limitation that adverse effects that have not required emergency care or hospital admission will not be considered.

As suggested from the editor this limitation was added as a bullet point in the "Strengths and limitations of this study" section: "*Only serious adverse events requiring emergency care or hospital admission were included.*"

1.3

Line 12 page 6: …unvaccinated or who received influenza vaccine during the 2021/2022 and 2022/2023 influenza vaccination campaigns and who were admitted to emergency care or hospital…
Comment: If vaccinated and unvaccinated are included, the records of the entire population of the 7 regions who have visited the emergency department or had a hospital admission for any of the outcomes of interest in the periods under evaluation will be evaluated. Is this the case?

Your interpretation is correct. We have highlighted in the protocol that including both vaccinated and unvaccinated cases in the study is a crucial aspect of the SCCS modified model distinguishing it from the SCCS standard model, which only considers vaccinated cases.

1.4

Line 19 page 6: …to the end of the observation period.
Comment: Data for the 2021/22 campaign will be collected in January 2023, while data for the 2022/23 campaign will be collected in April 2023, so the observation periods of the two campaigns will be different.

As you correctly point out, the study periods of the two campaigns are different, it was better specified in the Study period section:

"For the vaccination campaign 2021/2022: 1 September 2021 – 30 June 2022.
For the vaccination campaign 2022/2023: 1 September 2022 – 30 June 2023."

Data collection was delayed, due to organizational problems, therefore data collection for both vaccination campaigns started in May 2023 and will be completed in autumn 2023. Thus, the end of the study activities has been postponed to December 2023. We have amended the GANTT (now moved in supplemental Table 1) according to these new dates and we have harmonized the changes throughout the text.

1.5
Line 21 page 6: Participation in the study of the Italian Regions is voluntary.
Comment: Was an offer made to all regions of Italy, how was this offer made and why did only these 7 regions decide to participate?

We confirm that participation of the Italian regions was voluntary. We specified in the text that: "*The AIFA invited all the Italian regions to participate in the study but participation depended on the availability of the health care databases, data update and personnel to be dedicated to the study*."

1.6
Line 40 page 7: TheShinISS-Vax|Flu study will use a Self-Controlled Case Series (SCCS) design [12-17].
The SCCS design is best suited to evaluate the safety of vaccines and other medicinal products when the relationship between transient exposures and acute events is investigated. This method requires only data on individuals, vaccinated and unvaccinated, who have experienced an event (cases).
Comment: It is not clear to me that in a self-controlled case-control study it is necessary to include the unvaccinated in the study. Shouldn't the incidence of events in the vaccinated population be calculated when they have been exposed to the risk (incidence in the vaccination period) and when they have not been exposed to the risk (incidence in the control period)?

In SCCS model modified for event-dependent exposures, unlike the standard SCCS model, it is essential to include all cases, including those who were unvaccinated. This is discussed in (Ghebremichael-Weldeselassie et al. 19): "*In the standard SCCS model, inclusion of unvaccinated (or, more generally, unexposed) cases is not essential, as such cases do not contribute directly to the estimation of the vaccine effect. Such cases contribute primarily to the estimation of age or seasonal effects. Nevertheless, their inclusion is recommended when risk periods are long and there is potential for confounding between time since vaccination and age or season. Matters are quite different when it comes to the SCCS model for event-dependent exposures: for this model, it is necessary to include all or a random sample of unvaccinated cases. This is because lack of vaccination may indicate cancellation of vaccination, and may tend to occur more often for events that occur earlier (before they had the opportunity to be vaccinated). Thus, absence of vaccination may be informative about the timing of the event, and excluding unvaccinated cases may therefore introduce bias*".

We added a paragraph in the "Study design" section to better clarify this issue: "*In the modified SCCS model for event-dependent exposures, unlike the standard model, it is essential to include unvaccinated cases. This is because the absence of vaccination may indicate cancelled vaccination that occurs more often for events that occur earlier. As a result, the absence of vaccination can be informative on the timing of the event, and excluding unvaccinated cases may introduce bias [19].*"

1.7

Line 57 page 8: The outcomes of interest will be ascertained during an observation period, which is
defined as the time between the beginning of the vaccination campaign (1 October 2021 and 1 October 2022 for the first and second vaccination campaign, respectively) and the date of last regional health data update, for each individual alive;…
Comment: As previously mentioned, the evaluation periods for each campaign will be different.

We better clarified in point 1.4.

1.8
Line 5 page 9: This means that patients who have an emergency care admission or/and hospital discharge, for the same outcome, within the 365 days prior to the start of the study observation will be excluded
Comment: It would be necessary to explain a little more why patients who have had the event one year before are excluded and not 18 months, 2 years? What is the justification for this selected time period?

We realized that we make a typo in the text, we made the correction. We also added this sentence: "*A time-window of 5 years provides a sufficiently look back period to selectively identify incident cases*."

1.9
Line 3 page 12: Table 3. Definition of drugs
Comment: why have these few drugs been selected?

The list of drugs is not limited to those in table 3, but they are also reported in table 2 where drugs used for the definition of comorbidities are reported. We better clarify the definition of comorbidities in the "Definition of comorbidities and drugs" section: "*In tables 2 and 3 are reported the codes of drugs use, hospital discharges and exemptions derived from the local health care databases/registries, respectively, necessary for the definition of comorbidities*."

1.10
Line 26 page 12: The influenza vaccines will be categorized according to the available type of vaccines during the two vaccination campaigns, for example, quadrivalent vaccine, quadrivalent
and trivalent with MF59 adjuvant vaccine, live attenuated influenza vaccine (the nasal spray flu vaccine).
Comment: As it is already known which vaccines have been used in both campaigns, all the vaccines used could be listed, without the need to talk about 'examples'.

The type of vaccines actually administered were available only in June 2023, after the submission of the protocol (November 2022). The entire list of type of vaccines for both vaccination campaigns was reported in the "Definition of exposure" section: *": quadrivalent vaccine (egg-based and cell culture-based flu vaccine), quadrivalent and trivalent with MF59 adjuvant vaccine, live attenuated influenza vaccine (the nasal spray flu vaccine)*".

1.11
Line 32-34 page 12: If a combined influenza/COVID-19 vaccine is made available during the vaccination program 2022-2023, this will be considered an influenza vaccine exposure.
Comment: Would it be correct to establish a relationship of adverse events if both have been administered?
Would it not be more correct to consider patients who have received both vaccines as exclusion criteria?

During the vaccination campaign 2022/2023 the combined influenza and COVID-19 vaccine was not authorized. Thus, we eliminated this paragraph: "*If a combined influenza/COVID-19 vaccine is made available during the vaccination program 2022-2023, this will be considered an influenza vaccine exposure*". Our main analysis will not exclude cases with both influenza

and COVID vaccinations, administered separately. We moved from “Definition of exposure” section to “Method of analysis” section (sensitivity analyses): *“Moreover, we will carry out analyses on cases receiving only influenza vaccines, excluding those with both influenza and COVID-19 vaccines. This restriction will also be applied in cases where other vaccines are received concurrently”.*

1.12

Line 45-49 page 12: All remaining time within the individual observation period will define the non
Exposure period for each outcome of interest, and will represent the baseline period to which the exposure risk period will be compared
Comment: Will patients who have concurrently received other vaccines be excluded? For example, in the case of children aged 6 months to 14 years

We specified it in the “Method of analysis” section (see point 1.11).

1.13

Line 7 page 14: Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.
Comment: Why can't the public participate in research dissemination plans?

This project does not foreseen funds for activities to involve the public in the research planning and dissemination.

Reviewer: 2

2.1

The text starts with “The Italian Ministry of Health..” I think the introduction would improve if the first paragraph is dedicated to catch the readers attention. What is the problem that this study addresses? Why should I continue reading? Why is this study relevant for people living outside of Italy?

The Introduction was completely rephrased according to your suggestion. We have included two additional references, leading to a re-sequencing of all the references accordingly.

2.2

The study name is really long “TheShinISS-Vax|Flu” and the first part “TheShinISS” is *introduced too late to understand why this name was chosen.*

We prefer that the name of the study remains “TheShinISS-Vax|Flu” since it would be difficult at this point to change it considering that the “TheShinISS-Vax “ is the name of the project that research and regulatory community, including EMA, can easily recognize regarding vaccine safety. Additionally, “Flu” in the study name is relevant to identify the specific study on influenza vaccines.

2.3

The introduction lacks theory and previous findings:
a) Why is it important to access AESI of influenza?
b) What are common AEs following immunization with influenza vaccines (see: https://doi.org/10.1016/j.vaccine.2022.11.033 for more information)?
c) What are the most common AESIs (to ground your choice later in Table 1)?
d) What are the problems in recording AESIs/AEFIs, which make study designs like SCCS necessary? You should make a convincing point on why you think this study will help to understand adverse events.

a) We have now ensured that the importance of monitoring adverse events following influenza vaccines are highlighted in the introduction.
b/c) However, we prefer not to include in the introduction what are common AE/AESI following influenza vaccination since it is a matter of continuous research in diverse context, including the research presented in this protocol. On the other hand, as requested by the reviewer, we

found sensible to add in the methods section the selection criteria/process used to include the SAE in table 1 to ground our choice: "*We focus on 13 different outcomes considering the guidelines issued by the AIFA [26] and hypothetical concerns regarding analogous vaccines or complications associated with the disease itself*."
d) The importance to make study design like SCCS to investigate AESI is already reported in the Study design section of Methods.
Please noted that the ICD9-CM codes reported in Table 1 are now different from the submitted version. In fact, in the last months the ICD9-CM codes of the outcomes of the TheShinISS-vax project were revised, by a panel of experts (pharmacologists, clinicians, epidemiologist, data scientists in real world data) to improve the classification of the outcomes.

2.4
p. 6: Why are the diagnoses coded in ICD-9? ICD-10 is used since the 90s. If this is due to the predefined database structure, please discuss as a limitation

In Italy, diagnoses of emergency care admission or hospital discharge are coded by the ICD9-CM code. However, we prefer do not include this limitation in this protocol. First of all because the request of the Editor was to shorten "Strength and limitations" section. Secondly, we feel that the use of ICD9-CM code does not affect the robustness of our study but, in same limited cases, it could make difficult to compare our results with other studies using ICD10. This issue will be certainly considered in the discussion section of the future TheShinISS-vax|Flu publications.

2.5
Describing the SCCS study design and TheShinISS takes a lot of space without specifying how they work.
How is the SCCS design employed in this study?

We apologize for any confusion caused by our explanation of the SCCS design. To enhance the clarity of our methods section, we have included a diagram (Figure 2) that illustrates the risk and reference periods for the hypothetical subjects (cases) included in the study: *"Figure 2. Schematic presentation of SCCS method for hypothetical subjects included in the study. a) events occurring in vaccinated subjects during the risk period; b) events occurring in the reference period after vaccination; c) events occurring in the reference period before vaccination; d) events occurring in unvaccinated subjects."*

How was the SCCS modified to handle event dependent exposures?

Handling event-dependent exposures within the SCCS
model involves a complex mathematical and statistical framework, which poses a challenge to provide a concise summary in the methods section of an article. In line with other authors who have utilized the modified method, including a recent study that examined the modified SCCS for COVID-19 vaccine safety [19], we have chosen to briefly highlight the counterfactual approach underlying the modified SCCS method, adding a
new paragraph in the methods section and including three citations to the most relevant methodological papers addressing this topic including the book authored by the inventor and pioneer of the SCCS methodology, specifically "*The modified SCCS model addresses situations where the occurrence of an event affects the timing or the occurrence of subsequent exposures. It introduces a counterfactual scenario in which no exposure can occur after occurrence of an event [17-19]*".

When you write "TheShinISS allows to carry out distributed analyses in multi-database pharmacoepidemiological studies according to a Common Data Model strategy which is study tailored" --> How was the strategy tailored in this study? Figure 1 is not self-explanatory

In order to enhance the understanding of TheShinISS framework, we have included a more comprehensive explanation of Figure 1, providing more details on its steps: "*Going into further detail, Figure 1 illustrates the relational scheme of the study, including all the steps, which*

utilize TheShinISS to locally process healthcare databases structured according to a CMD: Step 0 - identification of the eligible population from the hospital discharges database and admissions to the emergency care database; Step 1 – extraction and preparation of the CDM of the vaccination registry and the population registry related to the eligible population identified in Step 0; Step 2 – identification of the study cohort by vaccination status, data quality control and descriptive analysis (by execution of TheShinISS); Step 3 - extraction and conversion of healthcare databases, and preparation of the CDM related to the cohort; Step 4 - execution of TheShinISS on CDM to perform: data quality control, linkage of the cohort with healthcare databases, anonymization, aggregation, and creation of a minimal set of exposure and outcome variables, and specific covariates of interest for the study, which will constitute the local anonymized analytical datasets."

Authors' corrections

a) To ensure compliance with the requirement of a maximum of five figures and tables, we have made the following adjustment: after adding Figure 2 to the manuscript, we decided to relocate the GANTT chart (Table 4) to Supplemental Table 1.
b) We corrected the first paragraph of the "Type of vaccine studied" section, specifically in the phrase
"*All influenza vaccines were administered to the study population*…"
c) In the first paragraph of the "Type of vaccine studied" section, we replaced "Italian Medicine Agency" with its acronym "*AIFA*".
d) In the fourth bullet point of the 'Data Source' section, we added *", Clinical Modification*" at the end of the phrase "International Classification of Diseases, 9th Revision".
e) In the sixth paragraph of the "Study Design" section, we replaced "Italian National Institute of Health" with its acronym "*ISS*" and "Common Data Model" with its acronym "*CDM*".
f) In the second paragraph of the "Definition of the study outcomes" section, we removed the phrase "International Classification of Disease, Ninth Revision, Clinical Modification", and instead used the acronym "*ICD9-CM code*".
g) In the fourth paragraph of "Definition of the study outcomes" section, we added the "*look back*" at the end of the phrase "…*within the 5 years days prior to the start of the study period (look back)…".*
h) In the fourth paragraph of the "Definition of the study outcomes" section, we appended the phrase "*look back"* to the end of the sentence to indicate the timeframe as "*within the 5 years prior to the start of the study period (look back)"*.
i) We corrected the fifth paragraph of the "Definition of the study outcomes" section, specifically in the phrase "*Table 1 lists the selected adverse events which are potentially associated with influenza vaccination and the corresponding ICD9-CM codes and the risk period, which are derived from the Brighton Collaboration [27] and the AIFA report [26*]."
j) In the title and header of the first column of Table 1, we replaced "vaccine" with its plural form.
k) We added "*Hospital discharge code*:" and "*Pharmacy claim code*:" as headers for the second and fourth columns of Table 2.
l) We specified that the information on comorbidities for COPD and Asthma was obtained from hospital discharge records within a period of 365 days, which is different from the other comorbidities reported in Table 2.
m) We added "*Pharmacy claim code*:" as header for the second column of Table 3.
n) We rephrased the first paragraph of the "Definition of exposure" section: "*The exposure variables will include the first or second dose of the influenza vaccines available in Italy during the vaccination campaigns 2021/2022 and 2022/2023*."
o) In the third paragraph of the "Definition of the exposure" section, we corrected a typo, specifically changing "For each outcome of interest, we will define specific risk periods (Table 1) which will be further subdivided in sub-risks periods of seven days (except for the anaphylaxis outcome)." into "*For each outcome of interest, we will define specific risk periods (Table 1) which will be further subdivided into 3 sub-risk periods*."
p) In the second paragraph of the "Methods of analysis" we corrected a typo, specifically changing "two years" to "*five years*".
q) At the beginning of the fourth paragraph of the "Methods of analysis" section, we change "method" in "*methodology*" and "adapted" in "*modified*".

r) In the sixth paragraph of the “Methods of analysis” section, we add “*modified*” before SCCS.
s) In the eighth paragraph of the “Methods of analysis” we corrected a typo, specifically changing “[(IR-1)/IR]” in *“[(RI-1)/RI]”.*
t) In the ninth paragraph of the “Methods of analysis” section, we delete “will be”.
u) We added a statistician colleague, *Flavia Mayer*, to the research team of TheShinISS-Vax|flu study, who joined the team in January 2023.
v) Over the past 6 months, the composition of TheShinISS network underwent changes, with the introduction of a new member (*Flavia Mayer*) and departure of two individuals (*Matilde Tenaglia* and *Francesca Romana Poggi*).

VERSION 2 – REVIEW

REVIEWER	Pérez Rivas, Francisco Javier Universidad Complutense de Madrid, Enfermería
REVIEW RETURNED	10-Jul-2023

GENERAL COMMENTS	The authors have done a fantastic revision, incorporating in a very adequate way all the contributions made and substantially improving the article. CONGRATULATIONS!

REVIEWER	Wilhelm, Marcel Philipps-Universität Marburg
REVIEW RETURNED	17-Jul-2023

GENERAL COMMENTS	My initial comments were all sufficiently addressed.