1. ABSTRACT

Title	An Observational Post-Authorisation Safety Study to Assess the Safety of Ad26.COV2.S Using European Healthcare Data through VAC4EU
Keywords	SARS-CoV-2; COVID-19; Ad26.COV2.S vaccine; safety; adverse events
Rationale and background	On 27 February 2021, the US Food and Drug Administration (FDA) granted an emergency use authorisation (EUA) for Ad26.COV2.S vaccine for use in individuals 18 years of age and older and on 11 March 2021 the European Commission granted conditional marketing authorisation (cMA) for Ad26.COV2.S vaccine for use in individuals 18 years of age and older, with the requirement to conduct further safety evaluation of the Ad26.COV2.S vaccine, including observational studies using real world data on the use of the vaccine. To fulfil these regulatory obligations, the sponsor initiated a post-authorisation safety study (PASS) to characterise and evaluate the safety profile of Ad26.COV2.S in a large population sample size to inform the scientific community on adverse events of special interest (AESIs) that could be associated with Ad26.COV2.S vaccine use.
Research question and objectives	This study had two consecutive aims: 1) to conduct a feasibility assessment to inform the feasibility of a safety evaluation study and 2) to assess the risk of developing specified and newly-identified AESIs following the administration of Ad26.COV2.S. The primary objective of the safety evaluation study was to assess the potential association between vaccination with Ad26.COV2.S. and the occurrence of prespecified and newly identified AESIs within disease-specific risk windows in individuals aged from birth to 80 years and older. The secondary objective was to assess the primary objective in specific sub-populations of interest, including immunocompromised individuals, pregnant women, individuals with a prior history of thrombotic events or thrombocytopenia, individuals with prior COVID-19, individuals with a prior history of the specific AESI more than a year before the start of follow-up.

Study design	This study was a retrospective observational study that used electronic healthcare data sources in Europe. Eligible individuals were included in the study from the date when vaccination with Ad26.COV2.S began in each country (21 April 2021 for The Netherlands and 22 April 2021 for Spain).
Study setting	For this final safety evaluation, three data expert and access partners part of the Vaccine Monitoring Collaboration for Europe (VAC4EU) participated with the following data sources: • PHARMO (PHARMO Data Network, PHARMO Institute for Drug Outcomes Research) (NL); • SIDIAP (Sistema d'Informació per el Desenvolupament de la Investigació en Atenció Primària) [Information System for Research in Primary Care] (ES); • VID (FISABIO, La Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana [The Foundation for the Promotion of Health and Biomedical Research of Valencia Region]) (ES).
Subjects and study size, including dropouts	This final report was based on data from a source population of 14 million individuals. The study population included 510,073 recipients of Ad26.COV2.S; 7,008,678 recipients of one of the COVID-19 mRNA vaccines, (Pfizer-BioNTech and Moderna) and 458,295 unique matched unvaccinated individuals.

Methods	**Study exposure** The exposure of interest for this study was the administration of Ad26.COV2.S and two other COVID-19 vaccines, Vaxzevria vaccine and COVID-19 mRNA vaccines. Data for vaccine administration, the date of vaccination, and vaccine batch numbers, when available, were obtained from all possible sources, such as pharmacy dispensing records, general practice records, immunisation registers, vaccination records, medical records, or other data banks. **Study outcomes** The AESIs studied for this final report were the prespecified 36 events assessed in our previous feasibility reports: Encephalitis, including acute disseminated encephalomyelitis (ADEM) and meningoencephalitis; Guillain-Barré syndrome (GBS); transverse myelitis; Bell's palsy; multiple sclerosis, including optic neuritis; sensorineural hearing loss; generalised convulsion with epilepsy; generalised convulsion without epilepsy; autoimmune thyroiditis; immune thrombocytopenia; type 1 diabetes mellitus; acute aseptic arthritis; anaphylaxis; exacerbation of asthma; cardiac inflammatory disorders, including myocarditis and pericarditis; microangiopathy; heart failure; stress cardiomyopathy; coronary artery disease, including acute myocardial infarction; arrhythmia; deep vein thrombosis (DVT); pulmonary embolism; disseminated intravascular coagulation; non-haemorrhagic stroke; haemorrhagic stroke; thrombocytopenia; peripheral thrombosis; cerebral venous sinus thrombosis; abdominal venous thrombosis, including splanchnic thrombosis; thrombosis with thrombocytopenia syndrome (TTS); composite venous thrombosis; composite arterial thrombosis; composite vessel type and mixed arterial and venous thrombosis; composite stroke; acute kidney failure; and acute hepatic failure. **Study period** The study period was aligned with the data availability and follow-up durations for each database. In PHARMO, the study period started on 21 April 2021 and ended on 30 June 2022. In SIDIAP, the study period started on 22 April 2021 and ended on 31 December 2022. In VID, the

	study period began on 22 April 2021 and ended on 22 March 2022. **Methods and data analyses** **Descriptive analyses:** The final analyses were conducted in a distributed manner using the ConcePTION common data model (CDM) and common analytic programs, based on routinely collected health data. The following transformation steps were implemented: syntactic harmonisation (extraction, transformation, and loading: ETL); semantic harmonisation; application of the epidemiological study design; statistical analyses; and pooling of results from all data sources. For this final report, we described COVID-19 vaccination patterns, demographics, follow-up, and reasons for censoring using descriptive statistics. Each AESI was identified using specific codes or algorithms, and the cumulative incidence (1-KM) per 100,000 persons with 95% confidence intervals (CIs) were depicted by exposure cohort. **Self-controlled risk interval (SCRI) analyses** The SCRI design assessed the risk of acute AESIs following Ad26.COV2.S vaccination. The study included vaccinated individuals who developed an AESI during the risk or post-vaccination control period, with follow-up starting at the beginning of the risk window and ending at the end of the 60-day control window, the AESI-specific risk window, or when censored. A 365-day clean look-back period was required for most AESIs, except for generalised epileptic convulsions and anaphylaxis, which both had a 90-day clean look-back period. A 30-day washout period was included between risk and control windows. Inclusion criteria were receiving a first dose of Ad26.COV2.S, having 12 months of prior data, at least one day of follow-up in both risk and control windows, and experiencing the AESI during either the control or the risk window. Exclusion criteria included prior administration of another COVID-19 vaccine, AESI occurrence during the clean look-back period. Follow-up was not censored at the AESI occurrence, allowing all person-time in relevant intervals to be included. Sensitivity analyses included censoring follow-up if a non-COVID-19 vaccine was received and the use of a pre-vaccination control window.

	Matched cohort study design The matched cohort design compared the Ad26.COV2.S recipients with those who received mRNA COVID-19 vaccines (Pfizer-BioNTech or Moderna) or were unvaccinated. Matching with Vaxzevria recipients was explored but ultimately not feasible due to differences in the age distribution across the vaccinated cohorts. Individuals were matched by age**,** sex, immunocompromised status, pregnancy status and certainty of the pregnancy, previous COVID-19, and presence of ≥1 risk factor for severe COVID-19. Inclusion criteria required a first dose of the corresponding COVID-19 vaccine within the study period and 12 months of prior data. Unvaccinated comparators were also required to have 12 months of prior data before their matched index date and no COVID-19 vaccination. Exclusion criteria included prior COVID-19 vaccine administration, missing key variables (i.e., exposure and matching variables) and relevant medical histories. Absolute standardised differences (ASDs) were calculated for each baseline characteristic to assess the imbalance between the Ad26.COV2.S and comparator cohorts. Variables with ASD values >0.1 were considered imbalanced. Inverse probability of treatment weighting (IPTW) from a propensity score model was used for adjustment of baseline variables. After matching, individuals with an AESI during the clean period were excluded together with their matched pair for the specific AESI analysis. The index date for each matched unvaccinated individual was the vaccination date of the matched vaccinated individual. Covariates for the propensity score model included outcome risk factors and matching variables, using logistic regression to estimate the score. Poisson regression models with IPTW adjustment to control for confounding were used to estimate the relative risk and its 95% CI. Negative control outcomes analyses were used to assess potential sources of bias. Sensitivity analyses included censoring at non-COVID-19 vaccine administration and estimating the vaccine direct effect on AESIs by censoring pairs when one member of the pair had a COVID-19 diagnosis. Special populations analysed included immunocompromised individuals, pregnant women, prior COVID-19 (a month before vaccination), those with prior thrombotic events more

	than 365 days before vaccination, and specific AESI history. In both designs, unadjusted and adjusted incidence rate ratios (IRRs) were estimated. For the SCRI design, we present the unadjusted effect estimates, since time variation of non-fixed covariates was minimal leading to instability of adjusted estimates. **Outcome validation** The following 11 AESIs were validated in VID: encephalitis (including ADEM), GBS, transverse myelitis, immune thrombocytopenia, cardiac inflammatory disorders (including myocarditis and pericarditis), DVT, pulmonary embolism, non-haemorrhagic stroke, haemorrhagic stroke, cerebral venous sinus thrombosis, and TTS. The two latter AESIs were also validated in SIDIAP. The strict positive predictive value (PPV) was calculated by dividing the number of confirmed cases with levels of diagnostic certainty (LOC) 1, 2, or 3 by the total number of validated cases with LOC 1, 2, 3, 4a, and 5. The broad PPV was calculated by dividing the number of confirmed cases with LOC 1, 2, 3, and 4a by the total number of validated cases with LOC 1, 2, 3, 4a, and 5.

Results	A total of 510,073 individuals were given an initial dose of Ad26.COV2.S vaccine, mainly during Q2- Q3 2021 in PHARMO and SIDIAP and Q2 2021 in VID. The majority (>80%) of COVID-19 vaccine doses administered were COVID-19 mRNA vaccines in all data sources. The percentages of vaccine brands and timing of administration were consistent with those reported in the ECDC Vaccine Tracker for the Netherlands and Spain. The first dose of Ad26.COV2.S vaccine was administered most frequently to individuals aged 40-49 years in SIDIAP, those aged 40-59 years in VID, and those aged 18-29 years in PHARMO. The percentages of females vaccinated with the Ad26.COV2.S vaccine were 36.9% in PHARMO, 44.1% in SIDIAP, and 46.2% in VID. Most individuals (72% in PHARMO, 45% in SIDIAP, 67% in VID) with the first dose of Ad26.COV2.S received a second dose of a COVID-19 mRNA vaccine about six months later. The median follow-up for Ad26.COV2.S cohorts was 7.3 months in PHARMO, 6.7 months in SIDIAP, and 6.0 months in VID. In all data sources, the primary reason for censoring among Ad26.COV2.S recipients was the administration of another COVID-19 vaccine booster dose, while the main reason for censoring among COVID-19 mRNA vaccine recipients was the end of the study period or enrolment termination. Individuals in the unmatched Ad26.COV2.S cohorts in PHARMO were younger (median age 27 years) and had fewer chronic conditions compared with those in SIDIAP (median age 45 years) and VID (median age 50 years). The prevalence of comorbidities varied by age group, with 23.7% in PHARMO having at least one risk factor for severe COVID-19 compared with 51.7% in SIDIAP and 45% in VID. **Safety analyses** The numbers of AESIs were low, particularly in PHARMO, due to the inclusion of a smaller population, without hospitalisation data, and the use of ICPC codes in general practice which lack specific codes for some of the events. This resulted in imprecise risk estimates, especially in subgroup analyses.

	Following recommendations from the American Statistical Association and the International Committee for Medical Journal Editors, we avoided relying solely on p-values and statistical significance. Instead, we used a quantitative framework that integrated the effect size, precision (confidence intervals), and potential data biases to provide a more comprehensive interpretation of our results. For risk estimates with low heterogeneity ($I^2 < 40\%$), we reported the results from the individual data sources as well as the results from the meta-analysis, while for those with high heterogeneity ($I^2 \geq 40\%$), we present results from the individual data sources. We considered AESIs frequent if at least one data source had ≥50 events, ensuring sufficient precision. For AESIs with fewer than 50 events, we emphasised the main analysis due to potential random variation in the subgroup results. IRRs >1 and ≤1.25 were considered suggestive of a slight increase in risk, IRRs between 1.26 and ≤2.00 a moderate increase, and IRRs >2.00 were considered suggestive of a strong increased risk. For frequently occurring events primary analyses did not suggest strongly increased risk for any AESI. Moderate risk increases were suggested for: • arrhythmia (IRR=1.27, 95% CI: 1.10–1.47) • peripheral thrombosis (IRR=1.56, 95% CI: 1.21–2.00) and • thrombocytopenia (IRR=1.60, 95% CI: 1.38–1.84). Slightly increased risks were suggested for: • composite venous thrombosis (IRR=1.24, 95% CI: 0.99–1.55) • DVT (IRR=1.23, 95% CI: 0.90–1.68) • coronary artery disease (IRR=1.18, 95% CI: 0.92–1.52) • Bell's palsy (IRR=1.10, 95% CI: 0.78–1.57) • sensorineural hearing loss (IRR=1.10, 95% CI: 0.85–1.43), and • pulmonary embolism (IRR=1.08, 95% CI: 0.63–1.85). Among users of the Ad26.COV2.S vaccine, no indication of an increased risk was seen for non-haemorrhagic stroke (IRR=0.97, 95% CI: 0.75–1.25), composite stroke

	(IRR=0.92, 95% CI: 0.72–1.17), and acute kidney failure ($I^2 > 40\%$). For less frequent AESIs, the results were suggestive of strong increased risk for anaphylaxis, GBS, immune thrombocytopenia, generalised convulsion (non-epileptic), disseminated intravascular coagulation, and cerebral venous sinus thrombosis after Ad26.COV2.S vaccine. The results suggested moderate risk increases for: abdominal venous thrombosis including splanchnic thrombosis, TTS, stress cardiomyopathy, and type 1 diabetes. The results also suggested potential slight risk increases for: cardiac inflammatory disorders including myocarditis and pericarditis and microangiopathy. However, the precision of these results was low due to the small number of events, particularly for anaphylaxis and generalised convulsions. Among Ad26.COV2.S vaccine recipients, no indication of an increased risk was seen for encephalitis (including ADEM and meningoencephalitis), haemorrhagic stroke, composite vessel type unspecified, mixed arterial and venous thrombosis, acute hepatic failure, or exacerbation of asthma. The risk for transverse myelitis, epileptic generalised convulsions, and acute aseptic arthritis in Ad26.COV2.S recipients could not be estimated due to the absence or insufficient numbers of events to conduct the analyses. Age- and sex-stratified analyses showed some differences in risk between categories for Bell's palsy, pulmonary embolism, DVT, composite venous thrombosis, autoimmune thyroiditis, multiple sclerosis and coronary artery disease, with discrepancies in results by sex and data source. Five out of the 11 AESIs had high PPVs (>80%), reflecting the low false positive rate of the algorithms used to identify cases of each event. For example, the PPV for pulmonary embolism in the Ad26.COV2.S cohort was 83.33% (95% CI: 67.19, 93.63%), indicating that the algorithm had accurately identified true positive cases.

	A moderate PPV of between 60% to 80% indicated that while most cases identified were likely to be true positives, there was a risk of some misclassification, meaning that some cases could be false positives. The low PPV of 55.17% (95% CI: 35.69–73.55%) for TTS in the Ad26.COV2.S cohort in SIDIAP suggested significant misclassification may have occurred. This contrasts with the PPV observed in VID, which was 100%, though only based on one event.

Discussion	This study has provided insights into the real-world safety profile of the Ad26.COV2.S vaccine, including SCRI analyses, matched cohort analyses comparing Ad26.COV2.S cohorts with COVID-19 mRNA-vaccinated and unvaccinated cohorts. For common AESIs (≥50 events), the study results did not suggest strongly increased risks (IRR > 2) for any event. However, moderate risk increases were observed for arrhythmia (IRR=1.27), peripheral thrombosis (IRR=1.56), and thrombocytopenia (IRR=1.60). Among the less frequent AESIs, (<50 events), the study results suggested a strongly increased risk (IRR > 2) for anaphylaxis, GBS, and immune thrombocytopenia.. We acknowledge several limitations that can be categorised as data source-related, methods-related, and study design-related factors. Diversity in the data sources, such as differences in coding systems or lack of codes, or hospitalisation data availability, may have affected the accuracy of the assessment of some AESIs. Methods-related limitations include censoring due to booster doses, which could have introduced differential censoring. Additionally, the SCRI study design relied on assumptions, such as independence of the probability of exposure following outcomes. To mitigate this limitation, we conducted sensitivity analyses using a cohort design. The PPVs in VID were greater than 80% for cardiac inflammatory disorders, pulmonary embolism, haemorrhagic stroke, cerebral venous sinus thrombosis, and TTS, indicating a low risk of event misclassification. However, the PPVs for TTS were lower in SIDIAP possibly due to a lack of access to original hospital data.
Marketing Authorisation Holder	Janssen-Cilag International NV
Name(s) and affiliation(s) of Principal Investigator(s)	 On behalf of the VAC4EU Ad26.COV2.S PASS Research Team