TITLE:

Risk of thromboembolic events and thrombocytopenia after vaccination against COVID-19

V2.0

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1. List of abbreviations

ACCESS vACinne COVID-19 monitoring readinESS

AESI Adverse Events of Special Interest

BIFAP Base de datos para la Investigación Farmacoepidemiológica en el Ámbito Público

BMI Body Mass Index

CMBD Conjunto Mínimo Básico de Datos

ECVM Early COVID-19-Vaccine Monitoring

EEA European Economic Area

EHR Electronic Health Records

EMA European Medicines Agency

EU European Union

HIV Human Immunodeficiency Virus

ICPC-2 International Classification of Primary Care

ICD-9 International Classification of Diseases 9th Edition

ICD-10 International Classification of Diseases 10th Edition

mRNA Messenger Ribonucleic Acid

NHS National Health System

PRAC Pharmacovigilance Risk Assessment Committee of the European Medicines Agency

SCCS Self-controlled Case Series

SCRI Self-Controlled Risk Interval

TIH Heparin Induced Thrombosis

TP Thrombocytopenia

TTS Thrombosis with Thrombocytopenia Syndrome

VITT Vaccine Induced Immune Thrombotic Thrombocytopenia

2. Summary

Title: Risk of thromboembolic events and thrombocytopenia after vaccination against COVID-19

Rationale and background: The new SARS-CoV-2 coronavirus, the cause of COVID-19, has led to a worldwide pandemic. The sequencing of its genome on 11 January 2020 triggered the development of vaccines against COVID-19. Following the emergency approval of some of these vaccines in December 2020, mass vaccination campaigns began worldwide. In March 2021, cases of thrombotic events associated with thrombocytopenia began to appear after the administration of AstraZeneca's vaccine. Subsequently, cases of thrombosis with thrombocytopenia were also observed after immunization with the Janssen vaccine. Some cases of venous thromboembolism have also been reported with Pfizer and Moderna vaccines. Although the risk following administration of AstraZeneca and Janssen vaccines for these thrombotic or embolic disorders with and without thrombocytopenia, as well as for thrombocytopenia without associated thromboembolism, has been established, it has not yet been fully characterised and quantified.

Objectives: To quantify the association between the occurrence of thromboembolic events and thrombocytopenia and the administration of COVID-19 vaccines. To quantify the association between the occurrence of thromboembolism together with thrombocytopenia and the administration of COVID-19 vaccines.

Data source: BIFAP (Base de datos para la Investigación Farmacoepidemiológica en el Ámbito Público).

Study design: A *self-controlled case series* (SCCS) design with pre- and post-vaccine control intervals as the main analysis. Also, two exploratory analyses will be conducted: firstly, using an SCCS design with a post-vaccine control interval only, and secondly, using a *self-controlled* risk *interval* (SCRI) design. The study period will be from September 1st, 2020, until death, patient exit from the database, or end of study (last data update in BIFAP at the time of the study).

Population: All individuals aged ≥5 years, registered with their primary care physician for at least 365 days, who have received one of the following COVID-19 vaccines: AstraZeneca, Pfizer, Moderna, or Janssen and for whom any of the defined outcomes of interest have been identified.

Events of interest: The first event of interest in the study period will be identified for each of the groups considered, i.e., venous thromboembolism, arterial thromboembolism, and thrombocytopenia, as well as concomitance of thromboembolism with thrombocytopenia.

Data analysis: Description of the study population characteristics, i.e., patients who have been vaccinated and have the event of interest will be described. For all study designs,

we will compare the event rates in the post-vaccination risk period with the control periods using conditional Poisson regression, by type of vaccine.

3. Rationale and background

3.1. Background

The new SARS-CoV-2 coronavirus, the cause of COVID-19, has led to a global pandemic. The sequencing of its genome on January 11th, 2020, triggered the development of vaccines against COVID-19. Following the emergency approval of some of these vaccines in December 2020, mass vaccination campaigns began worldwide. Millions of people have already received one of the four vaccines available so far in the EU. As of October 18th, 2021, 307 609 375 people had received at least 1 dose of these vaccines in the EU and EEA countries[1] .

3.2. Rationale

In early March 2021, thrombotic events associated with thrombocytopenia started to be reported after the administration of AstraZeneca's vaccine (Vaxzevria). Subsequently, cases of thrombosis with thrombocytopenia were also observed after immunization with Janssen's vaccine (COVID-19 Vaccine Janssen).

Following these reports, the European Medicines Agency (EMA) initiated successive evaluations after which it concluded that there was a causal relationship between Vaxzevria and the occurrence of thrombosis in combination with thrombocytopenia. Product information was updated with new information on Thrombosis with Thrombocytopenia Syndrome (TTS) and clotting disorders that had been observed very rarely following vaccination with Vaxzevria. This included severe cases of venous thrombosis, including unusual locations, such as cerebral venous sinus thrombosis, splanchnic vein thrombosis, as well as arterial thrombosis, occurring concomitantly with thrombocytopenia. Most of these cases occurred within the first three weeks after vaccination, mostly amongst women aged under 60. Some of them had a fatal outcome.

Also, a series of studies were conducted to identify the exact pathophysiological mechanism of these thrombotic events and to better evaluate the nature and magnitudes of the risk[2] .

A plausible explanation for the concomitance of blood clots and low blood platelets is an immune response leading to TTS, now also known as vaccine-induced immune thrombotic thrombocytopenia (VITT). It appears as a rare condition similar to heparin-induced thrombocytopenia (HIT), seen in patients treated with heparin (1).

At this time, risk factors for TTS not related to the vaccine have not been confirmed. The benefits of the vaccine continue to outweigh the risks and it is effective in preventing COVID-19 and reducing hospitalisations and deaths. In Spain, 31 suspected or confirmed cases of TTS had been reported up to August 8th, 2021, 7 of which were fatal. Most of the thrombus presented in unusual locations (venous sinuses or splanchnic veins). Thirty

[1] https://vaccinetracker.ecdc.europa.eu/public/extensions/COVID-19/vaccine-tracker.html#uptake-tab
[2] COVID-19 Safety update Vaxzevria vaccine - 14 April 2021 (europa.eu)

out of the 31 cases occurred after the first dose. About 9.6 million doses had been administered by that date[3] .

Regarding the second dose, there is limited exposure data to date. So far, the UK has administered the most second doses. Its data from reporting of spontaneous adverse events indicates that there is a lower incidence rate amongst younger adult groups after the second dose in comparison with older age groups (1 per million doses in people aged 18-49 years, after 8.2 million second doses administered, compared to 1.9 per million doses in people aged 50 years and over, after 15.9 million second doses administered). However, this data should be analysed with caution as second doses of this vaccine have been administered mainly to older people, which is a population group at a lower risk of developing this syndrome. Therefore, the risk after the second dose of Vaxzevria in people under 60 years of age is currently not fully known, as the available data is limited and inconclusive[4] .

TTS has also been included in the Janssen vaccine's product information as a very rare possible adverse reaction. The signal assessed by the Pharmacovigilance Risk Assessment Committee of the European Medicines Agency (PRAC) arose from the cases of this disorder identified so far in the US (8 in total, one of them fatal) in unusual locations (i.e., cerebral venous sinuses or the splanchnic veins) together with thrombocytopenia. Subsequently, there were some cases after vaccination with Janssen vaccine in the EEA; in Spain, as of August 8th, 2021, 5 confirmed or probable cases of TTS had been reported in persons vaccinated with COVID-19 Vaccine Janssen after 1.8 million doses were administered. Two of the patients died. The potential mechanism of action would be the same as described before for the Vaxzevria vaccine; it would be an immune-type reaction similar to that known for heparin-induced thrombocytopenia (HIT)[5] .

In addition, cases of thrombocytopenia, including immune thrombocytopenia, have been reported, usually within the first four weeks after administration of Vaxzevria and the Janssen vaccine, including cases associated with haemorrhage or fatal outcomes. Some of these have occurred in patients with a previous history of thrombocytopenia.

[3]https://www.aemps.gob.es/informa/boletines-aemps/boletin-fv/2021-boletin-fv/8o-informe-de-farmacovigilancia-sobre-vacunas-covid-19/

[4]https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting

[5]https://www.aemps.gob.es/informa/notasinformativas/medicamentosusohumano-3/seguridad-1/2021-seguridad-1/vacuna-frente-a-la-covid-19-de-janssen-conclusiones-de-la-evaluacion-del-riesgo-de-trombosis-junto-con-trombocitopenia/

[6] https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-covid-19-vaccine-janssen-6-october-2021_en.pdf;
https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-vaxzevria-previously-covid-19-vaccine-astrazeneca-6-october-2021_en.pdf

The product information for both vaccines will be updated to include this information, following evaluation and a subsequent decision by the PRAC (EMA)[6] .

On the other hand, the EMA is evaluating the evidence available so far on the potential association between the immunization with Vaxzevria and the reported cases of cerebral venous sinus thrombosis and venous thromboembolism with the Janssen vaccine, but in both cases, without the concomitant occurrence of thrombocytopenia. In the latter case, the safety issue is being closely monitored according to the Risk Management Plan, given the results of a clinical trial conducted by the company[7] .

The exact incidence of these thrombotic or embolic disorders with and without thrombocytopenia, as well as thrombocytopenia without associated thromboembolism, following administration of Vaxzevria or Janssen vaccines, is unknown but appears to be rare. Despite the low incidence, mass vaccination of millions of people has resulted in several hundred patients developing this condition and the present study is necessary to estimate the magnitude and better define the nature of this risk. In addition, COVID-19 disease itself has been associated with an increased risk of thrombotic complications. Incidence rates of stroke of 3% and venous thromboembolism of 20% have been reported in patients hospitalised with COVID-19[8] .

Cases of embolic and thrombotic events following administration of the two mRNA vaccines (Comirnaty and Spikevax) have also been reported to the Pharmacovigilance systems of EEA countries, with very few of them associated with thrombocytopenia. The EMA has closely monitored and evaluated these cases but does not consider that there could be a signal. This number is lower than what might be expected in the general population, and they do not appear to follow the same clinical pattern as the cases observed after the administration of Vaxzervria and Janssen vaccines[9] .

[7]https://www.aemps.gob.es/informa/boletines-aemps/boletin-fv/2021-boletin-fv/8o-informe-de-farmacovigilancia-sobre-vacunas-covid-19/
[8]DOI:10.1183/13993003.01111-2021; https://doi.org/10.1016/S1131-3587(21)00001-7
[9]https://www.ema.europa.eu/en/news/meeting-highlights-pharmacovigilance-risk-assessment-committee-prac-3-6-may-2021

4. Objectives

1) To quantify the association between the occurrence of thromboembolic events and thrombocytopenia and the administration of COVID-19 vaccines within pre-specified risk periods after vaccination, stratified by vaccine, age, sex and risk factor groups.

2) To quantify the association between the occurrence of thromboembolism together with thrombocytopenia and administration of COVID-19 vaccines within the pre-specified risk period after vaccination, stratified by vaccine, age, sex and risk factor groups.

5. Methods

5.1. Data source

For this study, we used BIFAP (Base de Datos para la Investigación Farmacoepidemiológica en el Ámbito Público)[10], a longitudinal population-based database of electronic medical records (EHR) from primary care physicians (PCP), who belongs to the Spanish National Health System (NHS), in 9 different Autonomous Communities (regions). The main use of BIFAP is to serve as a source of real-life data, mainly for the study of the utilization, safety, and effectiveness of drugs in the population.

The PCPs' electronic medical records contain fully anonymized demographic data (date of birth and sex), episodes of care (i.e., diagnoses, classified according to the International Classification of Primary Care (ICPC)-2 and ICD-9 coding systems), specialist referrals, lifestyle factors, drug prescriptions and dispensations incl. vaccinations, other additional health data (test results, medical procedures, etc.) and clinical notes registered as free text. BIFAP is embarked on the process of mapping all diagnoses recorded in the database to a highly granular and interoperable clinical terminology: SNOMED-CT; at the time of the study, the mapping of medical terms to this clinical terminology will still be ongoing. Additionally, information on hospital discharge diagnoses coded in ICD-10 terminology is linked to patients included in BIFAP for a subset of periods and regions participating in the database.

BIFAP has been updated yearly until 2020; however, from this year onwards, to be able to use it as a source of information for the COVID-19 research, data have been updated more frequently (bimonthly) in agreement with the participating Autonomous Communities. Specifically, as of today, BIFAP already includes information updated to April 30th, 2022, from the primary care clinical records of patients from 3 Autonomous Communities and up to December 31st, 2021 from 2 Autonomous Communities (approximately 15 million patients).

[10] http://bifap.aemps.es

BIFAP has participated in two EMA-funded studies on the safety of COVID-19 vaccines; the first one, the ACCESS project[11] , through the protocol "Background rates of Adverse Events of Special Interest for monitoring COVID-19 vaccines", in which baseline incidence rates of events of special interest (AESI) were estimated, including thromboembolic events and thrombocytopenia, and the second one, the study "Early COVID-19-Vaccine Monitoring (ECVM)"[11,] which monitors the incidences of AESI already evaluated in ACCESS in post- and pre-vaccination periods. This has confirmed that the information recorded on these events and COVID-19 vaccines is complete and valid for studies with this database.

5.2. Study design

A self-controlled case series (SCCS) design with pre- and post-vaccination control periods will be conducted as the main analysis. In addition, two exploratory (secondary) analyses will be performed consisting of two variants of the SCCS design: first, an SCCS study with a post-vaccination control period only, and second, a Self-controlled risk interval (SCRI) analysis.

Self-controlled case series designs require patients to present with the event of interest during the observation period, i.e., they are "case-only" designs (2). They are suitable for assessing acute exposures and events, with short latency or induction periods. Also, they implicitly control for all possible fixed (non-time-varying) confounders, such as sex or race (3), as each individual serves as his or her control but requires controlling for variables that may vary over time. To mitigate the effect of confounding by time-varying variables, a sufficiently short pre-exposure control period should be selected.

They are also subject to the “healthy vaccinee bias” when a pre-vaccination control period is included, because the occurrence of an event during the observation period may affect the probability of subsequent vaccination. To avoid this bias, a washout period will be established between the pre-vaccination control period and vaccination.

Only vaccinated cases will be included, thus minimizing potential misclassification bias due to incomplete data on vaccine exposure (3).

5.2.1 Self-controlled case series (SCCS) with pre- and post-vaccination control period (Main analysis)

The main analysis will be conducted using an SCCS design with pre- and post-vaccine control periods (Figure 1). Each individual contributes (in person-time) to both control and pre-specified risk periods, and the event rate during the risk period immediately after exposure is compared to the event rate during all other times (control intervals) (4,5).

The risk window is defined as an interval post-vaccination during which occurrence of the event may be associated with the vaccine exposure, based on biological plausibility

[11] https://vac4eu.org/covid-19-vaccine-monitoring/

and existing clinical information (6). In the control interval, the occurrence of the event is deemed unrelated to the exposure within each patient.

The observation period will extend from the first day of the pre-vaccination control period until 90 days post-vaccination. The pre-vaccination control period is defined as the follow-up time from the start of the study (September 1st, 2020) to the start of the washout period, which will be set as 28 days before vaccination. The risk window is the period from day 1 post-vaccination to day 28. The post-vaccination control period is defined as all follow-up time during the observation period following either the first or second COVID-19 vaccine dose (for two-dose COVID-19 vaccines) that is outside of the risk interval until day 90 post-vaccination, the end of the study period (last data draw down/data availability), patient exit from the database, or death, whichever occurs first.

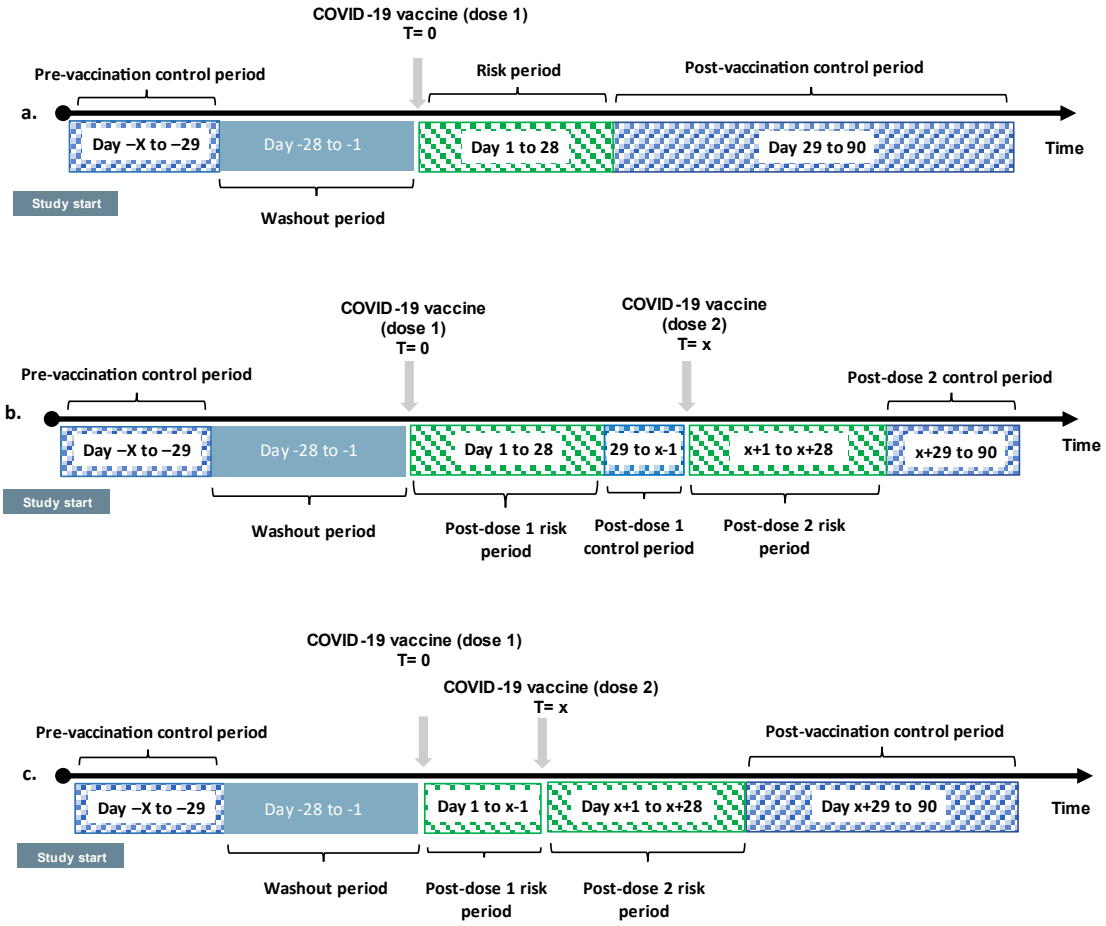


Figure 1. Study design Self-controlled case series (SCCS) with pre- and post-vaccination control periods. Description of three hypothetical scenarios: a. Dose 1 only; b. Dose 2 administered after the end of the risk period of dose 1; c. Dose 2 administered before the end of the risk period of dose 1.

5.2.2 SCCS with post-vaccination control period (Exploratory analysis)

The control interval will be limited to the post-vaccination interval because of the possible occurrence of previous events that may affect the likelihood of subsequent vaccination and the inclusion of the washout period would not be able to control for the potential "healthy vaccinee bias" (Figure 2).

However, this design has lower statistical power compared to the previous one, as it includes fewer cases of the events of interest due to the shorter observation period.

The observation period will start from the date of vaccination (day 0) of the first or single dose and extend to day 90 post-vaccination. The risk interval is the time from day 1 post-vaccination to day 28. The post-vaccination control interval is defined as all follow-up time during the observation period starting the day after the risk window and ending on day 90 post-vaccination, the last day of the study period, the patient exits the database, or they die, whichever occurs first.

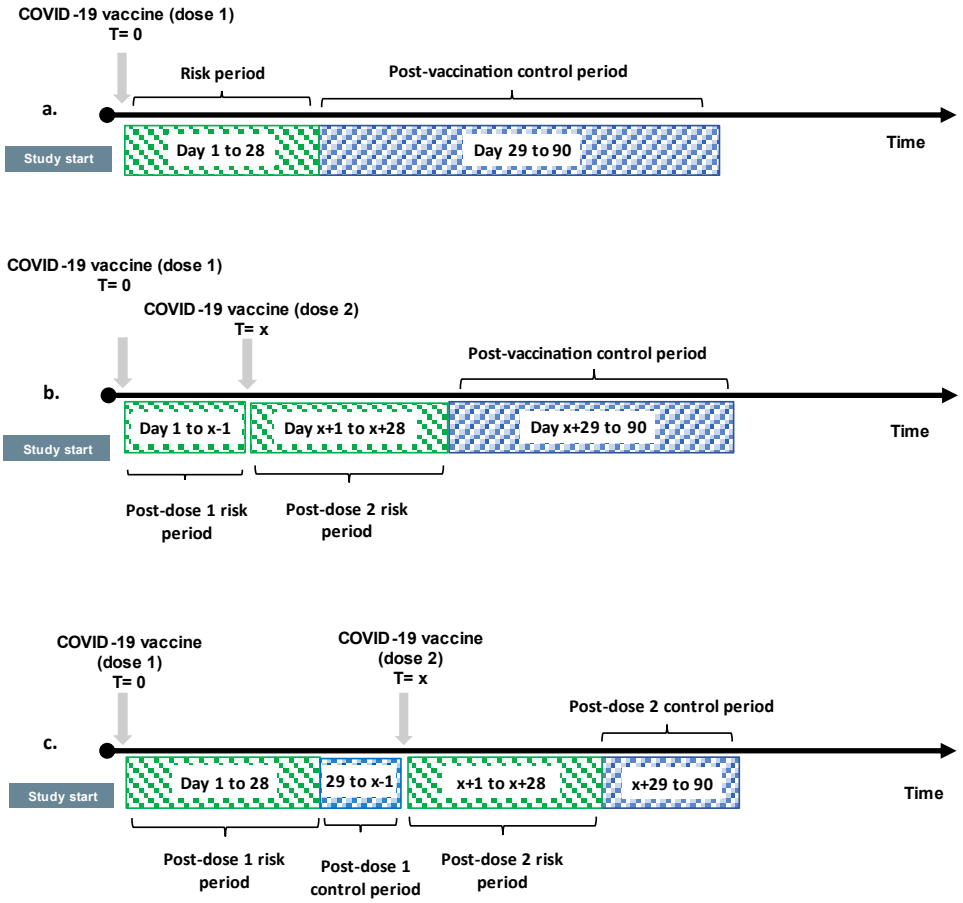


Figure 2. Study design Self-controlled case series (SCCS) with control interval after vaccination only. Description of three hypothetical scenarios: a. Dose 1 only; b. Dose 2 administered after the end of the risk period of dose 1; c. Dose 2 administered before the end of the risk period of dose 1.

5.2.3 Self-Controlled Risk Interval analysis (SCRI) (Exploratory analysis)

A 'Self-controlled risk interval' design will also be used as an exploratory analysis.

The observation period will start from day 90 before vaccination and extend to the end of the available risk interval, the date of death, the patient exits from the database, or the end of the study period, whichever occurs first.

We will establish the time from day 1 to day 28 as the risk window with day 0 being the day of vaccination and, before vaccination, a control interval of 62 days followed by a washout period of 28 days (Figure 3).

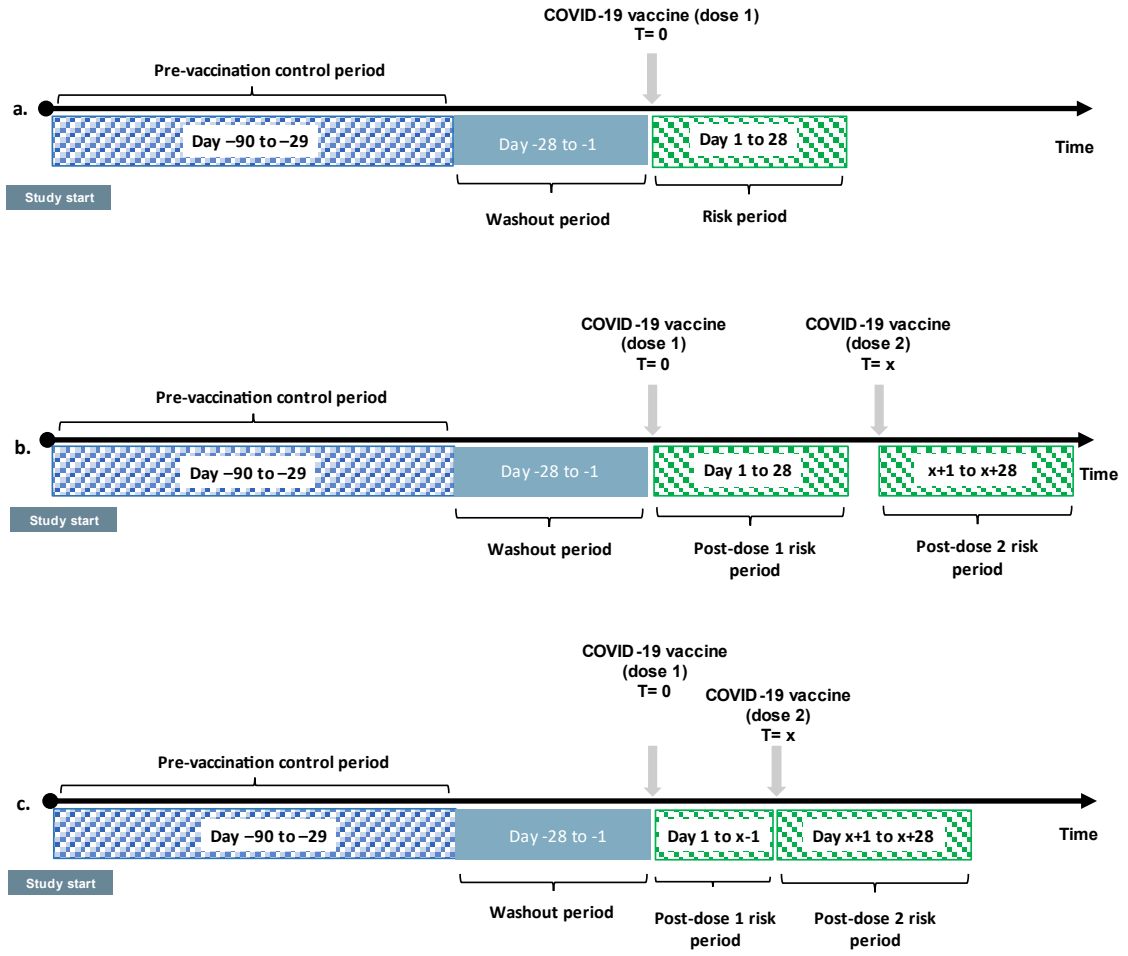


Figure 3. Study design Self-controlled risk interval (SCRI), with control period before vaccination. Description of three hypothetical scenarios: a. Dose 1 only; b. Dose 2 administered after the end of the risk period of dose 1; c. Dose 2 administered before the end of the risk period of dose 1.

5.3. Study period

From September 1st, 2020 until death, the exit of the patient from the database or end of study date (last data draw down/data availability at the time of the study).

5.4. Study population

5.4.1. Inclusion criteria

All individuals ≥5 years registered with their primary care physician for at least 365 days before cohort entry, who had received one of the following vaccines: ChAdOx1 from Oxford University and AstraZeneca (Vaxzevria), BNT162b2 from BioNTech and Pfizer (Comirnaty), mRNA-1273 from Moderna (Spikevax) or Ad26.CoV2.S from Janssen (COVID-19 Janssen Vaccine) and any of the events of interest during the study period will be included.

5.4.2. Exclusion criteria

For the analysis of each event, all patients who had any of the events of interest in the two years before the start date of the study period (September 1st, 2020) will be excluded.

5.5. Definition of risk and control intervals

The control and risk periods for the different designs in this study have already been defined individually for each of the designs to be applied in this study (main and exploratory analyses) in this document in section 5.2 *Study design*.

However, irrespective of the design applied, the following intervals (i.e., weeks) will be defined within the corresponding risk periods after vaccination: 0, 1-7, 8-14, 15-21, 22-28 days, for each of the doses identified. These intervals will be used to differentiate between acute and non-acute phases after vaccination.

If the administration of the second dose occurs within the risk period of the first dose, the risk window of the second dose prevails over the risk window of the first dose.

Initially, in this protocol, for the main analysis (SCCS design with control intervals before and after vaccination) and the exploratory analysis (SCRI design), we have included a time interval of 28 days before the date of vaccination, i.e., washout period. The rationale behind this is to account for the potential bias that would result if vaccination was temporarily affected by the occurrence of an event, e.g. in the case that patients with a thromboembolic event were not vaccinated or their vaccination was delayed, or in other words, vaccinated patients were those who had fewer medical problems in the previous days and became, therefore, "artificially" healthier patients.

However, histograms will be created to plot the frequency of the events of interest per week before and after vaccination (first dose, in case of a two-dose schedule, or single dose), to observe their distribution and thus check whether the events could be affecting the vaccination in any direction and then confirm the need for this washout period and its extension. We will also calculate the risk of events in this pre-vaccination period to assess whether the event conditions subsequent vaccination.

5.6. Exposure

Exposure will be defined as the presence of a record in the database of at least one dose of any of the COVID-19 vaccines (ATC code J07BX03) described before. The following data will be extracted for each vaccine record: brand, dose, and date of administration.

Quality control of the vaccine registry will be performed, and a set of criteria will be applied to exclude those doses/records that are not considered valid or of sufficient quality to be evaluated. Thus, only dose 1 will be considered when there are two doses recorded on the same day for the same patient, or when doses 1 and 2 are separated by less than 14 days; cases in which there are two different vaccines on the same day, or when consecutive doses do not appear in chronological order, or the same dose is repeated several times with more than 14 days between them will be excluded.

5.7. Events of interest

Events of interest (7,8) will be identified through their corresponding ICD-9, ICD-10 or SNOMED-CT codes as recorded in BIFAP database. Most of the events of interest to be assessed in this study had already been created in BIFAP (i.e., selection of codes based on clinical concepts under the event definition) and their incidence rates compared with those in other European databases in previous COVID-19 vaccine studies (ACCESS and ECVM), and therefore no validation will be performed.

The main events will be: thrombocytopenia, venous thromboembolism, and arterial thromboembolism (see section 5.7.1).

To study the concurrence of thromboembolism with thrombocytopenia, it will be identified whether arterial thromboembolism or venous thromboembolism is associated with thrombocytopenia (TP) when a TP record appears within 10 days before or after the corresponding diagnosis.

Venous thromboembolism, arterial thromboembolism, and thrombocytopenia will be studied separately, and within each of these, the first of the events recorded will be considered. The composition of each of the groups of events considered is described below:

5.7.1. Main events

1. **Venous thromboembolism**

1.1 Cerebral venous thrombosis

1.2 Deep Vein Thrombosis/Pulmonary Embolism

1.3 Other venous thromboses: Acute limb ischaemia, Splenic vein thrombosis, Splenic infarction, Hepatic vein thrombosis, Portal vein thrombosis, Intestinal infarction, Mesenteric vein thrombosis, or Visceral vein thrombosis.

2. **Arterial thromboembolism**

2.1 Ischaemic stroke

2.2 Acute myocardial infarction

2.3 Other arterial thromboses: Splenic artery thrombosis, hepatic artery thrombosis or celiac artery thrombosis.

3. **Thrombocytopenia (TP)**

Concurrence of arterial or venous thromboembolism with thrombocytopenia

Cerebral venous thrombosis + TP

Deep vein thrombosis/Pulmonary embolism + TP

Ischaemic stroke + TP

Acute myocardial infarction + TP

Other coagulation disorders will be studied as secondary events, such as:

Disseminated intravascular coagulation (DIC)

5.7.2. Control events

Gout and colonic diverticulitis will be included as negative controls (9), as they are not in principle associated with exposure to COVID-19 vaccines.

Anaphylaxis will be included as a positive control, as it could occur soon after vaccination with any vaccine (10).

5.8. Other variables of interest

They will be measured at baseline, i.e., when patients meet the age criteria (≥5 years), and have been registered with the physician for more than 365 days, since September 1st, 2020.

5.8.1. Demographic characteristics

Year of birth

Sex (male/female)

Age

Lifestyle factors: BMI, alcohol, and tobacco use (within two years before the study start date)

5.8.2. Co-morbidities

Risk factors for thrombocytopenia and thromboembolic events (7,11):

Cancer

Chronic kidney disease

Coronary artery disease (other than acute myocardial infarction)

Chronic obstructive respiratory disease

Obesity

Diabetes

Multiple sclerosis

Heart failure

Haemorrhagic stroke

HIV

Inherited blood clotting disorders (antithrombin III deficiency, protein C and S deficiencies, factor V Leiden (activated protein C resistance), or prothrombin gene mutations)

Bone marrow disease (aplastic anaemia, leukaemia, myelodysplastic syndromes)

Rheumatoid arthritis

Inflammatory Bowel Disease (IBD)

Acquired Antiphospholipid Syndrome

Acute liver failure

Chronic comorbidities will be measured any time before the start of the study while the acute ones 1 month before that date.

5.8.3 History of Covid-19

We will collect any COVID-19 infection recorded at any time before the start of the study (and during the study period) together with the recording date.

5.8.4. Medication (7,11)

At least one prescription of the following:

- In the previous month of the study start:

Antibiotics (J01)

Antiviral medicinal products (J05)

Non-steroidal anti-inflammatory drugs (M01)

Anticoagulants (B01A)

Acetylsalicylic acid (B01AC06)

- In the previous year, the use of the following will be identified:

Lipid-lowering drugs (C10)

Sex hormones (G03)

Tamoxifen (L02BA01)

Bevacizumab (L01XC07)

Glucocorticoids (H02AB)

Antidepressants (N06A)

Psychotropics (N05)

Immunosuppressants (L04A, H02)

Medicinal products for obesity (A08A)

Cardiovascular medicinal products (C01B, C01C, C01D, C01E, C01D, C01E)

Antihypertensive medicinal products (C02, C03, C07 , C08 , C09)

Medicinal products for sickle cell disease (L01XX05, B06AX)

Bronchodilators (R03, R07AA, R07AB)

Medicinal products for diabetes (A10B, A10A)

Anti-cancer medicinal products (L01, L02 , L03 , L04)

Antiepileptics (N03A)

Influenza vaccine (J07BB)

6. Analysis

6.1. Descriptive analysis

The characteristics of the study population will be described. Counts and percentages will be presented for categorical variables. Mean and standard error and median and interquartile range will be calculated for continuous variables.

- Demographics: age, sex, autonomous community
- Lifestyle variables
- Cases who had received an influenza vaccine (during the study period)
- Cases with prior COVID-19 infection
- Comorbidity
- Comedication

6.2. Measures of Association

For each vaccine in each of the designs, event rates in the post-vaccination risk intervals will be compared with those in the control intervals using conditional Poisson regression analysis. The risk period will be divided into weeks to adjust for seasonality, which is important given the potential time-varying influence of the pandemic on health-seeking behaviors and access to the health system (12). In fact, during the time of the study, the third, fourth, and fifth waves of COVID-19 occurred in Spain.

Time-fixed variables do not need to be included in the model as they are adjusted for by the design itself. However, an analysis including age and COVID-19 diagnosis in the model as time-varying variables will be carried out. A model without potential time-varying variables will be built first and, in subsequent models, these variables will be included. In addition, potential effect modification will be explored by first building models without interaction terms and then including all potential interactions with terms for age (groups 12-19, 20-29, 30-39, 40-49, 50-59, 60-65, 66-69, 70-79, >80 years[12]) and COVID-19, first.

Incidence Rate Ratios (incidence in the corresponding risk interval divided by incidence in the control interval(s)) and 95% confidence intervals will be calculated by conditional Poisson Regression.

Given that the strategy for the vaccination campaign in Spain has progressed in age-descending cohorts, where sample size allows, we will conduct analyses by age groups. Stratification by sex and comorbidities that increase the risk of severe COVID-19 disease (in the main analysis) will also be performed.

Analyses will be carried out by type of vaccine.

6.3. Sensitivity analysis (main analysis)

- A first sensitivity analysis will consist of censoring the follow-up on 10 March 2021, when the first regulatory communication on the immobilisation of Astrazeneca's vaccine due to thrombosis and pulmonary thromboembolism[13] was published, to study whether there was a possible over-reporting bias in case that increasing cases of thromboembolism were reported after the announcement of occurrence of these events.

- To avoid any potential under-reporting of any of the events in Primary Care, a sensitivity analysis will be carried out taking into account the information from the Conjunto Básico Mínimo de Datos- CMBD (i.e., hospital discharge diagnoses databank) linked to BIFAP's primary care information in the autonomous communities and time where this information is available. To this end, it is defined that, for the periods where information of the two databanks overlaps, any record of the event of interest from either BIFAP primary care or hospital discharge diagnoses will be identified and considered for the analysis.

- Further analysis will be performed by excluding those patients with a COVID-19 positive test at any time during the study period. In this way, the aim is to isolate the known effect of COVID-19 infection on the risk of thromboembolic events from that of the vaccine.

[12] COVID-19_Actualizacion8_EstrategiaVacunacion.pdf (mscbs.gob.es)

[13] https://www.aemps.gob.es/informa/notasinformativas/medicamentosusohumano-3/seguridad-1/2021-seguridad-1/inmovilizacion-en-austria-de-un-lote-de-la-vacuna-frente-a-la-covid-19-de-astrazeneca-la-evaluacion-preliminar-no-indica-relacion-con-los-acontecimientos-notificados/

- IRRs will be stratified by vaccine dose, if the sample size allows, for assessment of the assumption of constant risk across each of the risk intervals for two-dose regimen vaccines. The overall risk interval will be classified as first, dose one only; second, overlap of dose one and dose two, where the risk intervals of each dose overlap; and third, dose two only. The risk of the event occurring in these three risk interval categories will be compared separately with the control intervals (Figure 4).

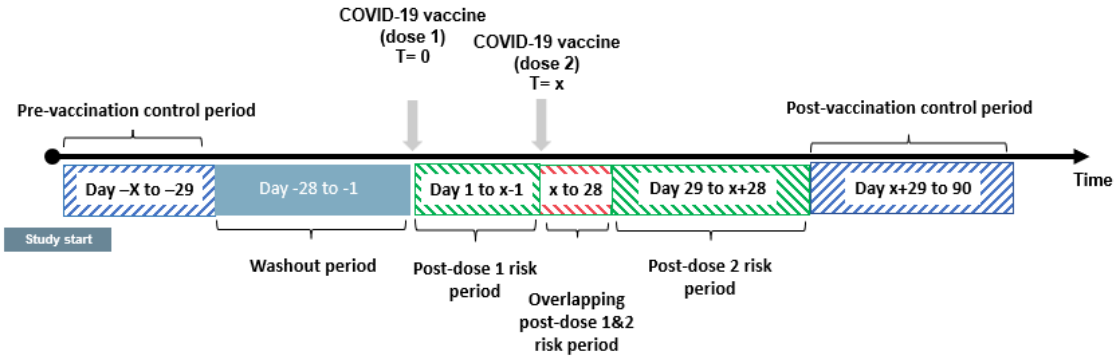


Figure 4. SCCS study design with control interval before and after vaccination.

6.4. SCCS assumptions assessment

To assess bias due to factors that may affect the likelihood of subsequent vaccination, the following analyses will be conducted to assess the validity and robustness of the risk estimates:

- An analysis will be conducted by excluding patients who die up to 30 days after the events of interest.
- Histograms of event occurrences per week before and after vaccination will be created to describe the distribution of events and to assess if the events could be affecting vaccination and therefore confirm the need for the washout period or its extension. We will also calculate the risk of events in the period before vaccination, to evaluate whether the event conditions the subsequent vaccination.

7. Limitations

The different study designs proposed are subject to limitations both by study design and by the use of secondary data from a medical record database.

Limitations related to the data include possible misclassification of events, both diagnosis, and date of onset. However, most of these events have been externally validated (i.e., by comparing IRs) with other databases within the ACCESS and ECVM

projects. There may also be some under-recording of the events of interest in the Primary Care records as most of them, due to their severity, require care from a specialist. For this purpose, a sensitivity analysis will be performed taking into account, in addition to the Primary Care information existing in BIFAP, the information from the CMBD (i.e., hospital discharge diagnoses) linked to BIFAP in the autonomous communities and the time where this information is available.

Identification of exposure is based on vaccination records, information that is available within the database to identify COVID-19 vaccines, and dates of vaccination. These vaccination records are not free from errors that may occur at the time of registration; however, these data are being validated in different studies so that the data used in this study are of sufficient quality to adequately assess exposure (ECVM, Covid-19 Vaccine Effectiveness Study).

The SCRI design allows for rapid assessment of suspected adverse events without the need to accumulate monitoring time in the post-risk period but is limited to acute events with short latency. In addition, because only pre-vaccination monitoring time is used, the approach is not appropriate for events that are a contraindication to vaccination. The impact of this can be limited by the inclusion of a pre-vaccination washout period if this period is known or can be accurately estimated.

The main strengths of self-controlled designs are the adjustment for time-invariant covariates and their suitability for assessing exposures and acute events.

The SCCS design has proven to be a valid alternative to traditional cohort or case-control study designs (13,14,15) for the study of adverse effects of vaccines.

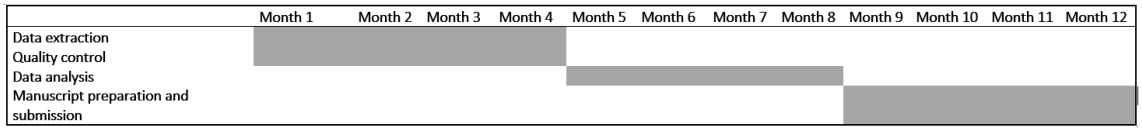
8. Protection of study subjects

Approval will be sought from a Medicines Research Ethics Committee in addition to the BIFAP Scientific Committee.

9. Plan for dissemination and communication of results

The results will be published in an indexed scientific journal.

10. Timeline



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