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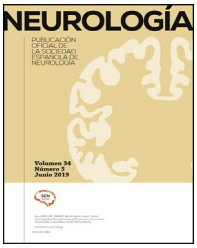

ORIGINAL

Thrombosis syndrome with thrombocytopenia associated with adenovirus vaccines against to COVID-19: Epidemiology and clinical presentation of the Spanish series6

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KEY WORDS
COVID-19;
Adverse drug effects and adverse reactions;

Summary
Introduction: We describe the epidemiological and clinical characteristics of cases of thrombosis syndrome with thrombocytopenia (TTS) reported in Spain.
Methods: Cases of venous or arterial thrombosis with thrombocytopenia following receipt of non-replicating adenovirus vector vaccine against COVID-19 (AstraZeneca and Janssen) from 1 February to 26 September 2021 *w e r e* included. Reporting rate (number of reported cases/number of doses administered), and observed vs. expected (O/E) case analysis are described. Predictors of mortality were assessed.

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Vaccinations; Embolism and thrombosis; Platelet disorders

Results: 61 cases were reported, 45 fulfilling the eligibility criteria, 82% female. The overall reporting rate was 4/1,000,000 doses and 14-15/1,000,000 doses among 30-49 year olds. The number of cases of cerebral sinus thrombosis observed was 6-18 times higher than expected in those under 49 years of age. Symptoms began 10 (interquartile range: 7-14) days after vaccination. Eighty per cent (95% confidence interval [CI] 65-90%) had thrombocytopenia at the time of their ED visit and 65% (95% CI 49-78%) had elevated D-dimer (>2000 ng/mL). Thrombosis was multi-site in 36% and fatal in 24% of patients. A nadir thrombocytopenia value < 50,000 /µL (*odds ratio* [OR]: 7.4; 95% CI: 1.2-47.5) and the presence of cerebral haemorrhage (OR: 7.9; 95% CI: 1.3-47.0) were associated with a fatal outcome. *Conclusions:* STT should be suspected in patients who present with symptoms about 10 days after vaccination and have thrombocytopenia and/or elevated D-dimer.

KEYWORDS
MeSH Terms;
COVID-19;
Drug-related side effects and adverse reactions; Vaccines;
Embolism and thrombosis;
Blood platelet disorders

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

Abstract
Background: We describe the epidemiological and clinical characteristics of thrombosis with thrombocytopenia syndrome (TTS) cases reported in Spain.
Methods: We included all venous or arterial thrombosis with thrombocytopenia following adenovirus vector-based vaccines (AstraZeneca or Janssen) to prevent COVID-19 disease between February 1st and September 26th , 2021. We describe the crude rate and the standardised morbidity ratio. We assessed the predictors of mortality.
Results: Sixty-one cases were reported and 45 fulfilled eligibility criteria, 82% women. The crude TTS rate was 4/1,000,000,000 doses and 14-15/1,000,000 doses between 30-49 years. The number of observed cases of cerebral venous thrombosis was 6-18 higher than the expected in patients younger than 49 years. Symptoms started 10 (interquartile range [IQR]: 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% (95% CI: 49-78%) had D-dimer >2,000 ng/mL. Patients had multiple location thrombosis in 36% and fatal outcome in 24% cases. A platelet nadir < 50,000/µL (odds ratio [OR]: 7.4; 95% CI: 1.2-47.5) and intracranial haemorrhage (OR: 7.9; 95% CI: 1.3-47.0) were associated with fatal outcome.
Conclusion: TTS must be suspected in patients with symptoms 10 days after vaccination and thrombocytopenia and/or D-dimer increase.

Introduction

The first vaccine against coronavirus disease virus 2019 (COVID-19), BNT162b2 (Comirnaty® , Pfizer BioNTech), was licensed on 21 December 2020 and the vaccination campaign began immediately in Spain in accordance with the vaccination strategy approved by the Interterritorial Council of the National Health System (Consejo Interterritorial del Sistema Nacio- nal de Salud[1] . On 29 January 2021 and 11 March 2021, the ChAdOx1-S nCov-19 vaccine (Vaxzevria® , AstraZeneca) and the Ad26.COV2-S vaccine (Janssen), respectively, were authorised. In parallel to their licensing, a safety surveillance plan for these vaccines was put in place[2] which, among other actions, included a comprehensive clinical review of clinically complex adverse events reported to the Spanish Pharmacovigilance System for Medicinal Products for Human Use (SEFV-H).

On 7 March 2021 the first two cases of STT in patients who had received Astra-Zeneca's vaccine were reported in Europe, specifically in Austria, and on 14 March 2021 the first case in Spain[3–5] . As a matter of urgency, the Spanish Agency for Medicines and Health Products (AEMPS) set up a multidisciplinary group of experts to evaluate these cases, monitor the reports registered in Spain and subsequently draw up specific guidelines with diagnostic and therapeutic recommendations on this serious adverse event[6] .

Thrombosis syndrome with thrombocytopenia (TTS) associated with vaccination is characterised by the presence of single or multiple thromboses, mainly venous but also arterial, with some predilection for affecting unusual locations, such as the splanchnic territory or cerebral venous sinuses[4,5,7–9] . The presence of antiplatelet factor 4 (anti-PF4) antibodies leads to aggregation of platelets.

platelet aggregation and micro- and macrothrombosis, causing marked thrombocytopenia and the characteristic thrombotic manifestations of the syndrome[8] . TTS has been associated with non-replicating adenovirus vector vaccines, such as the AstraZeneca vaccine or the Janssen vaccine[4–8,10] . The recognition of this clinical entity as a possible adverse reaction of adenovirus vaccines[11] was communicated by the AEMPS on 7 April 2021[12] and led to changes in the vaccination strategy in order to minimise this risk, such as limiting its use in younger age groups - where the risk seemed higher - and avoiding a second dose of AstraZeneca in this age group[13] . Shortly thereafter, on 26 May 2021, a working group of experts from the Federation of Spanish Scientific and Medical Associations (FACME) published diagnostic and therapeutic recommendations on the management of cerebral venous thrombosis related to COVID-19 vaccination[6] .

The aim of this article is to describe the epidemiological and clinical characteristics of all cases of TTS reported in Spain from 1 February to 26 September 2021 associated with SARS-CoV-2 non-replicating ade- novirus vector vaccines. These vaccines will be specifically studied, as they are the ones for which an association was observed and for which an alert situation was established by different national and international organisations. The demographic pattern of incident cases during the vaccination campaign (until 26 September 2021), the notification rate and the analysis of observed vs. expected cases are described. From the clinical point of view, the frequency of comorbidities, clinical presentation, laboratory findings, treatment used and prognosis are detailed. As exploratory objectives, the sensitivity of laboratory parameters at the time of presentation of patients in the ED and the variables that could be associated with a higher probability of death are analysed.

Methods

Observational, descriptive, case series design study conducted according to STROBE recommendations[14] . The study population consisted of patients who had suffered a venous or arterial thrombotic event within 100 days after administration of a non-replicating adenovirus vector vaccine against SARS-CoV- 2, the virus that causes COVID-19. The study population consisted of all cases with suspected diagnosis of TTS after administration of these vaccines reported to the SEFV- H, coordinated by the AEMPS. The study protocol was approved by the Medical Research Ethics Committee of the Hospital Clínico Universitario de Valladolid (PI 21-2450).

Eligibility criteria

The inclusion criteria, based on the World Health Organisation (WHO) and *Brighton Collaboration* criteria[15] were: 1) presence of at least one arterial and/or venous thrombosis; 2) presence of thrombocytopenia (defined as less than 150,000 platelets/µL or less than 150,000 platelets/µL); 3) presence of thrombocytopenia (defined as less than 150,000 platelets/µL or less than 150,000 platelets/µL). a decrease from baseline of more than 50%) at some point in their evolution; 3) exposure to a non-replicating adenovirus vector vaccine against COVID-19 within the previous 100 days. Cases were excluded if, in the opinion of the expert panel, they could be explained by another cause, and cases whose reported diagnosis was erroneous, equivocal, or lacked the necessary information.

Intervention

For the epidemiological analysis, we estimated the notification rate, defined as the number of cases with this adverse event in relation to the number of doses administered in the time period under study, classified by age, sex and number of doses administered (first or second). The number of doses administered per vaccine, age and sex was obtained from the Vaccination Register (REGVACU) of the Ministry of Health[16] . The analysis of observed (reported) cases in the population and over time, compared to the number of cases expected in the general population, was carried out following the methodology described at[17] globally and by age group. For this purpose, all cases were considered to have been reported - the most conservative assumption - and a risk window of 30 days was estimated, the period in which most of the registered cases made their debut. A ratio greater than unity indicates a numerical disproportion between observed and expected cases in the unvaccinated population, which is statistically s i g n i f i c a n t if the lower limit of the confidence interval (CI) exceeds 1. The calculation of observed/expected cases was performed only for cerebral venous sinus thrombosis (CSVT) as this is the only clinical entity that integrates the STT for which there is epidemiological information on its baseline incidence in the general unvaccinated population, from the hospital data of the FISABIO database[18] of the Valencian Community, there being no data on the incidence of other thromboses in atypical locations in our environment.

For the study of demographic, clinical and clinical characteristics, it is necessary to

The information available in the reported cases, anonymised at source, was reviewed and demographic data were analysed: sex, age, risk factors for thrombosis (Additional material 1) and history of COVID-19 infection; the type of vaccine administered and the number of doses; clinical data: presence of COVID-19 infection concomitant with STT, days from vaccination to onset of symptoms, days from onset of symptoms to diagnosis, presenting symptoms, location of thrombosis and, in cases with CVST, presence of headache at some point in the course, presence of intracranial haemorrhage and presence of alarm data (Additional material 2); analytical data: platelets at ED visit and nadir, D-dimer at ED visit and highest value, fibrinogen on admission and result of anti-PF4 antibody determination; treatment administered: immunoglobulins, heparin anticoagulant, non-heparin anticoagulant, platelet transfusion, steroid treatment[15] ; and prognosis: outcome of the case classified as recovered, fatal or unknown.

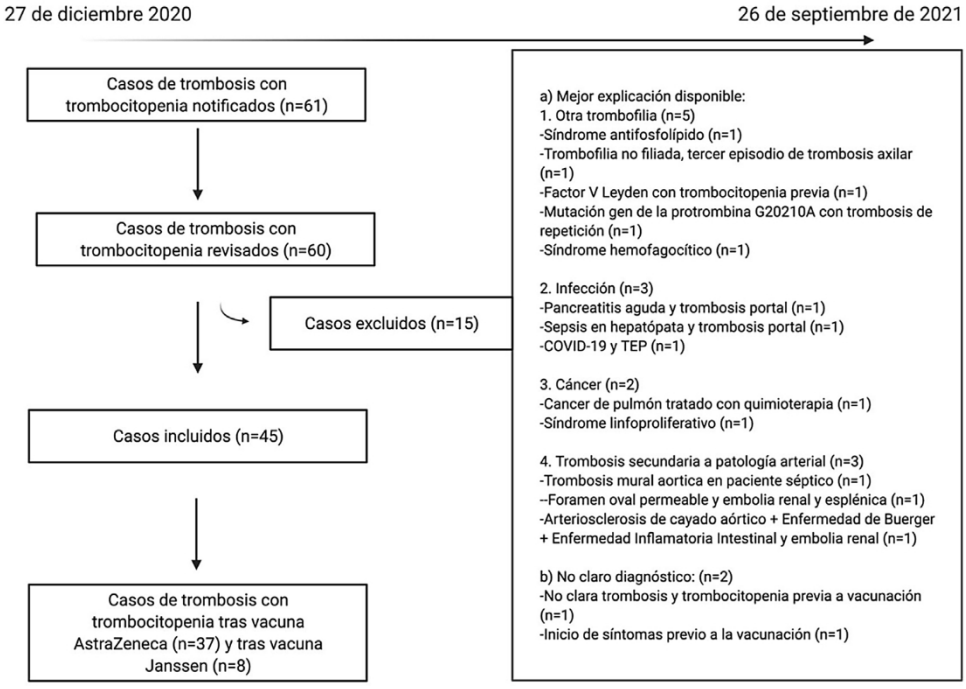

Figure 1 Reported, reviewed, excluded and included cases. There was one case reported and not reviewed as no information was available.

Study period

The study period covers the period from the date of administration of the first dose of non-replicating adenovirus vector vaccine against COVID-19 administered in Spain, from 1 February 2021 until 26 September 2021, when the administration of these vaccines became testimonial as most of the population eligible to receive them had been vaccinated.

Statistical analysis

A descriptive analysis was performed where qualitative and ordinal quantitative variables are presented as frequency and percentage. Continuous quantitative variables are presented as mean and standard deviation (SD) or median and interquartile range (IQR) according to their distribution. For the estimation of the number of cases, the 95% confidence interval (95% CI) was calculated. Fisher's exact test was used to compare qualitative or ordinal variables and the Student's *t-test* or Mann-Whitney U-test was used to test hypotheses between quantitative and qualitative variables depending on the distribution. An alpha error of 5% was accepted.

A subgroup comparison was made between patients vaccinated with the AstraZeneca vaccine vs. vaccinated with the Janssen vaccine, and between patients with positive anti-PF4 antibodies vs. patients with negative anti-PF4 antibodies, excluding those patients without this data available. Only patients who tested positive by ELISA or platelet functional study were considered positive. The sensitivity of the laboratory parameters was estimated using the following cut-off points, recommended in clinical practice guidelines[6,9,15] : 50,000, 100,000 and 150,000 PLATELETS/μL; 2,000 and 4,000 ng/mL D-dimer.

For the evaluation of variables associated with an increased risk of mortality, a logistic regression analysis was performed: firstly, univariate; and secondly, multivariate, including in the model those variables with a p value < 0.1 in the univariate analysis, presenting the OR with its 95% CI. Missing data were handled by analysis of complete cases.

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

Results

During the study period, 61 cases originally diagnosed with STT and attributed by their reporters to non-replicating adenovirus vector vaccines were reported to SEFV-H. Figure 1 represents the number of cases reported, reviewed, excluded and included. Figure 1 represents the number of cases reported, reviewed, excluded and included. Finally, 45 patients were included and analysed, 37 after vaccination with AstraZeneca and eight after vaccination with Janssen.

Analysis of observed cases vs. expected and notification rate

As of 26 September 2021, 9,771,767 doses of AstraZeneca's vaccine had been administered (5,103,885 first doses and

4,667,882 second doses) and 1,959,146 doses of AstraZeneca's vaccine had been administered by 26 September 2021.

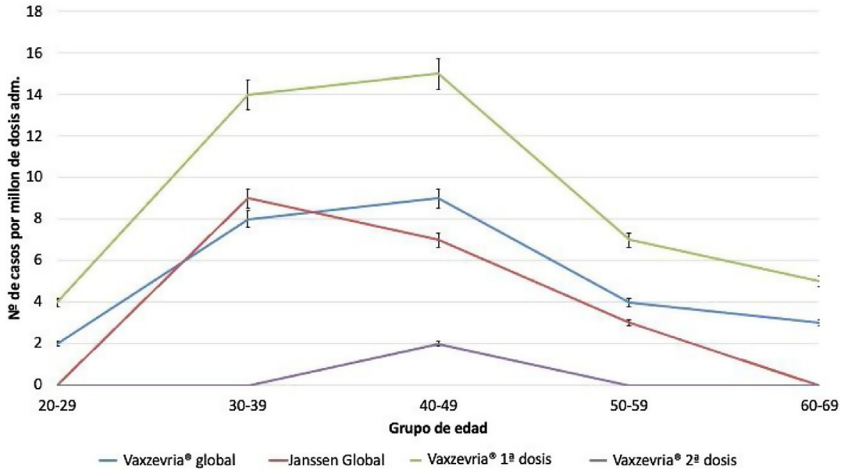

Figure 2 STT notification rate by vaccine administered and by age group, per million doses administered.

Janssen vaccine. The estimated overall notification rate was four cases/1,000,000 of vaccines administered with both AstraZeneca and Janssen, being higher in the 30-49 age group, where the rate is between eight and nine cases/1,000,000 doses administered of AstraZeneca. For the first dose of AstraZe- neca, the reporting rate of TST in the 30-39 and 40-49 age groups was 14 and 15 cases/1,000,000 population, respectively, while for the second dose it was zero and two cases/1,000,000 population, respectively (fig. 2).

Analysis of observed versus expected cases of CVST showed a statistically significant disproportion for the age groups 30-49 years immunised with AstraZeneca and 40-49 years with the Janssen vaccine (table 1).

Demographic aspects

Table 2 summarises the main characteristics of the sample. Only 2.2% of the patients reported a previous history of infection with COVID-19. In 15 patients, PCR for SARS-CoV-2 was reported on admission and in three patients antigen testing was reported, with negative results in all cases. In the comparison between subgroups, according to the type of vaccine and the presence or absence of anti-PF4 antibodies, statistically significant differences were only observed for a lower median age in patients with anti-PF4 antibodies. The median time between vaccination and clinical presentation of the event was 11 days (RIQ: 7-14, range 1-60 days).

Clinical presentation

Clinical information was available for 36 (80%) of the 45 cases. Figure 3 shows the most common presenting symptoms. Seventeen (37.8%) of the 45 patients presented with headache at some point in their evolution, with alarm data in all cases. Figure 4 presents the observed frequency of alarm data.

Laboratory parameters

At the time of the ED visit, the median platelet count was 81,000/µL (RIQ: 40,500-127,000; range: 8,000-212,000) and the median D-dimer was 21,000 ng/mL (RIQ: 5,360-35,612; range: 130-60,000).

Figure 5 shows the platelet value as a function of the time between vaccination and ED visit. In the comparison between the different vaccines, statistically significant differences were observed in the platelet value at the time of the ED visit and in the presence of anti-PF4 antibodies (supplementary material table 1, figure 1). In the comparison between patients with anti-PF4 antibodies and all other patients, no statistically significant differences were found (supplementary material table 2, figures 2 and 3).

Table 3 shows the sensitivity analysis of the cut-off points suggested in international guidelines according to the thrombocytopenia and D-dimer value, both by protocol (analysis of available information) and by intention to treat (including those cases with no available value).

Location of thrombosis

In 16 (35.6%) patients there was thrombosis in multiple locations. There were no statistically significant differences in the location and type of thrombosis according to the type of vaccine (table 4).

Therapeutic management

Thirty (66.7%) patients received anticoagulation therapy: non-heparinic in 29 (64.4%) cases and heparinic in eight (17.8%) cases. Seven (15.6%) patients received both types of anticoagulants. Immunoglobulins were treated in 19 (42.7%) patients and steroids in nine (20%), with at least one of the two treatments being used in 22 (48.9%) patients. No statistically significant differences were observed according to the vaccine administered or the presence of antiplatelet factor 4 antibodies (table 5).

Table 1 Analysis of observed versus expected cases of cerebral venous sinus thrombosis with adenovirus vaccines. Overall data and by age group

	Risk window: 30 days	30-39 years	40-49 years	50-59 years	60-69 years	Global
Vaccine AstraZeneca	N° of cases	4 cases	4 cases	1 case	2 cases	12 cases
	O/E ratio (IC 95%)	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)
Vaccine Janssen	N° of cases	0	3 cases	0	0	3 cases
	O/E ratio (IC 95%)	-	5,27 (1,09-15,40)	-	-	1,64 (0,34-4,78)

O/E: observed versus expected; CI: confidence interval. Values in bold represent statistically significant values (Ratio greater than unity if the lower limit of the confidence interval (CI) exceeds 1[17] .

Table 2 Demographic and clinical presentation variables

Variable	All (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	p-value	Anti-PF4 + (n = 20)	Anti-PF4 - (n = 15)	p-value
Age (median, RIQ)	53 (45,5-62,5)	60 (45,5-63)	48 (42,2-52)	0,121	46,5 (37,2-58,2)	61 (48-63)	0,009
Female sex (n, %)	37 (82,2%)	20 (54,1%)	6 (75%)	0,435	12 (60%)	8 (53,3%)	0,741
AstraZeneca	37 (82,2%)	37 (100%)	0 (0%)	-	13 (65%)	14 (93,3%)	0,015
Risk factors of thrombosis	13 (28,9%)	12 (32,4%)	1 (12,5%)	0,405	5 (25%)	6 (40%)	0,467
Dose	1ª: 43 (95.6%) 2ª: 2 (4,4%)	1ª: 35 (94.6%) 2ª: 2 (5,4%)	1ª: 8 (100%)		1ª: 20 (100%)	1ª: 14 (93.3%) 2ª: 1 (6.7%)	0,429
Days since the vaccination at 1er symptom (median, RIQ) n = 39	11 (7-14)	10 (7-14)	12 (8,7-17,5)	0,312	10 (7,2-13,7)	11 (8-19)	0,364
Days from 1er symptom to the diagnosis (median, RIQ), n = 37	1 (0-7)	1 (0-7)	1 (0-2)	0,312	1,5 (0,2-6,2)	1 (0-2)	0,158

IQR, interquartile range; PF4, platelet factor 4. The AstraZeneca variable was included for comparison of anti-PF4 antibodies according to vaccine type. Assessed by Fisher's exact test, or in the contrast between quantitative and qualitative variables, Student's *t-test (*normally distributed variables, presented as mean and standard deviation) or Mann-Whitney U test (non-normally distributed variables, presented as median and interquartile range).

Eleven (24.4%) patients were managed in an apparently suboptimal manner for the following reasons: performance of non-contrast cranial CT scan despite the presence of headache with alarm data and thrombocytopenia in seven (15.5%) cases, administration of heparin despite the presence of anti-PF4 antibodies in two cases (4.4%), platelet transfusion in the absence of indication in one (2.2%) case, and empirical diagnosis of possible COVID-19 infection without diagnostic test in one (2.2%) case.

Outcome and predictors of mortality

Eleven (24.4%) patients died and in two (4.4%) patients no information was available. A platelet value < 50,000/μL at the time of the ED visit or at nadir and the presence of intracranial haemorrhage were the variables associated with a higher probability of death in the univariate analysis. In the multivariate analysis, the presence of intracranial haemorrhage (OR: 7.9; 95% CI: 1.32-47.0) and a nadir platelet count < $50{,}000/\mu L$ (OR: 7.4; 95% CI: 1.16-47.6) were associated with increased morbidity (supplementary material tables 3-5).

Discussion

This paper presents the epidemiological and clinical data of the series of cases of TTS in association with non-replicating adenovirus vector vaccines reported to SEFV-H.

Figure 3 Presenting symptoms (n = 36). The figure describes the initial symptoms and depicts all patients, both those with neurological and non-neurological thrombosis. There were patients with non-neurological thrombosis who presented with neurological symptoms, such as headache.

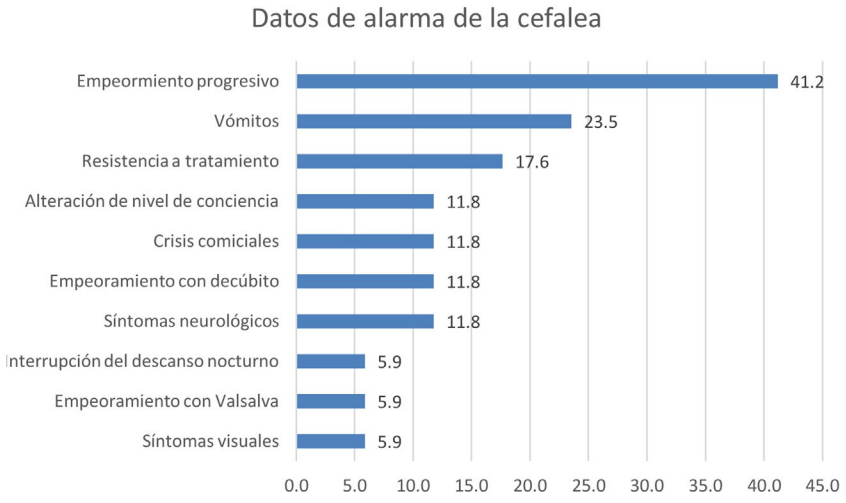

Figure 4 Headache alarm data (n = 17).

The frequency of reported cases of TST in Spain is between 1 case/1,000,000 and 1 case/100,000 doses administered, which is similar to other countries in the region, both for the AstraZeneca and Janssen vaccines[19—22], except in children under 30 years of age, probably due to the low immunisation rate with these vaccines in this age group[11,13]. The reporting rate of STT in other countries ranges from 0.3 to 1.02 cases/100,000 doses of the Janssen vaccine in the USA to 1.28 to 1.53 cases/100,000 doses after the AstraZeneca vaccine in European countries or the UK[19—22]. The reporting of adverse events with vaccines is the most rapid and universal procedure to identify potential new risks. The main limitation of pharmacovigilance is under-reporting, as only what is reported by healthcare professionals or citizens is received and processed. However, although reporting is subject to a number of limitations, it does not seem likely that such serious and typical cases would have been underreported due to their high media coverage and the work of dissemination of the AEMPS, the Ministry of Health of the Spanish Government, the Autonomous Communities and FACME. Therefore, the rates reported and described in this paper can be assimilated with a high degree of confidence to the actual incidence rate per number of vaccine doses administered.

The risk of TST associated with non-replicating adenovirus vector vaccines is highest in young people. Since the first analyses by the European Medicines Agency (EMA)[3,11] and the *Food and Drug Adminis- tration* (FDA)[10], it has been reported that the number of cases of CVST observed exceeded what would be expected in the general population, a fact that has been replicated in the present study and in other series[23—25]. This risk has been especially pronounced in young people, so the availability of other RNA vaccines, with no evidence of STT risk, led to a change in the vaccination strategy and the use of non-replicating adenovirus vector vaccines was no longer recommended for young people[13].

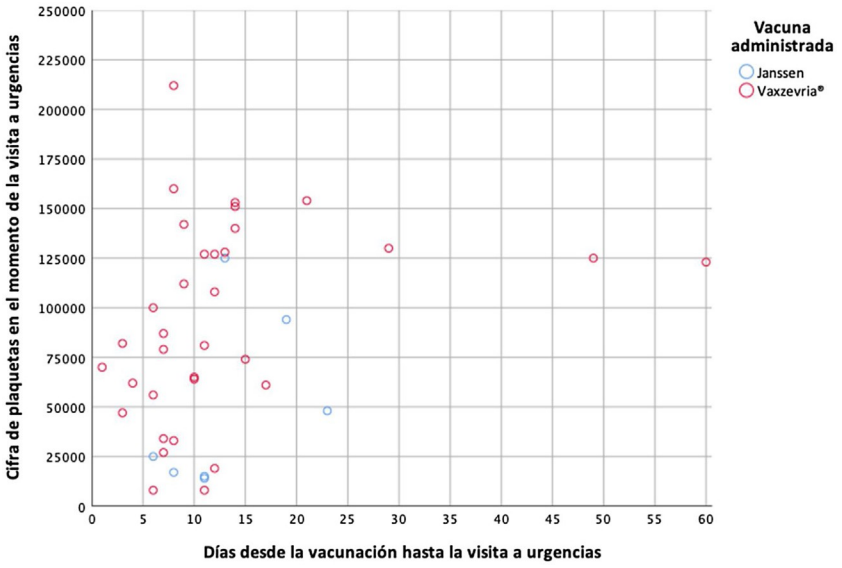

Figure 5 Platelet count at ED visit (Y-axis) as a function of time (in days) between vaccination and ED visit (X-axis). In blue, patients vaccinated with Janssen; in red, patients vaccinated with AstraZeneca.

Table 3 Sensitivity of platelet and D-dimer values in the ED and when the most extreme value (nadir and peak for platelets and D-dimer, respectively) is reached

Parameter	Moment	Type of analysis	Value	Sensitivity	95 % CI
Platelets	Emergencies	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	Emergencies	PP (n = 31)	2,000 ng/mL	93,5%	77,1-98,9
		ITT (n = 45)		64,4%	48,7-77,7
		PP (n = 31)	4,000 ng/mL	90,3%	73,1-97,4
		ITT (n = 45)		62,2%	46,5-75,8
	Peak	PP (n = 36)	2,000 ng/mL	94,4%	80,0-99,0
		ITT (n = 45)		75,6%	60,1-86,6
		PP (n = 36)	4,000 ng/mL	88,9%	73,0-96,4
		ITT (n = 45)		71,1%	55,5-83,1

PP: per-protocol analysis (only patients with available data); ITT: intention-to-treat analysis (including all patients and assuming that, if the data was not available, it was negative, which would indicate the minimum percentage of patients that would have this parameter).

After the first cases of TTS associated with the first dose of AstraZeneca were reported to pharmacovigilance systems, the effect of a second dose was unknown. However, based on our data and those from other countries[25,26] , the risk of TTS associated with this second dose appears to have been markedly lower, at around 0.3 cases/100,000 doses of AstraZeneca in the UK[25] . The median time to negativation of anti-PF4 antibodies in patients who developed anti-PF4 antibodies after the first dose of AstraZeneca has been reported to be 12 weeks[27] . This could have implications in countries where other vaccines are not available. Early series of TST survivors who have received a second dose of the same vaccine have not reported any new thrombotic events or thrombocytopenia after re-exposure[28] .

A unique feature of STT is the delayed onset of symptoms[29,30] . Adverse events in the first days after vaccination have been reported in a very high percentage of people, most of them mild, transient and similar to those observed with other vaccines[31-33] . However,

Table 4 Location of thrombosis according to type of vaccine administered and antiplatelet factor 4 antibody positivity

Variable	All (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	p-value	Anti-PF4 + (n = 20)	Anti-PF4 - Anti-PF4 (n = 15)	p-value
TSVC	15 (33,3%)	12 (32,4%)	3 (37,5%)	1.000	10 (50%)	5 (33,3%)	0,492
TE	12 (26,7%)	9 (24,3%)	3 (37,5%)	0,661	5 (25%)	4 (26,7%)	1.000
TEP	18 (40%)	15 (40,5%)	3 (37,5%)	1.000	8 (40%)	7 (46,7%)	0,741
DVT	7 (15,6%)	6 (16,2%)	1 (12,5%)	1.000	2 (10%)	3 (20%)	0,631
Brain haemorrhage	12 (26,7%)	8 (21,6%)	4 (50%)	0,181	8 (40%)	3 (20%)	0,281
Arterial embolism peripheral	4 (8,9%)	3 (8,1%)	1 (12,5%)	0,557	2 (10%)	1 (6,7%)	1.000
Ischaemic stroke	4 (8,9%)	1 (2,7%)	3 (37,5%)	0,014	4 (20%)	0 (0%)	0,119
Another	4 (8,9%)	4 (10,8%)	0 (0%)	1.000	1 (5%)	1 (6,7%)	1.000
Multiple	16 (35,6%)	10 (31,3%)	4 (50%)	0,427	8 (40%)	6 (40%)	1.000

CVST: cerebral venous sinus thrombosis; SVT: cerebral venous thrombosis; TE: splanchnic thrombosis; PE: pulmonary thromboembolism; DVT: pro-founding venous thrombosis; Anti-PF4: antiplatelet factor type 4 antibodies. Assessed by Fisher's exact test, or in the contrast between quantitative and qualitative variables, Student's *t-test* (normally distributed variables, presented as mean and standard deviation) or Mann-Whitney *U* test (non-normally distributed variables, presented as median and interquartile range).

Table 5 Treatments administered according to vaccine and presence or absence of antiplatelet factor 4 antibodies and outcome

Variable	All (n = 45)	AstraZeneca (n = 37)	Janssen (n = 8)	p-value	Anti-PF4 + (n = 20)	Anti-PF4 - (n = 15)	p-value
IGIV	19 (42,7%)	13 (35,1%)	6 (75%)	0,055	13 (65%)	5 (33,3%)	0,092
Anticoagulant heparin	8 (17,8%)	7 (18,9%)	1 (12,5%)	1.000	3 (15%)	4 (26,7%)	0,672
Anticoagulant no heparin	29 (64,4%)	22 (59,5%)	7 (87,5%)	0,226	16 (80%)	10 (66,7%)	0,451
Platelet transfusion	2 (4,4%)	1 (2,7%)	1 (12,5%)	0,327	2 (10%)	0 (0%)	0,496
Steroids	9 (20%)	7 (18,9%)	2 (25%)	0,651	5 (25%)	3 (20%)	1,000
Deadly outcome	11 (24,4%)	8 (21,6%)	3 (37,5%)	0,382	7 (35%)	2 (13,3%)	0,244

IVIG: intravenous immunoglobulins; PF4: platelet factor 4. Assessed by Fisher's exact test, or in the contrast between quantitative and qualitative variables, Student's *t-test* (normally distributed variables, presented as mean and standard deviation) or Mann-Whitney *U* test (non-normally distributed variables, presented as median and interquartile range).

the delayed onset of STT appears to be a distinctive clinical feature, as minimal time is required for the genesis of the causative antibodies. Ninety-three percent of patients presented in the interval between three and 30 days, initially proposed as the high-risk period[15] . In the present series, one case started with symptoms before this interval and in two others the picture appeared beyond 30 days, with negative anti-PF4 antibodies in all of them.

The present study is the first to assess the accuracy of the main laboratory parameters: platelet count and D-dimer. Up to one fifth of patients may not have thrombocytopenia at the time of their ED visit and the platelet parameters established as cut-off points by the WHO guidelines[15] appear to have a low sensitivity of less than 50% both at the time of the ED visit and at nadir. However, D-dimer appears to have a higher sensitivity, so a high index of suspicion and joint assessment of both parameters could improve diagnostic accuracy[9,30] . This is especially important because of the delay that the responsible physician may have to wait for the results of both tests.

anti-PF4 antibodies, which can vary from one to several days, which means that they cannot always be used when deciding on 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 anticoagulant therapy[8,17,18] . Early detection has led to a decrease in mortality[36] , from 40-50% in early cases to 10% in cases detected after the development of management recommendations and population-based campaigns[37] . It has been reported that some patients start with a headache with alarm data in combination with the other typical features of the syndrome, including anti-PF4 antibody positivity, in the absence of evident cerebral thrombosis[38—41] . Importantly, in our sample, headache was the most frequent presenting symptom, and was described by patients with non-cerebral thrombosis. In our study, the presence of intracranial haemorrhage and platelet count were associated with a different risk of mortality, similar to other studies[30,37,42] that have also described fibrinogen levels and age[42] .

Because of the analogy of TTS with heparin-induced thrombocytopenia syndrome, the use of non-heparin anticoagulants has been recommended[6,9,15] . However, in the series presented, the use of heparin anticoagulants was not found to be associated with increased mortality. Recent data indicate that heparin competes with anti-PF4 antibodies for the same platelet binding epitope in 95% of patients, which supports its safety[43] . Since it takes considerable time to perform the platelet function studies necessary for confirmation, the use of non-heparin anti-coagulants is recommended as a first choice if available[15,35] .

The present work has some limitations, as the data are derived from spontaneous notifications, so the information could be incomplete. To minimise this problem, conservative estimates were made and an intention-to-treat analysis was carried out. The reporting rate could be underestimated if some cases were indeed not reported or diagnosed, but this seems less likely given the high media attention this problem attracted. The follow-up period was variable and in some cases patients were still in the process of recovery, so the data reported in relation to prognosis may not fully reflect the reality of the population analysed.

Conclusions

STT has been reported to occur between 1 case/1,000,000 and 1 case/100,000 persons immunised with non-replicating adenovirus vector vaccines in Spain. Its occurrence is more frequent in young adults (< 49 years) and is characterised by the appearance of symptoms related to the location of the thrombosis that debut approximately 10 days after vaccination. All patients present with thrombocyto-penia at some point, although this may not be seen at the time of their ED visit. In these cases, the D-dimer level may be helpful in guiding the diagnosis. Treatment should be aimed at eliminating anti-PF4 antibodies and resolving the thrombosis, preferably with non-heparin anticoagulants. One in four patients died, with an association between severe thrombocytopenia and the presence of intracranial haemorrhage with an increased risk of death.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Annex. Additional material

Additional material to this article can be found in its electronic version available at doi:10.1016/j.nrl.2022.04.010.

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