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Post-marketing observational study on the safety of 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy: TheShinISS-Vax|Flu study protocol

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ABSTRACT

Introduction

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.

Methods and analysis

This is a Self-Controlled Case Series (SCCS) multiregional study using linked routinely collected data from regional healthcare databases of the participating Regions.

Study participants will be persons aged ≥6 months, unvaccinated or who have received influenza vaccine during the influenza vaccination campaigns in the seasons 2021/2022 and 2022/2023 in Italy and who have experienced the outcome of interest for the first time during the study period (from 01/10/2021 and 01/10/2022 for the first and second influenza season, respectively, to the last data update). Risk periods will be specifically defined for each outcome and further subdivided into periods of seven days. The exposures will be the first or second dose of the influenza vaccines administered during the two vaccination campaigns. Statistical analysis will be conducted separately for the data of the two campaigns. Exposure risk period will be compared to baseline risk period defined as any time of observation out of the risk periods. The modified SCCS method will be applied to handle event-dependent exposure and mortality and fitted using unbiased estimating equations to estimate relative incidences and excess of cases per 100,000 vaccinated by dose, age, sex, and type of vaccine. Calendar period will be included as time-varying confounder in the model, where appropriate.

Ethics and dissemination

The study received the approval from the National ethics committee for clinical trials of public research bodies and other national public institutions (PRE BIO CE n.0036723, 23/09/2022). Results will be published in peer-reviewed journals and reports in accordance with the publication policies of the Italian National Institute of Health and of the Italian Medicines Agency.

KEYWORDS

Influenza vaccines, post-marketing drug surveillance, Self-Controlled Case Series, adverse drug events, routinely collected health data.

ARTICLE SUMMARY

Strengths and limitations of this study

TheShinISS-Vax|Flu is an active surveillance of adverse events following immunization of influenza vaccines in Italy. An important strength of the present study is the coverage of a large population allowing the detection of rare safety outcomes. This will be made possible by TheShinISS, an R-based open-source statistical tool performing distributed analyses on Real-World Data.

The Self-Controlled Case Series study design allows to control for unknown or unmeasured individual confounders that do not vary over the time of the study.

The "modified" Self-Controlled Case Series methodology, adapted to handle event-dependent exposure and event-dependent mortality, contributes to the robustness of the findings for most outcomes.

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.

INTRODUCTION

The Italian Ministry of Health annually releases recommendations for the prevention and control of influenza. The recommendations on 2021/2022 and 2022/2023 influenza vaccination campaign [1,2] have expanded the vaccine eligible population comparing with the previous vaccination campaigns, also considering the challenges of the COVID-19 pandemic scenario. This context emphasizes the value of the continuous monitoring of the safety of influenza vaccines using both passive and active surveillance systems.

TheShinISS-Vax|Flu study, that is coordinated by the Italian National Institute of Health and the Italian Medicines Agency, is a post-marketing active surveillance of the adverse events following immunization of influenza vaccines in place in Italy. This is a collaborative project which aims to cover a large population using linked health care databases of the participating Italian Regions.

The Italian National Institute of Health has a long history of monitoring the safety of vaccines using ad hoc studies to collect and analyze data, also involving networks of local health authorities, general practitioners, and pediatricians [3-6]. These past experiences have offered the possibility to gain insights into areas where the existing surveillance system can be strengthened, and the development of large-linked database monitoring system has resulted a major challenge.

To address this challenge, the Italian National Institute of Health has pioneered a new model to conduct active surveillances of influenza and COVID-19 vaccines in Italy. This model applies a distributed analysis framework using TheShinISS, an R-based open-source statistical tool that locally processes data collected and updated periodically from regional healthcare databases according to a study-tailored, Common Data Model [7]. The advantages of this model consist of: the inclusion of a large population; the timely access and ease of regional data sharing with a reduction of workload of health professionals; and the enhancement in the quality control of the regional healthcare data. Recently, multi-regional studies have been conducted by TheShinISS using regional routinely collected and linked health data from vaccination registries, hospital discharges, and emergency care admissions and pharmacy claims databases [8-9].

The purpose of this study is to examine the association between influenza vaccines and rare, serious adverse events and adverse events of special interest (AESI) during the 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy.

METHODS AND ANALYSIS

Study population

Study population will include persons of ≥6 months of age of seven Italian Regions (Piemonte, Friuli Venezia Giulia, Emilia-Romagna and Toscana of Northen Italy; Lazio of Central Italy; Puglia and Campania of Southern Italy), 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 for at least one of the outcomes of interest from 1 October 2021 for the 2021-2022 and 1 October 2022 for the 2022-2023 vaccination campaign, to the end of the observation period. Participation in the study of the Italian Regions is voluntary.

Study period

September 2021 – July 2023

Type of vaccine studied

All influenza administered vaccines to the study population in the seven participating Italian Regions during the two campaigns in accordance with the recommendations of the Ministry of Health [1,2] and the provision of the Italian Medicines Agency decision [10,11].

Data sources

The following healthcare databases will be used:

- vaccination registry to identify influenza vaccination exposure and exposure to other vaccines which were administered from the 1 September 2021 and 1 September 2022 for the first and second influenza vaccination campaign, respectively, to the last data update;
- population registry to identify information on age, sex, date of registration and deregistration (where applicable) in the regional health care system, and vital status (causes of death are not recorded in this registry) to the last data update;
- pharmacy claims database to characterize the study population by obtaining information on the use of drugs (coded with Anatomical Therapeutic Chemical code) during the periods preceding the two influenza vaccination programs

(from 1 October 2020 and 1 October 2021 for the first and second influenza campaign, respectively, to the last data update);

- hospital discharges database to identify the outcomes of interest pre- and post-vaccination (from 1 October 2020 and 1 October 2021 for the first and the second influenza campaign, respectively, to the last data update) and also to obtain information on the comorbidities of the study population in the 5 years preceding influenza vaccination, (from 1 October 2016 and 1 October 2017 for the first and the second influenza campaign, respectively, to the last data update) coded with ICD-9 CM (International Classification of Diseases, 9th Revision);
- admissions to the emergency care database to identify the outcomes of interest (from 1 October 2020 and 1 October 2021 for the first and the second influenza campaign, respectively, to the last data update) coded with ICD-9 CM;
- exemptions from healthcare service co-payment database to obtain information on comorbidities of the study population (to the last data update).

Study design

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). Estimation is within individuals and consequently, any time-invariant unknown or unmeasured potential confounders are controlled for.

Therefore, it represents a valid epidemiological design alternative to the cohort and case-control study in the research on vaccine safety, particularly in situations where it is difficult to identify an appropriate comparison group, for example when vaccinated population has different characteristics from unvaccinated or most of the population has received the vaccine.

The SCCS model was originally developed to investigate the association between vaccines and adverse events with the key assumption that the occurrence of an event does not influence post-event exposures, for example by delaying or even cancelling the subsequent exposures. This assumption may be violated for vaccine safety studies when

the occurrence of the outcome of interest is a contra-indication to vaccination. To handle event-dependent exposures a modified SCCS method has been developed [15-17].

TheShinISS-Vax|Flu is a multiregional study using routinely collected data from regional healthcare databases/registries linked in each region at individual level. The study applies TheShinISS, the R-based open-source statistical tool which was developed by the researchers of the Italian National Institute of Health [7]. The tool is currently maintained and customized by the "TheShinISS Network" that includes researchers from the Italian National Institute of Health, the Department of Epidemiology of the Lazio Regional Health Service, and the Universities of Verona and Messina. TheShinISS allows to carry out distributed analyses in multi-database pharmacoepidemiological studies according to a Common Data Model strategy which is study tailored [18]. It has been already employed in large Real-World studies with different epidemiological designs [8,9,19-23].

Going more specifically, using the TheShinISS, at regional level, it is possible to (Figure 1):

a) upload the necessary archives;

b) check quality of data flows;

c) identify the study population;

d) make record-linkage between the study population and health care archives;

e) process data and create pseudonimised local analytical data-sets.

Definition of the study outcomes

The outcomes will be identified from the diagnosis of emergency care admission or hospital discharge using International Classification of Disease, Ninth Revision, Clinical Modification (ICD9-CM code).

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; conversely, when a case dies, the end of the observation period will be defined according to the SCCS methodology to deal with mortality [17].

Cases will be defined as those patients who have experienced the outcome for the first time during the study period (incident cases). 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. Deaths for any causes will be also considered.

Table 1 lists the selected adverse events which are potentially associated with influenza vaccination and the corresponding ICD9-CM code and the risk period, which is derived from the Brighton Collaboration [24] and the AIFA report [25]. The list will be updated in case of emerging signals on new adverse events potentially associated with influenza immunization and the participant Regions will be requested to provide further specific data.

Table 1. Definition of the adverse events potentially associated with influenza vaccine

Adverse events potentially associated with influenza vaccine	ICD9-CM	Risk period (days after the vaccination)
Bell's palsy	351	60
Acute hepatitis	570; 5714; 5733; 5739	60
Guillain-Barré Syndrome	3570	42
Encephalitis and encephalomyelitis	323.5; 323.8; 323.9	42
Thrombocytopenia	287.31	42
Vasculitis	273.2; 287.0; 362.18; 437.4; 443.1; 446; 447.6; 448.9; 710.0; 357.1; 357.82	42
Demyelinating diseases	323.81; 340; 341.1; 341.9; 341.2; 377.3	42
Convulsions	780.39	14
Anaphylaxis	995.0; 995.2	2
Neuritis (Optic neuritis, Brachial neuritis; Parsonage-Aldren-Turner syndrome; Other mononeuritis)	377.30; 723.4; 353.5; 354-355	28
Narcolepsy	347	42
Swelling of limb	729.81	
Syncope and collapse	780.2	

Definition of comorbidities and drugs

In tables 2 and 3 are reported the comorbidities and the use of drugs derived from the local health care databases/registries, respectively.

Table 2. Definition of comorbidities

	ICD-9-CM (in the last 5 years)	Exemptions code	ATC (in the last 12 months)
Chronic pulmonary disease	480-488; 491; 495; 518.81-518.84	024	J05AH
Chronic obstructive pulmonary disease	490; 492; 494; 496	057	R03
Asthma	493	007	
Cardiovascular and cerebrovascular diseases	390-398; 406-459	002; 021; 0A02; 0B02; 0C02; 036	B01AC; C01B; C01DA; C08DA; C08DB
Hypertension	401-405	031; 0A31	C02; C03; C07; C08; C09
Chronic kidney diseases	580; 582-585; 593; 753.12-753.14	023; 022; 061; 062	
Dementia/Alzheimer's disease	290, 294.1, 331.2	011; 029	N06DA; N06DX
Diabetes	250	013	A10
Rheumatic diseases	446.5; 710; 714; 720; 725; 696	006; 028; 030; 045; 054; 067	L04
Hematological disease	280-289 (excl. 285.1)	003	B01AA; B01AB; B01AE; B01AF; B01AX; B02BD; B03
Neurological diseases	238.7; 296.3; 311; 332; 345; 340; 348.39	017; 038; 044; 046	N03A; N04B; N05A; N06A
Neoplasms	140-209; V10	048	L01
Metabolic disorders	272; 278	025	C10
Moderate/severe hepatopathy	456.0-456.2; 571-572; 573.0	008; 016	-
Cystic fibrosis	277.0	018	R07AX
Ulcer disease	531; 532; 533		A02B
Colitis	555; 556	009	
HIV (Human Immunodeficiency Virus)	042	020	J05AE; J05AF; J05AG; J05AR
Infections	053; 599.0; 010-018; 031; 078.5; 052-054; 136.3; 117.5	055	J01, J02, J04, J05 (excl. J05AE, J05AF, J05AG, J05AH, J05AR)

Table 3. Definition of drugs

	ATC (in the last 12 months)
Other vaccines	J07 (excluding COVID-19 and J07BX03 flu vaccines J07BB)
Anti-COVID19 vaccines	J07BX03
Glucocorticoids	H02AB
NSAIDs (Non-Steroidal Anti-Inflammatory Drugs)	M01A
Estroprogestinics	G03

Definition of the exposure

The exposure variables will be the first or second dose of the influenza vaccines which are available in Italy during the vaccination campaign 2021/2022 and 2022/2023.

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).

If a combined influenza/COVID-19 vaccine is made available during the vaccination program 2022-2023, this will be considered an influenza vaccine exposure. Furthermore, we will compare results of the combined vaccine with those of the anti-influenza vaccination if data allow for it.

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).

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.

Methods of analysis

Statistical analysis will be conducted separately for the data of the two vaccination programs 2021/2022 and 2022/2023. Where appropriate, a pooled analysis will be conducted.

We will describe the characteristics of the cases as frequencies, percentages, medians, and Interquartile Ranges, in terms of age, sex, geographical areas, Charlson Index (based

on hospitalization in the five years prior vaccination), length of hospitalization, number of hospital admissions for any causes in the two years prior vaccination, number of drug prescriptions in the year prior vaccination, and comorbidities.

We will describe the data extraction process in a flowchart reporting number of individuals at each stage of the process, for example those individuals potentially eligible, included, analyzed and those excluded with reasons, indicating also numbers of individuals with missing or incoherent observations.

We will use the SCCS method [12-17], adapted to event-dependent exposures [16-18], to examine the association between influenza vaccine and each outcome of interest in individuals aged ≥6 months during the observation period. If patients died, the end of the observation period will be defined according to what is proposed by the SCCS methodology to handle mortality [18].

The SCCS model will be fitted using unbiased estimating equations to estimate Relative Incidences (RIs) and their 95% confidence intervals (95% CI) in the pre-defined risk periods compared to the baseline periods. Unbiased estimating equations theory generalizes likelihood theory to estimate the parameters of interest and it used when the likelihood function is difficult to obtain. Precision of the estimates can be calculated similarly to the methods of the maximum-likelihood estimate [26].

To account for possible seasonal variation in the baseline incidence of each outcome, temporal effects will be included in the model as time-varying covariate.

We will estimate, for each outcome of interest, the excess of cases per 100,000 vaccinated (EC) as the ratio of the number of excess cases due to the vaccine {[(IR-1)/IR] × n. events in the risks period} divided by the number of vaccinated × 100,000 [27]; while the 95% confidence intervals (CI 95%) will be calculated by nonparametric bootstrapping methodology (10,000 replications).

Subgroup analyses will be carried out by age group (<60 e ≥60 years), sex, and type of vaccine for each outcome of interest. Several sensitivity analyses will be performed to assess the assumptions of the SCCS model regarding the event-dependent exposure and observation period, the seasonality, and the pre-specification of risk periods.

Statistical analyses will be performed using R (R Core team 2021) with SCCS package [28] and STATA software.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

TIME SCHEDULE

Time schedule of the study is presented in Table 4.

Table 4. Time schedule of TheShinISS-Vax|Flu

GANTT	2022							2023						
	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pre-study procedures														
Protocol Drafting														
Customizing TheShinISS														
Study organization														
Ethics Committee														
Region participation														
2021-2022 campaign														
Data collection														
Interim analysis and report														
Analysis and final report														
2022-2023 campaign														
Data collection														
Interim analysis and report														
Analysis and final report														

LEGEND OF FIGURES

Figure 1. Diagram showing the data flow when using TheShinISS to locally process health care data structured according to a Common Data Model.

* Vaccination registry related to those registered in the regional population

ETHICS AND DISSEMINATION

The study received the approval from the National ethics committee for clinical trials of public research bodies and other national public institutions (PRE BIO CE n.0036723, 23/09/2022). Results will be published in peer-reviewed journals and reports in accordance with the publication policies of the Italian National Institute of Health and of the Italian Medicines Agency.

ADVERSE REACTION MANAGEMENT

The adverse reaction reporting is not required according to the Guideline on good pharmacovigilance practices (GVP) VI rev. 2 (VI.C.1.2.1.2. Non-interventional post-authorization studies with a design based on secondary use of data) [29].

AUTHORS CONTRIBUTIONS

SSA, CM, FMI, RDC, PF, PM, FP, ARM, and MM were involved in conception and study design. SSA, CM and MM were involved in drafting of the article. FMI, RDC, PF, PM, FP and ARM were involved in critical revision of the article for important intellectual content. All the authors were involved in final approval of the article. SSA, MM and CM provided statistical expertise.

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COMPETING INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data cannot be shared publicly under article 9 of Regulation (EU) 2016/679. Data are available from the Data Protection Officer of Istituto Superiore di Sanità- Dott. Carlo Villanacci, e-mail: responsabile.protezionedati@iss.it, for researchers who meet the criteria for access to confidential data.

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REFERENCES

1. Circolare del Ministero della Salute "Prevenzione e controllo dell'influenza: raccomandazioni per la stagione 2021-2022" https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2021&codLeg=79647&parte=1%20&serie=null [accessed 2 Nov 2022]
2. Circolare del Ministero della Salute "Prevenzione e controllo dell'influenza: raccomandazioni per la stagione 2022-2023" https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2022&codLeg=87997&parte=1%20&serie=null [accessed 2 Nov 2022]
3. Spila-Alegiani S, Salmaso S, Rota MC, et al. Reactogenicity in the elderly of nine commercial influenza vaccines: results from the Italian SVEVA study. Study for the evaluation of adverse events of influenza vaccination. *Vaccine* 1999;17(15-16):1898-904.
4. Spila Alegiani S, Alfonsi V, Bella A, et al. Vaccino antinfluenzale stagionale in Italia: misurare l'efficacia sul campo e la sicurezza. Stagione 2015-2016. Roma: Istituto Superiore di Sanità; 2017. (Rapporti ISTISAN 17/19).
5. Spila Alegiani S, Alfonsi V, Appelgren EC, et al. Active surveillance for safety monitoring of seasonal influenza vaccines in Italy, 2015/2016 season. *BMC Public Health* 2018;18(1):1401 doi: 10.1186/s12889-018-6260-5.
6. Galeotti F, Massari M, D'Alessandro R, et al. Risk of Guillain-Barré syndrome after 2010-2011 influenza vaccination. *Eur J Epidemiol* 2013;28(5):433-44 doi: 10.1007/s10654-013-9797-8.
7. Massari M, Spila Alegiani S, Da Cas R, et al. TheShinISS: un applicativo open-source per la conduzione di analisi distribuite in studi di farmacoepidemiologia di tipo multi-database ("TheShinISS": an open-source tool for conducting distributed analyses within pharmacoepidemiological multi-database studies). *Boll Epidemiol Naz* 2020;1(2):39-45 doi: 10.53225/BEN_006.
8. Spila Alegiani S, Morciano C, Belleudi V, et al. Valutazione postmarketing della sicurezza del vaccino antinfluenzale durante la campagna di vaccinazione antinfluenzale 2020-2021 in Italia: uno studio Self-Controlled Case Series sulla sindrome di Guillain-Barré (Post-marketing safety evaluation of flu vaccine during

the 2020-2021 flu vaccination campaign in Italy: a Self-Controlled Case Series study of Guillain-Barré syndrome). *Boll Epidemiol Naz* 2022;3(2):1-9 doi: 10.53225/BEN_042.

9. Massari M, Spila Alegiani S, Morciano C, et al. Post-marketing active surveillance of myocarditis and pericarditis following vaccination with COVID-19 mRNA vaccines in persons aged 12-39 years in Italy: a multi-database, self-controlled case series study. *PLoS Med* 2022;19(7):e1004056.
10. Determina dell'AIFA che autorizza l'aggiornamento, per la stagione 2021-2021, della composizione dei vaccini influenzali autorizzati secondo procedura di mutuo riconoscimento e decentrata (Determina AAM/PPA n. 654/2021). https://www.aifa.gov.it/documents/20142/1563396/Determinazione_AIFA_654-2021_GU.pdf [accessed 2 Nov 2022].
11. Determina dell'AIFA che autorizza l'aggiornamento, per la stagione 2022-2023, della composizione dei vaccini influenzali autorizzati secondo procedura di mutuo riconoscimento e decentrata (Determina AAM/PPA n. 652/2022). https://www.aifa.gov.it/documents/20142/1754982/Det_AIFA_652-2022.pdf [Accessed 2 Nov 2022].
12. Whitaker HJ, Farrington CP, Spiessens B, et al. Tutorial in bio-statistics: the self-controlled case series method. *Stat Med* 2006;25(10):1768–97.
13. Petersen I, Douglas I, Whitaker H. Self controlled case series methods: an alternative to standard epidemiological study designs. *BMJ* 2016;354:i4515 doi: 10.1136/bmj.i4515.
14. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. *Epidemiol Infect* 2011;139(12):1805–17.
15. Farrington CP, Whitaker HJ, Hocine MN. Case series analysis for censored, perturbed, or curtailed post-event exposures. *Biostatistics* 2009;10(1):3–16.
16. Farrington CP, Whitaker H, Weldeselassie YG. Self-Controlled Case Series Studies. A Modelling Guide with R. New York, Chapman and Hall/CRC Press 2018.
17. Ghebremichael-Weldeselassie Y, Jabagi MJ, Botton J, et al. A modified self-controlled case series method for event-dependent exposures and high event-related mortality,

with application to COVID-19 vaccine safety. *Stat Med* 2022;10;41(10):1735-50 doi:10.1002/sim.9325.

18. Gini R, Sturkenboom MCJ, Sultana J, et al. Different strategies to execute multi-database studies for medicines surveillance in real-world setting: a reflection on the European model. *Clin Pharmacol Ther* 2020;108(2):228-35 doi: 10.1002/cpt.1833.
19. Trifirò G, Massari M, Da Cas R, et al. Renin–Angiotensin–Aldosterone System Inhibitors and Risk of Death in Patients Hospitalised with COVID-19: A Retrospective Italian Cohort Study of 43,000 Patients. *Drug Safety* 2020;43:1297–308 doi: 10.1007/s40264-020-00994-5.
20. Spila Alegiani S, Crisafulli S, Giorgi Rossi P, et al. Risk of COVID-19 hospitalization and mortality in rheumatic patients treated with hydroxychloroquine or other conventional DMARDs in Italy. *Rheumatology* (Oxford). 2021;60(SI):SI25-SI36 doi: 10.1093/rheumatology/keab348.
21. Massari M, Spila Alegiani S, Fabiani M, et al. Association of influenza vaccination and prognosis in patients testing positive to SARS-COV-2 swab test: a large-scale Italian multi-database cohort study. *Vaccines* 2021;9(7):716 doi:10.3390/vaccines9070716
22. Trifirò G, Isgrò V, Ingrasciotta Y, et al. Large-scale post-marketing surveillance of biological drugs for immune-mediated inflammatory diseases through an Italian distributed multi-database healthcare network: the VALORE project. *BioDrugs* 2021;12:1–16 doi: 10.1007/s40259-021-00498-3.
23. Belleudi V, Rosa AC, Finocchietti M, et al. An Italian multicentre distributed data research network to study the use, effectiveness, and safety of immunosuppressive drugs in transplant patients: Framework and perspectives of the CESIT project. Front Pharmacol 2022;13:959267 doi: 10.3389/fphar.2022.959267.
24. Brighton Collaboration Case Definitions. https://brightoncollaboration.us/category/pubs-tools/case-definitions/ [accessed 2 Nov 2022].
25. Gruppo di lavoro sull'analisi dei segnali da vaccino, Agenzia Italiana del Farmaco. Guida alla valutazione delle reazioni avverse osservabili dopo vaccinazione. https://www.aifa.gov.it/sites/default/files/Guida_valutazione_reazioni_avverse_osservabili_dopo_vaccinazione_2.pdf [accessed 2 Nov 2022].

26. D. R. Cox. Unbiased estimating equations derived from statistics that are functions of a parameter. *Biometrika* 1993;80(4):905–9 doi: 10.1093/biomet/80.4.905

27. Wilson K and Hawken S. Drug safety studies and measures of effect using the self-controlled case series design. *Pharmacoepidemiol Drug Saf* 2013;22:108-10 doi: 10.1002/pds.3337.

28. Weldeselassie YJ, Whitaker H and Farrington P (2021). SCCS: The Self-Controlled Case Series Method. R. package version 1.5. https://CRAN.R-project.org/package=SCCS.

29. European Medicine Agency (EMA), Guideline on good pharmacovigilance practices (GVP) – 28 July 2017, EMA/873138/2011 https://www.ema.europa.eu/documents/regulatory-procedural-guideline/guideline-good-pharmacovigilance-practices-gvp-module-vi-collection-management-submission-reports_en.pdf [accessed 2 Nov 2022].

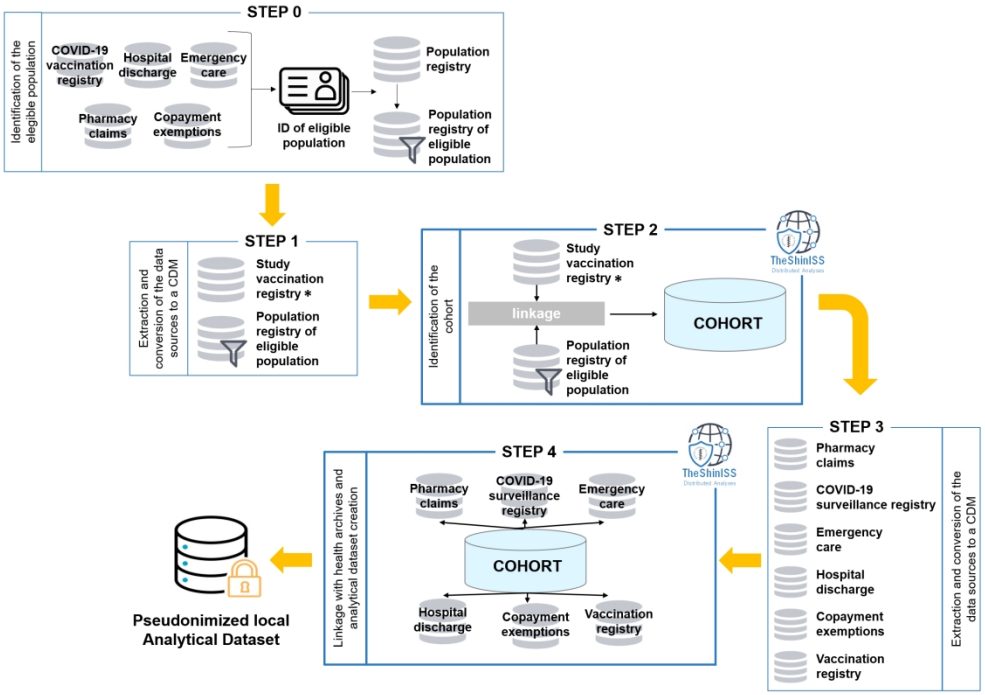

Figure 1. Diagram showing the data flow when using TheShinISS to locally process health care data structured according to a Common Data Model
* Vaccination registry related to those registered in the regional population

399x284mm (150 x 150 DPI)

BMJ Open

Post-marketing observational study on the safety of 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy: TheShinISS-Vax|Flu study protocol

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Post-marketing observational study on the safety of 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy: TheShinISS-Vax|Flu study protocol

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ABSTRACT

Introduction

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

Methods and analysis

This is a Self-Controlled Case Series (SCCS) multiregional study using linked routinely collected data from regional healthcare databases of the participating Regions.

Study participants will be persons aged ≥6 months, unvaccinated or who have received influenza vaccine during the influenza vaccination campaigns in the seasons 2021/2022 and 2022/2023 in Italy and who have experienced the outcome of interest for the first time during the study period (01/09/2021-30/06/2022 and 01/09/2022-30/06/2023 for the first and second vaccination campaign, respectively). Risk periods will be specifically defined for each outcome and further subdivided into periods of seven days. The exposures will be the first or second dose of the influenza vaccines administered during the two vaccination campaigns. Statistical analysis will be conducted separately for the data of the two campaigns. Exposure risk period will be compared to baseline risk period defined as any time of observation out of the risk periods. The modified SCCS method will be applied to handle event-dependent exposure and mortality and fitted using unbiased estimating equations to estimate relative incidences and excess of cases per 100,000 vaccinated by dose, age, sex, and type of vaccine. Calendar period will be included as time-varying confounder in the model, where appropriate.

Ethics and dissemination

The study received the approval from the National ethics committee for clinical trials of public research bodies and other national public institutions (PRE BIO CE n.0036723, 23/09/2022). Results will be published in peer-reviewed journals and reports in accordance with the publication policies of the Italian National Institute of Health and of the Italian Medicines Agency.

KEYWORDS

Influenza vaccines, post-marketing drug surveillance, Self-Controlled Case Series, adverse drug events, routinely collected health data.

ARTICLE SUMMARY

Strengths and limitations of this study

Large sample size and long follow-up for detecting rare adverse events of influenza vaccines.

An R-based statistical tool, TheShinISS, enabled distributed analyses on Real-World Data to overcome privacy issues.

Use of modified Self-Controlled Case Series method to handle event-dependent exposure and mortality, and to control for time-independent confounders.

It was not possible to validate outcomes through clinical records review.

Only serious adverse events requiring emergency care or hospital admission were included.

INTRODUCTION

Seasonal influenza is a viral respiratory disease in human, caused by A or B virus. Influenza epidemics occur annually worldwide with substantial burden of disease.

Influenza vaccination campaigns remain an important public health intervention to reduce influenza viruses' circulation during epidemic and pandemic. They are organized annually since the waning of immunity and the yearly changes in viral antigenic configuration requires annual updating of the vaccines [1].

Vaccines are rigorously evaluated in pre-registrative randomized clinical trials, but their wide scale introduction may provide the opportunity to identify rare adverse events that can be undetected in clinical trials. Therefore, it is essential the continuous monitoring of adverse events potentially associated with influenza vaccines using both passive and active surveillance systems, as a key element of any vaccination campaign [2].

New safety concerns may arise since composition of influenza vaccines changes yearly according to WHO recommendations (https://www.who.int/teams/global-influenza-programme/vaccines/who-recommendations).

The Italian National Institute of Health (ISS) and the Italian Medicines Agency (AIFA) coordinated TheShinISS-Vax|Flu study a post-marketing active surveillance of the adverse events following immunization of influenza vaccines in place in Italy. This is a collaborative project which aims to cover a large population using linked health care databases of the participating Italian Regions.

ISS has a long history of monitoring the safety of vaccines using ad hoc studies to collect and analyze data, also involving networks of local health authorities, general practitioners, and pediatricians [3-6]. These past experiences have offered the possibility to gain insights into areas where the existing surveillance system can be strengthened, and the development of large-linked database monitoring system has resulted a major challenge.

ISS has pioneered a new model to conduct active surveillances of influenza and COVID-19 vaccines in Italy. This model applies a distributed analysis framework using TheShinISS, an R-based open-source statistical tool that locally processes data collected and updated periodically from regional healthcare databases according to a study-tailored, Common Data Model (CDM) [7]. The advantages of this model consist of: the

inclusion of a large population; the timely access and ease of regional data sharing with a reduction of workload of health professionals; and the enhancement in the quality control of the regional healthcare data. Recently, multi-regional studies have been conducted by TheShinISS using regional routinely collected and linked health data from vaccination registries, hospital discharges, and emergency care admissions and pharmacy claims databases [8-9].

In Italy, the Ministry of Health annually releases recommendations for the prevention and control of influenza. The recommendations on 2021/2022 and 2022/2023 influenza vaccination campaign [10,11] have expanded the vaccine eligible population comparing with the previous vaccination campaigns, also considering the challenges of the COVID-19 pandemic scenario.

The purpose of this study is to examine the association between rare, serious adverse events and adverse events of special interest (AESI) and influenza vaccines during the 2021/2022 and 2022/2023 influenza vaccination campaigns in Italy.

METHODS AND ANALYSIS

Study population

Study population will include persons of ≥6 months of age of seven Italian Regions (Piemonte, Friuli Venezia Giulia, Emilia-Romagna and Toscana of Northen Italy; Lazio of Central Italy; Puglia and Campania of Southern Italy), unvaccinated or who received influenza vaccine during the 2021/2022 and 2022/2023 influenza vaccination campaigns (from October to March) and who were admitted to emergency care or hospital for at least one of the outcomes of interest from the beginning of the vaccination campaigns, to the end of the study periods. Participation in the study of the Italian Regions is voluntary. 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.

Study period

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

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

Type of vaccine studied

All influenza vaccines were administered to the study population in the seven participating Italian Regions during the two campaigns, in accordance with the recommendations of the Ministry of Health [10,11] and the provision of AIFA decision [12,13].

Data sources

The following healthcare databases will be used:

- vaccination registry to identify influenza vaccination exposure and exposure to other vaccines which were administered from the 1 September 2021 and 1 September 2022 for the first and second influenza vaccination campaign, respectively, to the last data update;
- population registry to identify information on age, sex, date of registration and deregistration (where applicable) in the regional health care system, and vital status (causes of death are not recorded in this registry) to the last data update;
- pharmacy claims database to characterize the study population by obtaining information on the use of drugs (coded with Anatomical Therapeutic Chemical code) during the periods preceding the two influenza vaccination programs (from 1 September 2020 and 1 September 2021 for the first and second influenza campaign, respectively, to the last data update);
- hospital discharges database to identify the outcomes of interest pre- and post-vaccination (from 1 September 2021 and 1 September 2022 for the first and the second influenza campaign, respectively, to the last data update) and also to obtain information on the comorbidities of the study population in the 5 years preceding influenza vaccination, (from 1 October 2016 and 1 October 2017 for the first and the second influenza campaign, respectively, to the last data update) coded with ICD-9 CM (International Classification of Diseases, 9th Revision, Clinical Modification);
- admissions to the emergency care database to identify the outcomes of interest (from 1 September 2021 and 1 September 2022 for the first and the second influenza campaign, respectively, to the last data update) coded with ICD-9 CM;

- exemptions from healthcare service co-payment database to obtain information on comorbidities of the study population (to the last data update).

Study design

TheShinISS-Vax|Flu study will use a Self-Controlled Case Series (SCCS) design [14-19].

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). Estimation is within individuals and consequently, any time-invariant unknown or unmeasured potential confounders are controlled for.

Therefore, it represents a valid epidemiological design alternative to the cohort and case-control study in the research on vaccine safety, particularly in situations where it is difficult to identify an appropriate comparison group, for example when vaccinated population has different characteristics from unvaccinated or most of the population has received the vaccine.

The SCCS model was originally developed to investigate the association between vaccines and adverse events with the key assumption that the occurrence of an event does not influence post-event exposures, for example by delaying or even cancelling the subsequent exposures. This assumption may be violated for vaccine safety studies when the occurrence of the outcome of interest is a contra-indication to vaccination. To handle event-dependent exposures a modified SCCS method has been developed [17-19].

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].

TheShinISS-Vax|Flu is a multiregional study using routinely collected data from regional healthcare databases/registries linked in each region at individual level. The study applies TheShinISS, the R-based open-source statistical tool which was developed by the researchers of ISS [7]. The tool is currently maintained and customized by the "TheShinISS Network" that includes researchers from ISS, the Department of Epidemiology of the Lazio Regional Health Service, and the Universities of Verona and

Messina. TheShinISS allows to carry out distributed analyses in multi-database pharmacoepidemiological studies according to a CDM strategy which is study tailored [20]. It has been already employed in large Real-World studies with different epidemiological designs [8,9,21-25].

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.

Definition of the study outcomes

We focus on 13 different outcomes considering the guidelines issued by AIFA [26] and hypothetical concerns regarding analogous vaccines or complications associated with the disease itself.

The outcomes will be identified from the diagnosis of emergency care admission or hospital discharge using ICD9-CM code.

The outcomes of interest will be ascertained during the study period. Each case will be follow up from the beginning of the vaccination campaign (1 September 2021 and 1 September 2022 for the first and second vaccination campaign, respectively) to the date of last regional health data update, for each individual alive; conversely, when a case dies, the end of the observation period will be defined according to the SCCS methodology to deal with mortality [19].

Cases will be defined as those patients who have experienced the outcome for the first time during the study period (incident cases). This means that patients who have an

emergency care admission or a hospital discharge, for the same outcome, within the 5 years prior to the start of the study period (look back) will be excluded. A time-window of 5 years provides a sufficiently look back period to selectively identify incident cases. Deaths for any causes will be also considered.

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]. The list will be updated in case of emerging signals on new adverse events potentially associated with influenza immunization and the participant Regions will be requested to provide further specific data.

Table 1. Definition of the adverse events potentially associated with influenza vaccines

Adverse events potentially associated with influenza vaccines	**ICD9-CM**	**Risk period (days after the vaccination)**
Bell's palsy	351.0	60
Acute hepatitis	570; 572.2; 573.3; 573.9	60
Guillain-Barré Syndrome	357.0; 357.8; 357.9	42
Encephalitis and encephalomyelitis	323; 348.3	42
Thrombocytopenia	283.0; 286.5; 287 (excl. 287.39); V83.01; V83.02	42
Vasculitis	136.1; 273.2; 287.0; 446.0; 446.2; 446.4; 446.5; 446.6; 446.7; 709.1	42
Demyelinating diseases	323.81; 340; 341.0; 341.1; 341.2; 341.9; 377.3; 377.49; 377.9; 725	42
Convulsions	780.39	14
Anaphylaxis	995.0; 999.4	2
Neuritis (Brachial neuritis; Neuralgic amyotrophy)	353.5; 723.4	28
Narcolepsy	347	42
Swelling of limb	729.81	
Syncope and collapse	780.2	

Definition of comorbidities and drugs

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.

Table 2. Definition of comorbidities

	Hospital discharge code: ICD-9-CM (in the last 5 years)	Exemptions code	Pharmacy claim code: ATC (in the last 12 months)
Chronic pulmonary disease	480-488; 491; 495; 518.81-518.84	024	J05AH
Chronic obstructive pulmonary disease*	490; 492; 494; 496	057	R03
Asthma*	493	007	
Cardiovascular and cerebrovascular diseases	390-398; 406-459	002; 021; 0A02; 0B02; 0C02; 036	B01AC; C01B; C01DA; C08DA; C08DB
Hypertension	401-405	031; 0A31	C02; C03; C07; C08; C09
Chronic kidney diseases	580; 582-585; 593; 753.12-753.14	023; 022; 061; 062	
Dementia/Alzheimer's disease	290, 294.1, 331.2	011; 029	N06DA; N06DX
Diabetes	250	013	A10
Rheumatic diseases	446.5; 710; 714; 720; 725; 696	006; 028; 030; 045; 054; 067	L04
Hematological disease	280-289 (excl. 285.1)	003	B01AA; B01AB; B01AE; B01AF; B01AX; B02BD; B03
Neurological diseases	238.7; 296.3; 311; 332; 345; 340; 348.39	017; 038; 044; 046	N03A; N04B; N05A; N06A
Neoplasms	140-209; V10	048	L01
Metabolic disorders	272; 278	025	C10
Moderate/severe hepatopathy	456.0-456.2; 571-572; 573.0	008; 016	-
Cystic fibrosis	277.0	018	R07AX
Ulcer disease	531; 532; 533		A02B
Colitis	555; 556	009	
HIV (Human Immunodeficiency Virus)	042	020	J05AE; J05AF; J05AG; J05AR
Infections*	053; 599.0; 010-018; 031; 078.5; 052-054; 136.3;	055	J01, J02, J04, J05 (excl. J05AE, J05AF, J05AG, J05AH, J05AR)

	117.5		

* ICD-9-CM in the 365 days

Table 3. Definition of drug use

	Pharmacy claims code: ATC (in the last 12 months)
Other vaccines	J07 (excluding COVID-19 and J07BX03 flu vaccines J07BB)
Anti-COVID19 vaccines	J07BX03
Glucocorticoids	H02AB
NSAIDs (Non-Steroidal Anti-Inflammatory Drugs)	M01A
Estroprogestinics	G03

Definition of the exposure

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.

The influenza vaccines will be categorized according to the available type of vaccines during the two vaccination campaigns: 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).

For each outcome of interest, we will define specific risk periods (Table 1) which will be further subdivided into 3 sub-risks periods.

All remaining time within the individual observation period will define the no-exposure period for each outcome of interest, and will represent the baseline period to which the exposure risk period will be compared (Figure 2).

Methods of analysis

Statistical analysis will be conducted separately for the data of the two vaccination programs 2021/2022 and 2022/2023. Where appropriate, a pooled analysis will be conducted.

We will describe the characteristics of the cases as frequencies, percentages, medians, and Interquartile Ranges, in terms of age, sex, geographical areas, Charlson Index (based on hospitalization in the five years prior vaccination), length of hospitalization, number

of hospital admissions for any causes in the five years prior vaccination, number of drug prescriptions in the year prior vaccination, and comorbidities.

We will describe the data extraction process in a flowchart reporting number of individuals at each stage of the process, for example those individuals potentially eligible, included, analyzed and those excluded with reasons, indicating also numbers of individuals with missing or incoherent observations.

We will use the SCCS methodology, modified to event-dependent exposures [14-19], to examine the association between influenza vaccine and each outcome of interest in individuals aged ≥6 months during the observation period. 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].

If patients died, the end of the observation period will be defined according to what is proposed by the modified SCCS methodology to handle mortality [19].

The SCCS model will be fitted using unbiased estimating equations to estimate Relative Incidences (RIs) and their 95% confidence intervals (95% CI) in the pre-defined risk periods compared to the baseline periods. Unbiased estimating equations theory generalizes likelihood theory to estimate the parameters of interest and it used when the likelihood function is difficult to obtain. Precision of the estimates can be calculated similarly to the methods of the maximum-likelihood estimate [28].

To account for possible seasonal variation in the baseline incidence of each outcome, temporal effects will be included in the model as time-varying covariate.

We will estimate, for each outcome of interest, the excess of cases per 100,000 vaccinated (EC) as the ratio of the number of excess cases due to the vaccine {[(RI-1)/RI] × n. events in the risks period} divided by the number of vaccinated × 100,000 [29]; while the 95% confidence intervals (CI 95%) calculated by nonparametric bootstrapping methodology (10,000 replications).

Subgroup analyses will be carried out by age group (<60 e ≥60 years), sex, and type of vaccine for each outcome of interest.

Several sensitivity analyses will be performed to assess the assumptions of the SCCS model regarding the event-dependent exposure and observation period, the seasonality,

and the pre-specification of risk periods. 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.

Statistical analyses will be performed using R (R Core team 2021) with SCCS package [30] and STATA software.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

TIME SCHEDULE

Time schedule of the study is presented in supplemental Table 1.

LEGEND OF FIGURES

Figure 1. Diagram showing the data flow when using TheShinISS to locally process health care data structured according to a Common Data Model.

* Vaccination registry related to those registered in the regional population

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.

ETHICS AND DISSEMINATION

The study received the approval from the National ethics committee for clinical trials of public research bodies and other national public institutions (PRE BIO CE n.0036723, 23/09/2022). Results will be published in peer-reviewed journals and reports in accordance with the publication policies of the Italian National Institute of Health and of the Italian Medicines Agency.

ADVERSE REACTION MANAGEMENT

The adverse reaction reporting is not required according to the Guideline on good pharmacovigilance practices (GVP) VI rev. 2 (VI.C.1.2.1.2. Non-interventional post-authorization studies with a design based on secondary use of data) [31].

AUTHORS CONTRIBUTIONS

SSA, CM, FMI, RDC, PF, PM, FP, ARM, and MM were involved in conception and study design. SSA, CM and MM were involved in drafting of the article. FMI, RDC, PF, PM, FP and ARM were involved in critical revision of the article for important intellectual content. All the authors were involved in final approval of the article. SSA, MM and CM provided statistical expertise.

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COMPETING INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data cannot be shared publicly under article 9 of Regulation (EU) 2016/679. Data are available from the Data Protection Officer of Istituto Superiore di Sanità - Dott. Carlo Villanacci, e-mail: responsabile.protezionedati@iss.it, for researchers who meet the criteria for access to confidential data.

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REFERENCES

1. Uyeki TM, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. Lancet. 2022 Aug 27;400(10353):693-706. doi: 10.1016/S0140-6736(22)00982-5.
2. Interim Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU, EMA/PRAC/222346/2014. https://www.ema.europa.eu/en/documents/scientific-guideline/interim-guidance-enhanced-safety-surveillance-seasonal-influenza-vaccines-eu_en.pdf [accessed 4 Jul 2023]
3. Spila-Alegiani S, Salmaso S, Rota MC, et al. Reactogenicity in the elderly of nine commercial influenza vaccines: results from the Italian SVEVA study. Study for the evaluation of adverse events of influenza vaccination. *Vaccine* 1999;17(15-16):1898-904.
4. Spila Alegiani S, Alfonsi V, Bella A, et al. Vaccino antinfluenzale stagionale in Italia: misurare l'efficacia sul campo e la sicurezza. Stagione 2015-2016. Roma: Istituto Superiore di Sanità; 2017. (Rapporti ISTISAN 17/19).
5. Spila Alegiani S, Alfonsi V, Appelgren EC, et al. Active surveillance for safety monitoring of seasonal influenza vaccines in Italy, 2015/2016 season. *BMC Public Health* 2018;18(1):1401 doi: 10.1186/s12889-018-6260-5.
6. Galeotti F, Massari M, D'Alessandro R, et al. Risk of Guillain-Barré syndrome after 2010-2011 influenza vaccination. *Eur J Epidemiol* 2013;28(5):433-44 doi: 10.1007/s10654-013-9797-8.
7. Massari M, Spila Alegiani S, Da Cas R, et al. TheShinISS: un applicativo open-source per la conduzione di analisi distribuite in studi di farmacoepidemiologia di tipo multi-database ("TheShinISS": an open-source tool for conducting distributed analyses within pharmacoepidemiological multi-database studies). *Boll Epidemiol Naz* 2020;1(2):39-45 doi: 10.53225/BEN_006.
8. Spila Alegiani S, Morciano C, Belleudi V, et al. Valutazione postmarketing della sicurezza del vaccino antinfluenzale durante la campagna di vaccinazione antinfluenzale 2020-2021 in Italia: uno studio Self-Controlled Case Series sulla sindrome di Guillain-Barré (Post-marketing safety evaluation of flu vaccine during the 2020-2021 flu vaccination campaign in Italy: a Self-Controlled Case Series study

of Guillain-Barré syndrome). *Boll Epidemiol Naz* 2022;3(2):1-9 doi: 10.53225/BEN_042.

9. Massari M, Spila Alegiani S, Morciano C, et al. Post-marketing active surveillance of myocarditis and pericarditis following vaccination with COVID-19 mRNA vaccines in persons aged 12-39 years in Italy: a multi-database, self-controlled case series study. *PLoS Med* 2022;19(7):e1004056.
10. Circolare del Ministero della Salute "Prevenzione e controllo dell'influenza: raccomandazioni per la stagione 2021-2022" https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2021&codLeg=79647&parte=1%20&serie=null [accessed 4 Jul 2023]
11. Circolare del Ministero della Salute "Prevenzione e controllo dell'influenza: raccomandazioni per la stagione 2022-2023" https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2022&codLeg=87997&parte=1%20&serie=null [accessed 4 Jul 2023]
12. Determina dell'AIFA che autorizza l'aggiornamento, per la stagione 2021-2021, della composizione dei vaccini influenzali autorizzati secondo procedura di mutuo riconoscimento e decentrata (Determina AAM/PPA n. 654/2021). https://www.aifa.gov.it/documents/20142/1563396/Determinazione_AIFA_654-2021_GU.pdf [accessed 4 Jul 2023].
13. Determina dell'AIFA che autorizza l'aggiornamento, per la stagione 2022-2023, della composizione dei vaccini influenzali autorizzati secondo procedura di mutuo riconoscimento e decentrata (Determina AAM/PPA n. 652/2022). https://www.aifa.gov.it/documents/20142/1754982/Det_AIFA_652-2022.pdf [Accessed 4 Jul 2023].
14. Whitaker HJ, Farrington CP, Spiessens B, et al. Tutorial in bio-statistics: the self-controlled case series method. *Stat Med* 2006;25(10):1768–97.
15. Petersen I, Douglas I, Whitaker H. Self controlled case series methods: an alternative to standard epidemiological study designs. *BMJ* 2016;354:i4515 doi: 10.1136/bmj.i4515.

16. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. *Epidemiol Infect* 2011;139(12):1805–17.

17. Farrington CP, Whitaker HJ, Hocine MN. Case series analysis for censored, perturbed, or curtailed post-event exposures. *Biostatistics* 2009;10(1):3–16.

18. Farrington CP, Whitaker H, Weldeselassie YG. Self-Controlled Case Series Studies. A Modelling Guide with R. New York, Chapman and Hall/CRC Press 2018.

19. Ghebremichael-Weldeselassie Y, Jabagi MJ, Botton J, et al. A modified self-controlled case series method for event-dependent exposures and high event-related mortality, with application to COVID-19 vaccine safety. *Stat Med* 2022;10;41(10):1735-50 doi:10.1002/sim.9325.

20. Gini R, Sturkenboom MCJ, Sultana J, et al. Different strategies to execute multi-database studies for medicines surveillance in real-world setting: a reflection on the European model. *Clin Pharmacol Ther* 2020;108(2):228-35 doi: 10.1002/cpt.1833.

21. Trifirò G, Massari M, Da Cas R, et al. Renin–Angiotensin–Aldosterone System Inhibitors and Risk of Death in Patients Hospitalised with COVID-19: A Retrospective Italian Cohort Study of 43,000 Patients. *Drug Safety* 2020;43:1297–308 doi: 10.1007/s40264-020-00994-5.

22. Spila Alegiani S, Crisafulli S, Giorgi Rossi P, et al. Risk of COVID-19 hospitalization and mortality in rheumatic patients treated with hydroxychloroquine or other conventional DMARDs in Italy. *Rheumatology* (Oxford). 2021;60(SI):SI25-SI36 doi: 10.1093/rheumatology/keab348.

23. Massari M, Spila Alegiani S, Fabiani M, et al. Association of influenza vaccination and prognosis in patients testing positive to SARS-COV-2 swab test: a large-scale Italian multi-database cohort study. *Vaccines* 2021;9(7):716 doi:10.3390/vaccines9070716

24. Trifirò G, Isgrò V, Ingrasciotta Y, et al. Large-scale post-marketing surveillance of biological drugs for immune-mediated inflammatory diseases through an Italian distributed multi-database healthcare network: the VALORE project. *BioDrugs* 2021;12:1–16 doi: 10.1007/s40259-021-00498-3.

25. Belleudi V, Rosa AC, Finocchietti M, et al. An Italian multicentre distributed data research network to study the use, effectiveness, and safety of immunosuppressive

drugs in transplant patients: Framework and perspectives of the CESIT project. Front Pharmacol 2022;13:959267 doi: 10.3389/fphar.2022.959267.

26. Brighton Collaboration Case Definitions. https://brightoncollaboration.us/category/pubs-tools/case-definitions/ [accessed 4 Jul 2023].

27. Gruppo di lavoro sull'analisi dei segnali da vaccino, Agenzia Italiana del Farmaco. Guida alla valutazione delle reazioni avverse osservabili dopo vaccinazione. https://www.aifa.gov.it/sites/default/files/Guida_valutazione_reazioni_avverse_osservabili_dopo_vaccinazione_2.pdf [accessed 4 Jul 2023].

28. D. R. Cox. Unbiased estimating equations derived from statistics that are functions of a parameter. *Biometrika* 1993;80(4):905–9 doi: 10.1093/biomet/80.4.905

29. Wilson K and Hawken S. Drug safety studies and measures of effect using the self-controlled case series design. *Pharmacoepidemiol Drug Saf* 2013;22:108-10 doi: 10.1002/pds.3337.

30. Weldeselassie YJ, Whitaker H and Farrington P (2021). SCCS: The Self-Controlled Case Series Method. R. package version 1.5. https://CRAN.R-project.org/package=SCCS.

31. European Medicine Agency (EMA), Guideline on good pharmacovigilance practices (GVP) – 28 July 2017, EMA/873138/2011 https://www.ema.europa.eu/documents/regulatory-procedural-guideline/guideline-good-pharmacovigilance-practices-gvp-module-vi-collection-management-submission-reports_en.pdf [accessed 4 Jul 2023].

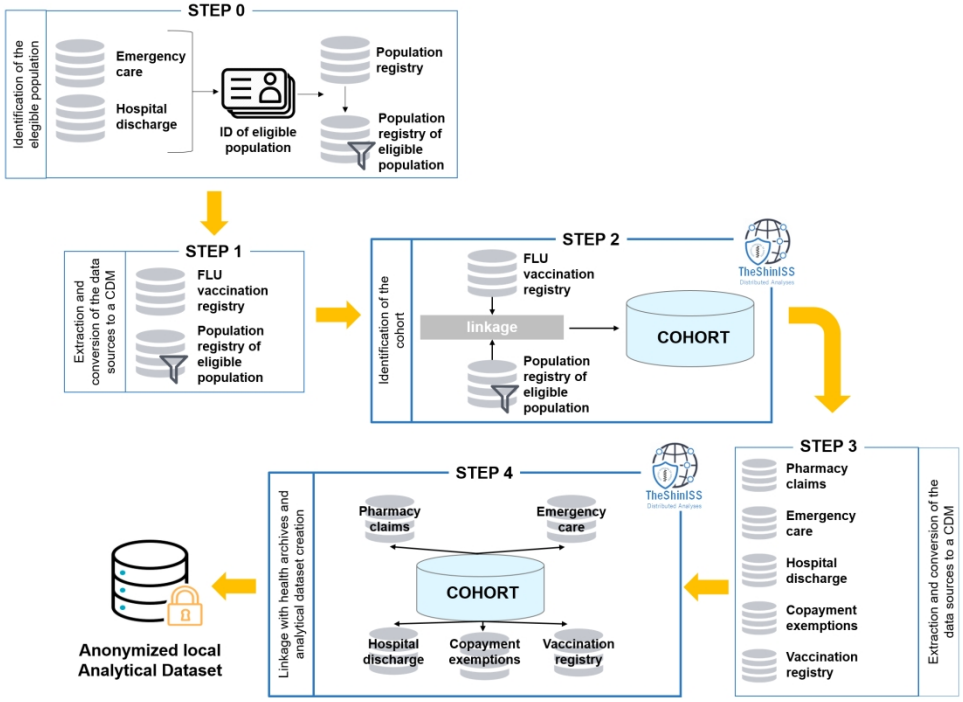

Figure 1. Diagram showing the data flow when using TheShinISS to locally process health care data structured according to a Common Data Model.
* Vaccination registry related to those registered in the regional population

380x284mm (150 x 150 DPI)

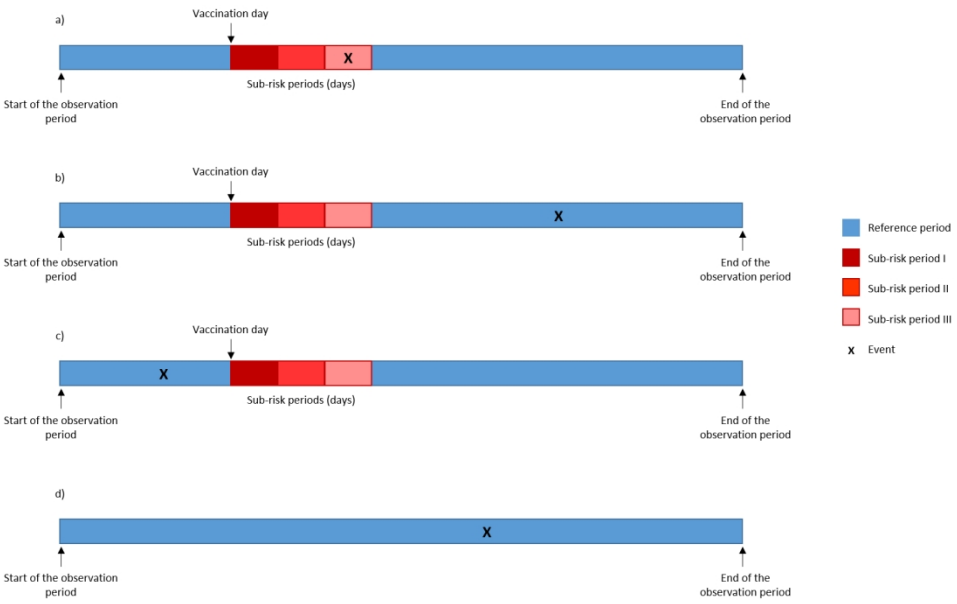

Figure 2. Schematic presentation of SCCS method for hypothetical subjects included in the study. a) events occurring in vaccinated subjects during the risk period; b) events occurring in the reference period after vaccination; c) events occurring in the reference period before vaccination; d) events occurring in unvaccinated subjects.

298x185mm (150 x 150 DPI)

Supplemental Table 1. Time schedule of TheShinISS-Vax|Flu

GANTT	**2022**							**2023**											
	Jun.	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Pre-study procedures																			
Protocol Drafting																			
Customizing TheShinISS																			
Study organization																			
Ethics Committee																			
Region participation																			
2021-2022 campaign																			
Data collection																			
Interim analysis and report																			
Analysis and final report																			
2022-2023 campaign																			
Data collection																			
Analysis and final report																			