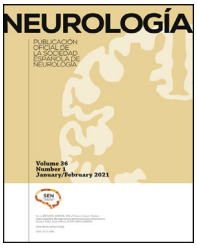
Neurología 39 (2024) 721—732

NEUROLOGÍA

www.elsevier.es/neurologia



ORIGINAL ARTICLE

Thrombosis with thrombocytopenia syndrome following adenovirus vector-based vaccines to prevent COVID-19: Epidemiology and clinical presentation in Spain☆

D. García-Azorín[a,*], E. Lázaro[b], D. Ezpeleta[c], R. Lecumberri[d,e], R. de la Cámara[f], M. Castellanos[g,h], C. Iñiguez Martínez[i,j], L. Quiroga-González[b], G. Elizondo Rivas[k,l], A. Sancho-López[m,n,o], P. Rayón Iglesias[b], E. Segovia[b], C. Mejías[b], D. Montero Corominas[b]

[a] *Servicio de Neurología, Hospital Clínico Universitario de Valladolid, Valladolid, Spain*
[b] *División de Farmacoepidemiología y Farmacovigilancia, Departamento de Medicamentos de Uso Humano, Agencia Española de Medicamentos y Productos Sanitarios, Madrid, Spain*
[c] *Servicio de Neurología, Hospital Universitario Quirónsalud Madrid, Pozuelo de Alarcón, Madrid, Spain*
[d] *Servicio de Hematología, Clínica Universidad de Navarra, Pamplona, Navarra, Spain*
[e] *CIBER-CV, Instituto de Salud Carlos III, Madrid, Spain*
[f] *Servicio de Hematología, Hospital de la Princesa, Madrid, Spain*
[g] *Servicio de Neurología, Complejo Hospitalario Universitario/Instituto de Investigación Biomédica de Coruña, Coruña, Spain*
[h] *Instituto de Salud Carlos III, Madrid, Spain*
[i] *Servicio de Neurología, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain*
[j] *Instituto de Investigación Sanitaria de Aragón (IIS Aragón), Zaragoza, Spain*
[k] *Centro de Farmacovigilancia de Navarra, Departamento de Salud, Gobierno de Navarra, Pamplona, Navarra, Spain*
[l] *Comité Técnico del Sistema Español de Farmacovigilancia, Spain*
[m] *Servicio de Farmacología Clínica, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain*
[n] *Sociedad Española de Farmacología Clínica (SECF), Spain*
[o] *Grupo de Vacunas de FACME, Madrid, Spain*

Received 15 February 2022; accepted 24 April 2022
Available online 31 October 2024

KEYWORDS
MeSH Terms;
COVID-19;
Drug-related side effects and adverse reactions;

Abstract
Background: We describe the epidemiological and clinical characteristics of thrombosis with thrombocytopenia syndrome (TTS) cases reported in Spain.
Methods: We included all cases of venous or arterial thrombosis with thrombocytopenia following administration of adenoviral vector vaccines (AstraZeneca or Janssen) against COVID-19 disease between 1 February and 26 September 2021. We describe the crude rate and the standardised morbidity ratio. We assessed the predictors of mortality.

DOI of refers to article: https://doi.org/10.1016/j.nrl.2022.04.010.
☆ Spanish working group for the study of thrombosis with thrombocytopenia syndrome associated with vaccines against SARS-CoV-2.
* Corresponding author.
E-mail address: dgazorin@ucm.es (D. García-Azorín).

https://doi.org/10.1016/j.nrleng.2024.10.001

Vaccines;
Embolism and thrombosis;
Blood platelet disorders

Results: Sixty-one cases were reported and 45 fulfilled eligibility criteria; 82% of patients were women. The crude TTS rate was 4 cases/1 000 000 doses, and 14-15 cases/1 000 000 doses among patients aged 30-49 years. The number of observed cases of cerebral venous thrombosis was 6-18 times higher than that expected in patients younger than 49 years. Symptoms started a median (quartiles 1 and 3 [Q_1-Q_3]) of 10 (7-14) days after vaccination. Eighty percent (95% confidence interval [CI]: 65%-90%) had thrombocytopenia at the time of the emergency department visit, and 65% (49%-78%) had D-dimer levels > 2000 ng/mL. Patients had thromboses affecting multiple locations in 36% of cases and fatal outcomea in 24%. Platelet nadir < 50 000/μL (odds ratio [OR]: 7.4; 95% CI: 1.2-47.5) and intracranial hemorrhage (OR: 7.9; 95% CI: 1.3-47.0) were associated with fatal outcomes.
Conclusion: TTS must be suspected in patients with symptoms 10 days after vaccination and thrombocytopenia and/or elevated D-dimer levels.

PALABRAS CLAVE
MeSH Terms;
COVID-19;
Efectos adversos de medicamentos y reacciones adversas;
Vacunas;
Embolia y trombosis;
Trastornos plaquetarios

Síndrome de trombosis con trombocitopenia asociado a vacunas de adenovirus frente a la COVID-19: Epidemiología y presentación clínica de la serie española

Resumen
Introducción: Se describen las características epidemiológicas y clínicas de los casos de síndrome de trombosis con trombocitopenia (STT) notificados en España.
Métodos: Se incluyeron los casos de trombosis venosa o arterial con trombocitopenia tras recibir vacuna con vectores de adenovirus no replicantes frente a la COVID-19 (AstraZeneca y Janssen) del 1 de febrero al 26 de septiembre de 2021. Se describe la tasa de notificación, (número de casos notificados/número de dosis administradas), y el análisis de casos observados frente a esperados (O/E). Se evaluaron los predictores de mortalidad.
Resultados: Se notificaron 61 casos, cumpliendo 45 los criterios de elegibilidad, 82% mujeres. La tasa de notificación global fue 4/1.000.000 dosis y 14-15/1.000.000 dosis entre los 30-49 años. El número de casos de trombosis de senos cerebrales observados fue 6-18 veces superior al esperado en menores de 49 años. Los síntomas comenzaron 10 (rango intercuartílico: 7-14) días tras la vacunación. El 80% (intervalo de confianza (IC) al 95%: 65-90%) tenía trombocitopenia en el momento de su visita a urgencias y el 65% (IC 95%: 49-78%) elevación del dímero D (> 2.000 ng/mL). La trombosis fue de múltiples localizaciones en 36% y fatal en 24% de los pacientes. Un valor nadir de trombocitopenia < 50.000/μL (*odds ratio* (OR): 7,4; IC95%: 1,2-47,5) y la presencia de hemorragia cerebral (OR: 7,9; IC95%: 1,3-47,0) se asociaron a un desenlace fatal.
Conclusiones: Debe sospecharse el STT en pacientes que presenten síntomas unos 10 días tras la vacunación y presenten trombocitopenia y/o elevación de dímero D.

Introduction

The first vaccine against the SARS-CoV-2 coronavirus, BNT162b2 (Comirnaty®, Pfizer BioNTech), was approved on 21 December 2020; Spain's vaccination campaign began immediately, in accordance with the vaccination strategy approved by the Inter-regional Council of the Spanish National Health System.[1] The ChAdOx1-S nCov-19 (Vaxzevria®, AstraZeneca) and Ad26.COV2-S vaccines (Janssen) were approved on 29 January and 11 March 2021, respectively. In parallel to the approval of the vaccines, a pharmacovigilance plan[2] was implemented which, among other actions, included comprehensive clinical review of clinically complex adverse events reported to the Spanish Pharmacovigilance System for Medicinal Products for Human Use (SEFV-H, for its Spanish abbreviation).

On 7 March 2021, the first cases were reported of thrombosis with thrombocytopenia syndrome (TTS) in patients who had received the AstraZeneca vaccine in Europe (specifically, Austria); the first case in Spain was reported on 14 March.[3–5] The Spanish Agency of Medicines and Medical Devices (AEMPS) acted urgently to create a multidisciplinary expert group to assess these cases, follow up cases reported in Spain, and subsequently develop specific guidelines with diagnostic and therapeutic recommendations on this severe adverse event.[6]

Vaccine-induced TTS is characterised by the presence of one or more thromboses, primarily affecting veins (as well as arteries), with a tendency to affect unusual locations, such as the splanchnic veins or the cerebral venous sinuses.[4,5,7–9] Presence of anti—platelet factor 4 (anti-PF4) antibodies promotes platelet aggregation and micro- and macrothrombosis, resulting in pronounced thrombocytopenia and the thrombotic manifestations that characterise the syndrome.[8] TTS has been associated with other non-replicating adenoviral vector vaccines, such as the AstraZeneca

and Janssen vaccines.[4—8,10] AEMPS reported the possibility that this clinical entity may appear as a potential adverse reaction to adenoviral vector vaccines[11] on 7 April 2021,[12] and modified the vaccination strategy with a view to minimising this risk, restricting their use in younger patients (in whom the risk appeared to be greater) and avoiding administration of a second dose of the AstraZeneca vaccine in this age group.[13] Soon after, on 26 May 2021, an expert working group from the Spanish Federation of Medical and Scientific Associations (FACME) published a set of diagnostic and therapeutic guidelines on the management of cerebral venous sinus thrombosis (CVST) associated with COVID-19 vaccination.[6]

This article describes the epidemiological and clinical characteristics of all cases of TTS associated with non-replicating adenoviral vector vaccines against SARS-CoV-2 reported in Spain between 1 February and 26 September 2021. These specific vaccines are addressed because they were the agents for which this association was observed and which were the subject of concerns raised by various national and international bodies. We describe the demographic pattern of incident cases during the vaccination campaign (up to 26 September 2021), the reporting rate, and the analysis of observed vs expected cases. Clinical data were analysed to study the frequency of comorbidities, clinical presentation, laboratory findings, treatment, and prognosis. As exploratory objectives, we analyse the sensitivity of laboratory parameters determined at the emergency department and the variables that may be associated with greater likelihood of mortality.

Methods

We conducted an observational, descriptive study of a series of cases, in accordance with the STROBE recommendations.[14] The study population included all patients presenting with venous or arterial thrombotic events due to suspected TTS in the 100 days after administration of a non-replicating adenoviral vector vaccine against SARS-CoV-2, and reported to the SEFV-H, coordinated by AEMPS. The study was approved by the medical research ethics committee of Hospital Clínico Universitario de Valladolid (code PI 21/2450).

Eligibility criteria

The inclusion criteria, based on the diagnostic criteria of the World Health Organization and the Brighton Collaboration,[15] were: *1)* presence of at least one venous and/or arterial thrombosis; *2)* presence of thrombocytopenia (platelet count < 150 000 platelets/μL or a > 50% reduction below baseline) at some point during clinical progression; and *3)* exposure to a non-replicating adenoviral vector vaccine against SARS-CoV-2 in the previous 100 days. We excluded cases that, in the opinion of the expert group, might be explained by another cause, and cases whose diagnosis was later reported to be incorrect or uncertain, or for which insufficient data were available.

Intervention

For the epidemiological analysis, we calculated the reporting rate, defined as the number of patients presenting this adverse event in relation to the number of doses administered during the study period; rates were calculated for different age ranges, sexes, and number of doses (first or second dose administered). Data on the number of doses administered of each vaccine, age, and sex were obtained from the Vaccination Register (REGVACU) of the Spanish Ministry of Health.[16] Analysis of the observed (reported) cases in the study population and period, compared to the expected number of cases in the general population, was conducted according to the method described by Mahaux et al.,[17] both for the total population and by age range. This calculation included all reported cases (the most conservative assumption) and assumed a time window at risk of 30 days, the period within which symptoms presented in the majority of registered cases. A ratio greater than one indicates a numerical disproportion between the number of cases observed and the expected number of cases in the unvaccinated population; this difference is statistically significant if the lower bound of the confidence interval (CI) is greater than one. Observed and expected cases were only calculated for CVST, which was the only clinical entity within the category of TTS for which sufficient epidemiological information is available on baseline incidence in the general unvaccinated population. These data were extracted from the FISABIO hospital database of the Valencian Community,[18] as no other incidence data on other thromboses in unusual locations are available in our setting.

For the study of demographic, clinical, and prognostic characteristics, we reviewed the available information on the reported cases, which were anonymised at source. The demographic variables analysed were sex, age, thrombotic risk factors (Supplementary material 1), history of COVID-19, vaccine administered, and number of doses. The clinical data studied were presence of COVID-19 concomitantly with TTS, time (days) from vaccination to symptom onset, time (days) from symptom onset to diagnosis, initial symptoms, localisation of the thrombosis; and (in patients with CVST) presence of headache at any time during progression, presence of intracranial haemorrhage, and presence of alarm signs (Supplementary material 2). The laboratory data included in the analysis were platelet count at the emergency department and platelet count nadir, D-dimer level at the emergency department and the highest value recorded, fibrinogen level at admission, and result of the anti-PF4 antibody determination. We also analysed the treatment administered (immunoglobulins, heparin anticoagulants, non-heparin anticoagulants, platelet transfusion, steroids[15]) and prognosis (recovery, death, or unknown).

Study period

The study period was from the date of administration of the first dose of a non-replicating adenoviral vector vaccine in Spain (1 February 2021) to 26 September 2021, when the majority of the eligible population had received these vaccines and their administration slowed.

Statistical analysis

In the statistical analysis, qualitative and ordinal variables are presented as frequency and percentage. Continuous variables are expressed as means and standard deviation (SD), or medians and quartiles 1 and 3 (Q_1-Q_3), depending on data distribution. The 95% confidence interval (CI) was calculated in the estimation of the number of cases. Qualitative and ordinal variables are compared using the Fisher exact test; associations between quantitative and qualitative variables were tested with the *t* test or Mann-Whitney U test, depending on data distribution. An alpha error of 5% was accepted.

A subgroup analysis made comparisons between patients receiving the AstraZeneca and the Janssen vaccines, and between patients with positive and negative anti-PF4 antibodies, excluding patients for whom this information was not available. Positivity for these antibodies was only established for patients who had undergone ELISA testing or a platelet function test. The sensitivity of laboratory parameters was estimated for the following cut-off points, recommended in clinical practice guidelines[6,9,15]: 50 000, 100 000, and 150 000 platelets/μL; 2000 and 4000 ng/mL of D-dimer.

To identify variables associated with greater risk of mortality, we initially conducted a univariate logistic regression analysis, followed by a multivariate analysis including variables showing *P*-values < 0.1 in the univariate model; results are expressed as odds ratios (OR) with 95% CI. We performed a complete case analysis to avoid bias due to missing data.

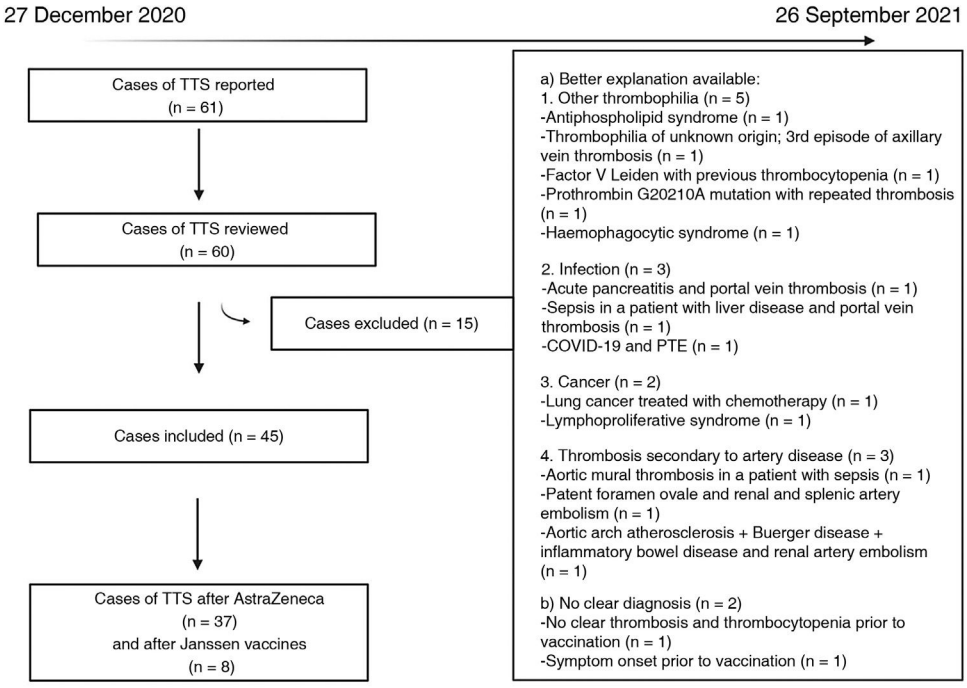


Figure 1 Cases reported, reviewed, excluded, and included in the series. One case was reported but not reviewed as no data were available.
PTE: pulmonary thromboembolism; TTS: thrombosis with thrombocytopenia syndrome.

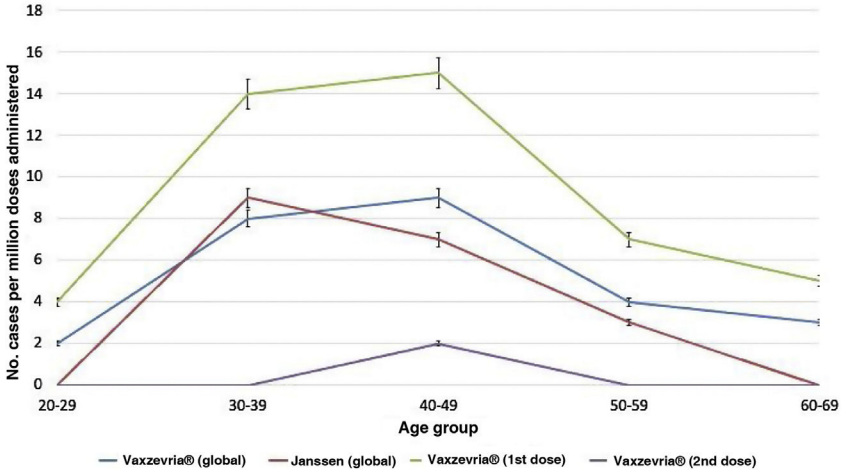


Figure 2 Reporting rate for thrombosis with thrombocytopenia syndrome according to the vaccine administered and age group, per million doses administered.

Sensitivity was calculated by intention-to-treat and per-protocol analysis, with 95% CI being calculated for each value. Statistical analysis was conducted using the SPSS statistics software, version 26.0 for Mac (IBM Corp; Armonk, NY, USA).

Results

During the study period, SEFV-H was notified of a total of 61 cases initially diagnosed as TTS, attributed by the notifying physicians to non-replicating adenoviral vector vaccines. Fig. 1 shows the number of cases reported, reviewed, excluded, and included. We finally included and analysed 45 patients, 37 who had received the AstraZeneca vaccine and 8 who had received the Janssen vaccine.

Analysis of observed and expected cases and reporting rate

As of 26 September 2021, a total of 9 771 767 doses (5 103 885 first doses and 4 667 882 second doses) of the AstraZeneca vaccine and 1 959 146 doses of the Janssen vaccine had been administered. The overall reporting rate was estimated at 4 cases/1 000 000 doses administered, for both vaccines; a higher rate was observed for the 30-49 years age group, with 8-9 cases/1 000 000 doses for the AstraZeneca vaccine. Reporting rates of 14 and 15 cases of TTS/1

Table 1 Analysis of the reported and expected cases of cerebral venous sinus thrombosis associated with adenoviral vector vaccines. Overall data from the series and by age group.

	Time window at risk: 30 days	30-39 years	40-49 years	50-59 years	60-69 years	Total
AstraZeneca vaccine	No. cases	4	4	1	2	12
	O/E ratio (95% CI)	18.84 (5.13-48.23)	6.38 (1.74-16.34)	1.26 (0.03-7.02)	0.17 (0.02-0.63)	1.53 (0.79-2.67)
Janssen vaccine	No. cases	0	3	0	0	3
	O/E ratio (95% CI)	—	5.27 (1.09-15.40)	—	—	1.64 (0.34-4.78)

CI: confidence interval; O/E observed vs expected. Statistically significant values are shown in bold (ratios greater than one, with the lower bound of the 95% CI being greater than one).[17]

Table 2 Demographic and clinical variables.

Variable	Total cases (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	*P*	Anti-PF4 + (n = 20)	Anti-PF4 — (n = 15)	*P*
Age in years, median (Q_1-Q_3)	53 (45.5-62.5)	60 (45.5-63)	48 (42.2-52)	.121	46.5 (37.2-58.2)	61 (48-63)	.009
Women, n (%)	37 (82.2%)	20 (54.1%)	6 (75%)	.435	12 (60%)	8 (53.3%)	.741
AstraZeneca	37 (82.2%)	37 (100%)	0 (0%)	—	13 (65%)	14 (93.3%)	.015
Thrombotic risk factors	13 (28.9%)	12 (32.4%)	1 (12.5%)	.405	5 (25%)	6 (40%)	.467
Dose	1st: 43 (95.6%) 2nd: 2 (4.4%)	1st: 35 (94.6%) 2nd: 2 (5.4%)	1st: 8 (100%)		1st: 20 (100%)	1st: 14 (93.3%) 2nd: 1 (6.7%)	.429
Days from vaccination to 1st symptom (median, Q_1-Q_3) (n = 39)	11 (7-14)	10 (7-14)	12 (8.7-17.5)	.312	10 (7.2-13.7)	11 (8-19)	.364
Days from 1st symptom to diagnosis (median, Q_1-Q_3) (n = 37)	1 (0-7)	1 (0-7)	1 (0-2)	.312	1.5 (0.2-6.2)	1 (0-2)	.158

PF4: platelet factor 4; Q_1-Q_3: quartiles 1 and 3. The variable ''AstraZeneca'' was included to compare anti-PF4 antibodies in patients receiving the different vaccines. Data were compared using the Fisher exact test; quantitative and qualitative variables were compared with the *t* test (normally distributed variables, expressed as mean [standard deviation]) or the Mann-Whitney U test (non—normally distributed variables, expressed as median [Q_1-Q_3]).

000 000 doses administered were observed in the 30-39 years and 40-49 years age groups, respectively, after the first dose of the AstraZeneca vaccine, with rates of 0 and 2 cases/1 000 000 doses administered after the second dose (Fig. 2).

The analysis of observed vs expected cases of CVST revealed a statistically significant disproportion among patients aged 30-49 years and receiving the AstraZeneca vaccine and among those aged 40-49 years and receiving the Janssen vaccine (Table 1).

Demographic characteristics

Table 2 summarises the main characteristics of the study population. History of SARS-CoV-2 infection was only reported in 2.2% of cases. PCR testing for SARS-CoV-2 was performed at admission in 15 patients, and antigen tests were performed in 3; results were negative in all cases. In the subgroup analysis for type of vaccine and presence/absence of anti-PF4 antibodies, the only statistically significant difference was a younger median age in patients with anti-PF4 antibodies. The median time from vaccination to clinical onset of TTS was 11 days (Q_1-Q_3: 7-14; range, 1-60).

Clinical presentation

Clinical descriptions were provided for 36 of the 45 patients (80%). Fig. 3 shows the most frequent initial symptoms. Seventeen of the 45 patients (37.8%) presented headache at some point during disease progression, with alarm signs in all cases. Fig. 4 shows the frequency of alarm signs.

Laboratory parameters

Median platelet count at the emergency department was 81 000/μL (Q_1-Q_3: 40 500-127 000; range, 8000-212 000); median D-dimer level was 21 000 ng/mL (Q_1-Q_3: 5360-35 612; range, 130-60 000). Fig. 5 shows platelet count as a function of the time between vaccination and emergency consultation. In the comparison between vaccines,

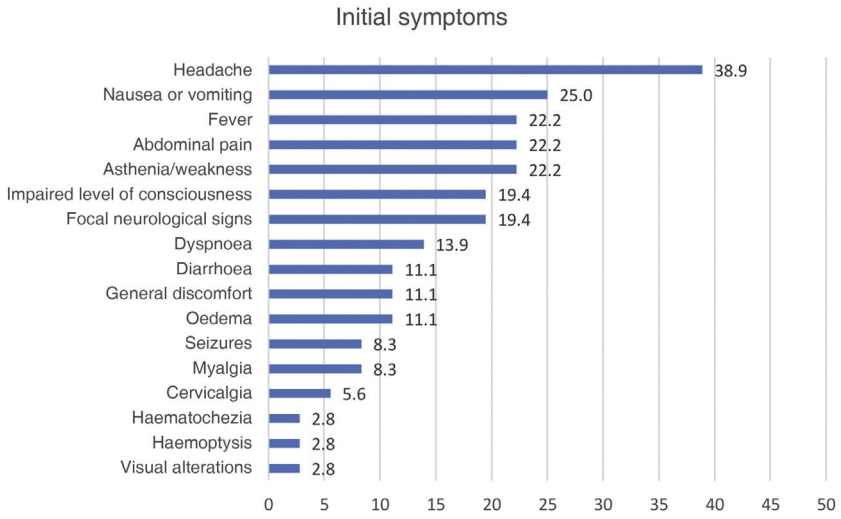


Figure 3 Symptoms at onset (n = 36). The figure includes all initial symptoms, and presents all patients (ie, those with neurological and non-neurological thrombosis location). Some patients with non-neurological thromboses presented neurological symptoms, such as headache.

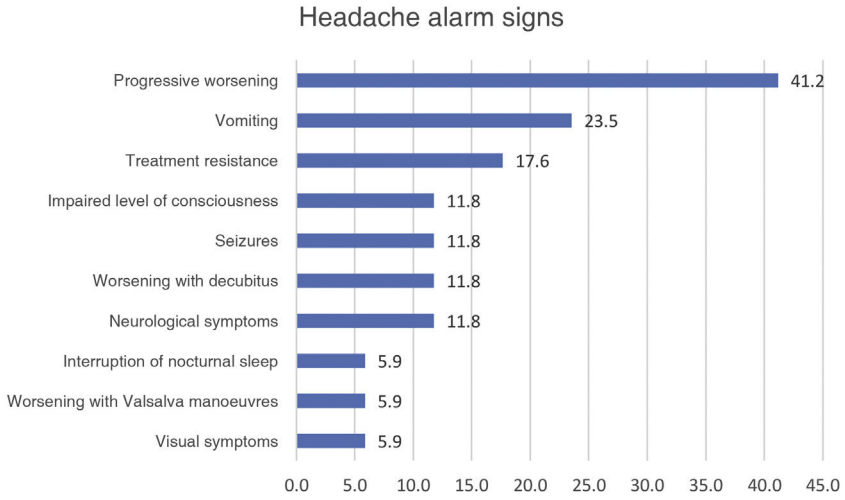


Figure 4 Headache alarm signs (n = 17).

statistically significant differences were observed in platelet count at the emergency department and in the presence of anti-PF4 antibodies (Supplementary material: table* 1 and Fig. 1). No significant differences were observed between patients with and without anti-PF4 antibodies (Supplementary material: table* 2, Figs. 2 and 3).

Table 3 shows the analysis of the sensitivity of the cut-off points for platelet count and D-dimer levels suggested in the international guidelines, both per protocol (analysis of cases with all data available) and by intention to treat (including cases with missing data).

Location of thromboses

Sixteen patients (35.6%) presented thromboses in multiple locations. No statistically significant differences in thrombosis location or type were observed as a function of the vaccine received (Table 4).

Therapeutic management

Thirty patients (66.7%) received anticoagulation therapy: non-heparin in 29 (64.4%) and heparin in 8 (17.8%). Seven patients (15.6%) received both types of drug. Immunoglobulins were administered to 19 patients (42.7%) and steroids to 9 (20%), with 22 patients (48.9%) receiving at least one of these 2 treatments. No statistically significant differences were observed in the comparison of patients receiving different vaccines or between patients with and without anti-PF4 antibodies (Table 5).

In 11 patients (24.4%), management was apparently suboptimal due to the following reasons: use of non-contrast CT scans, despite the presence of headache with alarm signs and thrombocytopenia (7 patients [15.5%]); administration of heparin despite the presence of anti-PF4 antibodies (2 [4.4%]); platelet transfusion in the absence of an indication (1 [2.2%]); and empirical diagnosis of possible COVID-19, without a diagnostic test (1 [2.2%]).

Outcomes and predictors of mortality

Eleven patients (24.4%) died; outcomes were not reported for 2 patients (4.4%). Platelet count < 50 000/μL at the emergency department or as the nadir value, and presence of intracranial haemorrhage were the variables associated with greater likelihood of mortality in the univariate analysis. The multivariate analysis

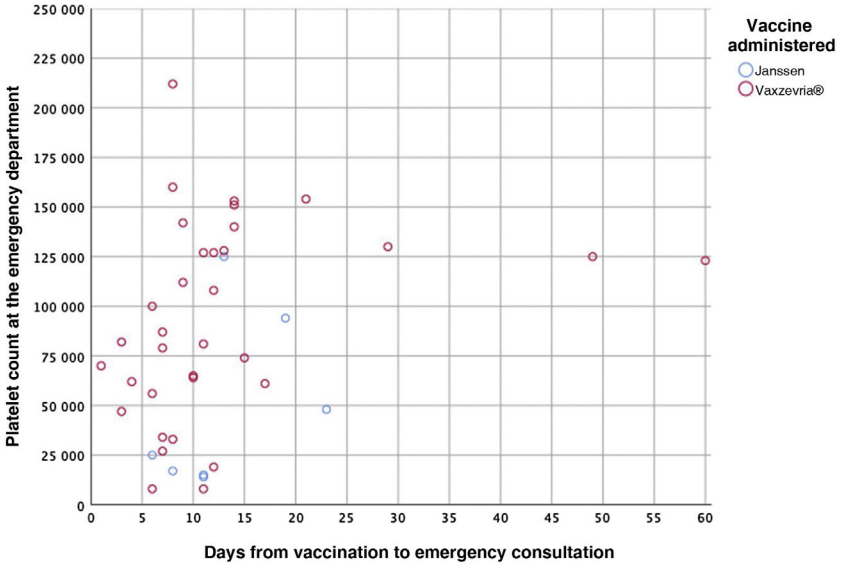


Figure 5 Platelet count at the emergency department (y-axis) as a function of the time between vaccination and consultation at the emergency department (x-axis). Patients immunised with the Janssen vaccine are shown in blue; patients immunised with the AstraZeneca vaccine, in red.

Table 3 Sensitivity of platelet count and D-dimer level at emergency consultation at the most extreme value (platelet count nadir and peak D-dimer value).

Parameter	Time	Type of analysis	Value	Sensitivity	95% CI
Platelet count	Emergency consultation	PP (n = 41)	50 000/μL	29.3%	16.6-45.7
		ITT (n = 45)		26.1%	14.7-41.4
		PP (n = 41)	100 000/μL	61.0%	44.5-75.4
		ITT (n = 45)		55.6%	40.1-70.0
		PP (n = 41)	150 000/μL	87.8%	73.0-95.4
		ITT (n = 45)		80.0%	64.9-89.9
	Nadir	PP (n = 44)	50 000/μL	45.4%	30.7-61.0
		ITT (n = 45)		44.4%	30.0-59.9
		PP (n = 44)	100 000/μL	72.7%	57.0-84.5
		ITT (n = 45)		71.1%	55.5-83.1
		PP (n = 45)	150 000/μL	100%	90.2-100
		ITT (n = 45)		100%	90.2-100
D-Dimer	Emergency department	PP (n = 31)	2000 ng/mL	93.5%	77.1-98.9
		ITT (n = 45)		64.4%	48.7-77.7
		PP (n = 31)	4000 ng/mL	90.3%	73.1-97.4
		ITT (n = 45)		62.2%	46.5-75.8
	Peak	PP (n = 36)	2000 ng/mL	94.4%	80.0-99.0
		ITT (n = 45)		75.6%	60.1-86.6
		PP (n = 36)	4000 ng/mL	88.9%	73.0-96.4
		ITT (n = 45)		71.1%	55.5-83.1

CI: confidence interval; ITT: intention-to-treat analysis (including all patients and assuming a negative result in the event of missing data, indicating that the lowest possible percentage of patients would present this parameter); PP: per-protocol analysis (only patients for whom data are available).

identified intracranial haemorrhage (OR: 7.9; 95% CI, 1.32-47.0) and platelet count nadir < 50 000/μL (OR: 7.4; 95% CI, 1.16-47.6) as predictors of mortality (Supplementary material: Tables 3—5).

Discussion

This study presents the epidemiological and clinical data from a series of cases of TTS associated with non-replicating adenoviral vector vaccines reported to SEFV-H.

The incidence of TTS in Spain is between 1 case/1 000 000 and 1 case/100 000 doses administered, a similar rate to those reported in other countries in our setting for both the AstraZeneca and the Janssen vaccine,[19—22] except among patients younger than 30 years, probably due to the low rate of immunisation with these vaccines in that age group.[11,13] The reporting rate for TTS in other countries ranges between 0.3-1.2 cases/100 000 doses administered for the Janssen vaccine in the United States, and 1.28-1.53 cases/100 000 doses for the AstraZeneca vaccine in European countries, such

Table 4 Thrombosis location as a function of the vaccine administered and positivity for anti—platelet factor 4 antibodies.

Variable	Total series (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	*P*	Anti-PF4 + (n = 20)	Anti-PF4 — (n = 15)	*P*
CVST	15 (33.3%)	12 (32.4%)	3 (37.5%)	1.000	10 (50%)	5 (33.3%)	.492
SVT	12 (26.7%)	9 (24.3%)	3 (37.5%)	.661	5 (25%)	4 (26.7%)	1.000
PTE	18 (40%)	15 (40.5%)	3 (37.5%)	1.000	8 (40%)	7 (46.7%)	.741
DVT	7 (15.6%)	6 (16.2%)	1 (12.5%)	1.000	2 (10%)	3 (20%)	.631
Cerebral haemorrhage	12 (26.7%)	8 (21.6%)	4 (50%)	.181	8 (40%)	3 (20%)	.281
Peripheral arterial embolism	4 (8.9%)	3 (8.1%)	1 (12.5%)	.557	2 (10%)	1 (6.7%)	1.000
Ischaemic stroke	4 (8.9%)	1 (2.7%)	3 (37.5%)	.014	4 (20%)	0 (0%)	.119
Other	4 (8.9%)	4 (10.8%)	0 (0%)	1.000	1 (5%)	1 (6.7%)	1.000
Multiple thromboses	16 (35.6%)	10 (31.3%)	4 (50%)	.427	8 (40%)	6 (40%)	1.000

Anti-PF4: anti—platelet factor 4 antibodies; CVST: cerebral venous sinus thrombosis; DVT: deep vein thrombosis; PTE: pulmonary thromboembolism; SVT: splanchnic vein thrombosis. Data were compared using the Fisher exact test; quantitative and qualitative variables were compared with the *t* test (normally distributed variables, expressed as mean [standard deviation]) or the Mann-Whitney U test (non—normally distributed variables, expressed as median [Q_1-Q_3]).

as the United Kingdom.[19—22] Notification of adverse events related to vaccines is the most agile and universal process for identifying potential new risks. The main limitation of pharmacovigilance systems is undernotification, as only those cases that are reported by healthcare professionals or members of the public are received and processed. However, while notification presents a series of limitations, it seems unlikely that physicians would fail to report such severe and distinctive cases, which received considerable media attention and were disseminated by AEMPS, the Spanish Ministry of Health, the autonomous communities, and FACME. Therefore, the cases reported and described in this study can be equated, with a high degree of confidence, to the real incidence rate.

The risk of TTS associated with non-replicating adenoviral vector vaccines is greater in younger patients. Since the first analyses by the European Medicines Agency (EMA)[3,11] and the United States Food and Drug Administration (FDA),[10] the number of cases of CVST observed was reported to exceed the number of cases expected in the general population; this observation is replicated in the present series and in other studies.[23—25] This risk is particularly great among the younger population; as a result, the availability of other vaccines using RNA technology, with no evidence of a risk of TTS, led to a modification in the vaccination strategy, and non-replicating adenoviral vector vaccines were no longer recommended at younger ages.[13]

After pharmacovigilance systems were notified of the first cases of TTS following administration of the first dose of the AstraZeneca vaccine, the effect of the second dose was unknown. However, our data and studies from other countries[25,26] show that the risk of TTS following the second dose is significantly lower, with an incidence of 0.3 cases/100 000 doses administered for the AstraZeneca vaccine in the United Kingdom.[25] Negativisation of anti-PF4 antibodies after the first dose of the AstraZeneca vaccine is reported to take a median of 12 weeks.[27] This may have implications in countries where other vaccines are not available. The first series of TTS survivors who received a second dose of the vaccine includes no cases of new thrombotic events or thrombocytopenia following re-exposure.[28]

A noteworthy characteristic of TTS is the delay in symptom onset.[29,30] Adverse events in the first days after vaccination are reported in a very high percentage of patients; they tend to be mild and transient, and to resemble those observed with other vaccines.[31—33] However, delayed onset appears to be a distinguishing feature of TTS, as some minimum time is needed for the responsible antibodies to be generated. Ninety-three percent of patients presented TTS within 3-30 days, the timeframe initially proposed as the period of greatest risk.[15] In our series, symptom onset occurred earlier than this in one patient, and beyond 30 days after vaccination in 2; all 3 were negative for anti-PF4 antibodies.

This is the first study to evaluate the precision of the main laboratory parameters, platelet count and D-dimer level. Up to one-fifth of patients may not present thrombocytopenia when they visit the emergency department, and the platelet values established as a cut-off point in the World Health Organization guidelines[15] appear to present low sensitivity (below 50%), both at the emergency department and as the nadir value. However, D-dimer level appears to be more sensitive; therefore, strong clinical suspicion and combined evaluation of both parameters may improve diagnostic accuracy.[9,30] This is particularly important in the light of the potential delay in the availability of anti-PF4 antibody results, which may take one or more days, meaning that this information cannot always be taken into account when selecting the optimal treatment and management for each patient.[9,15,34,35]

Treatment of this syndrome requires the elimination of anti-PF4 antibodies and the administration of anticoagulation therapy.[8,17,18] Early detection has reduced mortality rates,[36] which have decreased from 40% to 50% in early cases to 10% in cases detected after the creation of management recommendations and public awareness campaigns.[37] The syndrome sometimes presents with headache with alarm signs, in addition to the other characteristic features, including positivity for anti-PF4 antibodies, in the absence of clear cerebral thrombosis.[38—41] It should be noted that, in our sample, headache was the most common initial symptom, and was described by patients with thromboses in locations outside the brain. In our study, presence/absence of intracranial haemorrhage and platelet count were associated with differences in mortality risk, as reported by other authors,[30,37,42] who also report fibrinogen level and age.[42]

Given the similarity between TTS and heparin-induced thrombocytopenia syndrome, the use of non-heparin anticoagulants is recommended.[6,9,15] However, use of heparin anticoagulants was not associated with higher mortality in our series. Recent data indicate that, in 95% of patients, heparin competes with anti-PF4 antibod-

Table 5 Treatments administered and outcome, as a function of the vaccine and presence/absence of anti—platelet factor 4 antibodies.

Variable	Total cases (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	*P*	Anti-PF4 + (n = 20)	Anti-PF4 — (n = 15)	*P*
IVIG	19 (42.7%)	13 (35.1%)	6 (75%)	.055	13 (65%)	5 (33.3%)	.092
Heparin anticoagulants	8 (17.8%)	7 (18.9%)	1 (12.5%)	1.000	3 (15%)	4 (26.7%)	.672
Non-heparin anticoagulants	29 (64.4%)	22 (59.5%)	7 (87.5%)	.226	16 (80%)	10 (66.7%)	.451
Platelet transfusion	2 (4.4%)	1 (2.7%)	1 (12.5%)	.327	2 (10%)	0 (0%)	.496
Steroids	9 (20%)	7 (18.9%)	2 (25%)	.651	5 (25%)	3 (20%)	1.000
Mortality	11 (24.4%)	8 (21.6%)	3 (37.5%)	.382	7 (35%)	2 (13.3%)	.244

IVIG: intravenous immunoglobulins; PF4: platelet factor 4. Data were compared using the Fisher exact test; quantitative and qualitative variables were compared with the *t* test (normally distributed variables, expressed as mean [standard deviation]) or the Mann-Whitney U test (non—normally distributed variables, expressed as median [Q_1-Q_3]).

ies for the same binding site on platelet factor 4; this supports the safety of the treatment.[43] Due to the considerable delay in receiving results of the platelet function tests needed to confirm diagnosis, non-heparin anticoagulants are recommended as the option of first choice, if they are available.[15,35]

This study has certain limitations, as the data are taken from spontaneous notifications, and may therefore be incomplete. To minimise the impact of this problem, we made conservative estimates and conducted an intention-to-treat analysis. The reporting rate may be underestimated, with some cases not being reported or diagnosed; however, we consider this unlikely in the light of the great media attention that this complication attracted. The follow-up period was variable, and some patients were still recovering at the time of study inclusion; therefore, the data on prognosis may not fully reflect the reality of this population.

Conclusions

The incidence of TTS in Spain is between 1 case/1 000 000 and 1 case/100 000 people immunised with non-replicating adenoviral vector vaccines. It is more frequent in young adults (< 49 years), and is characterised by the appearance of symptoms related to the localisation of the thrombosis, with onset approximately 10 days after vaccination. All patients present thrombocytopenia at some point in disease progression, although this may not be observed at the time of their visit to the emergency department. In these cases, D-dimer level may be valuable in guiding diagnosis. Treatment should aim to eliminate anti-PF4 antibodies and resolve thrombosis, preferably through the use of non-heparin anticoagulants. One-quarter of patients died, and increased risk of death was associated with severe thrombocytopenia and presence of intracranial haemorrhage.

Funding

This study received no funding of any kind.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We are grateful to the healthcare professionals and members of the public who reported cases of adverse events, and the invaluable work and dedication of staff at the regional offices of the Spanish Pharmacovigilance System.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.nrleng.2024.10.001.

References

1. Grupo de Trabajo Técnico de Vacunación COVID-19, de la Ponencia de Programa y Registro de Vacunaciones. Estrategia de vacunación frente a COVID- 19 en Espan˜a. Versión 1, 2 de diciembre 2020. [Accessed 15 October 2021]. Available from: https://www.mscbs.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/covid19/docs/COVID-19_EstrategiaVacunacion.pdf.
2. División de Farmacoepidemiología y Farmacovigilancia. Agencia Española del Medicamento y Productos Sanitarios. Vigilancia de la Seguridad de las vacunas frente a la COVID-19, Versión 7, 25 de enero de 2021. [Accessed 15 October 2021]. Available from: https://www.aemps.gob.es/medicamentosUsoHumano/vacunas/docs/vigilancia_seguridad_vacunas_COVID-19.pdf?x11028.
3. European Medicines Agency. 29 March 2021 update. COVID-19 vaccine safety update VAXZEVRIA AstraZeneca AB. [Accessed 22 April 2021]. Available from: https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-vaxzevria-previously-covid-19-vaccine-astrazeneca-29-march-2021_en.pdf.
4. Schultz NH, Sorvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021;384:2124—30, http://dx.doi.org/10.1056/NEJMoa2104882.
5. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384:2092—101, http://dx.doi.org/10.1056/NEJMoa2104840.
6. FACME, multidisciplinary working group on the management of cerebral venous sinus thrombosis associated with COVID-19 vaccination. Diagnostic and treatment recommendations from the FACME ad-hoc expert working group on the management of cerebral venous sinus thrombosis associated with COVID-19 vaccination. Neurologia (Engl Ed). 2021;36:451—61, http://dx.doi.org/10.1016/j.nrl.2021.05.001.
7. Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021;384:2202—11, http://dx.doi.org/10.1056/NEJMoa2105385.
8. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after ''COVID-19 vaccine AstraZeneca'' exposure. J Clin Med. 2021;10:1599, http://dx.doi.org/10.3390/jcm10081599.
9. Thakur KT, Tamborska A, Wood GK, McNeill E, Roh D, Akpan IJ, et al. Clinical review of cerebral venous thrombosis in the context of COVID-19 vaccinations: evaluation, management, and scientific questions. J Neurol Sci. 2021;427:117532, http://dx.doi.org/10.1016/j.jns.2021.117532.
10. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325:2448—56, http://dx.doi.org/10.1001/jama.2021.7517.
11. European Medicines Agency. 14 April 2021 update. COVID-19 vaccine safety update VAXZEVRIA AstraZeneca AB. [Accessed April 2021]. Available from: https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-vaxzevria-previously-covid-19-vaccine-astrazeneca-14-april-2021_en.pdf.
12. Agencia Española de Medicamentos y Productos Sanitarios. Nota de seguridad 1, 7 de abril de 2021. Vaxzevria (vacuna frente a la COVID-19 de AstraZeneca): actualización sobre el riesgo de trombosis. [Accessed 15 October 2021]. Available from: https://www.aemps.gob.es/informa/notasInformativas/medicamentosUsoHumano/seguridad/2021/docs/NI_MUH_FV-04-2021-AZ-actualizacion.pdf?x11028.
13. Grupo de Trabajo Técnico de Vacunación COVID-19, de la Ponencia de Programa y Registro de Vacunaciones. Estrategia de vacunación frente a COVID- 19 en Espan˜a. Actualización 6, 20 de abril de 2021. [Accessed 15 October 2021]. Available from: https://www.mscbs.gob.es/profesionales/saludPublica/prev

Promocion/vacunaciones/covid19/docs/COVID-19_Actualizacion6_EstrategiaVacunacion.pdf.
14. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. STROBE initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007;4:e297, http://dx.doi.org/10.1371/journal.pmed.0040297.
15. World Health Organization. Guidance document for clinical case management of thrombosis with thrombocytopenia syndrome (TTS) following coronavirus disease (COVID-19) vaccination. Interim guidance 1 July 2021. [Accessed 15 October 2021]. Available from: https://apps.who.int/iris/bitstream/handle/10665/342999/WHO-2019-nCoV-TTS-2021.1-eng.pdf.
16. Registro de Vacunación (REGVACU) del Ministerio de Sanidad. [Accessed 15 October 2021]. Available from: https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/vacunaCovid19.htm.
17. Mahaux O, Bauchau V, Van Holle L. Pharmacoepidemiological considerations in observed-to-expected analyses for vaccines. Pharmacoepidemiol Drug Saf. 2016;25:215—22, http://dx.doi.org/10.1002/pds.3918.
18. Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana (Fisabio) [Accessed 15 Oct 2021]. Available from: http://fisabio.san.gva.es/datos-basicos.
19. Abbattista M, Martinelli I, Peyvandi F. Comparison of adverse drug reactions among four COVID-19 vaccines in Europe using the EudraVigilance database: thrombosis at unusual sites. J Thromb Haemost. 2021;19:2554—8, http://dx.doi.org/10.1111/jth.15493.
20. Cari L, Fiore P, Naghavi Alhosseini M, Sava G, Nocentini G. Blood clots and bleeding events following BNT162b2 and ChAdOx1 nCoV-19 vaccine: an analysis of European data. J Autoimmun. 2021;122:102685, http://dx.doi.org/10.1016/j.jaut.2021.102685.
21. Huh K, Na Y, Kim YE, Radnaabaatar M, Peck KR, Jung J. Predicted and observed incidence of thromboembolic events among Koreans vaccinated with ChAdOx1 nCoV-19 vaccine. J Korean Med Sci. 2021;36:e197, http://dx.doi.org/10.3346/jkms.2021.36.e197.
22. Taquet M, Husain M, Geddes JR, Luciano S, Harrison PJ. Cerebral venous thrombosis and portal vein thrombosis: a retrospective cohort study of 537,913 COVID-19 cases. EClinicalMedicine. 2021;39:101061, http://dx.doi.org/10.1016/j.eclinm.2021.101061.
23. Pottegard A, Lund LC, Karlstad O, Dahl J, Andersen M, Hallas J, et al. Arterial events, venous thromboembolism, thrombocytopenia and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study. BMJ. 2021;373:n1114, http://dx.doi.org/10.1136/bmj.n1114.
24. Krzywicka K, Heldner MR, Sánchez van Kammen M, van Haaps T, Hiltunen S, Silvis SM, et al. Post-SARS-CoV-2-vaccination cerebral venous sinus thrombosis: an analysis of cases notified to the European Medicines Agency. Eur J Neurol. 2021;28:3656—62, http://dx.doi.org/10.1111/ene.15029.
25. Medicines & Healthcare products Regulatory Agency. Coronavirus vaccine-weekly summary of Yellow Card reporting. Updated 2 December 2021. [Accessed 3 December 2021]. Available from: https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting.
26. Bhuyan P, Medin J, da Silva HG, Yadavalli M, Shankar NK, Mullerova H, et al. Very rare thrombosis with thrombocytopenia after second AZD1222 dose: a global safety database analysis. Lancet. 2021;398:577—8, http://dx.doi.org/10.1016/S0140-6736(21)01693-7.
27. Schönborn L, Thiele T, Kaderali L, Greinacher A. Decline in pathogenic antibodies over time in VITT. N Engl J Med. 2021;385:1815—6, http://dx.doi.org/10.1056/NEJMc2112760.
28. Lacy J, Pavord S, Brown KE. VITT and second doses of Covid-19 vaccine. N Eng J Med. 2022;386:95, http://dx.doi.org/10.1056/NEJMc2118507.
29. García-Azorín D, Do TP, Gantenbein AR, Hansen JM, Souza MNP, Obermann M, et al. Delayed headache after COVID-19 vaccination: a red flag for vaccine induced cerebral venous thrombosis. J Headache Pain. 2021;22:108, http://dx.doi.org/10.1186/s10194-021-01324-5.
30. Pavord S, Scully M, Hunt BJ, Lester W, Bagot C, Craven B, et al. Clinical features of vaccine-induced thrombocytopenia and thrombosis. N Engl J Med. 2021;385:1680—9, http://dx.doi.org/10.1056/NEJMoa2109908.
31. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397:99—111, http://dx.doi.org/10.1016/S0140-6736(20)32661-1.
32. Abu-Halaweh S, Alqassieh R, Suleiman A, Al-Sabbagh MQ, AbuHalaweh M, AlKhader D, et al. Qualitative assessment of early adverse effects of Pfizer-BioNTech and Sinopharm COVID-19 vaccines by telephone interviews. Vaccines (Basel). 2021;9:950, http://dx.doi.org/10.3390/vaccines9090950.
33. CDC COVID-19 Response TeamFood and Drug Administration. Allergic reactions including anaphylaxis after receipt of the first dose of Pfizer-BioNTech COVID-19 vaccine — United States, December 14—23, 2020. MMWR Morb Mortal Wkly Rep. 2021;70:46—51, http://dx.doi.org/10.15585/mmwr.mm7002e1.
34. Sachs UJ, Cooper N, Czwalinna A, Müller J, Pötzsch B, Tiede A, et al. PF4-dependent immunoassays in patients with vaccine-induced immune thrombotic thrombocytopenia: results of an interlaboratory comparison. Thromb Haemost. 2021;121:1622—7, http://dx.doi.org/10.1055/a-1535-9002.
35. Greinacher A, Langer F, Makris M, Pai M, Pavord S, Tran H, et al. Vaccine-induced immune thrombotic thrombocytopenia (VITT): update on diagnosis and management considering different resources. J Thromb Haemost. 2021;20:149—56, http://dx.doi.org/10.1111/jth.15572.
36. Thaler J, Jilma P, Samadi N, Roitner F, Mikušková E, Kudrnovsky-Moser S, et al. Long-term follow-up after successful treatment of vaccine-induced prothrombotic immune thrombocytopenia. Thromb Res. 2021;207:126—30, http://dx.doi.org/10.1016/j.thromres.2021.09.017.
37. van de Munckhof A, Krzywicka K, Aguiar de Sousa D, Sánchez van Kammen M, Heldner MR, Jood K, et al. Declining mortality of cerebral venous sinus thrombosis with thrombocytopenia after SARS-CoV-2 vaccination. Eur J Neurol. 2021;29:339—44, http://dx.doi.org/10.1111/ene.15113.
38. Salih F, Schönborn L, Kohler S, Franke C, Möckel M, Dörner T, et al. Vaccine-induced thrombocytopenia with severe headache. N Engl J Med. 2021;385:2103—5, http://dx.doi.org/10.1056/NEJMc2112974.
39. Kennedy VE, Wong CC, Hong JM, Peng T, Brondfield S, Reilly LM, et al. VITT following Ad26.COV2.S vaccination presenting without radiographically demonstrable thrombosis. Blood Adv. 2021;5:4662—5, http://dx.doi.org/10.1182/bloodadvances.2021005388.
40. Lavin M, Elder PT, O'Keeffe D, Enright H, Ryan E, Kelly A, et al. Vaccine-induced immune thrombotic thrombocytopenia (VITT) — a novel clinico-pathological entity with heterogeneous clinical presentations. Br J Haematol. 2021;195:76—84, http://dx.doi.org/10.1111/bjh.17613.
41. Waraich A, Williams G. Haematuria, a widespread petechial rash, and headaches following the Oxford AstraZeneca ChA-

dOx1 nCoV-19 vaccination. BMJ Case Rep. 2021;14:e245440, http://dx.doi.org/10.1136/bcr-2021-245440.
42. Hwang J, Park SH, Lee SW, Lee SB, Lee MH, Jeong GH, et al. Predictors of mortality in thrombotic thrombocytopenia after adenoviral COVID-19 vaccination: the FAPIC score. Eur Heart J. 2021;42:4053—63, http://dx.doi.org/10.1093/eurheartj/ehab592.
43. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia. Nature. 2021;596:565—9, http://dx.doi.org/10.1038/s41586-021-03744-4.