Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19 Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine AstraZeneca) (Other viral vaccines)

EPITT no:19683

Confirmation assessment report	12 March 2021
PRAC assessment report	17 March 2021
Adoption of first PRAC recommendation	19 March 2021
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AHEG remote meeting	29 March 2021 (1:30 pm-7 pm)
EMA EudraVigilance analysis	30 March 2021
PRAC assessment report on additional data	3 April 2021
Deadline for comments	5 April 2021 – 12 noon CET
Updated rapporteur assessment report	6 April 2021 am
Adoption of second PRAC recommendation	07 April 2021

Administrative information

Active substance (invented name)	Vaxzevria (previously COVID-19 Vaccine AstraZeneca)
Marketing authorisation holder	Astra Zeneca
Authorisation procedure	
☒ Centralised	
☐ Mutual recognition or decentralised	
☐ National	

Adverse event/reaction:[1]	embolic and thrombotic events

Signal validated by:	BE
Date of circulation of signal validation report:	11 March 2021
Signal confirmed by:	Belgium
Date of confirmation:	12 March 2021
PRAC Rapporteur appointed for the assessment of the signal:	**Jean-Michel Dogné (BE)**

Medicinal product no longer authorised

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[1] Please use MedDRA terminology whenever possible

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1. Background

Seriousness: 258 serious cases, 45 fatal.

Evidence: 269 cases in EudraVigilance for the SMQ 'Embolic and thrombotic events'

ROR: NA

Exposure: as of 11 March 2021, over 5.5 million doses of the AstraZeneca vaccine had been administered in EU/EEA countries [source: ECDC COVID-19 Tracker] [1]; in the UK, exposure was approximately 9.7 million doses as of 28 February 2021 [source: weekly summary of Yellow Card reporting] [2].

At the time of the PRAC meeting on 18 March 2021, it was noted that the latest overall exposure in EU/EEA and UK combined, as of 14 March 2021, was close to 20 million first doses administered [1].

Regulatory context: NA

COVID-19 Vaccine AstraZeneca is an adenovirus vector vaccine which received a conditional marketing authorisation in the EU on 29 January 2021 for active immunisation against COVID-19 in individuals 18 years of age and older.

Cases of thromboembolic events have been reported following administration of COVID-19 Vaccine AstraZeneca in several EEA countries, some leading to local suspensions of specific batches or to the use of the vaccine itself. An observed-to-expected analysis performed on 8 March 2021 identified no increased risk of thromboembolic events following administration of the vaccine although there were limitations.

Venous thromboembolism is an important potential risk included in the RMP of the recently approved COVID-19 Vaccine Janssen, another adenovirus vaccine, due to a numerical imbalance of cases reported in clinical trials. Natural infection with SARS-COV-2 has been associated with hypercoagulability, microangiopathy and venous or arterial thromboembolic events. One of the mechanisms hypothesised for the hypercoagulable state seen in patients with severe COVID-19 is related to the high-grade systemic inflammatory response.

2. Initial evidence

2.1. Signal validation

A search performed in EudraVigilance on 11 March 2021 for cases of 'Embolic and thrombotic events' (SMQ) yielded 269 cases, mostly from the UK (224 cases). Thrombotic thrombocytopenic purpura was not reported as such in any of the cases. Two additional cases were retrieved under the MedDRA PT 'coagulopathy', both from the UK, but were excluded from the review due to limited information. The majority (60%) of cases occurred in female patients. Median age was 70 years. Forty-five (45) cases had a fatal outcome.

Thirty (30) cases originated from the EEA: Germany (6), Sweden (5), Austria (5), Ireland (2), France (2), Denmark, Norway, Italy, Finland, Croatia, Latvia, Cyprus, Estonia, Greece, Czech Republic (1 each).

Of these, 19 were in women, 11 in male; 8/30 were consumer reports. Time-to-onset ranged from 0 to 16 days. Thrombotic events were reported in 10 cases, e.g. deep vein thrombosis, hepatic vein

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thrombosis, mesenteric vein thrombosis, portal vein thrombosis, carotid artery thrombosis, peripheral artery thrombosis, cerebral venous sinus thrombosis. Other reported events included pulmonary embolism (8), thrombocytopenia (6), disseminated intravascular coagulation (4), deep vein thrombosis (4), hepatic vein thrombosis (2). In 14 cases the vaccinee had risk factors for thromboembolic events such as hypertension, thyroiditis, obesity or chronic hepatitis B. Fourteen (14) cases have limited information. Seven (7) cases had a fatal outcome, in vaccinees were aged 24 to 60; disseminated intravascular coagulation occurred in 3 of them.

2.2. Signal confirmation

In total there were 269 cases of 'Embolic and thrombotic events' , median age was 70 years. Thirty (30) cases originated from the EEA. Seven (7) cases had a fatal outcome, in vaccinees aged 24 to 60; disseminated intravascular coagulation occurred in 3 of them. Thrombotic thrombocytopenic purpura was not reported as such in any of the cases. Although an observed-to-expected analysis performed on 8 March 2021 identified no increased risk of thromboembolic events following administration of the vaccine, further investigation is needed as these cases led to local suspensions of specific batches or the use of the vaccine itself. Besides, Venous thromboembolism is an important potential risk in the RMP of the recently approved COVID-19 Vaccine Janssen, another adenovirus vaccine, due to a numerical imbalance of cases reported in clinical trials.

Therefore the signal is confirmed.

2.3. Proposed recommendation

Following the suspension of a batch (number ABV5300) of COVID-19 Vaccine AstraZeneca [3] and the pause of the vaccination campaign with COVID-19 Vaccine AstraZeneca in Denmark and some other Member States [4], the MAH has been requested as part of a late breaking request for the Summary Monthly Safety Report (due date 15 March 2021) to provide:

A cumulative review of reports of Embolic and thrombotic events (SMQ Broad). The review should include at a minimum a discussion of fatal and serious events, if any batch clustering is observed with focus on ABV5300 batch, other risk factors – if they can be identified, an observed versus expected analysis, and risk-benefit considerations.

3. Additional evidence

3.1. Assessment of additional data

3.1.1. Introduction

The COVID-19 Vaccine AstraZeneca was granted a conditional marketing authorisation by the European Commission on 29 January 2021. Over 6.9 million doses have been administered in the EU/EEA countries [1] and over 9.7 million doses in the UK [2]. In EU countries, COVID-19 Vaccine AstraZeneca was administrated mainly in adults <60 years, while e.g. Comirnaty has been more evenly administered in all age groups and COVID-19 Vaccine Moderna more in adults >60 years [5].

The following events highlight the ongoing insights regarding the issue of thromboembolic events:

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- On 7 March 2021, the Austrian National Competent Authority suspended the use of one batch of the COVID-19 vaccine AstraZeneca (batch number ABV5300) as a precautionary measure following reports of events of thromboembolic events occurring with use of the vaccine[4].

- On 11 March 2021, the Danish Health Authority paused its vaccination campaign with COVID-19 Vaccine AstraZeneca. This was decided as a precautionary measure while a full investigation is ongoing into reports of blood clots in people who received the vaccine, including one case where a person died [4]. This has since been followed by suspension in multiple other countries, in the EU.

- On 13 March 2021, Norway issued a Rapid alert following a cluster of three healthcare professional cases reporting apparent immune thrombocytopenia in conjunction with cerebral venous sinus thromboses in young individuals (aged between 30-49) within 7 to 10 days of vaccination.

- On 15 of March, the Paul Ehrlich Institute issued a statement on the specifically noting cases of cerebral venous sinus thrombosis and thrombocytopenia. They received reports of cases of thrombotic events with concomitant thrombocytopenia in six women aged 20 to 49 years and one young man aged between 20-29 years who became symptomatic four to 16 days apart after receiving COVID-19 AstraZeneca vaccine. The six women developed central sinus vein thrombosis, two of which were fatal. A first Observed versus expected analysis concluded that more cases of sinus thrombosis have been reported than would be expected by statistical chance.

On 16 March, a preparatory PRAC meeting was held to discuss the ongoing signal of embolic and thrombotic events.

Based on above evolution and pattern of cases, the focus of the signal shifted from overall thromboembolic events to specific entities, specifically cerebral venous sinus thrombosis with thrombocytopenia and Disseminated intravascular coagulation.

This assessment is based on information which was available up to the 17th March 2021, including the EMA assessment of EudraVigilance (EV) data and preliminary information provided by the Biological Working Party (BWP).

The assessment report is structured as follows:

- Background on specific clinical entities

- Review of Clinical, non-clinical and Quality information

- Review of the Embolic and thrombotic events by the MAH, following the current signal request

- EMA evaluation of EV data and expert review

- MHRA conclusion

- Discussion on possible hypothesis

- Conclusions and recommendations

3.1.2. Background information on specific clinical entities

Cerebral venous sinus thrombosis (CVST) is a rare manifestation of thrombosis with an incidence that varies between studies (Capecchi, 2018) [6]. In adults, the annual incidence of CVST is 2 to 5 cases per million individuals, but it is likely to be underestimated because of the lack of well-designed epidemiological studies. Two recent studies in The Netherlands and southern Australia found a higher incidence than previously reported of 13.2 and 15.7 annual cases per million, respectively (Coutinho, 2012; Devasagayam, 2016) [7, 8].

At variance with arterial stroke that is more prevalent in the elderly, CVST typically affects young adults with a mean age of 35 years and is more common in women than in men (2.2:1) because of sex-specific risk factors (Capecchi, 2018) [6].

Because symptoms of CVST are variable and aspecific, diagnosis is often delayed to a median period of 7 days from the onset of clinical manifestations (Ferro, 2004) [9]. The most common presenting symptoms are: headache (88.8%), seizures (39.3%), paresis (37.2%), papilledema (28.3%) and mental status changes (22%).

Risk factors are associated with a multitude of acquired and inherited events, these include other central nervous system events such as intracranial neoplasias and infection, procedural events such as surgery and lumbar puncture as well as other systemic risk factors for thrombotic events eg: nephrotic syndrome, vasculitis, oral contraception and pregnancy (Alvis-Miranda, 2013) [10].

CVST is more common in women of reproductive age than in men, as a result of the use of oral contraceptives or hormone replacement therapy, pregnancy and the puerperium (Bousser, 2012) [11]. Oral contraceptive use is by far the most common risk factor, reported in more than 80% of women in various series and associated with a pooled estimate of approximately 6-fold increased risk of CVST (Dentali, 2006) [12]. In 85% of patients at least one risk factor is identified and 50% of events are triggered by the interaction of more risk factors (Capecchi, 2018) [6].

Cerebral venous sinus thrombosis, along with other paradoxical thromboembolic events, have been known to rarely occur in other immune thrombocytopenic states such as immune thrombocytopenia (ITP) (Hernandez, 2015; Sarpatwari 2010 [13]) and heparin-induced thrombocytopenic thrombotic syndrome (HITT). Plausible mechanisms for the clinical paradox associating immune thrombocytopenia particularly with venous thromboembolic events have been postulated, including increased platelet microparticle thrombogenicity following peripheral destruction, increased antiphospholipid antibody activity and increased levels of von Willebrand factor antigen (Rasheed, 2020) [14].

A recent literature review of CVST in COVID-19 identified 9 studies and 14 patients (Tu, 2020) [15]. The median age was 43 years and majority had no significant past medical conditions (60.0%). The time taken from onset of COVID-19 symptoms to CVST diagnosis was a median of 7 days.

Type 2 HITT is the more serious of the two types of HITT. It is a rare condition occurring in approximately 1- 3% of patients receiving heparin (Arepally, 2017) [16]. Events manifest secondary to an immunological response leading to thrombocytopenia, bleeding and thrombosis. The time to onset is typically 4-10 days following heparin therapy. Clinical events occur secondary to an immune response to PF4/heparin, although the immune response is recognised to occur more frequently than clinical manifestations of thrombocytopenia or thrombosis (Arepally, 2017) [16]. Patients can experience thrombocytopenia concurrent with thrombosis, with thrombosis being the more severe complication and can be life-threatening (Arepally, 2017) [16]. Thrombotic events primarily affect the venous system, although arterial involvement can also occur (Majeed, 2010) [17].

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Thrombotic thrombocytopenic purpura (TTP) presents with thrombocytopenia along with microvascular thrombosis and haemolytic anaemia with characteristic red cell fragments on peripheral smear (Louw, 2018) [18]. Secondary forms can result from extrinsic triggers including autoimmune disorders, pregnancy and viral infection. This arises from antibodies to ADAMTS13, a protease that cleaves von Willebrand factor (VWF) multimers into smaller ones. This protease's deficiency leads to VWF giant multimers that bind to platelets, and coagulation factors promote the coagulation cascade.

Thrombocytopenia and thrombosis have also been associated with **COVID-19 disease.** The incidence of thrombocytopenia in patients with COVID-19 has been variable across studies. Mild thrombocytopenia has been observed in up to one-third of these patients, with even higher rate in patients with severe disease (57.7%) compared with non-severe disease (31.6%). ITP has also been known to occur with onset occurring in 20% of cases 3 weeks after onset of COVID-19 symptoms, with reports occurring after clinical recovery (Bhattacharjee, 2020) [19]. Cases of TTP have also been reported to occur (Bhattacharjee, 2020) [19]. The proposed mechanisms of thrombocytopenia with COVID-19 involve inhibition of platelet synthesis due to direct infection of the bone marrow cells or platelets by the virus (possibly via CD-13 receptors) and dysfunctional marrow microenvironment; virus-mediated liver damage leading to decreased thrombopoietin production; pulmonary endothelial damage followed by platelet aggregation in the lungs, subsequent formation of microthrombi, and platelet consumption; and finally, the destruction of platelets by the immune system.

3.1.3. Clinical and non clinical (CHMP) and Quality (input from BWP)

Below is presented a summary of non-clinical and clinical data assessed within the marketing authorisation application and related to thromboembolic events .

- Clinical data were reviewed from clinical trial assessed as part of the marketing authorisation (MA) application and additional data submitted after the MA, including data currently under evaluation (EMEA/H/C/005675/II/0002), with DLP 7 Dec 2020. These data did not suggest an association of thrombotic events with the use of the AZ1222 vaccine.

- There were no adverse events of leukopenia, thrombocytopenia, or neutropenia reported for the AZD1222 group. A slightly higher frequency of platelet decrease was observed post second dose of the AZD1222 vaccine [3.1%] than post first dose [1.8%], however this was also true in the control group [0.6% and 1.1% respectively]. All clinical laboratory results in the AZD1222 group were within normal clinical range and were not considered Adverse Events.

- Available data from studies with other ChAdOx1 vectored vaccine candidates demonstrated the vector is well tolerated at all dose levels investigated, with no SAEs related to the vaccine reported. Local and systemic AEs were predominantly self-limiting and shortlived.

- Non-clinical data of AZ1222 show strong immunogenicity response following two dose administration in animals. In addition, available data show a favourable safety profile. Adverse effects are limited to the site of administration and all findings reported were reversible by the end of the recovery period.

- Regarding inflammatory response after vaccination, it was concluded that the only relevant signs observed following immunization in primary pharmacology studies in animal models are restricted to respiratory tract tissues following challenge and no apparent signs of inflammation are observed as a result of immunization.

Quality aspects were discussed at the BWP on 16 March 2021. It was concluded that there is no indication thus far that SAE are linked to quality of the vaccine. As a further follow-up, the MAH was

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requested to provide a full batch analysis for specific lots and batch data from UK supplied lots to understand if there are any clear differences between that and the EU product.

PRAC Assessment

Based on the input provided by the CHMP Rapporteur on the clinical and non-clinical data (DLP 7 Dec 2020), there was no evidence to suggest an association of thrombotic events with the use of the AZ1222 vaccine. A slightly higher frequency of platelet decrease was observed post second dose of the AZD1222 vaccine compared to post first dose, however this was also true in the control group. All clinical laboratory results in the AZD1222 group were within normal clinical range and were not considered as Adverse Events.

Moreover, the BWP concluded that there is no indication so far that SAE are linked to the quality of the vaccine (16 March 2021). Follow-up questions for specific batches were asked to the MAH.

3.1.4. MAH report

3.1.4.1. AstraZeneca response:

A search of the company's safety database was undertaken on 08 March 2021 for cumulative adverse event data (up to 08 March 2021) from spontaneous and solicited reporting sources using PTs under SMQ Embolic and thrombotic events (MedDRA version 23.1) in association with the use of post-marketing use of COVID-19 VACCINE ASTRAZENECA.

The search identified 267 cases involving 286 events (PTs) from post marketing sources. 279 events (PTs) were serious and 7 were non-serious. After the search was carried out, two additional cases of pulmonary embolism were identified, as of 10 March 2021. These 2 cases with 2 events (MedDRA Preferred Term [PT]) have been added to the analysis of pulmonary embolism. An additional case with PT Pulmonary Embolism was also included in the below analysis. The 288 events are listed Table 1 below:

Table 1 Post-marketing adverse event preferred terms identified in database search

AE Preferred Term	PT Count	PT Serious Count
Acute myocardial infarction	5	5
Amaurosis fugax	2	2
Aortic embolus	1	1
Blindness transient	3	3
Brain stem infarction	1	1
Brain stem stroke	1	1
Cerebral infarction	1	1
Cerebral thrombosis	2	2
Cerebral venous sinus thrombosis	4	4
Cerebrovascular accident	57	53
Cerebrovascular disorder	1	1
Deep vein thrombosis	15*	15*

Table 1 Post-marketing adverse event preferred terms identified in database search

AE Preferred Term	PT Count	PT Serious Count
Diplegia	8	8
Disseminated intravascular coagulation	1	1
Embolic stroke	1	1
Embolism	1	1
Embolism arterial	1	1
Haemorrhagic infarction	1	1
Haemorrhagic stroke	3	3
Hemiparesis	15	15
Hemiplegia	6	6
Hepatic vein thrombosis	1	1
Ischaemic stroke	11	11
Mesenteric vein thrombosis	1	1
Monoparesis	10	10
Monoplegia	31	31
Myocardial infarction	34	34
Paraparesis	2	2
Paraplegia	1	1
Paresis	3	2
Pelvic venous thrombosis	1	1
Portal vein thrombosis	1	1
Pulmonary embolism	22	22
Pulmonary infarction	1	1
Quadriplegia	1	1
Splenic infarction	1	1
Splenic vein thrombosis	1	1
Superior sagittal sinus thrombosis	1	1
Thrombophlebitis	1	1
Thrombophlebitis superficial	2	
Thrombosis	3	3
Transient ischaemic attack	28	28
Vascular stent occlusion	1	1
Total:	**288**	**281**

* After the listings were run, follow-up information was received on 10 March 2021 for one case . . Based on the follow-up information the initially reported PT of Deep vein thrombosis was no longer valid and removed. The case report remains included in the analysis, although no longer qualifying for the scope of the search. There was a duplicate case in the safety database. This case is not included in the analysis.

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The reports were received from United Kingdom (246), India (8), Austria (3), France (2), Germany (2), Poland (2), Bangladesh (1), Finland (1), Ireland (1), Latvia (1), Norway (1) and Sweden (1). The 269 cases described 166 females, 95 male and in 8 reports gender was not identified.

Two cases were from non-interventional/post-marketing sources and 267 were spontaneously reported.

Out of the 269 cases, 102 were medically confirmed. 40 events had a fatal outcome.

The case level outcomes were: Not Recovered (75), Recovered (44), Recovering (73), Recovered with Sequelae (13) Unknown (24) and Fatal (40). Follow-up information was received on 10 March 2021 for one case . Based on the follow-up information the initially reported PT of Deep vein thrombosis was no longer valid and this case should not have been part of this analysis. The case report remains included in the demographic and other information details, although no longer qualifying for the scope of the search.

PRAC Assessment

The search in the AstraZeneca global safety database (DLP 8 March 2021) using the SMQ Embolic and thrombotic events identified 269 cases, with 288 PTs. In decreasing order, these PTs were Cerebrovascular accident (n=57), Myocardial infarction (n=34), Pulmonary embolism (n=22), Monoplegia (n=31), Deep vein thrombosis (n=15), Ischemic stroke (n=11), [..], Cerebral venous sinus thrombosis (n=4),[…] DIC (n=1) […]. These cases originated mainly from the UK (91%) with female predominance (64%).

The estimated number of doses administered according the 2nd MSSR was 1,903,293 doses for the EU and 9,589,941 doses in the UK (DLP 28 February 2021).

3.1.4.2. Fatal case reports

There were 39 fatal cases occurring in 25 females and 14 males, aged between 30-99 years (mean 71 years). Age was not reported in 2 cases. The cases originated from the United Kingdom (32), India (6), and Austria (1). Of the 39 cases, 26 were medically confirmed.

There were 18 fatal cases with a cause of death of **Myocardial infarction (MI)**. Of the 18 cases, 10 occurred in vaccinees aged 70 years and older. A summary of the remaining 8 cases in age less than 70-year old is provided below:

- A total of 8 patients (7 males and 1 female), experienced an event of myocardial infarction with fatal outcome. Three reports were from the UK and 5 were reported from India. It was noted in each of the 5 reports of MI from India government officials, that the deaths were not related to the vaccine.

- The patients ranged in age from 42-67 years, with a mean age of 53 years. For 7 cases where the time to onset was reported, the range was from 1 to 9 days. The cause of death was reported as: MI or Heart attack in 7 reports, and Cardiogenic shock with myocardial infarction in 1 report.

- Five (5) of these 8 patients had a medical history of cardiac disease, aortic stenosis due for upcoming surgery, hypertension, diabetes and hypercholesterolemia, all of which were likely to have contributed to the fatal events of myocardial infarction. The remaining 3 reports lacked

sufficient information such as medical history concerning cardiovascular disease, diabetes, renal function, smoking, and obesity, to be able to assess a causal relationship.

There were 8 fatal cases of **Cerebrovascular accident**, all occurring in vaccines aged 79 years and older.

All other fatal events (8 events) were singular or were reported at two occurrences (**Error! Reference source not found.**) apart from 5 fatal cases of Pulmonary embolism/infarction that are described below.

	Case Medically Confirmed	Sex	Age groups	Cause of Death Disease PTs	AE Preferred Term	Time To Onset
1	YES	M	79+	Transient ischaemic attack; Lymphoproliferative disorder in remission; Asthenia	Transient ischaemic attack	Unknown
2	YES	M	60-69	Cardiac arrest	Acute myocardial infarction	Unknown
3	YES	F	30-39	Cerebral thrombosis	Cerebral thrombosis	1 week 5 days
4	YES	F	60-69	Cerebral thrombosis	Cerebral thrombosis	1 week 1 day
5	YES	M	30-39	Brain stem infarction; Superior sagittal sinus thrombosis; Haemorrhage intracranial; Cerebral haemorrhage	Brain stem infarction	1 week 3 days
					Cerebral venous sinus thrombosis	1 week 1 day
					Superior sagittal sinus thrombosis	Unknown
6	YES	M	70-79	Death; Aortic embolus	Aortic embolus	Unknown
7	NO	F	60-69	Haemorrhagic stroke	Haemorrhagic stroke	Unknown
					Hemiplegia	Unknown
8	NO	F	Unknown	Death	Acute myocardial infarction	2 days

PRAC Assessment

Review of the fatal case reports (n=39) indicated that the majority originated from the UK (n=32). There were 6 cases from India and 1 from Austria. Main cause reported was Myocardial infarction

(n=18), occurring in > 70 years for 10 cases and in 42-67 years for 8 cases.

Other causes reported were: Cerebrovascular Accident (n=8), all in patients > 79y, Pulmonary embolism (n=5) with 4 cases from the UK (70-89y) and 1 case from Austria (40-49y). Other PTs in remaining cases (8) include cerebral thrombosis (n=2), cerebral venous sinus thrombosis (n=1), aortic embolism (n=1), haemorraghic stroke (n=1).

3.1.4.3. Lot Numbers

Batch numbers were not reported in 139 cases or events. The three batches with the highest number of cases are: AB0006 (491,700 doses distributed, 17 SAEs reported), AB0001 (533,100 doses distributed, 12 SAEs), PV46662 (700,800 doses distributed, 10 SAEs). There was no cluster of events for any of these batches. In addition to the above batches, 2 cases from Austria have been received with batch no ABV5300 (1,060,200 doses distributed); no other thrombotic events were reported from this batch.

PRAC Assessment

The MAH stated that there was no cluster of events for any of the batches. The MAH should clarify in the next MSSR how this was concluded.

3.1.4.4. Observed versus Expected analyses for Pulmonary Embolism and Deep vein thrombosis

Observed-to-expected (O/E) analyses were performed by the MAH for deep vein thrombosis and pulmonary embolism. Background incidence rates were defined by the number of incident reports of a condition or event occurring naturally in the population, expressed in person-time and these background estimates were obtained from the literature. The observed number of cases were found to be significantly less than the expected number of events, the results of the O/E analysis are presented in Table 2. See section 3.2.2 of the Summary Safety Report for more information on process of O/E calculation.

Table 2   **Observed versus expected analysis for Pulmonary embolism and Deep vein thrombosis**

Medical Concept	Observed cases	Expected cases	Rate ratio (CI 95%)	Conclusion
Deep vein thrombosis	15	704.87	0.02 (0.01 - 0.04)	Observed significantly < expected
Pulmonary Embolism	22	861.7	0.02 (0.02 - 0.04)	Observed significantly < expected

CI Confidence Interval

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The observed number of cases for deep vein thrombosis and pulmonary embolism was well below the expected number of cases. This results in a rate ratio with a value clearly close to zero and an upper bound of the 95% confidence interval well below unity; that is, the results of this analysis show that the number of observed cases is statistically significantly lower than expected.

The findings of the O/E analysis are reassuring. However, O/E analyses are often subject to many important sources of uncertainty and the findings should be interpreted cautiously.

In this particular case, the following information was not provided: 1) there is no presentation of the literature search strategy or the results of the search for backgound incidences, 2) there is no discussion about the comparability between the source population of observed cases and the populations used to derive the background incidences, and there are no stratified analyses (by age, gender), 3) there are no sensitivity analyses presented to explore the impact of potential sources of uncertainty (e.g., under-reporting, background incidence). With regard to the latter, it should be noted that reasonable changes in the parameter values in the uncertainty analysis would probably not change the main conclusions, given the large difference between expected and observed figures and 4) there is no details on the risk period applied.

In conclusion, the analysis is reassuring but: 1) it should not be used as the only source of information for decision-making, 2) on the basis of the document, it is of limited value because questions remain to be answered in order to better assess the quality of the analysis (sources for incidence rates, lack of sensitivity analyses, lack of stratified analyses, assessment of the comparability of the source populations of the observed cases and for the calculation of incidence rates). The MAH is requested to provide a more detailed section to address these issues in the next MSSR.

3.1.4.5. Pulmonary embolism and pulmonary infarction

There were 22 cases of Pulmonary embolism, one of which also included Pulmonary infarction.

The cases originated in the United Kingdom (18), France (1) and Austria (3).

All three cases from Austria were reported in females.

Two cases from Austria were reported from the lot ABV5300 and one from the lot ABV3025.

All 22 cases were spontaneously reported and were serious. Five reports had fatal outcome and 14 of the 22 cases were medically confirmed.

Outcomes of the adverse events of Pulmonary embolism/Pulmonary infarction were: Recovering (8), Recovered with sequelae (2), Not recovered (1), Fatal (5) and Unknown (6).

The demographics and time to onset of the vaccinees are presented in Table 3:

Table 3   Cases with Pulmonary embolism by age interval

Age interval, years	Gender			Time to Onset			
	Male	**Female**	**Unknown**	**< 1 day**	**1-7 days**	**8-18 days**	**Unknown**
18 to <65	1	5	0	0	2	1	3
≥65	6	6	0	1	5	2	4
Unknown	0	2	2	1	1	0	2

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3.1.4.5.1. Fatal Pulmonary Embolism

There are 5 cases with fatal outcome. These are described below.

- A 79+ year-old female patient of unknown ethnic origin with unknown past medical history and concomitant medication of calcium carbonate, cholecalciferol for osteopenia received COVID-19 VACCINE ASTRAZENECA (batch number AB0007). On day 1, she collapsed while walking and was admitted to hospital. She was diagnosed with new atrial fibrillation, bilateral pulmonary embolisms, acute congestive cardiac failure, and was intubated. On day 2, the patient experienced multiple organ failure, mitral incompetence and pneumothorax. On day 2, the patient died. It is not known whether an autopsy was performed. Patient had not shown symptoms associated with COVID-19.

- A 79+ year-old female patient of unknown ethnic origin with past and current medical history of dementia and unknown concomitant medications received COVID-19 Vaccine AstraZeneca (batch number AB0001). On an unknown date, the patient experienced pulmonary infarction, deep vein thrombosis leg and pulmonary embolism. On day 5, the patient died from pulmonary infarction. An autopsy was performed which showed the cause of death was 1. Pulmonary infarction, 2. Left leg deep vein thrombosis (DVT) and 3. Ischaemic heart disease. The reporter concluded that the DVT may have occurred after a fall prior to receipt of her COVID vaccine.

- A 70-79 year-old female patient of unknown ethnic origin with past and current medical history of stroke, type 2 diabetes mellitus, essential hypertension and hypothyroidism and unknown concomitant products received COVID-19 Vaccine AstraZeneca (batch number AB0006). On day 3 post-vaccination, the patient experienced unilateral leg pain, leg swelling and deep vein thrombosis. On day 4, the patient experienced pulmonary embolism, hypotension, and hypoxia. On an unknown day, the patient died from the event of pulmonary embolism. An autopsy was not performed. The cause of death was pulmonary embolism. The history of stroke and the concomitant type 2 diabetes suggests that the patient was at risk of thrombotic events.

- A 70-79 year-old female vaccinee received COVID-19 Vaccine AstraZeneca (batch number unknown) and 4 days later experienced pulmonary embolism and malaise. The vaccinee died on the same day, cause of death was reported as pulmonary embolus. No other information was available.

- A 40-49 year-old female with a family history of fatal pulmonary embolism in immediate family received COVID-19 Vaccine AstraZeneca (batch number ABV5300). The patient experienced splenic vein thrombosis, mesenteric vein thrombosis. thrombocytopenia, pulmonary embolism, portal vein thrombosis and hepatic vein thrombosis. On an unknown date, the patient experienced gastrointestinal bleeding. The patient experienced asystole and died 10 days later. Cause of death was reported as pulmonary embolism, splenic vein thrombosis, portal vein thrombosis, asystole, gastrointestinal bleeding, hepatic vein thrombosis and vein thrombosis mesenteric. It was not known whether an autopsy was performed.

3.1.4.5.2. Non-Fatal Pulmonary Embolism

There are 13 cases reported with very limited medical information to evaluate/assess the case for relatedness/association.

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One case was related to a potential vaccination error and there was no information on pulmonary embolism from the narrative. It is not clear based on the available information if this a case of pulmonary embolism.

Three cases had confounding medical history:

- Case one has a past history of pulmonary embolism and was treated with Rivaroxaban but had missed a dose on the day of the event.

- Case two has a past history of unknown neoplasm which could have contributed to the pulmonary embolism.

- Case three experienced pulmonary embolism on the same day of receiving vaccine; the case was confounded as the vaccinee had suspected COVID-19.

3.1.4.6. Summary

In summary, a review of the cases of pulmonary embolism indicated no pattern of events and no clustering of risk factors were identified. Further, there were no trends seen for any batches that were included in this analyses.

The observed number of cases is significantly less than the expected number of events. Past history of pulmonary embolism, neoplasm, current history of deep vein thrombosis and missed dose of anti-coagulant were noted as risk factors for pulmonary embolism in cases where this information was available.

3.1.4.7. Deep vein thrombosis

There were 15 cases of Deep vein thrombosis occurring in 11 females and 4 males. All cases were from the United Kingdom. All cases were spontaneous, and serious and 7 were medically confirmed.

Outcomes of the adverse events of Deep vein thrombosis were: recovering (6), recovered with sequelae (1), not recovered (3), died (1) and unknown (4).

Lot/Batch numbers were available in 9 case reports, 1 report each from AB0001, AB0002, AB0005, AB0008, AB0011, AB0012, PV46652, and 2 reports from AB0006.

Table 4   Cases with Deep vein thrombosis by age group

Age group, years	Gender			Time to Onset			
	Male	**Female**	**Unknown**	**< 1 day**	**1-7 days**	**8-13 days**	**Unknown**
18 to <65	1	3	0	1	3	0	0
≥65	3	8	0	0	4	1	6

Follow-up information was received on 10 March 2021 for one Case (incorrectly considered to be a fatal DVT case). Based on the follow-up information this case did not involve a DVT. This case is nevertheless included in this analysis. In 2 cases, DVT was reported together with Pulmonary embolism and these cases were fatal and discussed above in Section 3.1.4.5.1.

In 4 cases the vaccinee had confounding medical history:

- Case : history of DVT

- Case : concurrent COVID-19 illness

- Case : history of testicular cancer with metastasis to liver
- Case : history of clotting disorder

In one of the 15 cases, there was a non-fatal case received which included a PT of DVT. However, reported verbatim was reported as "Suffered Nausea". No other details were provided in the narrative about DVT.

In the remaining 7 cases, available information was too limited to assess.

3.1.4.8. Summary

In summary, there was no trend seen after reviewing the cases of DVT. There were no cases of DVT associated with lot number ABV5300. There were no trends seen for any batches that were included in this analyses. The observed number of DVT cases is significantly less than the expected number of events.

Overall conclusion

Based on the data available from post-marketing experience, it is AstraZeneca's opinion that the information in the current document and the AstraZeneca Core Data Sheet continues to accurately reflect the known benefit-risks for COVID-19 Vaccine AstraZeneca.

PRAC Assessment

The MAH revised cases of Pulmonary embolism and Deep vein thrombosis. It is agreed that based on these cases, no specific pattern or clustering of risk factors could be identified.

However, based on the evolution of events, input from MS and analysis from EMA, the scope of the concerns should be directed to specific issues including Disseminated intravascular coagulation, haemorrhagic stroke, Cerebral venous sinus thrombosis and thrombosis with thrombocytopenia (see Global discussion and conclusion).

3.1.5. EMA analysis of EudraVigilance data

3.1.5.1. Observed to expected analysis

Based on data available in EudraVigilance up until 12 March 2021.

EMA provided an assessement of the signal of embolic and thrombotic events for COVID-19 Vaccine AstraZeneca, including an observed to expected analysis (DL 12 March 2021).

PRAC Assessment

An exploratory analysis for thromboembolic events was conducted based on EV data. There was a lower proportion of overall serious thromboembolic events for COVID-19 Vaccine AstraZeneca compared to the other COVID-19 vaccines. However, a higher fatality rate was noted.

The observed vs expected analysis for COVID-19 Vaccine AstraZeneca for events including DIC, Cerebral venous sinus thrombosis and Haemorrhagic stroke showed a disproportionality in younger

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age groups, specifically those aged 18-54 years. However several limitations for this type of analysis apply, and should be taken into account, as discussed in the report, including:

(i) Code lists used to identify expected cases may not match the MedDRA classification used to identify observed cases in EV;

(ii) Cases of cerebral venous sinus thrombosis from published article identified by review of medical records in 2008-2010; uncertainty on comparability of diagnostic criteria with 2021 cases;

(iii) Background incidence rates are from one database/country only;

(iv) There might be under-reporting when the observed number of cases is calculated from spontaneous reporting systems such as EV;

(v) There might be delay in reporting: during a mass vaccination some case reports might not have been submitted yet to EV;

(vi) There might be more intensive case ascertainment in vaccinated individuals.

It also has to be considered that the populations exposed to the different COVID-19 vaccines can vary overall and between Member States, for example in terms of age, gender and prevalence of relevant co-morbidities.

In conclusion, for overall thromboembolic events this analysis is reassuring. However, a signal of disproportionality was noted for rare events, such as DIC, Cerebral venous sinus thrombosis and Haemorrhagic stroke warranting further investigation.

Updated OE analyses performed with data (DLP including data available in Eudravigilance up to the 16th March 2021 at 6pm)

EMA performed an updated observed to expected analysis (DLP including data available in Eudravigilance up to the 16th March 2021 at 6pm).

Data sources used in the OE analysis

Data provided by the ACCESS consortium, stratified by age group, based on the average of the period from 2017-2020

- The databases used for the main analysis for the three events investigated are:
  o Coagulation disorder (this was used to compare with the SMQ Embolic and thrombotic events): ARS from Italy
  o Disseminated intravascular coagulation: FISABIO from Spain
  o Cerebral venous sinus thrombosis: ARS from Italy

- Different data sources were chosen according to the event considered, the reason was that when several incidence rates estimates existed (also considering other sources and literature), the data source with the median rate was chosen.

In addition a conservative sensitivity analysis was performed using the database with the lower incidence rate estimate.

Background rates used for the specific events (including age stratification)

- The rate are expressed by 100,000 person years

- Coagulation disorder: ARS from Italy

Age category	IR
20-29	40.14
30-49	85.08
50-59	200.73
60-69	427.56
70-79	912.00
80+	2055.95

- Disseminated intravascular coagulation: FISABIO from Spain

Age category	IR
20-29	0.60
30-49	1.09
50-59	3.07
60-69	4.67
70-79	8.37
80+	11.66

- Cerebral venous sinus thrombosis: ARS from Italy

Age category	IR
20-29	0.64
30-49	1.80
50-59	1.00
60-69	1.29
70-79	1.91
80+	1.55

Results

Disseminated intravascular coagulation	IR per 100,000 Person years From FISABIO	EEA			EEA and UK		
		Expected 14d	Observed 14d From EV	OE 14d with 95% c.i.	Expected 14d	Observed 14d From EV	OE 14d with 95% c.i.
20-29	0.60	0.04	1	23.26 (0.30 - 129.41)	0.04	1	23.26 (0.30 - 129.41)
30-49	1.09	0.78	4	5.12 (1.38 - 13.11)	1.99	4	2.02 (0.54 - 5.16)
50-59	3.07	1.48	1	0.67 (0.01 - 3.75)	4.38	1	0.23 (0.00 - 1.27)
60-69	4.67	1.84	1	0.54 (0.01 - 3.03)	9.24	1	0.11 (0.00 - 0.60)
70-79	8.37	0.83	0	0.00 (0.00 - 4.42)	11.30	0	0.00 (0.00 - 0.32)
80+	11.66	0.90	0	0.00 (0.00 - 4.09)	5.37	0	0.00 (0.00 - 0.68)
Total		5.87	7	1.19 (0.48 - 2.46)	32.31	7	0.22 (0.09 - 0.45)

Cerebral Venous Sinus Thrombosis	IR per 100,000 Person years From ARS	EEA			EEA and UK		
		Expected 14d	Observed 14d From EV	OE 14d with 95% c.i.	Expected 14d	Observed 14d From EV	OE 14d with 95% c.i.
20-29	0.64	0.05	1	21.80 (0.28 - 121.32)	0.05	1	21.80 (0.28 - 121.32)
30-49	1.80	1.29	11	8.55 (4.26 - 15.31)	3.27	12	3.67 (1.89 - 6.41)
50-59	1.00	0.48	1	2.07 (0.03 - 11.53)	1.43	2	1.40 (0.16 - 5.06)
60-69	1.29	0.51	0	0.00 (0.00 - 7.23)	2.55	0	0.00 (0.00 - 1.44)
70-79	1.91	0.19	0	0.00 (0.00 - 19.37)	2.58	0	0.00 (0.00 - 1.42)
80+	1.55	0.12	0	0.00 (0.00 - 30.74)	0.71	0	0.00 (0.00 - 5.14)
Total		2.63	13	4.94 (2.63 - 8.45)	10.58	15	1.42 (0.79 - 2.34)

** Based on cases retrieved using a search in Eudravigilance with the Preferred Terms,
"cerebral venous thrombosis" and "cerebral venous sinus thrombosis"*

Embolic and thrombotic events	IR per 100,000 Person years From ARS	EEA		
		Expected 14d	Observed 14d From EV	OE 14d with 95% c.i.
20-29	40.14	2.88	11	3.82 (1.91 - 6.84)
30-49	85.08	60.95	79	1.30 (1.03 - 1.62)
50-59	200.73	96.89	40	0.41 (0.29 - 0.56)
60-69	427.56	168.22	33	0.20 (0.14 - 0.28)
70-79	912.00	90.40	5	0.06 (0.02 - 0.13)
80+	2,055.95	158.30	8	0.05 (0.02 - 0.10)
Total		577.64	182	0.32 (0.27 - 0.36)

Of note, 9 cases without TTO assigned to the shortest risk period; 6 cases without age added only to the total, 1 cases with age group 10-19 not included in the analysis.

Exposure data used within the OE analysis including age stratification and source (i.e. ECDC)

Age category	Vaccinated in EEA with AZ
20-29	211,917
30-49	2,133,764
50-59	1,428,787
60-69	1,190,833
70-79	309,743
80+	226,536

Number of vaccinated with AZ (~5.5 millions) up to 7th March based on ECDC data extracted on Monday 15th March (data are publicly available at https://covid19-vaccine-report.ecdc.europa.eu/)

Most of the countries report data by detailed age groups to ECDC; however, some may report in a simplified way (<60 and 60 years and above) or do not provide details. When detailed data by age group was not available, information from countries where this information was provided was used to impute a distribution.

3.1.5.2. Specific Case assessement

Review of data received in EudraVigilance up to 12 March 2021

EMA conducted a search in the Eudravigilance database with a data lock point on 12 March 2021. The search terms were SMQ 'Embolic and thrombotic events', PT 'coagulopathy'. General results (Data lock point 12/03/2021).

A total of 293 case reports were retrieved. After exclusion of possible duplicates, non-serious cases and consumer reports, 202 serious cases from healthcare professionals were selected for further review.

Most of the 202 cases originate from outside the EEA, with UK accounting for the highest number (158); of note, 5 cases from India. The 37 EEA cases (18%) originate from Austria, Germany (8 each), France (4), Sweden (3), Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Greece, Ireland, Italy, Korea and Spain (1 each).

The majority of cases (122) are in female patients, 76 are in men and in 4 cases the gender is not reported. Age ranges from 16 to 99 years with a median of 47.

25 different batch numbers were reported in 106 cases with this information. The batch number most frequently reported was mentioned 14 times.

The 10 most frequently reported thromboembolic terms were cerebrovascular accident (49), myocardial infarction (30), pulmonary embolism (24), monoplegia (16), ischaemic stroke (15), transient ischemic attack (15), deep venous thrombosis (13), hemiparesis (12), monoparesis (9), and acute myocardial infarction (6).

In 45 cases (22%), the outcome was fatal.

Time-to-onset for the thromboembolic events was specified in 181 cases, ranging from 0 to 34 days.

In 44 cases, the TE events were preceded by flu-like symptoms. Thrombocytopenia was reported in 7 cases. In 120 cases, there were underlying risk factors for the reported events such as a history of hypertension, cerebrovascular accident, diabetes, cardiac disorders, pulmonary embolism or comedications such as oral contraceptives. In a further 9 cases, there was a history of confirmed or suspected COVID-19. In 55 cases there was insufficient information for causality assessment. In 18 cases, there were no clear alternative explanation for the events. Of note, a history of autoimmune disorders such as rheumatoid arthritis, multiple sclerosis or thyroiditis was reported in 29 cases.

Among the trombotic events of special interest: a cerebral venous sinus thrombosis (CVST) was reported 5 times, disseminated intravascular coagulation (DIC) was reported 4 times, and superior sagittal sinus thrombosis was reported once. (DLP 12/03)

Updated review including data available in Eudravigilance up to the 16th March 2021 at 6pm

A search in EudraVigilance for cases of 'embolic and thrombotic events' (SMQ) for the high level active substances COVID-19 VACCINE ASTRAZENECA CHADOX1 NCOV-19 and COVID 19 VACCINE (CHADOX1 S[RECOMBINANT]) was performed. The results included reports sent to EV up to 16 March 2021 18:00 CET.

A total of 469 reports was retrieved, which may include duplicates. Of these, 436 (93%) were serious, including 59 fatal (13%). One-hundred and ninety-one (191) reports originated from the EEA (41%) and 276 from the UK (59%). The majority of reports (312 [67%]) were from healthcare professionals. Reports were predominantly in female vaccinees (295 [63%]) with a median age of 60 years.

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Thrombocytopenia (and related PTs) was co-reported as a reaction in 26 cases. Disseminated intravascular coagulation was repored in the 6 of the retrieved cases, and a follow-up version of one of the previously submitted cases was being processed at the time the query was performed, thus there were 7 cases in DIC in total. There were 18 cases of cerebral venous sinus thrombosis including some reported as 'cerebral venous thrombosis' or 'cerebral thrombosis'. There were also 4 cases of mesenteric vein thrombosis.

Disseminated intravascular coagulation (DIC) (Data lock point including data available in EVDAS up to the 16th March at 6pm)

Six cases (+ 1 case re-submitted after cut-off) of DIC were received in Eudravigilance based on data available in Eudravigilance up to the 16th March 2021 18:00 CET) (table below). All cases originated from the EEA.

An overview of the 7 cases of DIC is presented within Table 6 below:

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Table 6: Overview of DIC cases

Case	1	2	3	4	5	6	7
Age group	20-29	30-39	60-69	40-49	30-39	50-59	30-39
Gender	F	M	F	F	F	F	F
Pre-existing condition	Not Applicable	Chronic hepatitis B Migraine BMI 32 Malaise, fainting, headache with normal tests	Hypertension Hypercholesterolaemia Hashimoto	Family history of polytropic thrombocytopenia	Allergy No family history of thrombosis		Not Applicable
Baseline treatment			Losartan K Levothyroxine Na Simvastatin	None	ethinyl estradiol and drospirenone (10 years) Desloratadine		Not Applicable
COVID-19	PCR neg Serology neg	27/01: PCR- 03/02: PCR- 27/02: False-pos Serology neg					
Initial reaction	Day 1: flu like syndrome vomiting, chills	Pyrexia, headache	Headache		Fever and myalgia during 1-2 days		
Clinical course	Day 7: recurrence of digestive symptoms and headache unresponsive to paracetamol Day 9: Thrombectomy for ischemic stroke - Hemiplegia and aphasia	Day 10: lost consciousness, hemiparesis, pyrexia (38.1 C)	Headache Day 5: Tiredness, Speech impairment Day 7: Hospital with Abdominal pain, Motor restlessness, Sytematic inflammatory response, Adrenal bleeding Day 8: one-sided paralysis, Cerebral artery occlusion, ischemic cerebral infarction, Thrombocytopenia Day 10: Increase intracranial pressure Day 12: Multiorgan failure	(dates unknwon) thrombosis in splenic vein and kidneys Necrotic intestine Pulmonary embolism	Day 6: fever, acute headache Visual disturbance in the lower right cadran Day 8: hospitalisaiton with Thrombosies of Venous sinuses, CID, thrombocytopenia Day 9: coma (Glasgow scale)	Day 11 CID, haemorrhagic stroke, pulmonary thromboembolism, coma, hemiplegia, aphasia, acute coronary syndrome	Headache vasomotor Thrombopenia Disseminated Intravscular Coagulation
Fatal		Yes	Yes	Yes	Yes		
Stroke	Yes	Yes	Yes	PE	CVST		
Thrombocytopenia	Yes: 58 G/L peripheral	Yes: 14 G/L	Yes	severe	22 G/L		Yes
DIC	resolved	Fatal	Yes	Fatal	Yes		Yes
Imaging	Sinusitis	CT scan: thrombosis	Cerebral artery		Day 8 CT, MRI:		

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		af areria carotis interna and areteria cerebri media. Small subarachnoid hemorrhage	occlusion (right internal carotid artery and arteria cerebri media ipsilateral)		Veous sinus thrombosis, of sigmoid and transverse sinuses, Thrombosis internal jugular vein (all lef side) Day 9: MRI: Sub-arachnoid haemorrhage + are reported Cerebellar oederna, cerebrellar haemaoma		
Labo	Myelogram normal AutoAb negative SAPL test, PNH negative	RBC 5,15x10*12/l; Hct 45,6%; PLT 14x10*9/l; IPF 9,9% (N 1,3-7%) - activation of thrombopoiesis. INR 1.09. D-dimers in plasma >35 (ref<0.5 mg/l), plasma fibrinogen 1.02 (ref 1.7...4.2 g/l).	Day 8: Systematic hypercoagulative disorder, DIC	Consumptive coagulopathy (DIC)	Day 8: Fibrin D Dimer >7 mg/L, Fibrinogen 1.3 g/L		
Diagnosis	Thombus left proximal M1	Ischemic stroke involving arteria carotis media, DIC	DIC Ischemic cerebral infarction Adrenal bleeding Systematic hypercoagulative disorder	Autopsy: pulmonary embolism, DIC Association with vaccine considered unlikely	CVST CID Important cerebral damage not compatible with life		
Expert opinion	Moderate thrombocytopenia unfrequent in stroke	Ischemic stroke withthrombocytopenia in the context of DIC	Ischemic stroke, Transfusions could contribute to DIC, Thombocytomenia in the context of DIC	Unclear case, poorly documented	Oral contraceptives increases 7x the risk of CVST in women. Expert 2: normal aPTT, normal INR and normal fibrinogen go against DIC. More like HIT-type clinical picture: Thrombocytopenia and thrombosis leading to treatment with heparin.	Acute coronary syndrome although troponin level can also be increased in PE [pulmonary emboly]. Haemorrhagic stroke and DIC with elevated D-Dimers, thrombocytopenia.	

Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19 Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine AstraZeneca) (Other viral vaccines)
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PRAC Assessment

Seven cases[2] reported with the PT "Disseminated Intravscular Coagulation" (DIC) were identified from the Eudravigilance report. Three were less documented.

The Disseminated Intravascular coagulation (DIC) events were identified mainly in women (6/7) and in young adults (aged 26 to 60 years). COViD-19 results were provided for two cases only and both test were negative (one of the case had 1 PCR positive on 4 and was considered as false positive) No information was found for the other cases. A fatal outcome was reported in four cases. In five cases, severe thrombocytopenia was reported.

An initial reaction to the vaccine is reported in four cases with no specific pattern. Those immediate reactions are followed by a period of improvement, most patients continuing their activities, before rapid deterioration 5 to 10 days after vaccination.

No risks factors for DIC can be clearly identified from this overview of cases. Clearly, the occurrence of neurological signs from 4 days after vaccination should alarm and request further imedical investigation. However, in the early phase of clinical deterioration, signs and symptoms can be difficult to interpret.

Cerebral venous sinus thrombosis (CVST)/Cerbral venous thrombosis (CVT) (DLP including data available in EVDAS up to 6pm on the 16th March 2021)

A total of 16 cases, reported under the MedDRA PTs 'cerebral venous thrombosis' or 'cerebral venous sinus thrombosis' were available in Eudravigilance up to the 16th March 2021 at 6pm. A further two cases, reported as 'cerebral thrombosis' were considered relevant following review by haematologist (expert opinion).

Of the 18 cases, six were fatal. In one of these fatal cases, an event of disseminated intravascular coagulation was also co-reported. Therefore, a total of 9 fatal cases were reported for events of DIC/CVST.

Cases originated from 5 countries: DE (7), IT (3), UK (3), IN (2), NO (2), ES (1). The majority (16) were in women, the median age was 42 (range: 22 to 64). The time-to-onset ranged from 1 to 14 days with a median of 8 days. Thrombocytopenia occurred in 12 cases (67%). Several cases possibly confounded by medical history and/or co-medications.

An overview of the 18 cases of CVT/CVST are presented in the table 7 below

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[2] Summary and DIC table updated by EMA on 19 March 2021

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Table 7: Overview of cases concerning CVT/CVST

Case	Age group	Gender	Medical history	Concomitant medication	TTO (days)	Other reactions	Fatal
1	40-49	F	Asthma, Intervertebral disc protrusion, Autoimmune thyroiditis		9	Acute respiratory failure, Cerebral venous sinus thrombosis, Chills, Coordination abnormal, Dizziness Gait disturbance, Headache, Hemiparesis, Pain in extremity, Pyrexia, Seizure, Thrombocytopenia Vomiting	Yes
2	30-39	F			7	Brain death, Haemorrhage intracranial, thrombocytopenia	Yes
3	40-49	F			13	Haemorrhage intracranial, headache, Immune thrombocytopenia, Peripheral artery thrombosis, Pulmonary embolism, pyrexia, tachycardia	
4	20-29	F			7	Epilepsy, Subarachanoid Haemorrhage	
5	30-39	F			8	Thrombocytopenia	
6	40-49	F			13	Cerebral haemorrhage, Conversion disorder, Headache, Hemianopia homonymous, language disorder	
7	40-49	F			6	Immune thrombocytopenia	
8	40-49	F			3	Thrombocytopenia	
9	50-59	M			12		

10	30-39	M			8	Fever, pyrexia, Headache, platelet count decreased, Brain stem infarction, cerebral haemorrhage, haemorrhage, haemorrhage intracranial, , Nervous system disorder, superior sagittal sinus thrombosis, CVST	Yes
11	60-69	F			8	Headache, vomiting, photophobia	Yes
12	30-39	F			12	Headache, vomiting, gastritis	Yes
13	30-39	F			8	Severe headache, vomiting, weakness, thrombocytopenia, haemorrhagic infarction	
14	40-49	F		Ethinylestradiol, Etonogestrel	12		
15	50-59	F			1	Thrombophlebitis superficial	
16	40-49	F	Factor II deficiency		12	Hepatic vascular thrombosis	
17	30-39	F	Hypersensitivity	Desloratadine, Drospirenone & Ethinylestradiol	6	Brain oedema, Cerebellar haematoma, Disseminated intravascular coagulation, subarachanoid haemorrhage, thrombocytopenia.	Yes
18	40-49	F		Ethinylestradiol, Etonogestrel	10	Cerebral haematoma, Haemorrhagic cerebral infarction, thrombocytopenia	

Other selected cases of interest (based on review of data received to EV up to 12 March conducted by EMA clinical experts in cardiology, haematology and neurology)

Of the 27 cases of interest, 16 concerned female patients, 8 concerned male patients and in 3 cases gender was not specified. Their age ranged from 20 to 89 years, with 3 persons in their twenties, 8 in their thirties, 3 in their fourties, 6 in their Fifties, 3 in their Sixties and one in their Eighties.

Among the 27 cases, 18 were not considered by the experts to be related to the central nervous system (CNS). Those includes cases of Venous thromboembolism (5), pulmonary embolism (7), deep vein thrombosis (2), portal vein thrombosis (1), splenic infaction (1), vena cava thrombosis (1), local site reaction (1), anxiety reaction (4). Most case of pulmonary embolism were associated with venous thromboembolism. DIC was identified as possible in two cases. Of note that patients could experience various of those terms and that the level of certainty of the diagnosis were variable.

Among CNS-related events, other causes were identified: in one patient, vascular condition (e.g. aneurysm) or effect of another drug (apixaban) could not be excluded, reaction at the injection site (1), a pseudo-relapse of multiple sclerosis due to fever, and one case not interpretable.

The PRAC selected five cases from this previous list. Two of those cases report stroke episodes (in yellow in the following table), two report CVST (in orange), and one haemorrhagic case (blue). For one of the latter, a haematologist expert suggested the possibility of Heparin-induced thrombocytopenia (HIT).

Case	Demographics and pre-existing conditions	Summary of clinical course	Experts' opinion
1	40-49 ♀ Hashimoto's thyroiditis, Asthma, Intervertebral disc prolapse	D0 Vaccination NA: headache + typical subacute vascular symptoms Thrombosis of venous sinuses	Neurologist: Pattern compatible with thrombosis of venous sinuses Cardiologist: Chills, fever, pain in limb, dizziness, headache, vomiting, gait instability, hemiparesis, seizure. – clinical picture could be of CVST (cerebral venous sinus thrombosis). Haematologist: Thrombocytopenia in the context of DIC
2	20-29 ♂ Concomitant medication was not reported	D0 Vaccination NA Death. Brain swelling (Confusion and Hemiparesis) but also stated intracerebral bleed and decompression craniomy and thrombopenia. Systemic symptoms present including Micturition urgency and Painful urination and Nausea and Vomiting and Petechiae Scleral hyperaemia and Macroscopic haematuria.	Neurologist: Personally, the most probable situation is a Haemorrhagic lobal stroke treated with craniectomy. There were also systemic symptoms so the CNS haemorrhagic may be in a broader context. Haematologist: In my opinion, the only case that could fit into the clinical presentation of TTP (although DIC is also likely)
3	40-49 (likely man) No cardiovascular risk factors.	D0 Vaccination D1 Flu-like syndrome + injection site reaction D2 One-sided hemiparesis + sensitive symptoms during few minutes. Hospitalization. NRL exploration normal but a micro-ischemia in DWI+ FLAIR- D4 NRL normal CSF high-proteins only. MRI same lesion now DWI- FLAIR+	Neurologist: Ischemic Stroke of unknown etiology in a young person without vascular risk factors and normal cardiac study (except for the finding of FOP but nothing supports the embolic etiology, so it is probably not related with the current stroke) Cardiologist: Clinical picture of possible TIA event in young patient with PFO. PFO is a risk factor for stroke or TIA within 24 hours after administration of vaccine.
4	30-39 ♂	D0 Vaccination D1 Fever, generalised aching Day9 Headache, thrombocytopenia (30 and fell to 10 – 15 x10⁹/l, clotting time: normal Blood test: no red cells fragments cerebral venous sinus thrombosis (CVST), intracranial haemorrhage D10 Superior sagittal sinus thrombosis, brain stem infarction, intracranial haemorrhage, cerebral haemorrhage D10 Death, no autopsy	Cardiologist: Clinical picture of rapidly developing thrombocytopenia of unknown trigger associated with CVST, brain stem infarction and intracranial haemorrhage in patient 9 days after administration of vaccine. Limited blood tests provided do not point to DIC (normal clotting time), do not point towards TTP (no red cell fragments). Unclear how the signs of inflammation the day after vaccination are linked to neurological picture that developed thereafter. The predominant etiologies of CVST in male patients are haematological disorders, a hypercoagulable state, trauma, cancer. Haematologist: intriguing because it fits very well with the "HIT-like theory". This patient had an inflammatory syndrome (aches, fever) followed by thrombocytopenia and thrombosis, but without any signs of DIC (normal clotting factors) or TTP, and ended up having bleeding, probably due to severe thrombocytopenia. Strong suspicion of HIT like reaction.
5	60-69 ♀ Psoriasis (no systemic treatment) Hypertension, dyslipidaemia Hypertensive heart disease amlodipine, valsartan and hydrochlorothiazide, Betaine,Potassium Hydrogen Carbonate, Nebivolol Rosuvastatin, nebivolol hydrochloride, Acetylsalicylic acid	D0 Vaccination D2 Stroke (carotid artery thrombosis) -> thrombolysis stopped due to thrombocytopenia worsening. Then decompressive craniectomy D2 Femoral artery thrombosis + embolectomy	Neurologist: Ischemic stroke The appearance of two events in different locations (CNS + Femoral) suggest that even atrial dilation was considered as mild by the Cardiologist, the origin may be embolic. Cardiologist: Clinical picture of possible AF with resulting emboli to CNS and leg (increased size of the atrium in echocardiography in patients with HTN, CHF [nebivolol] and hypercholesterolaemia). Haematologist: Agree, but also moderate thrombocytopenia, not very frequent in pts with stroke (0.3%)

PRAC Assessment

From the list of other cases selected in Eudravigilance report, 9 were identified as CNS-related. The PRAC selected five of those cases for further review, i.e. two cases of thrombosis, two cases of CVST and one case of cerebral haemorrhage. A possibility of DIC was mentioned for two of those patients, one with stroke and one with haemorrhage.

Those cases highlight the need for further research into occurrence of CVST and DIC in Eudravigilance.

Cases not reported in Eudravigilance at the time of the report

In addition to the cases identified in Eudravigilance, additional cases were communicated to the PRAC , with a recording date in the database after the data lock point of the report.

Cases are summarised here-after with special focus on the cases with a suspicion of CVST:

Case	1	2	3	4	5*
Age group	20-29	40-49	20-29	40-49	30-39
Gender	F	F	F	F	F
Pre-existing condition	No smoking, no contraception	Healthy		obesity, gestational diabetes, allergic rhinitis, psoriasis, and methrorragias associated to endometrial polypus	
Baseline treatment		etonogestrel/ethinylestradiol			
COVID-19	No information	No information	No information	No information	No information
Initial reaction		D1: headache			Severe reaction 7 hours after vaccination
Clinical course	D7: Convulsion (cerebral aneuvrism and tumor excluded)	D7: Headache, backpain, pain in the leg D10: found unconscious D11: hemiocraniotomy		Day 2: intense oppressive holocraneal cefalea with vomiing	D7: hospital admission with abdominal pain Persisting headache
outcome	Recovering	UKN - Cerebral status unclear		Recovering (enoxaparin)	Discharged
Thrombocytopenia	75 G/L	Yes	No	47 G/L D-Dimer 21,000	117 G/L
Diagnosis	Sinus venous thrombosis saggittlais superior and transversus) Cerebral haemorrhage	Haemorrhagic venous infarction Sinus venous thrombosis	Isolated venous sinus thrombosis	Extensive cerebral venous thrombosis, including longitudinal superior sinus, rectus sinus, transvers sinus, and left sigmoid sinus) Parieto-occipital infarction	Severe thrombosis in v. jugularis, the tisht sinus and in the sinus rectus, Cerebellar bleed
Expert opinion					

* Case submitted at the extraordinary PRAC (16 March 2021).

Three additional cases with **CVST** were reported on 16 March 2021:

Case 6 : 30-39-year-old woman who experienced sinus vein thrombosis with thrombocytopenia 8 days after vaccination . The patient is still in the hospital, and further data have been requested.

Case 7 : 40-49-year-old woman who experienced sinus vein thrombosis with thrombocytopenia (60,000/uL) 14 days after COVID-19 vaccination AstraZeneca. Further information is currently being sought.

Case 8 : 40-49 year old female developed sinus thrombosis and thrombocytopenia 6 days after vaccination with AstraZeneca.

Two other cases in women were communicated and were not identified in the Eudravigilance search:

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Case 9 : Female, 30-39. Fatal case involving hospital admission 10 days after vaccination with one sided hemiparesis, aphasia, and gradually reduced consciousness. CT showed cranial bleed. Thombocyts measures at 37. An autopsy report is requested.

Case 10 : Female , 50-59, with hypertension under treatment. Case of pulmonary embolism, with symptoms starting 7 days after vaccination.

One additional case **of portal vein thrombosis** was communicated on 16 March 2021:

Case 11 :M, 30-39. Diagnosis of portal vein thrombosis whose symptoms started 7 days after vaccination. Thrombocytopenia at 10 G/L. The patient developed abdominal purpura annd easy bleeding. The outcome is currently unknown.

PRAC Assessment

Eight additional cases of cerebral venous sinus thrombosis were identified after the data lock point of the EudraVigilance report. All patients were women with ages ranging from 22 to 46 years. Thrombocytopenia was reported in 7 cases of the eight cases. No result for COVID-19 testing is reported.

Those cases follow the same chronological pattern of events, with first reactions in the 2 or 3 days following vaccination and secondary degradation in the 6 to 14 days following vaccination.

Those cases are not sufficiently documented to identify potential risk factors beside the fact that all cases are women.

In the other cases communicated, we note one case of deep vein thrombosis, one case of portal vein thrombosis, one fatal case of cranial haemorrhage, and one case of pulmonary embolism.

The cases identified after the data lock point of the Eudravigilance search were reported ad hoc by member states because considered as potentially relevant to the assessment. Their identification does not result from a systematic search.

Review of the cases: Overall PRAC comment

The Eudravigilance search permitted to identify 202 serious cases from which 22 % were fatal. Most cases (122) were female.

In this report, the PRAC reviewed more specifically the cases of disseminated intravacular coagulation (DIC) and cerebral venous Sinus thrombosis (CVST). Several observations were made and should be further discussed.

1) A **chronological pattern** is observed, with a first reaction to the vaccine observed in the first days after vaccination. This episode usually last 2 or 3 days and is followed, often after a healthy interval, by a period of deterioration 6 to about 12 days after vaccination. The clinical evolution is rapid.

2) A high proportion of cases are **female**. This may be due to a higher proportion of women

vaccinated or a higher risk of thrombotic events in this population;

3) The persons affected are mainly **young adults**, with some cases in their twenties. Again, this could reflect the population currently vaccinated in many countries although many adverse events in older population are reported from UK. The identification of more cases in the younger population could reflect a higher risk in this population. Eventually, it could also reflects the fact that thrombotic events being less expected in this population, those cases attract more attention.

4) **Thrombocytopenia** is documented in most cases of thrombotic events. Thrombocytopenia is often severe and most likely peripheral. The association of cerebral thrombosis and thrombocytopenia is unusual and the pathophysiological mechanism of these thromboses may differ from classical ischemic strokes.

The review of the cases did not permit to clearly identify **risk factors** beside those already mentioned (i.e. potentially age and sex). It was also not possible to identify **prodromes**. High reactogenicity is documented for this vaccine and signs and symptoms reported here are not specific. Also, the first signs at the time of the degradation varied, although **neurological signs and a resurgence or increase of symptoms a few days after vaccination should be taken in high consideration.**

Finally, those cases did not permit to document a possible association with **COVID-19 infection**. However, when the results of the tests were made available, they were negative.

3.1.6. Information received from MHRA

Information received on 16th March.

Following the reports of temporarily pause of the vaccination programme with COVID-19 Vaccine AstraZeneca in several EU member states the MHRA conducted a review, focusing on specific UK data on venous thromboembolic events occurring more generally along with an evaluation of cases of thrombocytopenia, with and without venous thromboembolic events.

The report mentions the following:

With Astra Zeneca the pattern seen with venous thromboembolic events more generally is in keeping with expected background trends and usage of the vaccine. There have been a small number of cases reported of thromboses with thrombocytopenia which appear to be in keeping with the cases reported from the EU, however this remains at a low incidence given the usage to date. The available evidence is insufficient to establish a causal association, and MHRA continues to monitor cases reporting thrombocytopenia, immune thrombocytopenia and associated events.

In summary, venous thromboembolism (VTE) occurs naturally, in all ages, and is not uncommon. We have been closely reviewing reports of VTE and their consequences following vaccination with COVID 19 vaccines. Amongst the more than 24 million doses of both vaccines administered so far, several hundred cases of VTE are expected to have occurred by chance within a short time after vaccination. The analyses show no evidence that VTE, overall, is occurring more than what would be expected in the absence of vaccination, for either vaccine.

The action taken by some EU countries over the past week to temporarily pause the use of the AstraZeneca vaccine has been based mainly on isolated reports of cerebral sinus vein thrombosis concurrent with thrombocytopenia and bleeding shortly after vaccination. This form of blood clot can also occur naturally in the absence of vaccination, can occur in association with COVID disease and is extremely rare, and a causal association with the vaccine has not been established. The reporting rate

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of this following vaccination in Germany has been 4 per million doses of the vaccine. In the UK, 4 possible cases of this form of blood clot with low platelets after 11 million doses of the AstraZeneca vaccine have been identified so far.

Whilst this requires further review, a causal association with the vaccine cannot be established based on available information. Given the extremely rare rate of occurrence of these events, the benefits of the AstraZeneca COVID vaccine, with the latest data suggesting an 85% reduction in hospitalisation and death from COVID disease, far outweigh any possible risks of the vaccine.

3.1.7. Hypotheses and Comments from Member States

3.1.7.1. Background COVID 19 disease and thrombothic disease

Coagulation abnormalities (including thrombocytopenia) have been associated with COVID-19 disease, with variable incidence.

EMA report on Coagulation abnormalities in context of COVID-19 12 March 2021 (last update) Information overview v1.2

The EMA has released an analysis report on the COVID 19 coagulation abnormalities based on current status of knowledge in the information available in the literature and case studies.

Relevant sections of this report are summarised below.

Mechanistic and pathophysiological aspects

As regard mechanistic and pathophysiological aspects, coagulation abnormalities in COVID-19 are described as a consequence of both, cell and tissue damage that results from invasion of SARS-CoV-2 (in particular into type II pneumocytes with their ACE2 expression) with local inflammatory responses (endotheliitis), and also general changes such as associated with systemic inflammation, critical illness and reduced mobility (stasis). Some have referred to this as "viral coagulopathy", which in COVID-19 is notable for the high frequency and severity of microthrombosis, in addition to macro-thromboembolic events (such as also occurring in sepsis).

It seems to be held that microthromboses can lead to macrothrombosis and subsequently to clinical events (e.g., as in review by Ortega-Paz Luis et al. 2021; Manolis et al. 2021), in both the venous and arterial circulation.

The local mediator and link between inflammation, coagulation abnormalities and microthrombosis is reported to be neutrophil extracellular traps (NETs), in a process dubbed NETosis (reports predate COVID-19). This mediation occurs at both levels, local (microvascular) and systemic (macrovascular), for reasons including that NETs can enter vessels and cause platelet activation (through Toll-like receptors, TLR), factor V activation and thrombin generation. NETs are large extracellular, web-like structures of former cytosolic and granule proteins that assemble on a scaffold of chromatin, and these have been described to kill extracellular pathogens (Lee and Grinstein 2004).

These pathophysiological events can occur in the absence of an obvious systemic cytokine storm.

The coagulation abnormalities can lead to comorbidities and events such as VTE, stroke, diabetes, lung, heart attack, acute kidney injury and liver injury. Conversely, cardiovascular risk factors can exacerbate the dysfunction of endothelium cells and platelet and contribute to coagulopathy*.*

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Consistent laboratory findings of coagulation abnormalities in COVID-19 are: elevated factor VIII and elevated fibrinogen. Unusual findings are: bleeding, thrombocytopenia, "very long" (a)PTT (e.g., more than 6 s longer than normal [25-35 s] and / or aPTT is out-of-proportion longer than PT is longer). D-dimers are recommended to be measured (see below) and to be better reported to avoid misinterpretation [1].

Risk factors

In COVID-19 hospitalised patients, the "D-dimer level at presentation was independently associated with thrombotic events, consistent with an early coagulopathy", based on more than 3300 consecutive admissions in New York state [2]. Baseline D-dimer levels seem useful for stratification of investigational interventions (such as done in the REMAP-CAP trial), but at this time they have no systematic impact on clinical management.

Of note are estimated adjusted hazard ratios (HR) for a thrombotic event (ibidem, table 2): male 1.5, myocardial infarct 1.4, congestive heart failure 1.3 and D-dimer from 1.2 to 7, whereas HRs for age and BMI were around unity. (Reasons for the prognostic importance of sex can be addressed in an update of this document.) A systematic review quantified risk factors for VTEs in around 30,000 COVID-19 patients [table 4 in ,3], reporting that men at higher risk to develop VTE but finding no association between comorbidities or age with the risk of VTE, possibly because the high VTE risk associated with COVID-19 masks general VTE risk factors such as age.

Strong clinical risk factors for VTEs in the general population are high age, obesity, reduced mobility, history of cancer or active cancer, intensive care treatment, history of VTE or thrombophilia; inherited thrombophilia conditions may or may not be known at the time of a TE, but they concern up to 12% of the general population (considering for example factor V Leiden; further such conditions are prothrombin mutations, deficiency of AT or protein S or C, anti-phospholipid syndrome).

The ISTH scoring system is an important tool to synthesise risk factors in patients with conditions that predispose to disseminated intravascular coagulation [4], which in some patients predominates as thrombosis, arterial or venous thromboembolism, purpura fulminans associated with peripheral ischemia or vascular skin infarction. In the case of a predisposing condition, the score ranges from 3 (minimum) to 8 points (maximum)

- Platelet count: 1 point if 50,000 to 100,000/µl, 2 points if <50,000/µl

- PT: 1 point if 3 to 6 s, 2 points if more than 6 s prolonged

- Fibrinogen: 1 point if <100 mg/dL

- D-dimer: 2 points for moderate, 3 points for "strong" increase [cut-off values have not been defined but "3- to 4-fold increases" are proposed [5]]

COVID-19 as a severe infection should qualify as predisposing condition so that the score can be used to gauge the risks of patient for coagulation-related events. With respect to coagulopathy, it has been recommended to monitor PT, D-dimers, platelet counts and fibrinogen for determining the prognosis of Covid-19 patients [5].

Other risk factors for coagulation abnormalities and / or TEs in COVID-19 are currently suggested (e.g., deficiencies of vitamin D, magnesium, phosphate), with recommendations to correct obvious deficiencies.

In the paediatric population, the Multisystem inflammatory syndrome in Children (MIS-C) involves multi-organ function impairment according to a literature review [6]; thrombotic complications affected

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only 13 out of 928 paediatric patients with MIS-C but included serious events (2 splenic infarctions and 5 cerebral strokes during ECMO).

PRAC assessment

The association between coagulation abnormalities and COVID-19 disease is now rather well documented including in recent literature review papers. The literature review by *Raadsen M et al*. *(2021)** can be cited as an example. The authors discuss the Thrombocytopenia in Virus Infections and there is a paragraph on SARS-COV2. Consistently with the EMA report, the authors describe several mechanisms involving hypercoagulability and inflammation interact resulting in thrombotic phenomena, both in the microvasculature and in the larger, mostly pulmonary blood vessels.

Current recommendations in the EMA report for clinical management of coagulation abnormalities in COVID-19 are based on medicines and approaches that are used widely and generally for prophylaxis and treatment as for other critically ill or ICU-hospitalised patients.

*Raadsen, Matthijs et al. "Thrombocytopenia in Virus Infections." Journal of clinical medicine vol. 10,4 877. 20 Feb. 2021, doi:10.3390/jcm10040877.

3.1.7.2. Hypothesis based on cases:

1. Heparin induced thrombocytopenia- like syndrome (HIT)

This hypothesis is less documented and further information is still being collected.

The hypothesis is presented below. Based on this hypothesis, the etiology of the clinical presentation observed in patients would be a cascade of reactions triggered by the combination of heparin with Platelet Factor 4.

This hypothesis would be consistent with the HIT-like syndrome associated with thrombocytopenia and sometimes evolving into Disseminated intravascular coagulopathy or Cerebral venous sinus thrombosis also associated with thrombocytopenia.

The hypothesis of HITT like syndrome is supported by some thoughts and comments by EMA haematologist experts and by some Member States.

Analysis from EMA experts

Haematologist: A heparin-induced thrombocytopenia-like syndrome could be a possibility and explain the triad of thrombocytopenia, thrombosis and bleeding. Indeed, pseudo-HIT would fit better than TTP. Some factors supporting this theory are:

- HIT is more common in women than men.

- HIT is more frequent when there is some inflammatory background (surgery/trauma) than when heparin is administered in other contexts. I believe that something in common to all fatal cases from this series is a systemic inflammatory syndrome.

There are, however, some differences:

- HIT is a serious disease, with a mortality that can reach 20%, but the cause of death is typically

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thrombosis (e.g. stroke). DIC is a rare complication of HIT.

- The importance of thrombotic events explains why anticoagulation with an alternative agent (e.g. danaparoid) is a very important part of therapy. The fatal cases from this series all ended up having DIC, where anticoagulation would have been difficult/controversial.

- In HIT, heparin is administered continuously, and this continuous "antigen" administration self-perpetuates the immune response, whereas stopping the heparin typically improves the problem. Indeed, HIT is clearly associated with length of heparin therapy. The same applies to many other immune-related drug reactions. In contrast, here we are dealing with a single administration of a "drug", so the mechanism must be somewhat different. Or, for these patients this was not the first encounter with the antigen. This raises the question as to whether fatal cases went through COVID-19 disease before vaccination.

Cardiologist: Agree with these comments and in particular the difference related to the prolonged (or repeated) administration of antigen in immune-mediated HIT vs single administration of vaccine. Also the recovery from thrombocytopenia follows within day when the administration of heparin is stopped. But similar to what is observed with several cases here there is often in HIT the few days/weeks delay in development of thrombocytopenia/clinical picture (unless repeated exposure where the symptoms develop more rapidly).

Analysis from Member State

Some of the case stories including the Member State case, involve primarily younger, previously relatively healthy women (and two men) who developed thrombocytopenia, bleeding and thrombosis all within 7-10 days of vaccination with the AZ vaccine. A theory has now been raised that the mechanism behind the clinical presentation could be similar to what is seen in cases of heparin induced thrombocytopenia (HIT), except that the immunological response could be triggered by the AZ vaccine rather than heparin.

Heparin induced thrombocytopenia occurs very rarely and it is not possible to pinpoint in advance which patients are at increased risk of developing this condition. Heparin triggers an immunological response leading to thrombocytopenia, bleeding and thrombosis. The time to onset is typically 5-7 days. The cases with the AZ vaccine had time to onset of the serious adverse event within 7-10 days in patients with a clinical presentation very similar to that seen with HIT.

Given that the factors involved in the development of thrombocytopenia, bleeding and thrombosis, it is difficult, from quality perspective, to know what to look for. Although, at this stage, it does not seem likely, that the events seen is quality related, this should not be excluded. While it is clear that the AZ vaccine does not contain heparin, the question remains whether something else, related to the quality of the AZ vaccine, could have been involved in causing the events seen. Any possible option should be taken into consideration, such as: possible quality defects, a particular excipient, or a product- or process related impurity, originating from a particular step in the manufacturing process, and which may trigger a similar immunological response as seen in cases of HIT. It should also be considered if batches involved in the currently reported events is from a particular drug substance- or drug product site.

PRAC Assessment on HITT hypothesis

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It should be noted that the relevant information to substantiate this hypothesis is still being collected and more data is expected from experts and from MAH by the time of PRAC discussion of 18 March 2021.

The possibility of heparin-induced thrombocytopenia (HIT)-like syndrome triggered by a component in the vaccine is evaluated by experts. The clinical picture is quite similar. It seems that the pathophysiological mechanism involved may mimic the HIT entity, with platelets as a direct target. This remains to be demonstrated.

2. Other hypothesis:

- ### COVID-19 disease

As described above, COVID-19 is known to be associated with coagulation abnormalities (see section 3.1.7.1). Confounding effect or underdiagnosis of covid-19 disease cannot be excluded.

Comments from Member State

We note that in the 4 Member State cases, the time to onset is 10 days or less after what seems to be the first dose of the vaccine in the three that specify TTO. It takes 3 weeks for protection to start after the first AZ dose, hence these patients would not yet have immunity to COVID-19. We also noted the temporal relationship described in the fatal and complex cases in the signal AR. Case 1 had a fever when she presented with headache. Of note, in addition to thrombocytopenia, she also had elevated D-dimer.

Thrombotic events may be the presenting feature of COVID-19 which causes a hypercoaguable state, including thrombocytopenia, elevated D-dimer levels and prolonged PT (Jafari 2020) [20]. VTE in COVID includes PE, DVT and as well as 'immunothrombosis' in smaller pulmonary arteries and capillaries (BTS Guidance on Venous Thromboembolic Disease in patients with COVID-19, 2021) [21]. Thrombotic complications in COVID-19 also include MI and ischaemic stroke (Piazza & Morrow 2020) [22].

There are also numerous literature publications specifically concerning cerebral venous sinus thrombosis (CVST) in young patients with COVID-19 (e.g. Abouhashem 2020) [23], even without risk factors for VTE, and it has been described as a presenting feature (Hughes 2020) [24]. We note one case series in which one patient was on oral contraception additionally (Cavalcanti 2020) [25], and there is a further case in which CVST was accompanied by coagulopathy (Sugiyama 2020) [26]. There is also a further case which was accompanied by internal iliac vein thrombosis related to paucisymptomatic infection with COVID-19 (Beretta 2020). In this case, nasopharyngeal swab, repeated twice, tested negative for SARS-CoV-2 – it was serological tests that confirmed SARS-CoV-2 infection.

A recent literature review of CVST in COVID-19 identified 9 studies and 14 patients (Tu 2020) [15]. The median age was 43 years (IQR=36-58) and majority had no significant past medical conditions (60.0%). The time taken from onset of COVID-19 symptoms to CVST diagnosis was a median of 7 days (IQR=6-14). A significant proportion of patients had raised D-dimer (75.0%). Overall mortality rate was 45.5%. It also seems that women may be affected more frequently (Nwajei 2020) [27]. A further publication advises that COVID-19 patients with a preexisting hypercoagulable state, such as pregnant/ postpartum women, women using OCPs, those in a fasting state, and those with history of thrombophilia, malignancy, and chronic inflammatory diseases are high-risk groups (Shakibajahromi

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2020) [28]. Indeed, we note that three of the cases are on CHCs and in one, tranexamic acid is listed as a concomitant medication. This publication also highlights that elevated D-dimer, fibrinogen level, fibrin/fibrinogen degradation product (FDP), and thrombocytopenia are commonly reported laboratory abnormalities in COVID-19 patients, with higher rates in severe disease. It further noted that CVST and COVID-19 might present with haemorrhagic infarction as the first imaging manifestation and that the presence of any unexplained and atypical haemorrhagic lesion in the initial brain CT of these patients should raise the suspicion to CVST. A meta-analysis estimated the risk of CVST among hospitalised SARS-CoV-2 infected patients to be 0.08% (95% CI 0.01% - 0.5%) (Baldini 2021) [29].

 We further note that there are a number of published case reports describing portal vein thrombosis in previously healthy young males (Borazjani 2020 [30], Franco Moreno 2020 [31], Jafari 2020 [20]), and one publication describes COVID-19 as a novel aetiology of this condition (Hassan 2021) [32]. Mesenteric ischaemia can also be a presenting feature of COVID-19 or may occur at a late stage in the disease, as described in a recent case series (Singh 2021) [33].

 We further note that DIC has been described in association with COVID-19 (Asakura & Ogawa 2020) [34[34]. A recent paper has proposed a definition for COVID-19-associated coagulopathy (CAC), based on clinical and laboratory features (Iba 2021) [35]. This includes proven COVID-19 test with two or more of the following four criteria: (1) decrease in platelet count (less than 150×10^9/L); (2) increase in D-dimer (more than two times the upper limit of normal); (3) >1 s prolonged prothrombin time or INR > 1.2; (4) presence of thrombosis (macrothrombosis including deep vein thrombosis/venous thromboembolism, thrombotic stroke, acute coronary syndrome, limb artery thrombosis, mesenteric artery thrombosis, etc., and/or microthrombosis including skin acral lesions, etc.) (Iba 2021) [35]. If the patient meets one of the above 4 criteria and also one or more of following criteria: (i) increase in fibrinogen level; (ii) increased VWF (more than two times the upper normal limit); (iii) presence of lupus anticoagulant and/or high-titer antiphospholipid antibodies, they are defined as "risk of CAC".

 It may also be worth considering that the accuracy of viral RNA swabs in clinical practice varies depending on the site and quality of sampling. A positive test for SARS-CoV-2 on PCR generally confirms the diagnosis of COVID, and CDC indicates that confirmatory laboratory evidence is detection of SARS-CoV-2 RNA in a clinical specimen using a molecular amplification detection test. However, false negatives may occur (Woloshin 2020) [36]. Accuracy varies by site and stage of disease and degree of viral multiplication or clearance (Watson 2020) [37]. The CDC definition of severe Covid indicates RNA to be confirmatory laboratory evidence, but it can be diagnosed on presumptive laboratory evidence including detection of specific antigen in a clinical specimen or detection of specific antibody. However, antibody testing should be undertaken at least two weeks after onset of symptoms and the sensitivity and specificity of antibody tests vary over time, hence results should be interpreted in the context of clinical history and depends not only on the accuracy of the test itself, but also the pre-test probability of infection. At days 1-7, the sensitivity of IgM testing is only 23.2%, increasing to 58.4% at days 8-14. Similarly, for IgG, sensitivity at day 1-2 will be 29.7%, increasing to 66.5% day 8-14. A single negative test result may not be informative if the pre-test probability is high (Watson 2020) [38].

 We suggest that it may be warranted to consider COVID-19 an alternative aetiology, particularly in cases of VTE and ATE which are very unusual and/or associated with thrombocytopenia, elevated D-dimer levels and prolonged PT. It may also need to be considered that background rates of VTE and ATE including rare types of events measured prior to 2020 may not be reflective of the current background rate, with the virus in circulation.

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PRAC assessment on "COVID-19 disease" hypothesis

This hypothesis is mechanistically plausible. However this is not supported by the negative PCR and serologic results in some patients experiencing coagulation abnormalities.

It cannot be excluded that COVID-19 might be part of the possible explanations but it is hardly the only one.

Many of the people affected by this clinical picture suffer from auto-immune or allergic pre-existing conditions (asthma, psoriasis, drug allergies, auto-immune thyroiditis or other auto-immune disease) or a chronic hepatitis B (2 cases) that may play a role as chronic immune stimulation.

Taking into account the fact that COVID19 infection is associated with tremendous thrombotic complications, whose complete pathophysiology is not well understood, it is logical to suspect that the immune responses associated with COVID19 infection and COVID-19 vaccination may share some similarities that would increase the risk of thrombotic event. We could imagine that the S-specific T cell immune response may induce a cross reaction with a specific human protein or antigen involved in the hemostatic system (and leading to platelet activation and/or consumption) in people particularly sensitive because of a "reactive" immune system. The phenomenon of "molecular mimicry" in the situation of vaccines has been well reviewed by Segal et al. 2018 [39]. Regarding the timing of the immune response following vaccination, we know that the immune response starts quickly after vaccine administration (as evidenced by fever or systemic symptoms starting few hours after vaccination) and, even if 3 weeks are needed to achieve immunity against COVID19, the process is launched immediately and the production of antibodies is only part of the global immune response triggered by vaccination. However, this hypothesis would not explain why a particular occurrence of this clinical picture is described after the AstraZeneca vaccine compared to other non-adenovirus COVID19 vaccines (Pfizer, Moderna).

- ***Adenovirus vector***

Some evidence that thrombocytopenia has been reported following the administration of adenoviral gene transfer vectors. impact of the ChAdOx1 in this context.

Analysis from Member State

General clinical overview of cases:

For assessment of possible pathogenicity mechanisms, a general overview of the clinical issue(s) is obviously the first step. This general clinical overview is provided in the following.

It should be stressed that the information is preliminary and incomplete, as cases are still accumulating, several patients are in intensive care or recovering (outcomes unknown), and details for the diagnostic hematology workup for patients is lacking (translation of hospital records is pending).

So far, EMA/PRAC is aware of a total of approx. 15 cases of thromboembolic events combined with thrombocytopenia associated with use of the AZ COVID-19 vaccine.

The cases had the following in common:

Most cases occurred in individuals < 50 years of age, i.e. the younger age group (no sex predisposition).

Most cases occurred within 10 days of the 1st vaccine dose, in otherwise generally healthy individuals.

Specifically, COVID-19 is associated with thromboembolic events, but there was no history of active SARS-CoV-2 infection in these cases.

Many cases had fatal outcomes.

Most of the cases presented with one or more of the following symptoms: Thrombosis affecting different organ systems (CNS, lung, spleen, gastrointestinal tract) and including large vessels (cerebral venous sinus thrombosis in 5 individuals, in others thromboses affected portal vein, splenic vein, pelvic vein, femoral artery, common carotid artery), thrombocytopenia (presumably secondary to thrombosis, i.e. consumptive), bleeding, petechiae and multiorgan failure.

Platelet counts were in most cases in the 15 - 80 x 1E9/L range (i.e. above the very low levels often seen with drug-induced thrombocytopenia) [40].

The following observations are also considered relevant as regards evaluation of pathogenicity mechanisms:

In 3 of the apprx. 15 cases of thromboembolic events combined with thrombocytopenia, Hashimoto's thyroiditis was reported as co-morbidity.

In 3 of 13 other cases registered with monosymptomatic thrombocytopenia within 14 days of vaccination with AZ vaccine, comprising mostly older individuals (median age 63.5 years), idiopathic thrombocytopenic purpura (IPC) was reported as co-morbidity, and in these cases, flare-up of the purpura was seen after vaccination.

Both observations above suggest a possible auto-immune/immune component behind the thrombocytopenic and thromboembolic events.

In short, the cases appear to exhibit a general clinical presentation of moderate thrombocytopenia combined with pro-thrombotic predilection, affecting also large vessels.

Interestingly, this clinical presentation is considered to be quite similar to heparin-induced thrombocytopenia [40, 42]. It should be stressed, however, that there was no history of heparin treatment prior to thrombosis in these cases (some patients were treated with heparin after thrombosis was evident).

Description of vaccine (ChAdOx1 nCoV-19/AZD1222):
The active component in the vaccine is a replication incompetent chimpanzee adenovirus vector (ChAdOx1), encoding the full-length spike protein of SARS-CoV-2 (based on the original index Wuhan-1 sequence, codon-optimized, with a tissue plasminogen activator leader sequence).

The adenovirus particles are formulated in L-histidine (pH 6.6), NaCl, MgCl2, EDTA, sucrose, ethanol, polysorbate-80 (tween 80). One dose (0.5 mL) contains ≥ 2.5E8 infectious units of adenovirus particles.

Following intramuscular injection, the adenovirus vector is thought to transduce primarily muscle cells and fibroblasts at the injection site, and professional antigen presenting cells in draining lymph nodes, causing expression of the trimeric native-like prefusion form of the SARS-CoV-2 spike protein on cell surfaces (antigen-presenting cells also expected to display processed form of the spike antigen, not

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detailed) [42].

This mode and pattern of SARS-CoV-2 spike antigen presentation is essentially identical between ChAdOx1 nCoV-19/AZD1222 and eg mRNA-based vaccines. Also, all currently approved COVID-19 vaccines employ spike immunogen based on the sequence of the original SARS-CoV-2 isolate from the index human case in December 2019 (Wuhan-Hu-1 SARS-CoV-2 isolate) [43].

Possible vaccine-specific pathogenic mechanisms for combined thromboembolic/thrombocytopenic effects:

The following 3 pathogenic mechanisms were considered:

Hypothetical mechanism #1: Issue is caused by the SARS-CoV-2 spike protein immunogen encoded by the chimpanzee adenovirus vector. This mechanism was considered unlikely, as the mode and pattern of SARS-CoV-2 spike antigen presentation (on the surface of muscle cells and fibroblasts at intramuscular injection sites, and by antigen-presenting cells in draining lymph nodes) is not vaccine-specific (is shared with mRNA vaccines, which also employ very similar Wuhan-based spike immunogens).

Hypothetical mechanism #2. Issue is caused by product impurities and/or excipients: This mechanism was considered un-likely, because (i) the vaccine excipients (detailed above) are not considered to be associated with thromboembolic risk, and (ii) the issue has to our knowledge not associated with certain batches of product (which would have been expected if the cause was eg contamination with endotoxin or other pro-thrombotic compounds).

Hypothetical mechanism #3. Issue is caused by the chimpanzee adenovirus vector:

Replication in-competent adenovirus vectors based on different serotypes of human adenoviruses are known to be able to cause thrombosis, secondary (consumptive) thrombocytopenia and disseminated intravascular coagulation, potentially leading to multiorgan failure and death, especially after intravenous injection of virus particles. This has been consistently described in preclinical models eg nonhuman primates and rabbits, and one fatality has also been reported in a phase 1 clinical trial [44-49].

The pro-thrombotic effects of adenovirus vectors are considered to be caused by a combination of the following pathways/sub-mechanisms [44-49]:

Binding of adenovirus to platelets, causing platelet activation and thrombosis.

- Specifically, aggregation and activation of platelets is mediated by so called RGD motifs in the pentn base of adenovirus particles, binding to alphaIIb/beta3 integrins on platelet surfaces [44].

- Interestingly, adenovirus particles can also bind platelet factor 4, the platelet antigen considered to be responsible for triggering heparin-induced thrombocytopenia [40, 41, 50].

- Thus, it is likely that antibody production against vector and/or platelet self antigens can might aggravate platelet activation, as is known from eg histamine-induced thrombocytopenia [40, 41].

- Binding of adenovirus particles to endothelial cells, causing endothelial activation and thrombosis.

- Systemic inflammatory response syndrome (SIRS; cytokine storm), which in itself activated

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platelets and endothelia.

- Auto-amplification loops, where initial adenovirus vector-mediated injury to thrombocytes and endothelia self-amplify (as is also known from eg heparin-induced thrombocytopenia and disseminated intravascular coagulations) [40, 41].

None of pathways/sub-mechanisms above require active adenovirus replication in platelets or endothelia (all are triggered by simple binding of virus vectors to cell surfaces).

Due to the overall similarity in the build of adenovirus particles, it is considered likely that the abovementioned pro-thrombotic effects are also shared by chimpanzee adenoviruses such as the ChAdOx1 vector employed in the AZ vaccine.

At the same time, it is considered likely that due to the sequence differences in the RGD loops between ChAdOx1and human adenoviruses [51], the risk profile of the AZ vaccine may in this respect differ from vaccines based on human adenoviruses.

As mentioned, while the pathways/sub-mechanisms above are triggered by binding of virus vectors to cell surfaces, the thromboembolic mechanisms thus triggered can proceed and propagate due to self-amplifying feedback mechanisms; likely for this reason, the relationship between dose of vector and toxicity is non-linear, with dramatic differences in scope and severity of toxicity noted with only modest increases of vector dose at higher doses [49].

Also, there is substantial subject-to-subject variation in the toxicity of adenovirus vectors in humans [51]. The causes for this are unknown, but may relate to known genetic polymorphisms in eg alphaIIb/beta3 integrins or Fc receptors on platelet surfaces

It should be stressed that such pro-thrombotic effects of adenovirus vectors in humans is exceedingly rare [49].

For all the reasons above, it is concluded that if the AZ vaccine causes the observed thrombotic/thrombocytopenic events, this is most likely caused by the ChAdOx1 vector.

Finally, it should be mentioned that the mechanisms described above for adenovirus-mediated pro-thrombotic effects are very similar to the mechanisms described for heparin-induced thrombocytopenia ([40, 41, 44-51]; not further detailed here). This similarity in clinical presentations between thrombotic/thrombocytopenic events associated with AZ vaccine use and heparin-induced thrombocytopenia has also been remarked on in the latest EMA assessment.

PRAC Assessment on Adenovirus hypothesis

This hypothesis is mechanistically plausible but not supported by the fact that IV administration might be needed to observe the abnormalities. However the latter should be nuanced due to known challenges in the implementation of the "syringe-not-in-vessel" test in the context of massive vaccination.

The hypothesis is also consistent with the different O/E computed with non-adenovirus vaccines (Pfizer and Moderna) and compatible with the negative PCR and serologic results in some patients.

As mentioned by other experts, a thrombocytopenia following the administration of adenoviral gene transfer vectors has been described, involving von Willebrand factor and P-selectin. It is true that the

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"syringe-not-in-vessel" test is hardly ever realized by vaccinators and that the risk of intravenous injection may possibly increase the risk of activation of this thrombocytopenic mechanism. However, it is considered unlikely that the thrombotic complications possibly associated with the vaccine would be imputed to adenovirus and would have no pathophysiological link with the thrombotic complications observed with COVID19 infections.

- **ChAdOx coexpression Tissue plasminogen activator**

There might be a potential role of tissue plasminogen activator (tPA) leader sequence in a thrombotic/thromcytopenia disorder (The active substance consists of a recombinant, replication-deficient (E1 and E3 deleted) chimpanzee adenovirus (ChAdOx1) that encodes the SARS-CoV-2 (nCoV-19) spike protein combined with a tissue plasminogen activator (tPA) leader sequence).

Comment from Member State

The COVID-19 vaccine Astra Zeneca does not only code for the protein S but includes a tissue plasminogen activator (tPA) signal sequence, to increase immunogenicity [42]. tPA is used as a heterologous targeting signal sequence to traffic the protein to the cell secretion pathway to increase expression membrane. This has been shown to enhance the strength of the immune response against antigens having this signal sequence [52]. It may be hypothesised that such a supplement is likely to interfere with plasminogen activator, i.e. with the coagulation system.

The entire tPA sequence is available in GenBank [53]. The signal sequence is coded by bp 63 to 167, to yield after in silico translation a 36 aa peptide (i.e. MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARS). Considering the length of the peptide it has been hypothesised that it may play another role in addition to targeting cellular secretion pathways. However, it is unknown if it could cause thrombosis.

Furthermore, the strong expression and the secretion of a large amount of the spike protein, per se, may cause blood clots in predisposed individuals, especially in conjunction with calling a strong immune response to the S protein.

Finally, it is not known whether this sequence is cleaved from the product before it is secreted. Usually it is, but not always. It can be checked virtually if the junction of the tPA sequence and the S protein is susceptible to induce the production of antibodies.

CHMP comment on tPA

So far as we did not receive any (non-clinical) documentation from the company for the characterization of these specific antigens.

It is considered unlikely that a signal sequence, which is meant to translocate a protein to the cellular surface, interferes with the function of proteins who happen to possess the same signal sequence.

However, this is not considered the most important aspect. tPA initiates clot lysis (through plasminogen activation to plasmin). It does not directly interfere with the coagulation pathway itself (in other words, it does not inhibit DIC, consumption of coagulation factors, and other markers of a hypercoagulable state).

Most importantly, tPA/plasmin does not interfere with platelet function, and the defining new factor of

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the current signals/reports is thrombocytopenia. Thrombocytopenia is the result of platelet consumption or destruction (or decreased platelet formation, which in this case also considered unlikely).

As an aside, it is important to discriminate between coagulation in the strict sense (fibrin clot formation which involves coagulation factors II-XIII) and platelet function/activation (which involves vWF and various platelet receptors). Both result in what is loosely termed hemostasis, thrombus formation and (in pathological states) thrombosis – however, these two mechanisms are quite different.

PRAC Assessment on tPA

In general, leader sequences are meant to guide proteins to the endoplasmic reticulum (ER) for secretion. In principle the leader sequence is cleaved off. If cleavage does not occur, the fusion protein (tPA leader- Spike protein) remains anchored in the membrane of the cell and cannot be secreted. Furthermore, the tPA leader sequence has been shown to improve immunogenicity of an MVA vector vaccine by enhancing secretion of the antigen.

In principle, the leader sequence remains incorporated in the membrane and is recycled and degraded within the cell. Even if it would be released, there is no scientific evidence that this sequence could activate any pathways involved in hemostasis or coagulation.

Finally, one might speculate that the Spike protein itself could activate particular signalling pathways. However, there is no confirmed scientific rationale supporting this at this stage*.*

It could be also highlighted that the construct used in the COVID-19 vaccine AstraZeneca encodes for the Spike protein sequence which has not been modified. All other vaccines (Pfizer, Moderna, JNJ) have modified the sequence in order to stabilise the Spike protein in the pre-fusion state. However, this is not expected to have an impact on possible thrombosis events.

3.1.8. Conclusion

Based on the review of clinical and non-clinical data (DLP 7 Dec 2020), there is currently no evidence to suggest an association of thrombotic events with the use of COVID-19 Vaccine AstraZeneca.

The preliminary information from the BWP concluded that there is no indication so far that SAE are linked to the quality of the vaccine (16 March 2021). Follow-up questions for specific batches were made to the MAH.

Based on the available observed-to-expected analysis by the MAH and EMA, there is currently insufficient evidence to suggest there would be an increased risk of embolic and thrombotic events associated with COVID-19 Vaccine AstraZeneca.

However, a signal was noted for rare events, such as Disseminated intravascular coagulation, Cerebral venous sinus thrombosis and Haemorrhagic stroke warranting further investigation. This could be described as a heparin-induced thrombocytopenia (HIT)-like/heparin-induced thrombocytopenia with thrombosis (HITT)-like phenomenon. Available evidence is insufficient to establish a causal association and further assessement is needed.

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Given the uncertainty in the case definitions of interest as this issue has progressed, further investigation on a possible underlying mechanism is needed. Following gaps in knowledge and recommendations are currently identified:

1. Importance of ruling out COVID-19 disease as an alternative diagnosis in the cases reported as thrombotic events may be the presenting feature of COVID-19;

2. Further exploration of case definitions of interest for continued monitoring and expert review;

3. Further refinement of observed to expected analsyis at the level of background rates and exposure by e.g. age-group, including sensitivity analysis. Of note is also that the rates measured prior to 2020 may not be reflective of the current background rate, with the virus in circulation.

4. An ad hoc expert meeting for case review, possible causal association, development of hypothesis, pathophysiological mechanisms, possible underlying risk factors and risk minimisation measures.

3.2. PRAC proposed recommendation

The PRAC recommends an update of **section 4.4 of the SmPC**, which should include the following elements:

- Description of the clinical entity i.e. a heparin-induced thrombocytopenia (HIT)-like/heparin-induced thrombocytopenia with thrombosis (HITT)-like phenomenon;

- Statement that some cases have been presenting as cerebral venous sinus thrombosis, cerebral vein thrombosis and disseminated intravascular coagulation; that there is currently insufficient evidence to establish a definitive causal relationship with the vaccine; that cases mainly occurred i.e. women < 55 years

- Statement that the benefit/risk in individuals at increased risk of thromboembolic events (including autoimmune disease, oral contraceptive use, or prior history of thromboembolic events) should be evaluated before vaccination.

Moreover instructions to physicians and patients to be alert on specific signs and symptoms should be included.

This information needs to be **communicated** within the SmPC /PIL and additionally via a DHPC.

Finally, the following issues need to be adressed:

1. Importance of ruling out COVID-19 disease as an alternative diagnosis in the cases reported as thrombotic events may be the presenting feature of COVID-19;

2. Further exploration of case definitions of interest for continued monitoring and expert review;

3. Further refinement of observed to expected analsyis at the level of background rates and exposure by e.g. age-group, including sensitivity analysis. Of note is also that the rates measured prior to 2020 may not be reflective of the current background rate, with the virus in circulation.

4. Ad hoc expert group for development of hypothesis, pathophysiological mechanisms, and possible underlying risk factors.

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These recommendations were based on **a preliminary assessment** that was discussed during the extraordinary PRAC of 18 March 2021, where additional information was provided by the MAH, EMA and Member States.

3.3. Adopted PRAC recommendation

The PRAC has reviewed the available evidence on the occurrence of thromboembolic events following the administration of COVID-19 Vaccine AstraZeneca, using a wide range of sources including spontaneous case reports in EudraVigilance, quality, clinical, pre-clinical and literature data and additional data from the MAH. The review of EudraVigilance data included observed-to-expected analyses that pointed to possible signals of cerebral venous sinus thrombosis and disseminated intravascular coagulation, as well as an individual case review that suggested a possible pattern in women below 55 years with a time-to-onset within 1-2 weeks following vaccination. The PRAC has also explored possible pathophysiological explanations for the observed cases.

The PRAC has concluded that there may be a risk of rare thrombotic events accompanied by thrombocytopenia following receipt of COVID-19 Vaccine AstraZeneca that needs to be reflected in the product information, while further evidence is being collected.

The PRAC recommends by a majority of 31 out of 32 votes that the MAH for COVID-19 Vaccine AstraZeneca (AstraZeneca AB) should submit a variation by 19 March 2021 to amend the product information as described below (new text underlined/text to be removed with ~~strikethrough~~):

Summary of Product Characteristics

Section 4.4 Special warnings and precautions for use

Thrombocytopenia and coagulation disorders

A combination of thrombosis and thrombocytopenia, in some cases accompanied by bleeding, has been observed very rarely following vaccination with COVID-19 Vaccine AstraZeneca. This includes severe cases presenting as venous thrombosis, including unusual sites such as cerebral venous sinus thrombosis, mesenteric vein thrombosis, as well as arterial thrombosis, concomitant with thrombocytopenia. The majority of these cases occurred within the first seven to fourteen days following vaccination and occurred in women under 55 years of age, however this may reflect the increased use of the vaccine in this population. Some cases had a fatal outcome.

Healthcare professionals should be alert to the signs and symptoms of thromboembolism and or thrombocytopenia. Those vaccinated should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain following vaccination. Additionally, anyone with neurological symptoms including severe or persistent headaches or blurred vision after vaccination, or who experiences skin bruising (petechia) beyond the site of vaccination after a few days, should seek prompt medical attention.

Risk of bleeding with intramuscular administration

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As with other intramuscular injections, the vaccine should be given with caution in individuals receiving anticoagulant therapy or those with thrombocytopenia or any coagulation disorder (such as haemophilia) because bleeding or bruising may occur following an intramuscular administration in these individuals.

Package leaflet

Section 2 Warnings and Precautions

Talk to your doctor, pharmacist or nurse before you are ~~given COVID-19 Vaccine AstraZeneca~~ vaccinated:

…

Blood disorders

A combination of blood clots and low level of platelets, in some cases together with bleeding, has been observed very rarely following vaccination with COVID-19 Vaccine AstraZeneca. This included some severe cases with blood clots in different or unusual locations and excessive clotting or bleeding throughout the body. The majority of these cases occurred within the first seven to fourteen days following vaccination and mostly occurred in women under 55 years of age, however more women under 55 received the vaccine than other people. Some cases had a fatal outcome.

Seek immediate medical attention if you develop shortness of breath, chest pain, leg swelling, or persistent abdominal pain following vaccination.

Also, seek immediate medical attention if you experience after a few days severe or persistent headaches or blurred vision after vaccination, or experience skin bruising or pinpoint round spots beyond the site of vaccination which appears after a few days.

One PRAC member disagreed with the above-mentioned recommendation of the PRAC.

The MAH should distribute a direct healthcare professional communication (DHPC) according to the text and communication plan agreed with the CHMP.

The PRAC has also agreed that an *ad hoc* expert meeting should be convened to discuss hypotheses, pathophysiological mechanisms, and possible underlying risk factors.

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3.4. Assessment of additional data – round 2

3.4.1. Introduction

On 18 March 2021, a PRAC meeting was held to discuss the ongoing signal of embolic and thrombotic events. The focus of the signal shifted during evaluation from overall thromboembolic events to specific entities, specifically thromboembolic events and cerebral venous sinus thrombosis with thrombocytopenia.

The PRAC concluded that there may be a risk of rare thrombotic events accompanied by thrombocytopenia following administration of Vaxzevria (previously COVID-19 Vaccine AstraZeneca) that needs to be reflected in the product information, while further evidence is being collected (cfr. 3.4). The MAH was also requested to distribute a direct healthcare professional communication (DHPC) according to the text and communication plan agreed with the CHMP.

The PRAC also agreed that an Ad Hoc Expert Group meeting should be convened to discuss hypotheses, pathophysiological mechanisms, and possible underlying risk factors.

This assessment is based on the additional data analysis from EudraVigilance with individual case review review and O/E analysis, input from the Ad Hoc Expert Group and available literature.

The assesseement report is structured as follows:

- EMA evaluation of EV data and expert review (DL 23/03/2021)

- Ad Hoc Expert Group (29/03/2021)

- Information from the MHRA

- Hypothesis

- Additional studies

- Conclusion and Recommendations

3.4.2. EMA analysis of EudraVigilance data

EMA provided an assessment of the signal of embolic and thrombotic events for Vaxzevria (COVID-19 Vaccine AstraZeneca), including an observed to expected analysis (DL 23 March 2021, *Valid cases received on or before 22 March 2021 23:59*).

3.4.2.1. Review of cases

As of 22 March 2021 there were 1422 cases in Eudravigilance (EV) at the level of the SMQ '**Embolic and thrombotic events**', mostly from outside the EEA (974 [68.5%]), including UK (639 [44.0%])). Most of the cases (1292 [90.9%]) were serious and 132 (9.3%) were fatal. The median age was 61 years and 57% were female.

Thrombocytopenia was either co-reported or ascertained in the narrative in at least 57 cases, 19 of which were fatal. The female-to-male ratio was 43/14, and the median age was 46 years (range: 19-73, 2 missing).

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Table 5 – Fatality rate of thromboembolic cases (SMQ) with or without thrombocytopenia (cut-off: 22 Mar 2021)

	Fatal	Non-fatal	Fatality rate
Thrombocytopenia reported	19	38	33.3%
Thrombocytopenia not reported	113	1252	8.3%
Total	132	1290	9.3%

A total of 62 cases of cerebral **venous sinus thrombosis (CVST)** were ascertained based on reported terms and clinical expert review. Most CVST cases originated from EEA countries (44 [71%]). Median age was 42 years with a female/male ratio of 49/12. One or more risk factor(s) for thrombotic events were identified in 20 cases (32.3%), including oral contraceptives (9), hypertension (5), obesity (4), transplant (2), hormonal replacement therapy (1), atrial fibrillation (1), cerebrovascular accident (1), hypercholesterolaemia (1), postpartum (1), diabetes (1), ventricular septal defect (1), neoplasm (1). In 4 cases (6.5%) the vaccinee had underlying autoimmune condition(s): autoimmune thyroiditis (1), psoriasis (1), primary sclerosing cholangitis (1), autoimmune hepatitis (1), inflammatory bowel disease (1). These included 2 cases where the vaccinee had both thrombotic and autoimmune risk factors.

Information on COVID-19 testing was available in 17 cases: 6 cases reported a negative PCR test, 1 case a negative rapid diagnostic test, 9 cases a negative unspecified test, 1 vaccinee had positive SARS-CoV2 antibodies

Median time-to-onset was 9 days. Fourteen (14) cases had a fatal outcome (22.6%). Thrombocytopenia was either co-reported or ascertained in the narrative in at least 33 (53.2%) cases, 12 of which had a fatal outcome (36.4%). Haemorrhagic events, predominantly in the brain, were reported in 23 cases.

Table 6 - Fatality rate of CVST cases with or without thrombocytopenia (cut-off: 22 Mar 2021)

	Fatal	Non-fatal	Fatality rate
Thrombocytopenia reported	12	21	36.4%
Thrombocytopenia not reported	2	27	6.9%
Total	14	48	22.6%

The number of cases reporting **disseminated intravascular coagulation'** (DIC) has remained stable since the previous discussion at PRAC (7).

There were 24 cases of **splanchnic thromboses**, 7 of which were previously reviewed by PRAC. Median age was 47 years (range: 26-82), with a female/male ratio of 18/6. A young age and female predominance is unusual for splanchnic thromboses outside COVID-19. Furthermore, common risk factors for splanchnic thrombosis (cirrhosis and chronic liver disease, myeloproliferative disorders, etc) were not reported. One patient had a history of essential thrombocythaemia, a risk factor for thrombosis.

Four (4) cases were fatal. Thrombocytopenia was noted in 9 cases, CVST in 3. Overall 20 cases were considered suggestive of portal vein thrombosis, meaning that purely arterial thrombotic events were a minority.

Time-to-onset ranged from 0 to 32 days, with a median of 9 days.

Limitations: Only a limited subset of the 1422 case narratives retrieved at SMQ level were manually reviewed for relevant clinical information or platelet counts, hence there may be more cases of thrombocytopenia than the 58 identified.

The selection of CVST cases for the analysis was partly based on clinical expert judgement, which involved some assumptions.

The following table includes the distribution by age-group for the different entities. As different entities could occur in the same patient, a total number of cases (fatal and non fatal) is also presented.

Table 7a – Age-distribution of cases for the different entities and total cases combined (cut-off: 22 Mar 2021)

Thromboembolic and thrombocytopenia			
Age group	**Cumulative Nb Total (%)**	**Cumulative Nb EEA (%)**	**Cumulative Nb non- EEA (%)**
<55	39 (71%)	31 (74%)	8 (62%)
<60	44 (80%)	35 (83%)	9 (69%)
60+	55 (100%)	42 (100%)	13 (100%)
missing age	2	1	1
total	**57**	**43**	**14**
fatal	**19**	**14**	**5**

CVST			
Age group	**Cumulative Nb Total (%)**	**Cumulative Nb EEA (%)**	**Cumulative Nb non- EEA (%)**
<55	50 (85%)	37 (86%)	13 (81%)
<60	55 (93%)	41 (95%)	14 (88%)
60+	59 (100%)	43 (100%)	16 (100%)
missing age	3	1	2
total	**62**	**44**	**18**
fatal	**14**	**8**	**6**

Splanchnic thromboses			
Age group	**Cumulative Nb Total (%)**	**Cumulative Nb EEA (%)**	**Cumulative Nb non- EEA (%)**
<55	16 (70%)	14 (78%)	2 (40%)
60+	23 (100%)	18 (100%)	5 (100%)
missing age	1	0	1
total	**24**	**18**	**6**
fatal	**4**	**4**	**0**

	EEA	**Non-EEA**	**Total**
Fatal	16	7	23
Not fatal	59	18	77
Total	75	25	**100**

PRAC Assessment

This new search identified significantly more cases of CVST, from 18 cases in the previous search (16 March 2021) to 62 cases in this update (22 March 2021). Whether this increase is due to an increase of reporting or an increase of occurrence is not known. However, the main characteristics of those cases remain similar: a large majority of the cases are young and middle-aged women, with only 4 cases aged 60 and older. As previously observed, no clear risk profile may be identified. The risk factors for thrombosis, when identified, are varied. Other studies are needed to

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determine if those factors are distributed differently in these cases than in other similar populations.

Thrombocytopenia is reported in about half of CVST cases and represent an important risk factor for mortality. No potential association with concomitant COVID-19 infections is identified.

This new EudraVigilance study also identified splanchnic thrombosis as cases of interest. The median time to onset is the same as for CVST (9 days) and the profile of the patients is similar, with a majority of young and middle-aged women. This profile should be compared with the profile of vaccinees in EEA and non-EEA countries to check whether they are comparable or not. Exposure data by gender are needed for further interpretation.

The expert who reviewed the cases suggested that the term that would encompass all these events would be "splanchnic vein thrombosis" (SVT), which includes portal vein thrombosis, splenic vein thrombosis, mesenteric vein thrombosis and hepatic vein thrombosis. Alternativley, the term "portal vein thrombosis" (PVT), would capture almost all these events as well. "Splanchinc vein thrombosis" is also used by Valeriani (2019) to indicate the manifestation of unusual site venous thromboembolism and encompasses portal vein, mesenteric veins, splenic vein thrombosis and the Budd-Chiari syndrome.

No new information on DIC has been collected in this update.

Reference:

Valeriani E, Riva N, Di Nisio M, Ageno W. Splanchnic Vein Thrombosis: Current Perspectives. Vasc Health Risk Manag. 2019 Oct 22;15:449-461. doi: 10.2147/VHRM.S197732. PMID: 31695400; PMCID: PMC6815215.

3.4.2.2. Observed to Expected analysis

Updated observed-to-expected analyses confirmed previous findings, with a higher number of observed cases of CVST than expected in the EEA, especially in the younger age groups. Results (Table 8) showed that the observed number of cases in EV who experienced an event of CVST within 14 days (n=34) is higher than what was expected according to the background rates. The difference between observed and expected is higher in the younger age groups, specifically those aged 18-59 years. Restricting the analysis to cases of CVST with thrombocytopenia and without thrombocytopenia revealed a similar picture.

Table 8 – Observed to expected analysis for CVST, CVST with thrombocytopenia, and CVST without thrombocytopenia

	CVST	CVST with thrombocytopenia	CVST without thrombocytopenia
Age group	OE 14d with 95% c.i.	OE 14d with 95% c.i.	OE 14d with 95% c.i.
18-29	**33.61 (10.83 - 78.44)**	**6.72 (0.09 - 37.40)**	**26.89 (7.23 - 68.84)**
30-49	**9.38 (5.46 - 15.02)**	**6.07 (3.03 - 10.86)**	**3.31 (1.21 - 7.20)**
50-59	**14.55 (7.26 - 26.04)**	**6.62 (2.13 - 15.44)**	**7.94 (2.90 - 17.28)**
60-69	1.11 (0.01 - 6.16)	1.11 (0.01 - 6.16)	0.00 (0.00 - 4.06)
70-79	0.00 (0.00 - 6.89)	0.00 (0.00 - 6.89)	0.00 (0.00 - 6.89)
80+	0.00 (0.00 - 14.83)	0.00 (0.00 - 14.83)	0.00 (0.00 - 14.83)

	Total	7.73 (5.35 - 10.80)	4.09 (2.42 - 6.47)	3.64 (2.08 - 5.91)

For DIC and embolic and thrombotic events in general, the overall observed number of cases is lower than expected; however, it is higher in younger age groups.

Limitations: The limitations of the analysis are discussed in the full report and include the comparability between data sources used to calculate the expected and the observed number of cases, the representativeness of the two databases used for background incidence rates, the underestimation of observed case, and the overestimation of observed cases if there is more intensive case ascertainment in vaccinated individuals than in routine.

Precaution: Observed to expected, however, is not an aetiological study to substantiate or confirm the strength of an association.

PRAC Assessment

This new analysis did not confirm a signal for disseminated intravascular coagulation (DIC). Embolic and thrombotic events were higher than expected in the younger population (<50 years) as well as cases of cerebral venous sinus thrombosis (CVST) (<60 years). Of note that the signal is confirmed whether CVST is associated to thrombocytopenia or not. However, the same background rate was used for both situations, which may not correspond to reality.

It is unknown how CVST affect O/E findings for embolic and thrombotic events in the younger population. Performing O/E analysis for embolic and thrombotic events with and without CVST as sensitivity analysis could be of interest.

More details are needed to document this signal. For example, observed to expected studies per gender are needed to investigate whether the strength of the signal is higher in women. Although a formal calculation is needed, it is very likely that the strength of the signal is higher in females, considering that almost all cases reported occurred in women, and they account for around 60%-65% of the given doses, according to information provided by several Member States. A follow-up period of 14 days was chosen because the vaccination with Vaxzevria started on 8th February in EEA countries where the O/E was conducted. Using a longer risk period would mean that most of the vaccinees could be followed for a fraction of the risk period. This is acknowledged but O/E analysis should be repeated using longer risk period (e.g. 42 days) when permitted by the duration of the vaccination campaigns. Similarly, at the time of the analysis, vaccinees received one dose only while two doses are necessary for a full schedule. Additional analysis taking into account the second doses should be conducted when possible.

A similar analysis should be conducted for splanchnic venous thrombosis. Background rates from the ACCESS project could be used.

Finally, the PRAC acknowledges the limitation of this analysis. Eventually, background rates from other databases should be considered to increase the representativeness of those rates. Nevertheless, these data stongly support the signal.

3.4.2.3. Literature review

> **PRAC Assessment**
>
> EMA provided an updated overview of relevant literature articles.
>
> The majority of them concern thromboembolism or platelet disorders observed in COVID-19 infection (or not).
>
> Interesting articles regarding vaccinations and secondary immune thrombocytopenia syndrome have been taken on board for the description of the different hypotheses that could explain thrombotic events after vaccination (see section 3.5.5).
>
> In particular, articles describing immunity-induced by adenovirus and HIT-like syndrome have been considered.

3.4.3. Ad Hoc Expert Group

3.4.3.1. MAH presentation during Ad Hoc Expert Group meeting

> **PRAC Assessment**
>
> The MAH presented their views on the questions raised by PRAC regarding the mechanisms of action, medical management of the events and data gaps and lines of research.
>
> The Safety update presented (DLP 24 March 2021) identified 66 case reports of thromboembolic events co-reported with thrombocytopenia in the AstraZeneca global safety database. Overall exposure in Europe (EU/EEA and UK) was estimated at 28.5 million administered doses. Based on the UK exposure data, it was estimated that these events in the age group 18-49 years occured more frequently in men (3.7/million) compared to women (2.7/million), while in the age group 50-64 years these occurred more frequently in women. These estimates should be interpreted with caution, as these are based on spontaneous reporting, but highlight the uncertainities about gender/age group distribution of these events depending on the region and exposure patterns.

3.4.3.2. AHEG meeting

On 31 March 2021, the Ad Hoc Expert Group (AHEG) gathered 24 experts and two patients' representatives. The questions posed to the group were about 1) the plausible mechanisms of action behind the occurrence of thrombocytopenia combined with coagulation disorders, 2) considerations for diagnosis, treatment, risk factors and prevention of those episodes, and 3) additional studies that would be relevant to further characterise the risk.

The first question on the plausible mechanism of action is further discussed laterwhere the hypothesis behind this mechanism are discussed. The experts considered that an atypical heparin induced thrombocytopenia (aHIT) like disorder was the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients. It was considered

extremely likely that the syndrome, which resembles aHIT, concerns autoantibody against PF4 which exhibits a high binding affinity.

In response to question 2, the consensus was that, at this point in time, the optimal case definition is not known but it should not be restricted to those cases of CVST with thrombocytopenia. Tests to detect anti-PF4 antibodies were discussed with the conclusion that this type of assay is typically restricted to specialised laboratories. Patients should not be routinely screened for theses antibodies unless they present decrease in platelet count and thrombosis complications within 4-29 days post-vaccination.

Regarding treatment, it was considered that heparin should not be administered until a diagnosis of HIT has been definitively excluded. Once a diagnosis of HIT has been excluded, it is still unclear whether patients with aHIT-like syndrome could be treated with heparin.

The optimal treatment of CVST is heparin. No firm conclusion could be provided regarding the optimal choice for alternative anticoagulants. Treatments with intravenous Immunoglobins (IVIG) were also considered.

In terms of prevention and risk factors it was noted that at this point in time there is insufficient data to make any reliable conclusions.

In response to question 3, on additional studies that may be needed, there was a general consensus that are many gaps in knowledge for which further research is deemed necessary. In-vitro assays, non-clinical and clinical studies, and epidemiologic studies were discussed. Further collaboration with the Brighton collaboration is need in order to finalize the case definition to be used in epidemiological studies.

PRAC Assessment

Overall, the discussion among the experts were in line with the other discussions previously held at the PRAC level, leading to the preliminary conclusion that an atypical heparin induced thrombocytopenia (aHIT) like disorder is the most plausible hypothesis. While mechanisms linked to the adenovirus vector should not be discarded, other hypothesis are less likely.

The PRAC fully supports the AHEG conclusions on the mechanisms and gaps in knowledge. The PRAC also agrees with the AHEG conclusion on the need for additional studies.

3.4.4. Information from MHRA

Information from the last update of the MHRA's Coronavirus vaccine - weekly summary of Yellow Card reporting, covering the period from 9 December 2020 to 21 March 2021 is provided below.

At the DLP of 21 March 2021, an estimated 10.8 million first doses of the Pfizer/BioNTech vaccine and 15.8 million doses of the Oxford University/AstraZeneca vaccine had been administered, and around 2.2 million second doses, mostly the Pfizer/BioNTech vaccine, had been administered.

For both vaccines the overall reporting rate is around 3 to 6 Yellow Cards per 1,000 doses administered.

The report indicates that the rigorous review into the UK reports of rare and specific types of blood clots is ongoing. Up to and including 24 March, the MHRA has received 22 reports of cerebral venous sinus thrombosis (CVST) and 8 reports of other thrombosis events with low platelets, out of a total of

18.1 million doses of Vaxzevria given by that date. There were no reports for the Pfizer/BioNTech vaccine. To note, the current analysis prints include data up to and including the 21 March 2021.

The MHRA considers that, on the basis of the ongoing review, the benefits of the vaccines against COVID-19 continue to outweigh any risks.

PRAC Assessment

Updated MHRA's weekly report indicates that 22 cases of CVST and 8 cases of other thrombosis with low platelets, out of a total of 18.1 million doses of Vaxzevria given, have been reported to the MHRA at the DLP of 24 March 2021. Those data have been briefly presented orally during the preparatory PRAC meeting on 31. March 2021.

The MHRA concludes that on the basis of the ongoing review, the benefits of the vaccines against COVID-19 continue to outweigh any risks.

The MHRA also refers to the guidance[3] issued by the British Society for Haematology on thrombosis and thrombocytopenia possibly occurring after vaccination with COVID-19 vaccines, which includes information on presentation and typical laboratory features, and treatment recommendations. The guidance also includes advice on recommended investigations for possible cases.

Additional information regarding UK cases should be sought, as well as any further information on events reported after the second dose. Liaison with the MHRA should be continued as frequently as possible and necessary.

3.4.5. Hypothesis

3.4.5.1. Immunological syndrome ((atypical) HIT-like syndrome) Following Coronavirus-19 Vaccination

The clinical picture of patients with moderate to severe thrombocytopenia and thrombotic complications at unusual sites beginning approximately one week after vaccination against SARS-CoV-2 by AZD1222 suggests a disorder clinically resembling heparin-induced thrombocytopenia.

Heparin-induced thrombocytopenia (HIT)

HIT is a well-recognized prothrombotic iatrogenic disorder caused by immunoglobulin G antibodies that target multimolecular complexes of PF4 and heparin and activate platelets via FcγIIa receptors (FcγRIIa). These antibodies recognize multimolecular complexes between cationic PF4 and anionic heparin. The end result of these changes is a profound thrombotic tendency. Thrombosis occurs in one-third to one-half of patients with HIT and may be venous, arterial, or microvascular. Rates of amputation are ~1% to 3%, and rates of death associated with HIT are ~5% to 10%.[1] Although common venous thrombotic events (lower-limb deep-vein thrombosis [DVT], upper-limb catheter-associated DVT, pulmonary embolism) are most often seen, unusual venous thrombi can occur, such as cerebral venous sinus thrombosis (presenting as hemorrhagic cerebral infarction) and splanchnic vein thrombosis (mesenteric vein thrombosis presenting as gut infarction with gastrointestinal bleeding; adrenal vein thrombosis presenting as adrenal hemorrhage). Large-artery thrombosis in HIT affects artery distribution (limb artery > stroke > myocardial infarction). Microvascular thrombosis can

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[3] https://b-s-h.org.uk/about-us/news/guidance-produced-from-the-expert-haematology-panel-ehp-focussed-on-syndrome-of-thrombosis-and-thrombocytopenia-occurring-after-coronavirus-vaccination/

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also result in association with severe HIT-associated disseminated intravascular coagulation (DIC) [Overview: CLINICAL PRACTICE. Heparin-Induced Thrombocytopenia. Greinacher A. N Engl J Med. 2015 Jul 16;373(3):252-61. doi: 10.1056/NEJMcp1411910. PMID: 26176382).

Physiopathology

The HIT antigen is situated on the platelet factor 4 (PF4), a chemokine that is contained in platelet α-granules. PF4 is not immunogenic in its primary form. Conformational PF4 changes are needed to expose a neo-epitope, which is the HIT antigen. These changes occur by the formation of complexes between PF4 and heparin. The size and the charge of the complexes play a central role in pathogenicity. These two parameters depend on the relative amounts of PF4 and heparin. Similarly, the immunogenicity of a high PF4/heparin ratio also explains the high incidence of HIT in patients with high amounts of circulating PF4 (e.g., orthopedic and vascular surgery) and prophylactic doses of unfractionated heparin[2].

The adverse drug effect known as heparin-induced thrombocytopenia shows many similarities to a bacterial host defence mechanism. Platelet factor 4 (PF4) that is released from platelet αgranules binds to polyanions such as heparin or polyanions on the surface of bacteria and undergoes a conformational change. This results in immunogenic PF4–polyanion (heparin) or PF4–polyanion (bacteria) complexes. After activation, B lymphocytes (probably marginal zone B cells) generate anti–PF4–polyanion IgG. These antibodies can bind to different PF4-coated bacteria and opsonize them. However, these antibodies also bind to PF4–heparin complexes, forming immunocomplexes. The Fc parts of the IgG bind to platelet Fcγ RIIa receptors, resulting in Fcγ-receptor clustering and consequent strong platelet activation and aggregation. This intravascular platelet consumption causes a decrease in the platelet count and the production of platelet-derived microparticles that accelerate thrombin generation. In addition, HIT antibodies activate monocytes (by means of the Fcγ RI) and (directly or indirectly) endothelial cells, inducing additional tissue factor expression. The resulting increase in thrombin generation leads to an increased risk of thrombosis among patients with HIT, providing a rationale for treatment that reduces thrombin generation.

From Greinacher et al. New Eng J Med. 2015.

The antigenic complex formation occurs on the platelet surface in a dynamic and potentially reversible manner. In presence of PF4, increasing heparin initially leads to an increasing antigen-complex size. HIT antibodies will then bind to these unfractionated heparin. With further increase, heparin would then displace PF4 from the platelet surface and diminish the size of the antigen-complexes, thus decreasing their capacity to activate platelets. The antigen–antibody binding on the platelet surface induces platelet activation via FcγRIIa (CD32, the low affinity IgG receptor) and leads to platelet degranulation and aggregation. Degranulation increases the available PF4 concentration for further antigen-complex formation. Besides these "classical" platelet activation endpoints, platelet activation also induces the production of procoagulant platelets and platelet-derived procoagulant microparticles, dramatically enhancing thrombin generation.

HIT is then a complex immune-mediated pathology. Its mechanisms depend on the concentrations of PF4 and heparin, particularly their ratio to each other and involve platelets, monocytes, endothelial cells, and neutrophils as well. The activation of these cells induces, besides thrombocytopenia, a coagulation cascade activation leading to a severe hypercoagulant state.

Atypical HIT or HIT-like syndrome or autoimmune HIT

In recent years, it has been recognized that triggers other than heparin can rarely cause a disorder that strongly resembles heparin-induced thrombocytopenia on both clinical and serological grounds, including certain polyanionic drugs (e.g., pentosan polysulfate, antiangiogenic agent PI-88, hypersulfated chondroitin sulfate, DNA and RNA (including DNA/RNA-based aptamers), polyphosphates, infections (viral, bacterial), or knee replacement surgery.

These various scenarios in which an apparent non-heparin trigger has been invoked have been called spontaneous or autoimmune HIT.

A brief comparison between HIT and aHIT is presented by Marchetti et al (2021):

Table 1. Comparison of classical and autoimmune HIT.

	Classical HIT	**Autoimmune HIT**
Antigen	Neoepitope on PF4, revealed by a conformational change due to binding to a negatively-charged surface	
Negatively-charged molecule	Heparin	Other polyanions: chondroitin sulfate, polyphosphates, nucleic acid, bacterial components
Pathogenesis	Similar (see text: part 1a-d)	
Therapy	Heparin avoidance, alternative non-heparin anticoagulation	Intravenous immunoglobulins (IVIG), plasmapheresis

Besides heparin, other polyanions, such as hypersulfated chondroitin sulfate, DNA and RNA (including DNA/RNA-based aptamers), polyphosphates, and bacterial wall components (e.g. lipid A), have been shown to induce the conformational changes in PF4 required to expose the HIT antigen(s). Indeed, a large population study showed a close correlation between chronic periodontal infection and the presence of anti-PF4/H antibodies within a (non-heparin-exposed) general population. Furthermore, binding of high concentrations of PF4 to platelets may also induce exposure of HIT antigen(s) in the absence of added polyanions. In this case, polyanions on the platelet surface probably augment the close proximity of PF4. For the phenomenon of aHIT, this clearly shows that the antigens can be exposed by factors other than heparin.[6]

However, such 'heparin-independent' platelet-activating properties are not unique to spontaneous HIT syndrome, but are also found in sera of a minority of (heparin-dependent) typical HIT patients. Moreover, patients who show this in vitro reactivity profile are more likely to have unusual HIT syndromes such as delayed-onset HIT, persisting HIT, fondaparinux-associated HIT, and HIT induced by exposure to heparin 'flushes'. Such patients often show unusual clinical features, such as severe thrombocytopenia that can persist for weeks, often accompanied by disseminated intravascular coagulation (DIC) and microvascular thrombosis.

Clinical presentation of (atypical) HIT

The clinical diagnosis of HIT rests on demonstrating thrombocytopenia and/or thrombosis in temporal association with heparin therapy while excluding other causes of thrombocytopenia.

The cardinal manifestation of HIT is the occurrence of thrombocytopenia, which occurs in 95% patients in temporal association with heparin therapy.

Thrombocytopenia can manifest either as an absolute drop in platelet count or a relative decline of 30% to 50% from baseline platelet counts. Absolute thrombocytopenia in HIT is often moderate and typically not associated with bleeding complications. Severe thrombocytopenia can occur as a manifestation of fulminant thrombotic disease and consumptive coagulopathy.

In most patients, thrombotic complications occur concurrently with thrombocytopenia and can affect any vascular bed. Venous thromboses predominate, particularly at sites of vascular injury from catheters. Atypical presentations, such as bilateral adrenal haemorrhage, venous limb gangrene, and skin necrosis should prompt diagnostic consideration of HIT.

Interestingly, not all patients developing HIT antibodies develop HIT, and not all patients with HIT develop thromboembolic complications. The mechanisms underlying these variable responses are not completely understood. However, specific gene polymorphisms could be involved, especially in the risk to develop thromboembolic complications in HIT. Indeed, an association between the polymorphism of FcγRIIIA 158VV and occurrence of HIT and between the polymorphism of FcγRIIA 131R and thromboembolic complications in HIT has been observed in different studies. Further genetic polymorphisms could be identified in the future, explaining the different responses to HIT antibodies[2].

Clinical studies indicate also that certain serologic features, such as IgG isotype, capacity to activate platelets and high antibody levels (as measured by optical density [OD] and/or titer) are associated with thrombotic risk. The contribution of IgA or IgM isotypes to thrombosis in HIT is likely subordinate to established pathogenic mechanisms directly linking IgG-mediated activation of platelet and monocyte FcgRIIa receptors to thrombin generation[5].

Temporal features

The most important diagnostic element of HIT is timing of complications relative to heparin therapy. In heparin-naïve individuals, PF4/heparin antibodies become detectable at a median of 4 days from start of heparin therapy. Clinical manifestations of thrombocytopenia and/or thrombosis develop 5 to 14 days after initial heparin therapy, and on average +/- 2 days (range 1-5 days) after antibody detection. In patients with recent heparin exposure (<100 days), thrombocytopenia occurs precipitously within 24 hours of drug reexposure because of circulating anti-PF4/heparin antibodies.[5]

Immunological syndrome ((atypical) HIT-like syndrome) Following Coronavirus-19 Vaccination

The clinical presentation of patients experiencing the immunological syndrome events after Vaxzevria vaccine are very similar to what is described for (atypical) HIT including thrombocytopenia and thrombosis in atypical sites that can complicate in DIC, secondary bleeding and/or CVST. Serological studies using sera from four patients who developed thrombocytopenia and thrombosis (three of them at unusual sites) following vaccination showed strong reactivity in anti-PF4/heparin enzyme-immunoassay, and also showed strong positive testing for platelet-activating antibodies. According to Greinacher et al.[6], the clinical picture observed in these patients suggests a disorder clinically resembling heparin-induced thrombocytopenia. However, in contrast to patients with typical heparin-induced thrombocytopenia, the laboratory conditions that results in patient serum-induced platelet activation differed. Notably, patient serum strongly activated platelets when PF4, rather than heparin, was added to a washed platelet assay. A similar phenomenon has been observed with some sera from patients who have typical heparin-induced thrombocytopenia. It appears that the platelet-activating antibodies induced by vaccination bind to non-complexed PF4 alone, also noted in some sera from patients with heparin-induced thrombocytopenia. Whether these antibodies are autoantibodies against PF4 induced by the strong inflammatory stimulus of vaccination or if the vaccine itself triggers the

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formation of platelet activating antibodies cannot be distinguished by this study. Enhanced reactivity of the sera *in-vitro* in the presence of Vaxzevria could be explained by direct binding of the virus to platelets. Adenovirus binds to platelets and can cause platelet preactivation. However, we cannot exclude other cofactor(s) or confounders that could also induce procoagulant platelets *in-vivo*.

Most importantly, as indicated in the minutes of the AHEG, at this point in time the optimal case definition is not known.

Diagnosis

There is no sufficient data at this stage to have a commonly endorsed diagnostic method for the clinical entity of interest.

The following symptoms have been encountered in patients:

- Reduction in platelet count;

- Events occurring within a typical risk window (i.e. 4-20 days post vaccination);

- Thrombotic complications including but not restricted to CSVT-it was discussed that the case definition could be broadened to include all thrombotic events);

- High titers of anti-PF4 antibodies;

- Exclusion of other aetiologies for VTE and thrombocytopenia;

The need and the characterization of the most appropriate functional tests are still to be established.

In conclusion, it is considered that the optimal diagnostic test (strategy) was currently not known and further research is needed to this regard.

Pharmacological treatment

In terms of pharmacological treatment, specifically anticoagulant therapy, it was considered that treatment with heparin should not be administered until a diagnosis of HIT has been definitively excluded. Alternative agents include fondaparinux or argatroban. Once a diagnosis of classical HIT has been excluded, it still is unclear whether patients with vaccination induced HIT-like syndrome could be treated with heparin, mostly because there is little evidence.

Also as indicated by the AHEG, no firm conclusion could be provided at the current point in time regarding the optimal choice for pharmacological treatment. More research is also needed.

3.4.5.2. Viral Vector

COVID-19 Oxford-AstraZeneca Vaccine (Vaxzevria) is a recombinant replication-defective chimpanzee adenovirus expressing the SARS-Cov-2 S surface glycoprotein (Spike protein) with a leading tissue plasminogen activator (TPA) signal sequence. S is a type I, trimeric, transmembrane protein located at the surface of the viral envelope, giving rise to spike shaped protrusions from the virion.

The S protein subunits are responsible for cellular receptor ACE-2 binding via the receptor-binding domain and fusion of cirus and cell membranes, thereby mediating the entry of SARS-CoV-2 into the target cells. The S protein has an essential role in virus entry and determines tissue and cell tropism, as well as host range. The role of S protein in receptor binding and membrane fusion make it a good target for vaccines, being chosen as such by most of vaccines developed up to date. Differently from

the other vaccines authorised in the EU, the <u>unmodified</u> S protein (i.e. no pre-fusion stabilized) form is present.

This viral vector (ChAdOx1) is replication-deficient as the essential E1 and E3 gene regions have been deleted from the chimpanzee adenovirus Y25, so the virus is unable to replicate within vaccinated animals or humans.

<u>Pre-Clinical Findings</u>

According to the Summary of Key Findings from Nonclinical Data, repeat-dose Good Laboratory Practice (GLP) toxicity study with AZD1222 in mice has been conducted, with preliminary findings (not including recovery data) indicating that there were no clinically relevant observations considered to be related to administration of AZD1222. Furthermore, as the ChAdOx1 platform technology used for AZD1222 is well characterised, non-clinical toxicology findings with the ChAdOx1 MERS-CoV vaccine expressing the full-length spike (S) protein in mice are also considered of direct relevance to the non-clinical safety profile of AZD1222. Additionally, results from toxicology studies on similar replication-defective ChAd vaccines (ChAdOx1 NP+M1 and AdCh63 MSP-1) are also considered to be of significance. Results from repeat-dose mouse toxicology studies with vaccines ChAdOx1 NP+M1 and AdCh63 MSP-1 were consistent with ChAdOx1 MERS, and demonstrated that these vaccines were well tolerated with no associated adverse effects. Toxicity data (and toxicity in the target organs) from the ChAdOx1- and ChAd63-based vaccines follow the same pattern, with findings consistent with a predicted response to vaccine administration (eg, observed changes in the intramuscular (IM) injection site and immune system response).

A reassessment of data from the pivotal repeated dose toxicity study in mice did not reveal clear differences regarding platelet levels between dosed and control animals by the end of dosing or recovery periods regardless sex. In addition, no relevant significant differences have been reported in clinical pathology examinations assessing clotting and platelet clumps. Fibrinogen levels, partial thromboplastin time (PTT) or prothrombin time were not measured in pivotal toxicity studies.

Regarding specifically respiratory and cardiovascular safety pharmacology, a single safety pharmacology study has been performed to date, designed to investigate the potential effects of AZD1222 on respiratory parameters in conscious male mice for at least 4 hours following administration, in addition to assessment of arterial blood pressure, heart rate and body temperature for up to 24 hours post-dose. Single IM dose levels of zero (control), and 2.59×10^{10} vp (AZD1222) were administered, with an interval of 3 days between the 2 treatment sessions. There were no changes in arterial blood pressure, heart rate, body temperature or respiratory parameters considered to be AZD1222-related. The non observed effect level (NOEL) for cardiovascular and respiratory assessment was 2.59×10^{10} vp. The only relevant signs observed following immunization with the vaccine AZD1222 in animal models were restricted to respiratory tract tissues following challenge and no apparent signs of inflammation were observed as a result of immunization.

In conclusion, non-clinical data of AZ1222 showed strong immunogenicity response following two dose administration in animal models. In addition, available data showed a favourable safety profile. Adverse effects reported were limited to the site of administration and all findings were reversible by the end of the recovery period.

<u>Quality considerations</u>

No adjuvant, stabilisers or preservatives included in the AZD1222 formulation were deemed to influence the safety profile of the final vaccine product. Host cell proteins may remain as a contaminant

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as a result of the manufacturing process; however, levels are controlled by biological product deviation (BPD) release criteria, and are therefore not of relevance.

As a result of the investigations performed in the context of this safety issue, the Biologicals Working Party (BWP) concluded that the affected batches were within pre-defined specifications, the results of the GMP investigation were satisfactory, the release results within normal ranges. OMCL (NL) is independently testing batch ABV5300 and batches ABV6096, ABV5811, ABV2856, ABV3374.

Regarding possible differences in the UK batches due to different manufacturing sites at some steps, no relevant differences on manufacturing process have been found, just some minor differences on pre-defined specifications. However, released data provided from a number of batches showed results aligned with EU-released batches and the supply was anyway from EU-approved manufacturing sites.

In conclusion, based on the review of the quality data provided, the BWP considered that there was no indication that the observed SAEs (signal of embolic and thrombotic events) are linked to quality of the vaccine. It should be noted as well that some quality defects in the vaccine should probably led to a more generalized consequences for the population that the events related with this safety issue.

Clinical Safety Data

In the clinical development programme for this vaccine, an imbalance in the unsolicited Adverse Events (AEs) within the SOC Vascular Disorders was not identified. With the data used for the Conditional Marketing Authorisation (CMA), AEs within this SOC were reported by 50 of 10,069 participants (0,5%) in the active arm vs 57 of 9,902 participants (0,6%) in the control arm, that can be either MenACWY vaccine or placebo. There is a variation still under assessment with data cut-off date of 7th December and the figures are still similar: 62 of 10,317 participants (0,6%) vs 65 of 10,141 participants (0,6%), respectively. With this latter cut-off date, no thrombotic or thromboembolic events were reported within Vascular disorders PT within 28 days of administration of AZD1222 SD dose or placebo.

Table 1.5.2.2.2 Unsolicited Adverse Events by System Organ Class and Preferred Term (Dose1 SD for Safety Analysis Set)

System Organ Class Preferred Term (MedDRA version 23.1)	Number (%) of Participants[a]	
	AZD1222 (N = 10317)	Control (N = 10141)
Vascular disorders	62 (0.6)	65 (0.6)
Blood pressure fluctuation	0	1 (<0.1)
Capillary fragility	1 (<0.1)	0
Cyanosis	1 (<0.1)	0
Diastolic hypertension	0	1 (<0.1)
Flushing	3 (<0.1)	6 (0.1)
Haematoma	4 (<0.1)	1 (<0.1)
Hot flush	4 (<0.1)	2 (<0.1)
Hyperaemia	11 (0.1)	25 (0.2)
Hypertension	25 (0.2)	22 (0.2)
Hypertensive crisis	0	1 (<0.1)
Hypotension	6 (0.1)	4 (<0.1)
Orthostatic hypotension	4 (<0.1)	1 (<0.1)
Pallor	2 (<0.1)	0
Peripheral coldness	2 (<0.1)	1 (<0.1)
Peripheral venous disease	0	1 (<0.1)
Systolic hypertension	1 (<0.1)	1 (<0.1)
Vasodilatation	0	1 (<0.1)
White coat hypertension	1 (<0.1)	1 (<0.1)

Regarding Adverse Events of Special Interest (AESI) by SOC *Thrombotic/Thromboembolic Events,* there are less events in the active arm vs control arm. With the data used for the Conditional Marketing Authorisation (CMA), AESIs within this category were reported by 3 of 10,069 participants (<0,1%) in the active arm vs 7 of 9,902 participants (0,1%) in the control arm. In the variation still under assessment with data cut-off date of 7th December, the differences remain similar: 6 of 10,317 participants (0,1%) vs 17 of 10,141 participants (0,2%), respectively. None of these events were considered SAEs.

Table 1.5.4.2 Adverse Events of Special Interest by Special Interest Category and Preferred Term (Dose1 SD for Safety Analysis Set)

	Number (%) of Participants [a]	
Special Interest Category Preferred Term (MedDRA version 23.1)	AZD1222 (N=10317)	Control (N=10141)
Thrombotic, thromboembolic, and neurovascular events	6 (0.1)	17 (0.2)
Acute myocardial infarction	0	2 (<0.1)
Amaurosis fugax	0	1 (<0.1)
Blindness transient	1 (<0.1)	1 (<0.1)
Coronary artery occlusion	1 (<0.1)	1 (<0.1)
Deep vein thrombosis	0	2 (<0.1)
Hemiparesis	0	1 (<0.1)
Mesenteric vein thrombosis	0	1 (<0.1)
Monoparesis	0	1 (<0.1)
Myocardial infarction	1 (<0.1)	1 (<0.1)
Pulmonary embolism	1 (<0.1)	1 (<0.1)
Thrombophlebitis	0	1 (<0.1)
Thrombophlebitis superficial	0	1 (<0.1)
Thrombosis	1 (<0.1)	0
Transient ischaemic attack	1 (<0.1)	4 (<0.1)

However, regarding thrombocytopenia, the proportion of participants with platelet decreases was slightly higher in the AZD1222 group compared with control within 30 days post 1st dose and within 30 day post 2nd dose taking into account the data cut-off 4th November (used for CMA), as no new data has been submitted at cut-off December 7th.

Table 1.6.1.1 Summary of Haematology laboratory results by Maximum Toxicity Grade (Any Dose for Safety Analysis Set)

Toxicity Criteria	Grade	Baseline		Within 30 Days Post First Dose		Within 30 Days Post Second Dose	
		AZD1222	Control	AZD1222	Control	AZD1222	Control
White Blood Cells-Elevated	Total	1054	770	977	693	294	95
	Normal n (%)	1041 (98.8)	763 (99.1)	963 (98.6)	680 (98.1)	285 (96.9)	91 (95.8)
	Grade 1 n (%)	13 (1.2)	7 (0.9)	14 (1.4)	13 (1.9)	9 (3.1)	3 (3.2)
	Grade 2 n (%)	0	0	0	0	0	1 (1.1)
	Grade 3 n (%)	0	0	0	0	0	0
	Grade 4 n (%)	0	0	0	0	0	0
White Blood Cells-Decreased	Total	1054	770	977	693	294	95
	Normal n (%)	1042 (98.9)	757 (98.3)	926 (94.8)	674 (97.3)	282 (95.9)	93 (97.9)
	Grade 1 n (%)	12 (1.1)	13 (1.7)	49 (5.0)	19 (2.7)	12 (4.1)	2 (2.1)
	Grade 2 n (%)	0	0	2 (0.2)	0	0	0
	Grade 3 n (%)	0	0	0	0	0	0
	Grade 4 n (%)	0	0	0	0	0	0
Platelets-Decreased	Total	1054	770	977	693	294	95
	Normal n (%)	1045 (99.1)	766 (99.5)	959 (98.2)	689 (99.4)	285 (96.9)	94 (98.9)
	Grade 1 n (%)	6 (0.6)	3 (0.4)	12 (1.2)	3 (0.4)	5 (1.7)	1 (1.1)
	Grade 2 n (%)	2 (0.2)	1 (0.1)	4 (0.4)	1 (0.1)	2 (0.7)	0
	Grade 3 n (%)	1 (0.1)	0	2 (0.2)	0	2 (0.7)	0
	Grade 4 n (%)	0	0	0	0	0	0

As the current safety issue comprises a number of cases in which these rare thrombotic events are accompanied by thrombocytopenia, this finding merits further discussion. Furthermore, clinical trials with other vaccines developed using this same chimpanzee adenovirus non-replicating vector reported similar findings, as studies with Ebola vaccine in paediatric patients (Keshinro et al, 2017), and the adenoviruses themselves are already recognized and known to cause thrombocytopenia in some degree (Ahi, Bangari, and Mittal, 2011; Atasheva, Yao, and Shayakhmetov , 2019; Xu et al, 2010; Yasuda et al, 2019).

According to the AHEG meeting held the 29th March, this effect could be linked most probably to an immunological syndrome, resembling an atypical Heparin-Induced Thrombocytopenia-like syndrome, as described elsewhere in this report. However, the vaccine component that could act as triggering factor activating platelets and originating the anti-PF4 identified in some patients, remains to be elucidated.

The viral vector as the triggering factor

Several non-clinical *in-vitro* and *in-vivo* studies (i.e. conducted in monkeys, mice, rats, and humans) have demonstrated that Ads interact with coxsackie and adenovirus receptor (CAR) resulting in platelet

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activation (Lillicrap et al, 2009) showing CAR on human platelets and their subsequent activation. When seen in vivo, platelet activation has been demonstrated primarily within the context of IV injections of adenoviruses and has been associated with severe thrombocytopenia. Although this vaccine is administered IM, the possibility of inadvertent IV administration in some cases should not be ruled out, but unlikely to explain all reported cases. It is of note that the biodistribution studies by the usual IM route with this specific vaccine are not fully finished. However, the results from some other vaccines with this same vector, IM injection of E1-deficient non-replicating ChAdOx1 nCoV19 did not show a biologically significant biodistribution distant from injection site and draining lymph nodes (non-infectious PCR or trace amounts only). The pending on-going study is already a legally binding obligation and final results are expected by the end of April 2021.

The AHEG also noted that other types of Ads interact with CD46, a complement regulatory protein, and can also induce platelet activation. Furthermore, data from *in-vitro* and *in-vivo* studies have shown that Ads can activate VCAM on endothelial cells, causing activated platelets, which also bind to other leukocytes, to induce clotting. It was also noted that Ads interact with a number of different extracellular proteins (in particular coagulation factors, complement, antimicrobial peptides) and can be taken up by other cells at the site of injection by a number of different pathways using different receptors (including Toll-like receptor 4, a pattern recognition receptor). The experts specified that it is poorly understood what is occurring with the majority of the Ad vector at the site of injection. While the Ad vector may result in activation of platelets and endothelial cells via this mechanism, the dose of AZ vaccine would likely be too low to elicit the events observed, provided that the vector is non-replicating.

CAR is present on erythrocytes from humans and rats, but not monkeys and mice (mice were the models mostly used by the MAH in the non-clinical development). ChAdOx1 and Ad type 5 can bind to CAR and complement receptor 1 (CR1). The reaction with CR1 is antibody-mediated and can result in complement activation. Infection by wild-type Ads has been associated with disseminated intravascular coagulation (DIC) and thromboses. However, a direct effect of the ChAdOx1 vector to induce thrombocytopenia is unlikely due to the dose administered in the vaccine, provided that this is a non-replicating vector itself.

In conclusion, as this is a non-replicating viral vector, IM injected, the potential link could only be connected with the expressed proteins.

Role to the TPA leader sequence

Some concerns have been raised regarding the tissue plasminogen activator (tPA) leader sequence contained in the vector. This is a 34 amino acid sequence, used in ChAdOx1-vectored vaccine antigen Spike protein to enable Spike expression on the cell surface. Previous studies have shown that encoding tPA upstream of recombinant antigens enhanced immunogenicity. According to data presented by the MAH, in transfected cells, tPA cannot be detected. Once inside the cell, tPA lead is rapidly degraded. Moreover, as it is cleaved from the spike protein, according to data from *in-vitro* studies, this should not be a concern. tPA leader sequence has been used in several vaccines and has not been associated with these events previously, so this seems an unlikely hypothesis based on existing available data.

Role of the Spike protein

The expressed part of the genome corresponds to the SARS-Cov-2 S surface glycoprotein (Spike protein). Differently from the other SARS-Cov-2 vaccines approved in the EU, this vaccine has a complete spike protein (unmodified, wild type) whereas other vaccines have modified S proteins. This means that whilst for AZ vaccine both forms (pre-fusion and post-fusion) can be present, for the other

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vaccines, the S protein is stabilized in the pre-fusion conformation, and just this type of S protein is present. *In* vitro expression of the unmodified S protein present in AZ vaccine has not been tested.

It has been postulated that the accumulation of misfolded proteins that are unable to form proper three-dimensional structures or complexes in the endoplasmic reticulum (ER) causes ER stress, leading to an adaptive cellular response known as the unfolded protein response (UPR) (Aoe, 2020). An alternative mechanism that could be a potential cause for the recent thrombosis cases observed in humans immunized with AZD1222 could be as a result of a potential unfolded protein response. This issue could be further investigated from a non-clinical perspective since studies in this regard are easy and relatively fast to perform. This proposal has been already posed in the AHEG.

Viral glycoproteins can induce Endoplasmic reticulum (ER) stress during infection due to an incorrect folding or accumulation in the ER lumen. Cells can respond in several ways to reduce the burden imposed by unfolded proteins in the ER, ways that are collectively known as the unfolded-protein response (UPR). Induction of UPR results in transcriptional activation of genes encoding ER-resident molecular chaperones to increase protein-folding activity and repression of protein synthesis. Three different pathways can be activated in response to UPR: activation of protein kinase R-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). Inhibition of PERK activity has been associated with thrombotic events.

Conclusion:

- The MAH should submit the results of the pending biodistribution study with for Vaxzevria on the agreed date .

- The MAH should propose a study to test in-vitro expression of the S protein of Vaxzevria (these are already available for the other already authorised SARS-CoV-2 vaccines with a different S protein without showing concerns).

- The MAH should propose any further non-clinical studies aimed at elucidating the mechanism that trigger platelet activation and subsequent thrombotic effects.

3.4.5.3. COVID-19 disease

The association between coagulation abnormalities and COVID-19 disease is well-documented, including in recent literature review papers. Several mechanisms, including hypercoagulability and inflammation, interact and finally cause thrombotic phenomena, both in the microvasculature and in the larger, mostly pulmonary blood vessels (Raadsen M et al., 2021). However, thromboembolic complications of the nervous system with subsequent cerebrovascular stroke have also been increasingly reported, including cerebral venous sinus thrombosis (CVST) in young patients with COVID-19 (Abouhashem et al., 2020), even without the presence of risk factors (Hughes et al., 2020). Elevated D-dimer, fibrinogen level, fibrin/fibrinogen degradation product, presence of antiphospholipid antibodies and thrombocytopenia are commonly reported laboratory abnormalities in COVID-19 patients, with higher rates in severe disease (Mowla et al., 2020).

In summary, one hypothetical mechanism could be that the thromboembolic events occur in patients with undiagnosed/previous COVID-19 disease. This hypothesis is mechanistically plausible. Yet, this is not supported by the negative PCR and serologic results in some patients experiencing coagulation abnormalities. Then again, one should keep in mind that testing accuracies may vary (Watson et al., 2020 and Woloshin et al., 2020).

A second hypothetical mechanism is that, taking into account the fact that COVID-19 infection is associated with thrombotic complications, whose complete pathophysiology is yet not well understood,

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it is logical to suspect that the immune responses associated with COVID-19 infection and COVID-19 vaccination may share some similarities that would increase the risk of a thrombotic event.

Another possibility within this context is the phenomenon of molecular mimicry in the situation of vaccines, which has been reviewed by Segal et al., 2018. One could imagine that the spike protein-specific immune response may induce a cross-reaction with a specific human protein or antigen involved in the haemostatic system (and leading to platelet activation and/or consumption) in people particularly sensitive because of a "reactive" immune system.

3.4.5.4. Other hypotheses

Antiphospholipid syndrome

A possible role of the antiphospholipid syndrome ((potentially life-threatening thrombophilia in which patients develop pathogenic autoantibodies targeting phospholipids and phospholipid-binding proteins (= aPL antibodies)) has also been hypothesized.

The role of the antiphospholipid syndrome in the observed cases seem to be unlikely as overall no reported antiphospholipid antibodies, no complement activation, no coagulopathy and normal blood smear without haemolysis observed.

Rare hereditary conditions
Rare hereditary conditions such as congenital ADAMTS13 deficiency could predispose for thrombosis and thrombocytopenia in response to e.g. an immune response.

Assessment comment on hypotheses:

It is considered, in agreement with the AHEG, that an atypical heparin induced thrombocytopenia (aHIT) like disorder is the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients. It is considered extremely likely that the syndrome, which resembles aHIT, concerns a severe autoantibody against PF4 which exhibits a high binding affinity. It was hypothesised that the antibody itself is changing the structure of PF4, similar to what has been shown for aHIT. It was noted that high titres of anti-PF4 antibodies were observed in all patients whose biomaterial was analysed which substantiates this hypothesis.

A role of adenovirus might explain (I) the fact that similar cases of CVST were observed with the other adenovirus-vector based vaccine from Janssen and (II), the difference in O/E ratio calculated for non-adenovirus vaccines (Pfizer and Moderna) versus the AstraZeneca adenovirus vaccine. However, intravenous administration might be needed to observe these events and we are not able to verify this for the cases perceived. Also, as noticed by the AHEG, currently it is poorly understood what is occurring with the adenovirus vector at the site of injection and the dose of the AstraZeneca vaccine Vaxzevria could be too low to elicit the events observed.

Other alternative triggers of the immunological syndrome ((atypical) HIT-like syndrome) have been suggested but none seems very convincing at this point in time, mostly due to lack of strong supporting evidence and lack of mechanistic plausibility. They include: potential roles of tissue plasminogen activator (tPA) leader sequence, antiphospholipid antibodies or rare hereditary auto-immune conditions.

New studies are needed to address the current uncertainties in the definition of the clinical entity and related aetiology, diagnostic and treatment.

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Following gaps in knowledge have been identified:

• Characterisation of the mechanisms and identification of the triggers of platelet activation after vaccination;

• Characterisation of laboratory profile of the entity and recommendation of diagnostic method;

• Characterisation of risk factors;

• Implication of administering the second dose.

There has been increasing reports associating COVID-19 disease with a thromboembolic phenomenon including ischemic strokes, venous thromboembolism and more specifically, CVST. Regarding this "viral coagulopathy", COVID-19 is notable for the high frequency and severity of micro thrombosis, in addition to macro-thromboembolic events (such as also occurring in sepsis). Also, SARS-CoV-2 virus is able to bind to platelets, amongst others, via integrins (Sigrist et al., 2020). Taking into account that virus-platelet binding might trigger thrombocytopenia and platelet activation (Gresele et al., 2017), a possible platelet activation by SARS-CoV-2 cannot be excluded. However, these hypotheses related to the COVID disease would not explain why this clinical picture particularly occurs after Vaxzevria vaccination, compared to other non-adenovirus COVID-19 vaccines (Pfizer, Moderna). Furthermore, in a number of cases reported, previous COVID-19 infection was ruled out and in the majority of cases there is explicit information confirming negative serology. Therefore, the COVID disease-related hypotheses seem very unlikely to underlie the observed thromboembolic events, although they might comprise an additional explanation or risk factor.

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24) *Segal Y, Shoenfeld Y. Vaccine-induced autoimmunity: the role of molecular mimicry and immune crossreaction. Cell Mol Immunol. 2018 Jun;15(6):586-594. doi: 10.1038/cmi.2017.151. Epub 2018 Mar 5. PMID: 29503439; PMCID: PMC6078966.*

3.4.6. Additional Studies

3.4.6.1. Additional studies in the context of the RMP of Vaxzevria

Some strategies should be put in place in order to elucidate some uncertainties about this new clinical entity.

In the currently approved version of the RMP of Vaxzevria (version 1 – succession number 5)[4], there are some interventional and non-interventional clinical studies that are ongoing or are planned and that could be adapted to address this issue.

<u>Interventional studies</u>

Currently, there are 7 ongoing clinical studies that are included in the RMP as additional pharmacovigilance activities. All but two (COV004 and D811C00002) were category 2 studies:

- COV001 - Ongoing Phase 1/2 study (UK) randomised single-blinded, controlled trial in healthy adults

- COV002 - Ongoing Phase 2/3 study (UK) randomised, single-blind, controlled trial in adults

- COV003 - Ongoing Phase 3 study (Brazil) randomised, single-blind, controlled trial in adults

- COV005 - Ongoing Phase 1/2 study (South Africa) randomised, double-blind, placebo controlled trial in adults with and without HIV

- D8110C00001 - Ongoing Phase 3 study (US, Chile, Peru) randomised, double-blind, placebo controlled trial in adults

- COV004 - Ongoing Phase 1B/2 study (Kenya) randomised, single-blinded, controlled trial in adults

- D8111C00002 - Ongoing Phase 1/2 study (Japan) randomised, double-blind, placebo controlled trial in adults

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PRAC Assessment

In these ongoing studies, the cases of thrombosis, especially those multiple or with unusual locations (e.g. CVST, mesenteric thrombosis, portal thrombosis, arterial thrombosis) should be evaluated in detail measuring platelet levels, D-dimer and fibrinogen levels.

Additional tests to rule out other aetiologies of thrombotic events and/or thrombocytopenia are also proposed: complete blood count (haemoglobin level, white blood cells with complete formula, thrombocyte level), haemolysis parameters (schistocytes, reticulocytes, haptoglobin levels), ADAMTS13 activity, PTT, TCA, fibrinogen, D-dimers, lupus anticoagulant research (including anti-cardiolipin antibodies IgG + IgM and anti-B2GPI antibodies IgG + IgM).

In the cases with low level of platelets, titration of anti-PF4 antibodies should also be performed.

Additionally, those cases of thrombosis with thrombocytopenia and/or bleeding, should also be investigated more generally with a search for anti-platelets antibodies (with specific target to identify). A deep exploration of platelet function is needed to understand the thrombotic and maybe bleeding phenotype (possibly not always associated with thrombocytopenia). And more generally, it would be interesting to investigate the "immune background" of the patients who experience this kind of side effect. Parameters of auto-immunity should be investigated (e.g. anti-nuclear factor, ANCA, rheumatoid factor, HLA B27, hypersensitivity markers,…).

The protocols of these ongoing studies should be modified accordingly.

Regarding clinical trials already finished from the clinical development program, the MAH should further explore the feasibility of analysing HIT-antibodies from samples collected during the

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clinical studies (without symptoms apparently) soon after Vaxzevria vaccination should they are available; immunological testing such as ELISA would suffice.

Non-interventional studies

The current version of the RMP does not include thrombotic and embolic events in the safety concerns. However, some of those events are listed as Adverse Event of Special Interest (AESI). At this point, AESIs considered for routine and additional pharmacovigilance activities include, among others, Stroke and other cerebrovascular events, Venous thromboembolism, and Myocardial infarction.

The MedDRA PTs which are intended to aid in the identification and retrieval of possible individual reports for those specific AESI are as followed:

AESI	MedDRA PTs
Stroke and other cerebrovascular events, Venous thromboembolism	• Basal ganglia stroke • Brain stem infarction • Brain stem stroke • Cerebellar infarction • Cerebellar stroke • Cerebral infarction • Cerebral microinfarction • Cerebral venous sinus thrombosis • Cerebrovascular accident • Choroidal infarction • Deep vein thrombosis • Embolic cerebellar infarction • Embolic cerebral infarction • Embolic stroke • Embolism venous • Haemorrhagic infarction • Haemorrhagic stroke • Haemorrhagic transformation stroke • Ischaemic cerebral infarction • Ischaemic stroke • Lacunar stroke • Pituitary infarction • Post procedural stroke • Pulmonary embolism • Spinal stroke • Stroke in evolution • Strokectomy • Thrombotic stroke • Vertebrobasilar stroke

Myocardial infarction	• Acute cardiac event
	• Acute coronary syndrome
	• Acute myocardial infarction
	• Angina unstable
	• Blood creatine phosphokinase MB abnormal
	• Blood creatine phosphokinase MB increased
	• Coronary artery embolism
	• Coronary artery occlusion
	• Coronary artery reocclusion
	• Coronary artery thrombosis
	• Coronary bypass thrombosis
	• Coronary vascular graft occlusion
	• Kounis syndrome
	• Myocardial infarction
	• Myocardial necrosis
	• Myocardial reperfusion injury
	• Myocardial stunning
	• Papillary muscle infarction
	• Periprocedural myocardial infarction
	• Post procedural myocardial infarction
	• Postinfarction angina
	• Silent myocardial infarction
	• Troponin I increased
	• Troponin increased
	• Troponin T increased

The pharmacovigilance activities for the monitoring of AESIs include both routine pharmacovigilance (i.e. signal detection and monthly summary safety reports) and additional activities. More specifically, AESI will be further assessed in the two main non-interventional studies, i.e. the Enhanced Active Surveillance (EAS) and the post-marketing observational study using existing secondary health data sources.

Enhanced Active Surveillance (EAS)

Three EAS studies (D8111R00003 [EU], D8110R00001 [US], and ESR 21-21121[UK; conducted by the DSRU]) are currently proposed. The EAS studies will be single arm cohort studies of adult volunteers, conducted at participating primary care or general practices or other centres with an ability to administer Vaxzevria and follow-up participants. Participants will be identified and enrolled at the receipt of their first dose of AZD1222 in a real-world setting.

The protocol of the EAS to be conducted in the European Union (Germany, France, Spain, Sweden) has been recently evaluated. Target enrolment in the EU is 15,000 participants. A similar study will be conducted in the UK (10,000 participants), and, pending approval, a third similar study in the United States (US; 15,000 participants).

The primary objective of the EAS studies is to assess the safety and tolerability of at least one intramuscular dose of AZD1222 for 3 months after vaccination.

The secondary objectives are (i) to assess the longer-term safety and tolerability for 18 months after the first dose of AZD1222, and (ii) to assess the safety and tolerability of at least one IM dose of AZD1222 in participants ≥ 65 years of age and in other key subgroups, (iii) to estimate the frequency of select pregnancy outcomes in women vaccinated with AZD1222 during pregnancy or within 45 days of the estimated conception date, and (iv) to estimate the frequency of select outcomes in neonates/infants born to mothers vaccinated with AZD1222 during pregnancy or within 45 days of the estimated date of conception.

The outcome measures for the primary objective are serious adverse events, AESI and medically-attended AEs following immunisation.

Medically attended adverse events following immunisation will be recorded for the initial period. AESIs and safety concerns will be recorded for up to 18 months post first dose of AZD1222.

The first interim report of this study is expected for the Q3 2021.

Post-marketing observational study using existing secondary health data sources

This PASS is a retrospective, longitudinal cohort study using population-based automated health care data to ascertain vaccination details, patient characteristics, and outcomes of interest. The study will be performed in the US and in EU/UK.

The study objectives are (i) to estimate the incidence of safety concerns and AESIs in recipients and non-recipients of AZD1222, among all populations targeted for vaccination and in the specific populations considered as missing information, (ii) to estimate the relative risk (comparing exposed and unexposed person time) of safety concerns including AESIs among all populations targeted for vaccination and in the specific populations considered as missing information, and (iii) to characterise the use of AZD1222 among all populations targeted for vaccination and in the specific populations considered as missing information.

Outcomes will be identified using algorithms based on codes for diagnoses, procedures, and treatments in electronic data. Operational case definitions from the ACCESS project[5] will be implemented for the AESIs for which they have been developed.

The study design will be a matched cohort design. The matched exposed cohort will consist of subjects receiving at least one dose of Vaxzevria during the study period. The unexposed cohort will consist on subjects not vaccinated with any COVID-19 vaccine on or before the data of the initial Vaxzevria dose in the exposed person.

For the matched cohort design, cohort entry (index date) among exposed individuals will be defined as the date of the first Vaxzevria dose. Cohort entry (index date) in unexposed individuals will be assigned based on the calendar date of vaccination in matched exposed individuals. Follow-up of exposed and unexposed individuals will be censored if death, leaving the database, occurrence of event of interest, or end of the study period, whichever comes first. Additionally, persons in the unexposed cohort will be censored if they have received any dose of Vaxzevria or other COVID-19 vaccine during the study period.

As a secondary approach for objective (ii), a self-control case series (SCCS) will be used to assess relative risks for AESIs meeting criteria for the design (i.e., acute onset, short latency, risk intervals that are relatively well known, and event not affecting the probability of vaccination). The SCCS will be a case-only study that includes individuals vaccinated with Vaxzevria who have experienced an event during the study period (Weldeselassie et al. 2012). The incidence rate ratio comparing the rate of the adverse event in a period hypothesized to be at increased risk due to exposure ("risk period" or "exposed person time") will be compared to that in all other time within an individual's observation period that does not fall within the risk period ("unexposed person time").

The study observation period will be of 2 years.

According to the MAH, data-related limitation of this study is the reliance on the accuracy of codes and algorithms to identify outcomes. To the extent feasible, outcomes and their dates of occurrence will be

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[5] https://drive.google.com/drive/folders/1Y_3cuGRN1gjBv2ec1fC0aYcpxEjtrY9

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validated. Besides, design-related limitation of both the matched cohort and SCCS designs is that any uncertainty about risk periods will lead to misclassification and attenuation of risk estimates.

To note that the current protocol is not evaluated yet.

PRAC Assessment

The RMP of Vaxzevria (Version: 1; Succession number: 5) comprises studies which could partially addressed the current safety issue. The two observational PASS include the assessment of AESI in their objectives. However those studies present some limitations which precludes a complete and comprehensive evaluation of the rare events of thrombosis with or without thrombocytopenia as they were observed in post-marketing.

First of all, the **list of AESIs** currently included in the RMP for routine and additional PhV is uncomplete and unprecise. Only the AESIs "Thrombocytopenia", "Stroke and other cerebrovascular events, venous thromboembolism" and "Myocardial infarction" are AESIs linked with the safety issue under investigation. Some other thromboses like splanchnic vein thrombosis are not included in the list of AESIs, but should also be investigated. Moreover, the list of AESIs included in the RMP of Vaxzevria contains –"Thrombocytopenia" and "Stroke and other cerebrovascular events, venous thromboembolism"/"Myocardial infarction" as separate AESIs and therefore, this may pose difficulties in identifying cases with both thrombosis and thrombocytopenia concomitantly.

Besides the list of PTs related to the AESI should be broaden:

- The list of PTs considered relevant to identified cases of thrombosis is described in the table below;

- PTs related to thrombocytopenia should also include "Platelet count decreased, Heparin-induced thrombocytopenia, Platelet count abnormal, Platelet disorder" in addition to the current list of terms (i.e. immune thrombocytopenia, thrombocytopenia, thrombocytopenic purpura).

Table A - PTs to be included for the identification of cases of thrombosis

'Embolic and thrombotic events, arterial'	'Embolic and thrombotic events, venous'	'Embolic and Trhombotic events, vessel type unspecified and mixed arterial and venous'
Acute aortic syndrome[3]	Budd-Chiari syndrome	Basal ganglia stroke[2]
Acute myocardial infarction[3]	Cavernous sinus thrombosis	Brain stem infarction[2]
Arterial thrombosis	Cerebral venous sinus thrombosis	Brain stem stroke[2]
Cerebellar artery occlusion	Cerebral venous thrombosis[2]	Cerebellar infarction[2]
Cerebellar artery thrombosis	Deep vein thrombosis[2]	Cerebral infarction[2]
Cerebral artery embolism	Embolism venous[2]	Cerebral ischaemia
Cerebral artery occlusion	Hepatic vein occlusion	Cerebral microembolism
Cerebral artery thrombosis	Hepatic vein thrombosis	Cerebral microinfarction[2]
Coronary artery embolism[3]	Iliac vein occlusion	Cerebral thrombosis
Coronary artery occlusion[3]	Inferior vena caval occlusion	Cerebral vascular occlusion
Coronary artery reocclusion[3]	Jugular vein occlusion	Cerebrovascular accident[2]
Coronary artery thrombosis[3]	Jugular vein thrombosis	Choroidal infarction[2]
Coronary vascular graft occlusion[3]	Mesenteric vein thrombosis	Coronary bypass thrombosis[3]
Embolism arterial	Mesenteric venous occlusion	Disseminated intravascular coagulation[1]
Femoral artery embolism	Ophthalmic vein thrombosis	Embolic cerebellar infarction[2]
Hepatic artery embolism	Pelvic venous thrombosis	Embolic cerebral infarction[2]
Hepatic artery occlusion	Portal vein occlusion	Embolic stroke[2]
Hepatic artery thrombosis	Portal vein thrombosis	Haemorrhagic adrenal

Iliac artery embolism	Pulmonary embolism[2]	infarction
Iliac artery occlusion	Pulmonary infarction	Haemorrhagic cerebral
Ischaemic cerebral infarction[2]	Pulmonary microemboli	infarction
Ischaemic stroke[2]	Pulmonary thrombosis	Haemorrhagic infarction[2]
Mesenteric arterial occlusion	Pulmonary vein occlusion	Haemorrhagic stroke[2]
Mesenteric arteriosclerosis	Pulmonary venous	Haemorrhagic transformation
Mesenteric artery embolism	thrombosis	stroke[2]
Mesenteric artery thrombosis	Renal vein occlusion	Heparin-induced
Myocardial infarction[3]	Renal vein thrombosis	thrombocytopenia
Myocardial necrosis[3]	Retinal vein occlusion	Microembolism
Papillary muscle infarction[3]	Retinal vein thrombosis	Pituitary infarction[2]
Peripheral artery occlusion	Splenic vein occlusion	Post procedural stroke[2]
Peripheral artery thrombosis	Splenic vein thrombosis	Spinal stroke[2]
Peripheral embolism	Superior sagittal sinus	Stroke in evolution[2]
Post procedural myocardial	thrombosis	Strokectomy[2]
infarction[3]	Superior vena cava occlusion	Thrombotic stroke[2]
Pulmonary artery occlusion	Transverse sinus thrombosis	
Pulmonary artery thrombosis	Venous thrombosis	
Renal artery occlusion		
Renal artery thrombosis		
Renal embolism		
Retinal artery embolism		
Retinal artery occlusion		
Retinal artery thrombosis		
Silent myocardial infarction[3]		
Spinal artery embolism		
Spinal artery thrombosis		
Splenic artery thrombosis		
Splenic embolism		
Subclavian artery embolism		
Subclavian artery occlusion		
Subclavian artery thrombosis		
Thromboembolectomy		
Thrombotic microangiopathy		
Thrombotic thrombocytopenic		
purpura		
Transient ischaemic attack		
Vertebral artery occlusion		
Vertebral artery thrombosis		

Codes: 1 – PT already listed for AESI 'Vaccine associated-disease'; 2 – PT already listed for AESI 'Stroke and other cerebrovascular events Venous thromboembolism'; 3 – PT already listed for AESI 'Myocardial infarction', no code = selection by PRAC RAP expert

Secondly, regarding the **Enhanced Active Surveillance**, the study has several limitations including mainly the limited sample size which will not provide a sufficient power to detect very rare events. Other limitations are possible misclassification due to self-reporting of AEs, potential of missing some SAEs due to possible inability of participants to report, and the difficulties in identifying cases with both thrombosis and thrombocytopenia concomitantly. With these limitations, it will be very difficult to recruit the cases described in the signal from this study.

Finally, regarding the **Observational study using existing secondary health data sources**, one of the limitations relates to the definition of the outcomes. In this study, cases of thrombocytopenia reported concomitantly with thrombosis may not be easily identified. The MAH should include search strategies to identify such cases. Moreover, the outcomes will be identified using case definitions from the ACCESS project[6] which may not fully address the safety concern which require further investigation. For example, the proposed event definition form is currently

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[6] https://drive.google.com/drive/folders/1Y_3cuGRN1gjBv2ec1fC0aYcpxEjtrY9

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for coagulation disorders in general. Liaising with the ACCESS project, but also with the Brighton Collaboration should help to provide operational case definition for the events of thrombosis with or without thrombocytopenia.

Non-clinical studies

To date, the RMP does not include any non-clinical studies as part of the pharmacovigilance plan.

PRAC Assessment

Proposal for new pre-clinical studies after assessment of the possible mechanistic explanations (see Section 3.5.5.2. Viral vector role for further details)

The MAH should submit the results of the pending biodistribution study with for the AZ vaccine on the agreed date.

The MAH should propose a study to test in-vitro expression of the S protein of AZ vaccine (these are already available for the other already authorised SARS-CoV-2 vaccines with a different S protein without showing concerns).

The MAH should propose any further non-clinical studies aimed at elucidating the mechanism that trigger platelet activation and subsequent thrombotic effects.

Proposal for new pre-clinical studies after AHEG meeting

In addition, after the AHEG some other studies are suggested to be performed.

In-vitro data: to test the interaction of the AZ vaccine with blood components (i.e. thrombocytes, erythrocytes, leucocytes etc., coagulation factors, natural IgM antibodies) both in the presence and absence of pre-existing immunity to ChAdOx1

Non-clinical data: more extensive non-clinical data namely but not limited to animal models: The MAH should provide further animal data namely on chimpanzee adenovirus. The potential effects of chimpanzee adenovirus to human should also be addressed.

Overall conclusion on the RMP

PRAC Assessment

Considering the results of the O/E analysis, the clinical and laboratory patterns of reported cases and the plausible biological mechanism, the risk of 'Thrombosis with or without thrombocytopenia' should be included in the RMP as important identified risk for further characterisation.

The PASS currently included in the RMP (i.e. EAS and observational study using secondary health data sources) try to address the current important potential risks and missing information. The objectives of both studies, mainly the study using secondary databases should be updated to address the new important identified risk.

Despite adaptation of the study protocols should be proposed, it seems very unlikely that the enhanced active surveillance would identify cases that report thrombosis and thrombocytopenia concomitantly. For the protocol of the study using secondary databases, the MAH should discuss how the new safety concern could be addressed.

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Besides, additional laboratory testing should be proposed for the ongoing clinical trials included in the RMP, as well as for already finalised studies from the clinical development program.

However, even if adapted, both interventional and non-interventional studies included in the current pharmacovigilance plan would be insufficient to fully characterise the new important identified risk.

Additional studies are clearly needed, as also highlighted by the AHEG:

- Clinical study required for CVST cases observed following vaccination (i.e. this cohort should be compared to other CVST cases or post-COVID-19 CVST cases). This will inform on risk factors, optimal treatment, diagnosis and prognosis of these patients;

- One expert considered that there is a requirement for broad screening procedure to identify relevant cases and characterize them with high precision clinically. They also proposed a biobank to facilitate further investigation of clinical data. One expert agreed that further sampling was required;

- -In-vitro data: to test the interaction of the AZ vaccine with blood components (i.e. thrombocytes, erythrocytes, leucocytes etc., coagulation factors, natural IgM antibodies) both in the presence and absence of pre-existing immunity to ChAdOx1;

- Non-clinical data: overall consensus that the MAH should provide more extensive non-clinical data namely but not limited to animal models: AZ should bring more animal data namely on ChAdOx1. Also, the effect of ChAdOx1 to human is needed;

- Further studies to elucidate the role of the spike antigen in these events specifically whether the Spike protein is antigenic (i.e. taking the role of heparin and binding with platelet factor 4);

- Further studies on the optimal diagnostic test and the definition of the disease. One expert considered that it would be useful to have a type of workshop to test the sera in screening tests. They noted that working is currently ongoing (i.e. to ascertain whether tests have a high false negative rate due to the assay design);

- Further collaboration with the Brighton collaboration in order to finalise the case definition which can be used in epidemiological studies (e.g. case control, cohort studies). One expert specified that there are several useful registries in the Nordic countries which can be used. They also noted that work needs to be done to facilitate ascertainment of relevant cases. They discussed ongoing work in a Member State whereby an algorithm is being utilized that searches for TROM words in the medical files of patients in order to identify relevant cases, including those that have a milder disease picture. Expected vs. observed incidences of this clinical entity or group of entities are needed to better understand the role of vaccination;

- Parallel in-vitro studies which inform on the cellular response for all vaccines including the mRNA vaccines (e.g. in-vitro studies in which cellular components are incubated with the vaccines, measuring cytokine responses etc.)

In conclusion, the MAH should proposed an update of the pharmacovigilance plan, including adaption of planned and ongoing studies (both observational PASS and clinical trials) and new mechanistic non-clinical studies.

Besides, additional epidemiological studies are also needed to further assess the association

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between vaccination and the new safety concern. However, considering the limitations of the PASS run at MAH level, a better option seems to conduct those studies by consortia supported by EMA. This will allow for more powered studies and a more comprehensive evaluation of COVID-19 vaccines.

3.4.6.2. Epidemiological studies

Current and planned EMA-funded studies on the risk of thromboembolic events in patients vaccinated with COVID-19 vaccines

There are several <u>methodological considerations</u> to take into account when discussing possible studies:

- **Objective(s):** need(s) to be clearly defined as there is an important impact on the study design and the sample size if the objective is to study the *absolute risk* (stratified by age and gender and potential risk factors), versus *measuring an association*, which requires comparisons between groups to be adequately defined, e.g. vaccinated vs. non-vaccinated groups, groups of persons receiving different vaccines, or pre-/post-vaccination periods.

- **Outcome**: all thromboembolic events or specific well-defined events; currently the entity is still to be clearly defined and there is a need to establish case definitions and select code lists for database studies (if such studies are appropriate). Moreover, diagnosis, coding and recording accuracy for rare thromboembolic events in databases may need validation.

- **Study design:**

  - Not all study designs are currently feasible. For example, a sufficient follow-up period beyond the risk window after the vaccination is needed for a traditional self-controlled case series analysis (SCCS), but a self-controlled risk interval (SCRI) method would be possible as it requires only a pre-vaccination reference time window of reference. Moreover, spontaneous reports cannot be used as a source of cases in a case-control study as a suspected relation of the adverse reaction with the vaccine is a reason for reporting.

  - A cohort study with nested case-control analysis requires a very large study population due to rarity of some thromboembolic events such as DIC (~1.45/100,000 person-years) and CVTS (~1.26/100,000 person-years)[7]; multiple databases or large national databases may be needed.

  - There is need for hospital data with availability of vaccine exposure data for different vaccines, however linkage to vaccination registers is in different stages of development and does not exist for some hospital databases.

  - Availability or linkage to laboratory data (thrombocytopenia, heparin/platelet factor 4 (PF4)) would be important.

  - Several types of bias should be considered: e.g. selection bias and confounding factors, including characteristics of vaccinated persons over time (age, morbidity, Covid-19 infection).

- **Time constraints**

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[7] (http://www.encepp.eu/phact_links.shtml

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Identification of cases for case-control studies could be based on the identification of potential cases in electronic databases followed by a screening of medical records; besides the challenges of applying diagnostic criteria at each stage and accessing the medical records, the time needed to complete such study may not support timely regulatory decision-making.

Options that are already available or may be considered are:

A first step in an epidemiological investigation would comprise a comprehensive case finding from haematologists and other clinical specialists; it would allow individual case description, mechanistic study and identification of potential risk factors. An European database of vaccinated persons and presented with well-defined thrombotic events could be established through medical associations or learned societies and could be supported by EMA or Member States. Possible use of the EudraVigilance database could be explored. A full-blown epidemiological study would however require exhaustive identification and reporting of cases.

Large electronic health care databases (with linkage to hospital data):

- These databases would be most useful for measuring absolute risks of some defined adverse events with stratification by potential risk factors. A potential issue is the lag time needed to diagnose and code appropriately new clinical entities and to update databases.

- These databases would allow comparisons with non-vaccinated cohorts or historical periods or between available vaccines

- Several databases would be suitable in the EU depending on the availability of vaccination data and specific outcome data

- Several EU databases including hospital data are available to EMA contractors: Utrecht University, Erasmus MC-Oxford University and IQVIA. The OHDSI network could provide access to US databases.

On-going EMA funded studies:

- The EU PE&PV Research Network has conducted the ACCESS study with the provision of background incidence rates for a list of 26 AESIs and 9 embolic and thrombotic events (EUPAS37273, http://www.encepp.eu/phact_links.shtml); it could provide by 31/04/2021 background incidence rate for additional adverse events to be provided, stratified by age and sex.

- The study **"Natural history of coagulopathy and use of anti-thrombotic agents in COVID-19"** (SC02 to FWC EMA/2018/21/PE) is an EMA-funded study performed by the Erasmus Medical Centre in association with University of Oxford. It has two main objectives:

  o to estimate the incidence of venous and arterial thromboembolic events and their consequences among patients with COVID-19;

  o in these patients, to evaluate how arterial and venous thromboembolic events are associated with patient characteristics that promote stasis of circulation, vascular endothelial injury and hypercoagulability, taking into account factors such as treatments.

The study protocol has been developed in collaboration with the US FDA and the same study protocol is used in databases in 7 European countries and in the US Sentinel system. The final results are due end of August 2021. However, following the concern of thromboembolic events in persons vaccinated with Vaxzevria, the protocol was amended on 22 March to add two objectives:

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- background incidence rates for adverse events of interest (to be provided), stratified by age and sex, by 31/04/2021

    - incidence rate of embolic and thrombotic events (to be defined) within 7-, 14-, 21- and 28 days following COVID-19 vaccination, stratified by age, sex, vaccine, relevant risk factors for embolic and thrombotic events (e.g. BMI, diabetes, hypertension, pregnancy, malignancy), and use of anti-thrombotic/anticoagulant medication and other relevant medication (e.g. oral contraceptives, hormonal replacement therapy) at time of vaccination). The deadline for this deliverable is 31/06/2021.

- The one-year (2021) EMA-funded study "Early safety monitoring of COVID-19 vaccines in EU Member States" (ECVM) is conducted by the EU PE&PV Research Network to complement routine pharmacovigilance and other EU-level safety monitoring activities by:

    - generating prospective data on the incidence of suspected adverse reactions following vaccination of different population groups in seven EU Member States (the Netherlands, Belgium, Luxembourg, Italy, France, Germany, Croatia) and the United Kingdom. The study uses a web-based application to collect self-reported information from vaccinated individuals and the results will be submitted through monthly reports. These reports will be communicated to the PRAC Rapporteurs.

    - monitoring the incidence of adverse events of special interests (AESIs) and COVID-19 diagnoses in cohorts of vaccinated individuals using electronic healthcare databases, including but not limited to databases which participated in the ACCESS project. Results for AESI incidence rates study in 4 Electronic Health Record databases (with access to GP and hospital data) are expected end of April; with O/E analyses as applicable and potentially additional background rates.

EMA-funded studies – planned (amendment of protocol is possible)

- The EMA-funded study **"Safety monitoring of COVID-19 vaccines in the EU"** (ROC20) will be a two-year (2021-2022) EMA-funded study extending the ECVM study with two objectives:
    - to design and implement a large, two-year, prospective cohort study to estimate the incidence of suspected adverse drug reactions (ADRs) and adverse events of special interest (AESIs) following immunisation with COVID-19 vaccines; this cohort study will be ideally performed in a target number of at least 10 EU Member States not included in ROC19 and will include special populations (e.g. pregnant women);
    - to conduct in electronic health care databases signal strengthening activities for potential safety concerns emerging from active surveillance.
  The objectives stated above may be amended to respond to specific concerns raised by the COVID-19 vaccination campaign, including concerns about thromboembolic events.

- The EMA is considering launching a call among its framework contractors to perform an additional study to measure the association between thromboembolic events/coagulopathies and COVID-19 vaccines, within defined time periods after vaccination, stratified by vaccine brand, age and gender. Depending on data availability, analyses should be adjusted by relevant covariates, such as personal characteristics (e.g. age and gender, venous thromboembolism risk), COVID-19 infection, co-morbidities (e.g. obesity, hypertension, diabetes, underlying autoimmune disease), or co-medication (e.g. oral contraceptives, hormonal therapies). Considerations could be given to availability of laboratory data to support mechanism of action for the combination of thrombocytopenia and coagulation disorders. If sufficient insight into plausible biological

mechanisms is available at the time of study design, exploratory analyses could include the effect of genetic polymorphisms.

The call could be launched by 16th April. Depending on the objectives and study design, the study may need 6-8 months to be performed.

Comment PRAC

There are several studies current/planned **EMA-funded studies** that could help assess the potential risk of thromboembolic events (TEEs) in patients vaccinated with COVID-19 vaccines.

Some of the ongoing/planned studies seem appropriate to assess the potential risk of serious thromboembolic events associated with Vaxzevria (and well as with other COVID19 vaccines). In particular, the EMA-funded database study "Natural history of coagulopathy and use of anti-thrombotic agents in COVID-19" (amended protocol) performed by the Erasmus Medical Centre in association with University of Oxford, and the study under consideration, planned to measure the association between TEEs/coagulopathies and COVID-19 vaccines (call planned to be launched by 16th April) appear to be the most interesting to provide relevant data on this topic. These studies could bring data on background incidence rates and incidence rates after vaccination of outcomes of interest, as well as on demographics and risk factors. However, there are a lot of important challenges identified and many details remain to be defined (study design, outcome definition and ascertainment, data capture, confounders, exposure, lag time, etc.).

Studies based on self-reported primary data collection can have complementary value but appear less suitable for examining the association between Vaxzevria and specific clotting events because they are prone to selection and misclassification bias (and study size may not be appropriate for very rare events).

With regard to the possibility of the MAH conducting pharmaco-epidemiological studies, it seems unlikely that the MAH would have access to better data sources or expertise than the academic groups with which the EMA and MHRA already work (Erasmus MC, Utrecht University, University of Oxford, DSRU, LSTHM). Therefore, the MAH might be asked to primarily focus on **pre-clinical/mechanistic studies**.

3.4.7. Conclusion

The review of additional data analysis from EudraVigilance with individual case review review and O/E analysis, input from the AHEG and available literature pointed to signals of Embolic and thromboembolic events, Cerebral venous sinus thrombosis, and Splanchnic vein thrombosis, with or without thrombocytopenia, mainly occurring in women below 55 years and with a time-to-onset within 2 weeks following vaccination.

Clinical entity

It is considered, in agreement with the AHEG, that an atypical heparin induced thrombocytopenia (aHIT) like disorder is the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients. It is considered extremely likely that the syndrome, which resembles aHIT, concerns a severe autoantibody against PF4 which exhibits a high binding affinity. It was hypothesised that the antibody itself is changing the structure of PF4, similar to what has been shown for aHIT. It was noted that high titres of anti-PF4 antibodies were observed in all patients whose biomaterial was analysed which substantiates this hypothesis.

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Greinacher et al. (1) suggests to name this prothrombotic thrombocytopenic disorder 'vaccine induced prothrombotic immune thrombocytopenia (VIPIT)' and based on this, an algorithm was proposed by the German Society for Thrombosis and Haemostatis Research (GTH) (2) and the Ontario COVID-19 Science Advisory Table (3). The British Society for Haematology published a guidance for the syndrome of thrombosis and thrombocytopenia after coronavirus vaccination, including a case classification (4).

In line with the AHEG it is agreed that at this point in time the optimal case definition is not known. It was highlighted that until the specific presentations of the VTEs would be better defined, case definition should not be restricted to those cases of CVST with thrombocytopenia, and could be broadened to include all thrombotic events.

Causality

The AHEG agreed that there likely are various clinical entities explaining the incidence of thrombocytopenia, thrombotic complications and/or a combination of these two. There was general consensus amongst the experts that there is a strong relationship between these events and vaccination with Vaxzevria.

Using Bradford-Hill criteria (5), there are several arguments to support that a causal relationship between the vaccination with Vaxzevria and the adverse events is at least a reasonable possibility:

- *Plausibility*: an immunological pathophysiology is described (heparin independent antibody mediated platelet activation via platelet Fc gamma RIIA receptors) which appears to be temporarily associated with a unique vaccine technology. The very low numbers explain why this signal could never be detected in a trial;

- *Consistency*: There has a been a consistency of clinical entities (e.g. CVST occurring in conjunction with thrombocytopenia or splanchnic vein thrombosis in a young female population without risk factors) and laboratory findings across cases and across multiple countries;

- *Temporality*: median time to onset was 9 days for Thromboembolic events with thrombocytopenia , CVST and splanchnic vein thrombosis cases after first dose of Vaxzevria (Eudravigilance, DL 23/03/2021);

- *Specificity*: A cluster of clinical and laboratory features which are very rarely seen in clinical practice;

- *Change in risk factor*: Recognition of rare cases in multiple countries associated with the increased number of vaccinations in younger age groups. Not identified so far with other vaccine technologies;

- *Analogy*: an atypical heparin induced thrombocytopenia (aHIT) like disorder was considered by the AHEG the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients.

Second dose

So far, the reported cases occurred after administration of the first dose of Vaxzevria. Experience of exposure to the second dose is still limited. According to British Society for Haematology, those either affected by, or under investigation for this complication should not receive their second vaccine until the stimulant for this condition is clear (4). Other experts stated that a second dose of another COVID-19 vaccine may be safe (3).

References

Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19
Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine
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1. Greinacher A, Thiele T, Warkentin TE, Weisser K. A Prothrombotic Thrombocytopenic Disorder Resembling Heparin-Induced Thrombocytopenia Following Coronavirus-19 Vaccination. :1-8.

2. GTH. Updated GTH statement on vaccination with the AstraZeneca COVID-19 vaccine, as of March 22, 2021. 2021;(45).

3. Pai M, Grill A, Ivers N, et al. Vaccine - Induced Prothrombotic Immune Thrombocytopenia (VIPIT) Following AstraZeneca COVID - 19 Vaccination. 2021:1-7. https://covid19-sciencetable.ca/sciencebrief/vaccine-induced-prothrombotic-immune-thrombocytopenia-vipit-following-astrazeneca-covid-19-vaccination/.

4. Guidance Produced from the Expert Haematology Panel (EHP) Focussed on Syndrome of Thrombosis and Thrombocytopenia Occurring after Coronavirus Vaccination Updated Guidance on Management. https://b-s-h.org.uk/about-us/news/guidance-produced-from-the-expert-haematology-panel-ehp-focussed-on-syndrome-of-thrombosis-and-thrombocytopenia-occurring-after-coronavirus-vaccination/. Accessed April 2, 2021.

5. Hill AB. The environment and disease: association or causation? J R Soc Med. 2015;108(1):32-37. doi:10.1177/0141076814562718

3.5. Updated proposed Recommendations – round 2

Taking into consideration all the assessed data and MS comments, the PRAC recommends to amend the product information, in sections 4.4 and 4.8 to reflect these adverse reactions.

Furthermore, a number of studies should be put in place aimed at identifying the exact pathophysiological mechanism for the occurrence of these thrombotic events and at quantifying the magnitude of the risk.

Proposals for changes in the product information areas described below (new text underlined/text to be removed with strikethrough):

Summary of Product Characteristics :

Section 4.4 Special warnings and precautions for use

Thrombocytopenia and coagulation disorders

Thrombosis and a combination of thrombosis and thrombocytopenia, in some cases accompanied by bleeding, has been observed very rarely following vaccination with COVID-19 Vaccine AstraZeneca. This includes severe cases presenting as venous thrombosis, including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis mesenteric vein thrombosis, as well as arterial thrombosis, concomitant with thrombocytopenia. Some cases had a fatal outcome. The majority of these cases occurred within the first seven to fourteen days following vaccination and occurred mostly in women under 55 years of age, however this may reflect the increased use of the vaccine in this population. Some cases had a fatal outcome. No specific risk factors have been identified. *Benefit-risk should be considered* taking into account the availability of the alternatives and epidemiological local data.

Healthcare professionals should be alert to the signs and symptoms of thromboembolism and or thrombocytopenia. Those vaccinated should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain following vaccination. Additionally, anyone with neurological symptoms including severe or persistent headaches or blurred vision after vaccination, or who experiences skin bruising (petechia) beyond the site of vaccination after a few days, should seek prompt medical attention.

Section 4.8 Undesirable effects

Table 1 - Adverse drug reactions

MedDRA SOC	Frequency	Adverse Reactions
Blood and lymphatic system disorders	Common	Thrombocytopenia*
Vascular disorders	[Frequency to be proposed by the MAH]	Thrombosis (e.g. Cerebral venous sinus thrombosis, splanchnic vein thrombosis, Arterial thrombosis) *

And at the end of the table the following statement should be added:

* Serious and rare cases, some of them fatal, of venous thrombosis (including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis (including portal, splenic, mesenteric and hepatic vein thrombosis), as well as arterial thrombosis) have been reported post-marketing that may be associated with thrombocytopenia (see section 4.4).

Package leaflet

Section 2 What you need to know before you use <product name>

Talk to your doctor, pharmacist or nurse before you are vaccinated:
…
…

Blood disorders

Blood clots or a combination of blood clots and low level of platelets, in some cases together with bleeding, has been observed very rarely following vaccination with VAXZEVRIA/COVID-19 Vaccine AstraZeneca. This included some severe cases with blood clots in different or unusual locations and excessive clotting or bleeding throughout the body. The majority of these cases occurred within the first ~~seven to~~ fourteen days following vaccination and mostly occurred in women under 55 years of age, ~~however more women under 55 received the vaccine than other people~~. Some cases had a fatal outcome.

Seek immediate medical attention if you develop shortness of breath, chest pain, leg swelling, or persistent abdominal pain following vaccination.

Also, seek immediate medical attention if you experience after a few days severe or persistent headaches or blurred vision after vaccination, or experience skin bruising or pinpoint round spots beyond the site of vaccination which appears after a few days.

Section 4 Possible side effects

Common

- low level of platelets

[Frequency to be proposed by the MAH]

- blood clots in unusual locations (brain, bowel, liver, spleen)

In addition, the MAH should distribute a **direct healthcare professional communication (DHPC)** according to the text and communication plan as proposed.

The PRAC considered that given the serious and unpredictable nature of the risks, and that effective risk minimisation is key to support a positive benefit-risk balance, and should update **the risk management plan and the pharmacovigilance plan according to the following:**

1. The MAH should update the RMP to include 'Thrombosis in combination with thrombocytopenia' in the list of safety concerns as an important identified risk, while 'Thrombosis' should be added as an important potential risk.

2. The additional pharmacovigilance measures section should also be updated to include/update the following studies:

Study type	Objective	Description	Category
Non-clinical study	To further characterize the possible mechanisms and to identify the possible triggers of platelet activation after vaccination	• The MAH should submit the results of the pending biodistribution study with for the AZ vaccine on the agreed date. • The MAH should propose a study to test in-vitro expression of the S protein of AZ vaccine (these are already available for the other already authorised SARS-CoV-2 vaccines with a different S protein without showing concerns).	1.
Non-clinical study	To further characterize the possible mechanisms and to identify the possible triggers of platelet activation after vaccination	In addition, the MAH should propose a plan to respond the following considerations: • The MAH should consider to perform in-vitro study to test the interaction of the AZ vaccine with blood components (i.e. thrombocytes, erythrocytes, leucocytes etc., coagulation factors, natural IgM antibodies) both in the presence and absence of pre-existing immunity to ChAdOx1. • The MAH should consider to collect more extensive non-clinical data namely but not limited to animal models. The MAH should provide further animal data namely on chimpanzee adenovirus. The potential	3

		effects of chimpanzee adenovirus to human should also be addressed.	
		• The MAH should propose further studies to elucidate the role of the spike antigen in these events specifically whether the Spike protein is antigenic (i.e. taking the role of heparin and binding with platelet factor 4).	
		• The MAH should propose any further non-clinical studies aimed at elucidating the mechanism that trigger platelet activation and subsequent thrombotic effects.	
		• The MAH should discuss the negative predictive value of a negative test for anti-PF4 antibodies, and whether the existence of such cases might also suggest other possible mechanisms.	

On-going clinical studies	To assess the aetiologies of the thrombotic with thrombocytopenia and/or bleeding events	A deep exploration of platelet function is needed, as well as investigation of the patients immunological background. The following tests should be considered, but not limited to:
		
		• Measurement of platelet levels, D dimer and fibrinogen levels;
		
		• Additional laboratory testing: complete blood count (haemoglobin level, white blood cells with complete formula, thrombocyte level), haemolysis parameters (schistocytes, reticulocytes, haptoglobin levels), ADAMTS13 activity, PTT, TCA, fibrinogen, D-dimers, lupus anticoagulant research (including anti-cardiolipin antibodies IgG + IgM and anti-B2GPI antibodies IgG + IgM);
		
		• In case of low level of platelets: titration of anti-PF4 antibodies;
		
		• Additional search for anti-platelets antibodies (with specific target to identify) and deep exploration of platelet function;
		
		• Investigation of patient "immune background": anti-nuclear factor, ANCA, rheumatoid factor, HLA B27, hypersensitivity markers,…
		
		• Other analysis to consider include:
		
		  – Faktor V Leiden; Faktor II (prothrombin) variant; Inflammatory markers: TNFa, IL-1, IL-4, IL-6, IL-10, IL-13,
		
		  – Platelet activation markers: sCD40L, soluble glycoproteins, degranulation markers (PF4, vWF, P-selectin, annexin V).
		
		  – Cell adhesion: VCAM, ICAM, E-Selectin
		
		  – Immunology: C3, C4, antinuclear IgG, anti-doublestranded DNA IgG, anti-Smith IgG, anti-SSA IgG, anti-SSB IgG, anti-Jo1 IgG, anti-MPO
			Cat. 2 for those already in that category, if not Category 1.

		IgG, anti-PR3 IgG, anti-glomerular basement menbrane IgG – Complement activation markers: Complement Complex C5b-9, C5a – Adeno virus serology – Tissue type (genetics) – Serology to be considered: Cytomegalovirus (IgG and IgM,) Epstein-Barr virus (IgG and IgM), HIV, Parvo virus B19 COVID-19 testing, including PCR and serology: in describing relevant cases, information on the type of testing performed, the timing of such testing, and the site from which the PCR swabs were taken should be provided	
Completed clinical studies	To assess the aetiologies of the thrombotic with thrombocytopenia and/or bleeding events	Feasibility of analysing HIT-antibodies from samples (without symptoms) collected during the clinical studies already finished from the clinical development program.	3
Monthly Safety Reports, O/E	To estimate the risk of thrombosis with or without thrombocytopenia	• PTs related to thrombocytopenia should also include "Platelet count decreased, Heparin-induced thrombocytopenia, Platelet count abnormal, Platelet disorder" in addition to the current list of terms (i.e. immune thrombocytopenia, thrombocytopenia, thrombocytopenic purpura). • The list of PTs related to embolic and thrombotic events should include: see list below	3
PASS: Enhanced active surveillance	To describe thrombosis with or without thrombocytopenia	The protocol should be updated to better capture and describe thrombosis with or without thrombocytopenia	3
PASS: Post-marketing observational study using existing secondary health data sources	To estimate the frequency of thrombosis with or without thrombocytopenia	The protocol should be updated to better capture and describe thrombosis with or without thrombocytopenia	3

List: Preferred Terms for embolic and thrombotic events

'Embolic and thrombotic events, arterial'	'Embolic and thrombotic events, venous'	'Embolic and Thrombotic events, vessel type unspecified and mixed arterial and venous'
Acute aortic syndrome[3] Acute myocardial infarction[3] Arterial thrombosis Cerebellar artery occlusion Cerebellar artery thrombosis	Budd-Chiari syndrome Cavernous sinus thrombosis Cerebral venous sinus thrombosis Cerebral venous thrombosis[2] Deep vein thrombosis[2]	Basal ganglia stroke[2] Brain stem infarction[2] Brain stem stroke[2] Cerebellar infarction[2] Cerebral infarction[2]

Cerebral artery embolism	Embolism venous[2]	Cerebral ischaemia
Cerebral artery occlusion	Hepatic vein occlusion	Cerebral microembolism
Cerebral artery thrombosis	Hepatic vein thrombosis	Cerebral microinfarction[2]
Coronary artery embolism[3]	Iliac vein occlusion	Cerebral thrombosis
Coronary artery occlusion[3]	Inferior vena caval occlusion	Cerebral vascular occlusion
Coronary artery reocclusion[3]	Jugular vein occlusion	Cerebrovascular accident[2]
Coronary artery thrombosis[3]	Jugular vein thrombosis	Choroidal infarction[2]
Coronary vascular graft occlusion[3]	Mesenteric vein thrombosis	Coronary bypass thrombosis[3]
Embolism arterial	Mesenteric venous occlusion	Disseminated intravascular coagulation[1]
Femoral artery embolism	Ophthalmic vein thrombosis	Embolic cerebellar infarction[2]
Hepatic artery embolism	Pelvic venous thrombosis	Embolic cerebral infarction[2]
Hepatic artery occlusion	Portal vein occlusion	Embolic stroke[2]
Hepatic artery thrombosis	Portal vein thrombosis	Haemorrhagic adrenal infarction
Iliac artery embolism	Pulmonary embolism[2]	Haemorrhagic cerebral infarction
Iliac artery occlusion	Pulmonary infarction	Haemorrhagic infarction[2]
Ischaemic cerebral infarction[2]	Pulmonary microemboli	Haemorrhagic stroke[2]
Ischaemic stroke[2]	Pulmonary thrombosis	Haemorrhagic transformation stroke[2]
Mesenteric arterial occlusion	Pulmonary vein occlusion	Heparin-induced thrombocytopenia
Mesenteric arteriosclerosis	Pulmonary venous thrombosis	Microembolism
Mesenteric artery embolism	Renal vein occlusion	Pituitary infarction[2]
Mesenteric artery thrombosis	Renal vein thrombosis	Post procedural stroke[2]
Myocardial infarction[3]	Retinal vein occlusion	Spinal stroke[2]
Myocardial necrosis[3]	Retinal vein thrombosis	Stroke in evolution[2]
Papillary muscle infarction[3]	Splenic vein occlusion	Strokectomy[2]
Peripheral artery occlusion	Splenic vein thrombosis	Thrombotic stroke[2]
Peripheral artery thrombosis	Superior sagittal sinus thrombosis	
Peripheral embolism	Superior vena cava occlusion	
Post procedural myocardial infarction[3]	Transverse sinus thrombosis	
Pulmonary artery occlusion	Venous thrombosis	
Pulmonary artery thrombosis		
Renal artery occlusion		
Renal artery thrombosis		
Renal embolism		
Retinal artery embolism		
Retinal artery occlusion		
Retinal artery thrombosis		
Silent myocardial infarction[3]		
Spinal artery embolism		
Spinal artery thrombosis		
Splenic artery thrombosis		
Splenic embolism		
Subclavian artery embolism		
Subclavian artery occlusion		
Subclavian artery thrombosis		
Thromboembolectomy		
Thrombotic microangiopathy		
Thrombotic thrombocytopenic purpura		
Transient ischaemic attack		
Vertebral artery occlusion		
Vertebral artery thrombosis		

Codes: 1 – PT already listed for AESI 'Vaccine associated-disease'; 2 – PT already listed for AESI 'Stroke and other cerebrovascular events, Venous thromboembolism'; 3 – PT already listed for AESI 'Myocardial infarction', no code = selection by PRAC RAP expert

Issues to be addressed in the Monthly Summary Safety Reports:

1. The MAH should discuss possible implications regarding the second dose and commit to timely and closely monitor this issue. Additional information regarding UK cases should be sought, as well as any further information on events reported after the second dose. Liaison with the MHRA should be continued as frequently as possible and necessary.

2. According to the EPAR no formal dose-finding study have been performed. The MAH should comment if the observed thromboembolic adverse reactions potentially could be related to a dosing issue.

3. Non-clinical: In a non-clinical study in monkeys, one animal (26Z) presented with very low (zero) thrombocytes (and other haematological parameters) following vaccination with ChAdOx nCOV-2 vaccine and before viral challenge. The MAH should comment on this finding in relation to the current issue of rare thrombosis and thrombocytopenia in humans following vaccination with Vaxevria.

Epidemiological studies with possible role of EMA supervision

Following proposals were made by the AHEG:

-   Clinical study required for CVST cases observed following vaccination (i.e. this cohort should be compared to other CVST cases or post-COVID-19 CVST cases). This will inform on risk factors, optimal treatment, diagnosis and prognosis of these patients.

-   Broad screening procedure to identify relevant cases and characterize them with high precision clinically: a biobank to facilitate further investigation of clinical data.

-   Further studies on the optimal diagnostic test and the definition of the disease. One expert considered that it would be useful to have a type of workshop to test the sera in screening tests. They noted that work is currently ongoing (i.e. to ascertain whether tests have a high false negative rate due to the assay design).

-   Further collaboration with the Brighton collaboration in order to finalise the case definition which can be used in epidemiological studies (e.g. case control, cohort studies). One expert specified that there are several useful registries in the Nordic countries which can be used. They also noted that work needs to be done to facilitate ascertainment of relevant cases. They discussed ongoing work in a Member State whereby an algorithm is being utilized that searches for TROM words in the medical files of patients in order to identify relevant cases, including those that have a milder disease picture. Expected vs. observed incidences of this clinical entity or group of entities are needed to better understand the role of vaccination.

-   Parallel in-vitro studies which inform on the cellular response for all vaccines including the mRNA vaccines (e.g. in-vitro studies in which cellular components are incubated with the vaccines, measuring cytokine responses etc.).

In addition, there are several studies current/planned EMA-funded studies that could help assess the potential risk of thromboembolic events (TEEs) in patients vaccinated with COVID-19 vaccines. In particular, the EMA-funded database study "Natural history of coagulopathy and use of anti-thrombotic agents in COVID-19" (amended protocol) performed by the Erasmus Medical Centre in association with University of Oxford, and the study under consideration, planned to measure the association between thromboembolic events/coagulopathies and COVID-19 vaccines (call planned to be launched by 16th April) appear to be the most interesting to provide relevant data on this topic. These studies could bring data on background incidence rates and incidence rates after vaccination of outcomes of interest, as well as on demographics and risk factors.

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A regular <u>update of the EMA analysis of Eudravigilance data</u> (3.5.2), using background incidence data for additional adverse events as generated within the ACCESS project, is of great importance to further monitor and characterise these signals.

3.6. Comments from other PRAC members and MAH – Round 2

MS1

We generally endorse the comments from the rapporteur and thank the rapporteur's team for this impressive and massive amount of work.

Overall, we endorse the update of 4.4 and 4.8 and LoQs for MAH. However, we have an additional comment regarding the proposed SmPC text. Please see below.

As the Rapporteur, we fully support the AHEG conclusions on the mechanisms and gaps in knowledge and also agree with the conclusion on the need for additional studies. However, we support and endorse rapporteurs view that the COVID disease-related hypotheses seem unlikely and focus may be directed towards the viral vector especially considering the novel signal on COVID-19 Janssen Vaccine (Ad26.COV2-S [recombinant]) which hypothetically could involve a similar mechanism. Finally, we acknowledge that the underling mechanisms are far from fully investigated and that several factors may contribute to the development of the phenomenon.

Updated the protocols of ongoing studies to include additional laboratory testing allowing for an in-depth assessment of the aetiologies of the thrombotic with thrombocytopenia and/or bleeding events, including additional search for anti-platelets antibodies in patients enrolled in ongoing studies, as suggested in the LoQs is also fully endorsed. The following analysis should be considered conducted in the ongoing clinical studies (Some are already mentioned):

Faktor V Leiden

Faktor II (prothrombin) variant

Inflammatory markers: TNFa, IL-1, IL-4, IL-6, IL-10, IL-13,

Platelet activation markers: sCD40L, soluble glycoproteins, degranulation markers (PF4, vWF, P-selectin, annexin V).

Cell adhesion: VCAM, ICAM, E-Selectin

Immunology: C3, C4, antinuclear IgG, anti-doublestranded DNA IgG, anti-Smith IgG, anti-SSA IgG, anti-SSB IgG, anti-Jo1 IgG, anti-MPO IgG, anti-PR3 IgG, anti-glomerular basement menbrane IgG

Complement activation markers: Complement Complex C5b-9, C5a

Adeno virus serology

Tissue type (genetics)

Serology to be considered: Cytomegalovirus (IgG and IgM,) Ebstein-Barr virus (IgG and IgM), HIV, Parvo virus B19

Given the available data and conclusions we support the suggestions from the preparatory PRAC meeting to discuss the option to start a new procedure investigating parts of the benefit/risk balance for the vaccine. Either subsequent to the current procedure, in parallel or handling the further progress of the signal as an integrated part of the new procedure.

Finally, we would like to draw the attention towards novel data from two Member States that we have just shared with the PRAC . The paper and data are still not submitted or peer reviewed but the authors conclude that "Among recipients of the Oxford-AstraZeneca COVID-19 vaccine, and within 28 days from receiving the

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first dose, increased rates of cardiovascular and haemostatic events were observed, in particular venous thromboembolism events, including cerebral venous thrombosis, and thrombocytopenia. However, the absolute risk of such events was low." Even if the data are preliminary and including a relatively low number of individuals, this study may also be taken into consideration. The paper supports findings in the AR (e.g. the EMA O/E analysis based on spontaneous reporting) with additional data on observed vs. expected including evaluation of the standardized morbidity ratio in a population-based cohort study. The findings further strengthen the signal, including updates to the SmPC, and considering the seriousness of the reported events, the study supports the need for a discussion of the benefit/risk either in the current signal procedure or in an upcoming new procedure.

Summary of Product Characteristics, Package Leaflet and Labelling

The proposal for updates in section 4.4 and 4.8 is generally endorsed. We agree with the last sentence proposed in section 4.4 that "Benefit-risk should be considered taking into account the availability of the alternatives and epidemiological local data." This wording suggests a more individual assessment of the benefit/risk at national level. In such a setting, it is unusual that this information is only present in section 4.4 of the SmPC and not in accordance with what is usually done when it is up to national authorities to decide if a given drug is 1st or 2nd line treatment. Therefore, it is suggested to include this information in section 4.1 as well with a cross reference for section 4.4.

The proposal for changes in the product information is as described below (new text underlined):

4.1 Therapeutic indications

Vaxzevria is indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2, in individuals 18 years of age and older.

The use of this vaccine should be in accordance with official recommendations. Benefit-risk should be considered taking into account the availability of the alternatives and epidemiological local data (see section 4.4).

PRAC Rapporteur

The endorsement regarding the hypothesis, mechanisms, gasps in knowledge and need for additional studies are noted.

The comment regarding the additional laboratory testing in ongoing clinical studies is endorsed and added to the recommendations, as analysis to consider.

The comment to discuss the procedure is noted and is proposed for discussion at the PRAC meeting.

The additional data shared are appreciated. These confirm the signal and do not change the conclusions of the assessment report. Moreover, it could be explored if analysis by age group and sex has been or could be performed.

The proposal regarding update of 4.1 is not endorsed as a formal assessment of the benefit-risk to change the indication has not been performed.

MS2

We generally endorse the PRAC Rapp´s and CoRapp´s assessment, however, we have some additional

comments.

Regarding the SmPC wording in section 4.4 we don´t support to delete the information that higher occurrence of thrombosis and combination of thrombosis and thrombocytopaenia in women under 55 years may reflect increased use in this population. Exposure of Vaxzevria is more than 2 times higher in population under 60 years than in older persons according to the AR. Deletion of that information could lead to the false feeling that these reactions couldn´t occur in older persons and that is something we really couldn´t be sure about. In addition, we would like to propose to update the age limit to 59 years of age according to the age stratification used in EV analyses.

Regarding to the DHPC, the currently proposed summary is basically the same as it was in the previous DHPC. We propose to update the summary of DHPC with new important information about AHEG meeting (e.g. that AHEG meeting took place on the 29th March, the general consensus was that there is a strong relationship between these events and vaccination with Vaxzevria and that the mechanism is not clear at the moment, however, the most plausible hypothesis is atypical heparin induced thrombocytopaenia) in addition to the shortened information about events which was already stated in the first DHPC.

The information about higher vaccination of population bellow 60 years should also remain in the DHPC text.

We support to update list of safety concerns with thrombosis and thrombocytopaenia as well as update of the protocols of ongoings studies with the laboratory testing.

With respect to the non-interventional studies, it seems questionable if update of EAS could be useful when AEs are self-reported and the size of the study is limited (EU – 15 000 participants, UK – 10 000 participants and US 15 000 participants).

We support the proposal on the EMA funded studies. It is unlikely that the MAH could acquire better information that the academic groups collaborating with EMA.

PRAC Rapporteur

The endorsement regarding the overall assessement, update of the safety concerns and EMA funded studies are noted.

The comment regarding 4.4 regarding the deletion of use of the vaccine is not supported, as this doesn't reflect factual information. However, it is proposed to add 'occurred <u>mostly</u> in women under 55 years of age' to reflect that these reactions also occurred in men and in older age-categories.

The comment to update the age limit to 59 years of age is proposed for discussion at plenary PRAC

.Regarding the DHPC: this includes the conclusions of the AHEG. In line with the SmPC proposal, the wording '<u>mostly</u> in women under 55 years of age' is proposed.

Regarding the EAS, the comment is endorsed and it is proposed for this study "to *describe* cases of thromboses and/or thrombocytopenia"

MS3

Regarding the "EMA analysis contribution", we have the following comment: it is stated that one Member State cases are poorly documented. Narratives of the cases are extensively documented in the reports sent to EVDAS. The narratives of the cases were already re-sent last week so that the table could be updated, but this

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has not happened. Please, find attached the mail with the narratives of the cases included in the report "EMA analysis contribution". We kindly request to the EMA colleagues to remove the sentence that these cases are poorly documented.

EMA Comment

The reason for the missing narratives is being investigated with colleagues. Meanwhile, a corrected version of the EMA report was provided and included, without the comments that the cases were poorly documented.

MS4

We are very grateful for all the work achieved by the Rapporteur's team and EMA colleagues in such a limited amount of time. We generally support the conclusions of the rapporteur but have some additional comments:

• We would like to highlight that we have now identified 5 cases where anti-PF4 antibody were searched for but were negative.We would suggest that a discussion is included on the negative predictive value of a negative test for anti-PF4 antibodies, and whether the existence of such cases might also suggest other possible mechanisms.

• We especially support the addition in section 4.4. of the SmPC of the sentence "No specific risk factors have been identified. Benefit-risk should be considered taking into account the availability of the alternatives and epidemiological local data."

• Considering the current level of evidence, and that it is a slightly different clinical presentation from "thrombosis in unusual sites" we consider that disseminated intravascular coagulation (DIC) should also appear in sections 4.4 and 4.8 of the SmPC (please also refer to our below comments on EMA's OE analysis).

• Considering the very strong concerns and uncertainty regarding the impact of the second dose on these serious adverse effects, and the likely immunological mechanism, we would suggest that the MAH provide a discussion and very closely monitor this issue. It would also be very reassuring to receive confirmation that EMA colleagues will be in a position to readily provide a comparison between the first and second dose in their analysis. Similar work from MHRA colleagues would also be very much appreciated. The involvement of the CHMP as part of a referral procedure would also be very helpful to address this issue as part of a more general benefit-risk review.

Regarding the very helpful observed/expected analysis performed by EMA colleagues, we have the following comments:

• the number of observed cases on 22nd of March seems to have been compared to expected cases using incidence rates on the number of vaccinated people at the same date (22nd March). However, most cases appear within one or two weeks, and not immediately. Therefore, people vaccinated on the 22nd of March might not "have had the time" to develop the studied event. This might lead to the denominator and the expected numbers being overestimated, and OE differences underestimated.

• the analysis includes a number of "expected DICs" and it was highlighted during the preparatory PRAC that this should be acceptable considering that some populations at risk of DICs might also be vaccinated. Nevertheless, it should be highlighted that this assumption does not seem to match the reported cases, as at least some cases were reported in healthy and rather young adults without any obvious risk factor or cause of DIC. We are therefore concerned that we are comparing two different populations: one at risk of DICs due to

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comorbidities, and one without risk factors. We would therefore strongly suggest that the analysis includes a discussion of this in the section related to the OE analysis for DICs, as well as in the limitations section.

PRAC Rapporteur

Regarding the anti-PF4 antibody that were found negative in some cases: it is agreed that other possible mechanisms should be explored. This request is added to the recommendations to the MAH.

Endorsement of additions to 4.4 is noted.

Regarding the impact of a second dose: this is agreed and a question to discuss this has been added to the Recommendations.

Regarding the addition of DIC to 4.4 and 4.8, this is not agreed as the updated EMA Eudravigilance report did not confirm a signal, however it is agreed that in an updated report this issue of possibly comparing two different populations should be further explored and discussed.

EMA comment regarding the O/E analysis: we agree with the comment. The data from ECDC are up to Sunday 21st March. Person time at risk has been calculated on a weekly basis and people vaccinated in the week 15-21 March have been considered to add only 3.5 days on average to the person time, rather than the full seven days.

Please also consider that the more the vaccination campaign progresses, the less is the weight of the vaccinated during the last week (for the current analysis the vaccinated in the last week represented 12.5% of the total vaccinated), and therefore the less the extent of overestimation even without the adjustment showed.

EMA comment regarding the DICs analysis: we agree with what stated. The background incidence rate was calculated on the overall population, that includes also people at risk and healthier people. While the vaccination campaign in the young groups might have prioritised healthier social care workers that are likely to be healthier than the average population.

This is reflected in the limitation section of the OE analysis where it is stated that "The background incidence rates were calculated for the overall population, while the vaccination campaign in some countries might have prioritised specific groups (e.g. frail individuals)". In the examples we will add "or healthier social care workers that are likely to be healthier than the average population".

MS5

We would like to acknowledge the work done by the rapporteurs within this very limited timeframe. In general we endorse the recommendations and have the following comments:

- We support the updated SmPC section 4.4 recommendation but propose to ad "mostly in women under 55 years" in line with PIL and the facts that some cases have been seen in women over this age and in men. We particular agree to the information that no risk factors have been identified

- We agree to the proposed update of SmPC section 4.8 but are open for a refinement of the PTs. We support the important information that thrombosis and thrombocytopenia have been seen in combination

- We agree that thrombosis with- or without thrombocytopenia is included as an important identified risk in the RMP. The overall study proposal is agreed on. It should be mentioned that the MAH must be particular observant to publications (both peer- and non-peer reviewed) on the issue in question

- According the EPAR no formal dose-finding study have been performed. The MAH should comment if

the observed thromboembolic adverse reactions potentially could be related to a dosing issue

Non-clinical:

In a non-clinical study in monkeys one animal (26Z) presented with very low (zero) thrombocytes (and other haematological parameters) following vaccination with ChAdOx nCOV-2 vaccine and before viral challenge. The MAH should comment on this finding in relation to the current issue of rare thrombosis and thrombocytopenia in humans following vaccination with Vaxevria.

Public Health England

Study 6284 Final Report

7.14. Appendix 14: IDEXX haematology analyser data

	Group 1 - ChAdOx1 nCoV-19						Group 2 - Controls					
	17Z	13Z	45Z	29Z	26Z	36Z	22Z	31Z	4Z	18Z	24Z	53Z
Platelets (K/ul)	312	244	212	156	0	319	179	246	151	32	205	237
WBC(K/uL)	7.99	12.42	10.24	4.09	1.56	5.36	5.91	12.11	9.89	5	6.15	11.66
NEUT#(K/uL)	4.53	7.74	7.96	2.49	0.56	2.93	3.84	4.33	5.25	2.5	2.47	8.14
LYMPH#(K/uL)	3.1	4.18	1.64	1.29	0.96	2.21	1.69	6.54	3.62	2.31	3.23	2.9
MONO#(K/uL)	0.33	0.49	0.64	0.31	0.04	0.22	0.33	1.24	0.32	0.19	0.45	0.6
EO#(K/uL)	0.03	0	0	0	0	0	0.04	0	0.08	0	0	0.01
BASO#(K/uL)	0	0.01	0	0	0	0	0.01	0	0.01	0	0	0.01
M:L	0.106452	0.117225	0.390244	0.24031	0.041667	0.099548	0.195266	0.189602	0.157761	0.082251	0.139319	0.206897
N:L	1.46129	1.851675	4.853659	1.930233	0.583333	1.325792	2.272189	0.66208	1.335878	1.082251	0.764706	2.806897

Medicinal product no longer authorised

PRAC Rapporteur

Endorsement of additions to 4.4 is noted and the proposition to add "<u>mostly</u> in women under 55 years" is supported.

Refinement of PT's in 4.8 is acknowledged, but for the time being, it is preferred to capture these under the entitiy of 'thromboses'.

Regarding the dose-relationship and the discussion of findings in pre-clinical studies: this is agreed and added to the recommendations.

MS6

We agree with the inclusion of 'Thrombosis with or without thrombocytopenia' as a safety concern in the risk management plan, but we are of the view that this should be as an important potential risk (please see comment on SmPC below).

We support the Rapporteur's recommendation to update the protocols of ongoing studies to include additional laboratory testing allowing for an in depth assessment of the aetiologies of the thrombotic with thrombocytopenia and/or bleeding events. We recommend that investigations in this regard should also include COVID-19 testing, including PCR and serology. In describing relevant cases, information on the type of testing performed, the timing of such testing, and the site from which the PCR swabs were taken should be provided.

In view of the known association of COVID-19 with thromboembolic events, the potential for the disease to be asymptomatic, and the emergence of new variants, we remain of the view that it is important to understand how rates of these events following vaccination compare with rates observed in the general population in the COVID-19 era. Indeed, we note that the rate of CVST in one MS among 20-29 year olds appeared to increase in 2020, from less than 8 cases per million in the preceding 3 years, to 17 cases per million (confidence

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interval ranging from 4 up to 68 cases per million). In particular there were no cases among males in this age group in 2020, while the rate in females tended to increase, to 35 cases per million (confidence interval 8.7 – 139.5 cases per million). The EMA-funded database study "Natural history of coagulopathy and use of anti-thrombotic agents in COVID-19" might be an opportunity to further explore this.

Summary of Product Characteristics, Package Leaflet and Labelling

We consider that there are important gaps in evidence to support inclusion of labelling as proposed in section 4.8 of the SmPC for a number of reasons:

• In particular, we believe that it is premature to conclude that the criterion of plausibility has been met. While it is acknowledged that the syndrome is immunological, a temporal relationship with the vaccine does not demonstrate plausibility or confirm the causal relationship. Mechanistic studies will be required to demonstrate whether this is indeed an immunological response to the vaccine, and the MAH should be asked to perform these without delay. In the interim, while these studies are being conducted, we consider that a robust and clear warning in SmPC section 4.4 and in the patient leaflet that these events have been observed remains sufficient.

• The rationale for inclusion of thrombosis, in the absence of thrombocytopenia/reduced platelet count, in 4.8 is unclear, in light of the proposed biological mechanism being aHIT like syndrome.

• The rationale for including thrombocytopenia as a 'standalone' adverse reaction at the current time in context of this procedure is unclear. Furthermore, with regard to the proposed frequency, at baseline in the trials, it appears that there were some patients with existing thrombocytopenia, particularly in the group randomized to the vaccine. It appears that the proportion of patients in the control group who underwent assessment of platelet levels during the trial was lower than that in the group randomized to the vaccine. The validity of the numerical difference in the incidence of thrombocytopenia in the trials is therefore uncertain.

With regard to the proposed warning in 4.4:

• We question the inclusion of thrombotic events in the absence of thrombocytopenia. It is not known whether the same diagnosis of a syndrome resembling HIT applies to cases occurring in the absence of thrombocytopenia or a relative reduction in platelet count. We understand that the tests for activating antibodies to platelet factor IV have been conducted in patients who had thrombocytopenia. O/E analyses have not indicated an overall risk of thromboembolic events. Moreover, they have not been stratified according to arterial and venous events. Furthermore, with regard to the cases of CVST that have not been reported with thrombocytopenia, it is unclear whether this is because thrombocytopenia was not a feature of these cases, or was simply not reported with the event, i.e. was missing data.

• Concerning the statement that the 'benefit-risk should be considered taking into account the availability of the alternatives and epidemiological local data', we are of the view that a more fulsome assessment of the benefit of the vaccine, relative to what is known about this risk, would be required to provide the substantive evidence to support inclusion of an informed statement in the product license.

Based on the comments above, further discussion and revision of the proposed wording for the product information is necessary and warranted. This also has important implications for the proposed DHPC.

Communication

We also consider that the public health communication and DHPC should include relevant information on the state of knowledge on the frequency of and risk factors for these events based on the most up-to-date data including limitations and uncertainties, in order to support public health agencies, physicians and patients in clinical decision-making.

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Other Aspects

Given O/E analysis is a key feature of the assessment at this stage, we support the Rapporteurs proposal for further sensitivity analysis and would also like to highlight further areas for exploration. The data from ACCESS indicate substantial variability in rates between Member States concerning CVST. Rates in Italy are 2-3 fold higher than those in Spain, despite both databases capturing inpatient diagnoses. The differences are even higher for younger age groups, with a more than 4-fold higher rate in Italy in those aged 40 – 49 and a more than 6 fold higher rate for those aged 30-39. Selection bias may be a limitation in comparing numbers observed in the EEA with those expected in the database.

However, we also note that rates of CVST in Italy, which were used to calculate the expected rate of CVST for the O/E analyses, are estimated with poor precision and very wide confidence intervals. In the 30-39 year age group, the rate of CVST in Italy is 17 per million, with a wide confidence interval ranging to 27 per million. The rate in the 40-49 year old age group is 19 per million, again confidence interval ranging up to 27 per million. The validity of the O/E analyses when the baseline rate is itself imprecisely estimated seems uncertain. Further sensitivity analyses could be undertaken to explore these issues.

PRAC Rapporteur

Regarding the additional testing, to include COVID-19 testing, including PCR and serology, this is endorsed and added in to the recommendations.

Regarding 4.8, we respectfully disagree and conclude there is sufficient evidence for a causal association, as discussed above:

Using Bradford-Hill criteria (5), there are several arguments to support that a causal relationship between the vaccination with Vaxzevria and the adverse events is at least a reasonable possibility:

- *Plausibility*: an immunological pathophysiology is described (heparin independent antibody mediated platelet activation via platelet Fc gamma RIIA receptors) which appears to be temporarily associated with a unique vaccine technology. The very low numbers explain why this signal could never be detected in a trial;

- *Consistency*: There has a been a consistency of clinical entities (e.g. CVST occurring in conjunction with thrombocytopenia or splanchnic vein thrombosis in a young female population without risk factors) and laboratory findings across cases and across multiple countries;

- *Temporality*: median time to onset was 9 days for Thromboembolic events with thrombocytopenia , CVST and splanchnic vein thrombosis cases after first dose of Vaxzevria (Eudravigilance, DL 23/03/2021);

- *Specificity*: A cluster of clinical and laboratory features which are very rarely seen in clinical practice;

- *Change in risk factor*: Recognition of rare cases in multiple countries associated with the increased number of vaccinations in younger age groups. Not identified so far with other vaccine technologies;

- *Analogy*: an atypical heparin induced thrombocytopenia (aHIT) like disorder was considered by the AHEG the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients.

Regarding the addition of thrombocytopenia and thrombosis, this is considerd to be sufficiently supported by the Observed to Expected analysis and case review provided in the EV EMA report. The frequency of thrombocytopenia is supported by the observations from the clinical studies. It is agreed these EMA

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Eudravigilance analysis should further refined and updated, including sensitivity analysis, updates of background incidence data and future EMA funded research.

Regarding the Communication, this is proposed to be further discussed at the PRAC plenary meeting.

EMA comment regarding the rates used and COVID-19: Regarding the year 2020, it may be only the pre-pandemic period, but this would need to be checked in the protocol. We do not think this would have an important impact on the results anyway.

EMA comment regarding the O/E analysis: we agree on the need for further sensitivity analyses, we will receive background rates from additional databases in the next coming weeks. It is reassuring to highlight how the results from the Italian database are very close to the ones from the study from Coutinho et al (The incidence of cerebral venous thrombosis: a cross-sectional study. Stroke. 2012;43(12):3375-7) and from a large study in Australia performed between 2005 and 2011 (Devasagayam et al.). Regarding "poor precision", it can be stressed that this is not linked to the method but to the medical condition with a very small number of events.

Moreover, we would also like to highlight as OE analysis using spontaneous reports should remain a tool for signal detection and refinement, and how now additional evidence should come from epidemiological analyses using electronic health records and epidemiological studies that focus on estimating associations between occurrence of thromboembolic events and vaccination.

MS7

Overall, we agree with the conclusions of the well written preliminary second round report of this signal evaluation, and again want to express out gratefulness for all the work that has been undertaken within such short time frame.

We endorse the conclusion of the rapporteur that there is sufficient support to infer a causal relationship between the Covid 19 vaccine AstraZeneca and the occurrence of thrombosis in combination with thrombocytopenia.

However, we do not find there is sufficient support to conclude that thrombosis solely is an identified risk with the vaccine. This needs further review and data before final conclusions can be drawn.

Concerning additional comments on the product information and the DHPC, see comments below.

Regarding additional research we support the proposals made, although further work on e.g. feasibility is needed as pointed out in the AR.

Risk Management Plan/ Post-authorisation Safety Studies/ Conditions

We agree that thrombosis in combination with thrombocytopenia should be added to the RMP as an important identified risk, while thrombosis solely should be added as an important potential risk.

Benefit-Risk Assessment

We agree that the benefit/risk balance remains positive for the present indication, provided that the epidemiological situation, estimates of Sars-Cov-2 related risks in the individual subjects, and the availability of alternative vaccines, are considered.

Summary of Product Characteristics, Package Leaflet and Labelling

Section 4.4

We support updating section 4.4, however, we do not support to add thrombosis solely (see above). Further,

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we do not agree to add the statement: No specific risk factors have been identified.

Although it can be agreed that there is insufficient evidence to draw firm conclusion on risk factors, we still consider that the available data point to that subjects of younger age have a higher risk for developing these very infrequently occurring events, compared with those of higher age. In addition, but a little less clear, that women appear to be at higher risk than men. Further, it is questionable to add such type of statement to the PI at all; i.e. that 'nothing' has been identified. We therefore propose this sentence being removed from the PI.

We also support the added sentence regarding benefit/risk in this section.

Section 4.8

An update of section 4.8 is warranted, to describe the combination of thrombosis and thrombocytopenia. We do not support the proposal in the AR i.e. to add thrombocytopenia and thrombosis separately in two different SOCs. Although the precise wording to describe the relevant syndrome can be further discussed, the following is a proposal:

Vascular SOC : Thrombosis in combination with thrombocytopenia*

And add a description under the table in line with :

* Serious and rare cases, some of them fatal, of venous thrombosis (including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis (including portal, splenic, mesenteric and hepatic vein thrombosis), as well as arterial thrombosis) in combination with thrombocytopenia have been reported post-marketing (see section 4.4).

Other Aspects

Concerning the proposed DHCP, see attachment. Our main concern with the draft is the lack of expression of causality for these infrequently occurring events of thrombosis in combination with thrombocytopenia, and the AZ- vaccine.

This fact also needs to be reflected in further communication activities, to adequately describe the current knowledge.

Regarding the communication plan, we consider that also "specialists in internal medicine, haematology and emergency medicine" may be part of the target groups for the DHPC.

PRAC Rapporteur

As discussed during the PRAC plenary, we agree that thrombosis in combination with thrombocytopenia should be added to the RMP as an important identified risk, while thrombosis solely should be added as an important potential risk.

Regarding the SmPC and Other aspects, this is further discussed at the PRAC plenary meeting.

MS8

We mostly endorse the Rapporteurs' comprehensive assessment and fully appreciate the well written assessment report despite challenging time lines, however we have additional comments.

Risk Management Plan/ Post-authorisation Safety Studies/ Conditions

We agree that thrombosis solely should be added to the RMP as an important potential risk and only

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thrombosis in combination with thrombocytopenia should be added as an important identified risk.

We support the Rapporteur's recommendation to update the protocols of ongoing studies to include additional laboratory testing allowing for an in depth assessment of the aetiologies of the thrombotic with thrombocytopenia and/or bleeding events. We recommend that investigations in this regard should also include COVID-19 testing, including PCR and serology. In describing relevant cases, information on the type of testing performed, the timing of such testing, and the site from which the PCR swabs were taken should be provided.

Although it is acknowledged that preceding/asymptomatic COVID-19 cannot be the exclusive cause (otherwise more cases would have been reported with other COVID-19 vaccines), the rapporteurs' dismissal of this alternative aetiology (as risk factor) based on the currently available evidence is not endorsed: SARS-CoV2 test results are only available in a minority of cases. For instance, out 62 CVST cases (present in EV, Table 15; cut-off: 22 Mar 2021) in only 16 cases a PCR negative result is reported. In addition, details regarding SARS-CoV2 serological status at baseline are lacking in 61 out of 62 CVST cases in EV. In only one UK caseresults from SARS-CoV2 serostatus is available (positive).

Moreover, in view of the known association of COVID-19 with thromboembolic events, the potential for the disease to be asymptomatic, and the emergence of new variants, we remain of the view that it is important to understand how incidence rates of these events following vaccination compare with rates observed in the general population during the COVID-19 pandemic. O/E comparisons using historic (pre-pandemic) expected rates should be interpreted cautiously. The EMA-funded database study "Natural history of coagulopathy and use of anti-thrombotic agents in COVID-19" might be an opportunity to further explore this.

Benefit-Risk Assessment

Summary of Product Characteristics, Package Leaflet and Labelling

With regard to the proposed warning in 4.4:

In our opinion, the sentence: Benefit-risk should be considered taking into account the availability of the alternatives and epidemiological local data. should not be included in the warning as:

• Section 4.4 should be limited to warnings and not contain statements on B/R assessments.

• Section 4.1 already includes a statement that the vaccine should be uses according to official recommendations.

• Evidently benefits should always be weight against the risks for individual patients. The overall B/R of Vaxzevria remains positive.

• It is in the remit the NITAGs to make recommendations for the use of specific vaccines for specific demographics, taking into account the local epidemiology and the availability of alternative products.

• We do not support 'passing the problem' to HCPs

• With regard to the proposed changes to warning in 4.4: the proposed inclusion of Thrombosis without thrombocytopenia is not agreed. As also remarked by other MSs, there is too much uncertainty surrounding the reported cases to, at this point in time, signal a potential risk of thrombosis without thrombocytopenia. Notably, O/E analyses have not indicated an overall risk of thromboembolic events. Therefore we should not communicate that there is an association between AZ vaccine and Thrombosis as there is no good evidence to support this at this time.

• The rarity of the events should be clearly stated.

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- Whether or not the overrepresentation of women under 55 is the result of an actual real increased risk, or has other possible causes, remains to be confirmed. The definite statement that "No specific risk factors have been identified" might be confusing / ambiguous. In many reports the case was insufficiently documented, and it is unknown whether known risk factors for the "normal" TE events (pregnancy, pill use, asthma, surgery) also contributed or not.

With regard to section 4.8:

• The proposal to include Thrombocytopenia with frequency Common is not agreed as this requires further justification and/or quantitative clarification regarding rarity and seriousness of the events.

• The rationale for inclusion of thrombosis in general, in the absence of thrombocytopenia/reduced platelet count, in 4.8 is unclear, in light of the proposed biological mechanism being aHIT like syndrome and the overall O/E analysis not being increased. A causality assessment of individual cases would be warranted to determine whether there is a causal association of the broad PT thrombosis, before inclusion in section 4.8 can be recommended.

PRAC Rapporteur

As discussed during the PRAC plenary, we agree that thrombosis in combination with thrombocytopenia should be added to the RMP as an important identified risk, while thrombosis solely should be added as an important potential risk. Regarding the additional COVID-19 investigations for the ongoing studies, this has been added to the recommendations. Regarding preceding/asymptomatic COVID-19 infection, it is agreed that this could be a risk factor or an additional explanation and should not be dismissed.

Regarding the SmPC, this is further discussed at the PRAC plenary meeting.

MAH

Summary of Product Characteristics (SmPC)

Section 4.4 Special warnings and precautions for use

Thrombocytopenia and coagulation disorders

A combination of thrombosis and thrombocytopenia, in some cases accompanied by bleeding, has been observed very rarely following vaccination with COVID-19 Vaccine AstraZeneca. This includes severe cases presenting as venous thrombosis, including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis mesenteric vein thrombosis, as well as arterial thrombosis, concomitant with thrombocytopenia. The majority of these cases occurred within the first seven to fourteen days following vaccination and occurred in women under 55 years of age, however this may reflect the increased use of the vaccine in this population. Some cases had a fatal outcome. No specific risk factors have been identified. The benefits and potential risks should be considered taking into account the availability of alternative vaccines and local epidemiological data.

Healthcare professionals should be alert to the signs and symptoms of thromboembolism and or thrombocytopenia. Those vaccinated should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain following vaccination. Additionally, anyone with neurological symptoms including severe or persistent headaches or blurred vision after vaccination, or who experiences skin bruising (petechia) beyond the site of vaccination after

a few days, should seek prompt medical attention.

AZ accept the PRAC's preliminary proposals as an imposition, with the exception of:

• AZ do not accept changes to first sentence as there was no imbalance in thrombosis in the clinical trials. AZ propose "A combination of thrombosis and thrombocytopenia…"

• AZ wish to make minor edits to the benefit-risk sentence: "The benefits and potential risks should be considered taking into account the availability of alternative vaccines and local epidemiological data."

Section 4.8 Undesirable effects

There were no imbalance in trials for events of thrombocytopenia or thrombosis. Additionally, to date, there is insufficient evidence to confirm causal relationship between the vaccine and these events. On this basis, AZ does not accept the inclusion of these events in Section 4.8 of the SmPC.

Package leaflet

Section 2 What you need to know before you use <product name>

Talk to your doctor, pharmacist or nurse before you are vaccinated:

Blood disorders

Blood clots or a combination of blood clots and low level of platelets, in some cases together with bleeding, has been observed very rarely following vaccination with Vaxzevria/COVID-19 Vaccine AstraZeneca. This included some severe cases with blood clots in different or unusual locations and excessive clotting or bleeding throughout the body. The majority of these cases occurred within the first ~~seven to~~ fourteen days following vaccination and mostly occurred in women under 55 years of age~~, however more women under 55 received the vaccine than other people~~. Some cases had a fatal outcome.

Seek immediate medical attention if you develop shortness of breath, chest pain, leg swelling, or persistent abdominal pain following vaccination.

Also, seek immediate medical attention if you experience after a few days severe or persistent headaches or blurred vision after vaccination, or experience skin bruising or pinpoint round spots beyond the site of vaccination which appears after a few days.

AZ accepts these revisions.

In addition, taking on board feedback received last week, AZ has reformatting this section. Please see accompanying QRD with tracked changes.

Section 4 Possible side effects

AZ does not accept these additions. Please see comment above addressing SmPC section 4.8.

DHPC

Astra Zeneca has reviewed the revised version of a DHPC concerning Thromboembolic events with Thrombocytopenia. It is AstraZeneca's view that the comparatively limited amount of new verified information

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provided does not justify the dissemination of a new DHPC at this time. Rather, the communication could be at risk of being perceived as repetitive and disregarded by the recipients. This in turn could undermine the receptiveness for forthcoming communications, should such be considered needed.

Should PRAC, however, decide to progress the distribution of a new DHPC, AstraZeneca would like to provide comments as per the attached - mostly in line with the PI comments and also proposed as first bullet point of the summary:

- *Vaxzevria/COVID-19 Vaccine AstraZeneca benefits outweigh the risks despite possible link to very rare blood clots with low blood platelets.*

  Text included in previous DHPC. AZ opinion is that this statement is still relevant.

PRAC Rapporteur

Regarding the comments on the SmPC, we respectfully disagree and conclude there is sufficient evidence for a causal association, as discussed above:

Using Bradford-Hill criteria (5), there are several arguments to support that a causal relationship between the vaccination with Vaxzevria and the adverse events is at least a reasonable possibility:

- Plausibility: an immunological pathophysiology is described (heparin independent antibody mediated platelet activation via platelet Fc gamma RIIA receptors) which appears to be temporarily associated with a unique vaccine technology. The very low numbers explain why this signal could never be detected in a trial;

- Consistency: There has a been a consistency of clinical entities (e.g. CVST occurring in conjunction with thrombocytopenia or splanchnic vein thrombosis in a young female population without risk factors) and laboratory findings across cases and across multiple countries;

- Temporality: median time to onset was 9 days for Thromboembolic events with thrombocytopenia , CVST and splanchnic vein thrombosis cases after first dose of Vaxzevria (Eudravigilance, DL 23/03/2021);

- Specificity: A cluster of clinical and laboratory features which are very rarely seen in clinical practice;

- Change in risk factor: Recognition of rare cases in multiple countries associated with the increased number of vaccinations in younger age groups. Not identified so far with other vaccine technologies;

- Analogy: an atypical heparin induced thrombocytopenia (aHIT) like disorder was considered by the AHEG the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients.

Regarding the addition of thrombocytopenia and thrombosis, this is considerd to be sufficiently supported by the Observed to Expected analysis and case review provided in the EV EMA report. The frequency of thrombocytopenia is supported by the observations from the clinical studies.

Regarding the DHPC and Communication, this is proposed to be further discussed at the PRAC plenary meeting.

3.7. Adopted PRAC recommendation

The review of additional data analysis from EudraVigilance with individual case review (conclusions and recommendations are based on the EV search with cut-off date: 22 March 2021) and O/E analysis, input from the AHEG and available literature pointed to signals of Embolic and thromboembolic events, Cerebral venous sinus thrombosis, Splanchnic vein thrombosis and Arterial thrombosis, with or without

thrombocytopenia, mainly occurring in women below 60 years and with a time-to-onset within 2 weeks following vaccination.

Following input from experts, it is considered that an atypical heparin induced thrombocytopenia (aHIT) like disorder is the most plausible hypothesis for the events of thrombosis in combination with thrombocytopenia, given the similarities observed in both the serological profile and clinical presentation of affected patients. It is considered likely that the syndrome, which resembles aHIT, concerns a severe autoantibody against PF4 which exhibits a high binding affinity. It was hypothesised that the antibody itself may change the structure of PF4, similar to what has been shown for aHIT. It was noted that high titres of anti-PF4 antibodies were observed in all patients whose biomaterial was analysed, which contributes to this hypothesis.

PRAC is of the view that a causal relationship between the vaccination with Vaxzevria and the adverse events is at least a reasonable possibility.

So far, the reported cases occurred after administration of the first dose of Vaxzevria. Experience of exposure to the second dose is still limited.

Furthermore, a number of studies will be put in place to identify the exact pathofysiological mechanism for the occurrence of these thrombotic events and define the precise magnitude of the risk.

The PRAC recommends that the MAH for Vaxzevria (AstraZeneca AB) should submit a variation to amend the product information as described below (new text underlined/text to be removed with strikethrough):

Summary of Product Characteristics :

Section 4.4 Special warnings and precautions for use

Thrombocytopenia and coagulation disorders

A combination of thrombosis and thrombocytopenia, in some cases accompanied by bleeding, has been observed very rarely following vaccination with Vaxzevria. This includes severe cases presenting as venous thrombosis, including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis mesenteric vein thrombosis, as well as arterial thrombosis, concomitant with thrombocytopenia. Some cases had a fatal outcome. The majority of these cases occurred within the first seven to fourteen days following vaccination and occurred mostly in women under 55 60 years of age, however this may reflect the increased use of the vaccine in this population. Some cases had a fatal outcome.

Healthcare professionals should be alert to the signs and symptoms of thromboembolism and or thrombocytopenia. Those vaccinated should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain following vaccination. Additionally, anyone with neurological symptoms including severe or persistent headaches or blurred vision after vaccination, or who experiences skin bruising (petechia) beyond the site of vaccination after a few days, should seek prompt medical attention.

Section 4.8 Undesirable effects

Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19
Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine
AstraZeneca) (Other viral vaccines)
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Table 1 - Adverse drug reactions

MedDRA SOC	Frequency	Adverse Reactions
Blood and lymphatic system disorders	Common	Thrombocytopenia
Vascular disorders	Very rare	Thrombosis in combination with thrombocytopenia*

And at the end of the table the following statement should be added:

*Severe and very rare cases of thrombosis in combination with thrombocytopenia have been reported post-marketing. These included venous thrombosis such as cerebral venous sinus thrombosis, splanchnic vein thrombosis, as well as arterial thrombosis (see section 4.4)

Package leaflet

Section 2 What you need to know before you use <product name>

Talk to your doctor, pharmacist or nurse before you are vaccinated:

…

…

Blood disorders

Very rare blood clots, often in unusual locations (e.g. brain, bowel, liver, spleen), in combination with low level of blood platelets A combination of blood clots and low level of platelets, in some cases together with bleeding, has been observed very rarely following vaccination with Vaxzevria. This included some severe cases with blood clots in different or unusual locations and excessive clotting or bleeding throughout the body. The majority of these cases occurred within the first seven to fourteen days following vaccination and occurred mostly in women under 55 60 years of age, however more women under 55 received the vaccine than other people. Some cases had a fatal outcome.

Seek immediate medical attention if you develop shortness of breath, chest pain, leg swelling, or persistent abdominal pain following vaccination.

Also, seek immediate medical attention if you experience after a few days severe or persistent headaches or blurred vision after vaccination, or experience skin bruising or pinpoint round spots beyond the site of vaccination which appears after a few days.

Section 4 Possible side effects

Common

- low level of blood platelets

Very rare

- blood clots often in unusual locations (e.g. brain, bowel, liver, spleen) in combination with low level of blood platelets

ANNEX II

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

- **Risk management plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;

- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete within the stated timeframe, the below measures:

Description	Due date
In order to elucidate the possible mechanisms of platelet activation after vaccination and to identify the possible triggers, the MAH should submit the final report for the biodistribution study for Vaxzevria	30 April 2021
In order to elucidate the possible mechanisms of platelet activation after vaccination and to identify the possible triggers, the MAH should conduct and submit the final report for a non-clinical study to test in-vitro expression of the S protein of Vaxzevria	7 July 2021
In order to ensure that all reported thrombotic events with thrombocytopenia and/or bleeding events are investigated by performing an in-depth exploration of platelet function in the interventional study in	30 November 2023

Description	Due date
immunocompromised subjects, the MAH should submit the clinical study report, in accordance with a revised and agreed study protocol.	

In addition, the MAH should distribute a direct healthcare professional communication (DHPC) according to the text and communication plan agreed with the CHMP (see Annexes below).

The PRAC considered that given the serious and unpredictable nature of the risks, and that effective risk minimisation is key to support a positive benefit-risk balance, the MAH should submit a variation within 14 days of this recommendation, to update **the risk management plan (RMP) as follows:**

1) Add 'Thrombosis in combination with thrombocytopenia' as an important identified risk, and 'Thrombosis' as an important potential risk. Consequential changes in the RMP should be performed, as needed.

2) The Pharmacovigilance Plan should be updated as follows:

   a) The MAH shall include two imposed non-clinical studies to further characterize the possible mechanisms and to identify the possible triggers of platelet activation after vaccination:

      i) The pending biodistribution study for Vaxzevria; due date for the final study report: 30 April 2021

      ii) A study to test in-vitro expression of the S protein of AZ vaccine; due date for the final study report: 07 July 2021;

   b) The MAH should propose a plan for non-clinical studies (Category 3) to further elucidate the hypotheses of the events of concern. This should include the assessment of the interaction of the AstraZeneca vaccine with blood components (i.e. thrombocytes, erythrocytes, leucocytes, coagulation factors, natural IgM antibodies, etc.) both in the presence and absence of pre-existing immunity to ChAdOx1; the role of the Spike antigen and/or vector in these events and specifically whether the Spike protein is antigenic (i.e. taking into account the role of heparin and binding with PF4).

   c) The MAH should investigate the feasibility and propose amendments to the following clinical trials: *COV001, COV002, COV003, COV004, COV005, D8110C00001*, so that all reported thrombotic events with thrombocytopenia and/or bleeding events are investigated by performing an in-depth exploration of platelet function as needed, as well as investigation of the patients immunological background. The following tests should be considered, but not limited to:

      i) Measurement of platelet levels, D dimer and fibrinogen levels;

      ii) Additional laboratory testing: complete blood count (haemoglobin level, white blood cells with complete formula, thrombocyte level), haemolysis parameters (schistocytes, reticulocytes, haptoglobin levels), ADAMTS13 activity, PTT, TCA, fibrinogen, D-dimers, lupus anticoagulant research (including anti-cardiolipin antibodies IgG + IgM and anti-B2GPI antibodies IgG + IgM);

      iii) In case of low level of platelets: titration of anti-PF4 antibodies;

      iv) Additional search for anti-platelets antibodies (with specific target to identify) and deep exploration of platelet function;

v) Investigation of patient "immune background": anti-nuclear factor, ANCA, rheumatoid factor, HLA B27, hypersensitivity markers.

vi) Other analysis to be considered include:

(1) Faktor V Leiden; Faktor II (prothrombin) variant; Inflammatory markers: TNFa, IL-1, IL-4, IL-6, IL-10, IL-13,

(2) Platelet activation markers: sCD40L, soluble glycoproteins, degranulation markers (PF4, vWF, P-selectin, annexin V).

(3) Cell adhesion: VCAM, ICAM, E-Selectin

(4) Immunology: C3, C4, antinuclear IgG, anti-doublestranded DNA IgG, anti-Smith IgG, anti-SSA IgG, anti-SSB IgG, anti-Jo1 IgG, anti-MPO IgG, anti-PR3 IgG, anti-glomerular basement menbrane IgG

(5) Complement activation markers: Complement Complex C5b-9, C5a

(6) Adeno virus serology

(7) Tissue type (genetics)

(8) Serology: Cytomegalovirus (IgG and IgM,) Ebstein-Barr virus (IgG and IgM), HIV, Parvo virus B19

vii) COVID-19 testing, including PCR and serology: in describing relevant cases, information on the type of testing performed, the timing of such testing, and the site from which the PCR swabs were taken should be provided

d) The study *D8111C0000A*, in immunocompromised, should be upgraded to Category 1 (imposed), and protocol amended to take into account all the requirements in point c) above and the due date in Annex II of the marketing authorisation.

e) For completed Clinical Trials, the MAH should evaluate the feasibility of analysing HIT-antibodies from samples collected during the studies, and propose an evaluation plan/study as a Category 3 study.

f) The protocols of studies *D8111R00003 (EU) - D8110R00001 (US) - ESR 21-21121 (UK; DSRUsponsored))- A Phase IV Enhanced Active Surveillance Study of People Vaccinated with AZD1222* and *Post-marketing observational study using existing secondary health data sources D8111R00006 [EU/UK]* should be modified to include the AESI of concern, as described below.

List: Preferred Terms for embolic and thrombotic events

'Embolic and thrombotic events, arterial'	'Embolic and thrombotic events, venous'	'Embolic and Thrombotic events, vessel type unspecified and mixed arterial and venous'
Acute aortic syndrome[3]	Budd-Chiari syndrome	Basal ganglia stroke[2]
Acute myocardial infarction[3]	Cavernous sinus thrombosis	Brain stem infarction[2]
Arterial thrombosis	Cerebral venous sinus thrombosis	Brain stem stroke[2]
Cerebellar artery occlusion	Cerebral venous thrombosis[2]	Cerebellar infarction[2]
Cerebellar artery thrombosis	Deep vein thrombosis[2]	Cerebral infarction[2]
Cerebral artery embolism	Embolism venous[2]	Cerebral ischaemia
Cerebral artery occlusion	Hepatic vein occlusion	Cerebral microembolism
Cerebral artery thrombosis	Hepatic vein thrombosis	Cerebral microinfarction[2]
Coronary artery embolism[3]	Iliac vein occlusion	Cerebral thrombosis
Coronary artery occlusion[3]	Inferior vena caval occlusion	Cerebral vascular occlusion
Coronary artery reocclusion[3]	Jugular vein occlusion	Cerebrovascular accident[2]
Coronary artery thrombosis[3]	Jugular vein thrombosis	Choroidal infarction[2]

Coronary vascular graft occlusion[3]	Mesenteric vein thrombosis	Coronary bypass thrombosis[3]
Embolism arterial	Mesenteric venous occlusion	Disseminated intravascular coagulation[1]
Femoral artery embolism	Ophthalmic vein thrombosis	Embolic cerebellar infarction[2]
Hepatic artery embolism	Pelvic venous thrombosis	Embolic cerebral infarction[2]
Hepatic artery occlusion	Portal vein occlusion	Embolic stroke[2]
Hepatic artery thrombosis	Portal vein thrombosis	Haemorrhagic adrenal infarction
Iliac artery embolism	Pulmonary embolism[2]	Haemorrhagic cerebral infarction
Iliac artery occlusion	Pulmonary infarction	Haemorrhagic infarction[2]
Ischaemic cerebral infarction[2]	Pulmonary microemboli	Haemorrhagic stroke[2]
Ischaemic stroke[2]	Pulmonary thrombosis	Haemorrhagic transformation stroke[2]
Mesenteric arterial occlusion	Pulmonary vein occlusion	Heparin-induced thrombocytopenia
Mesenteric arteriosclerosis	Pulmonary venous thrombosis	Microembolism
Mesenteric artery embolism	Renal vein occlusion	Pituitary infarction[2]
Mesenteric artery thrombosis	Renal vein thrombosis	Post procedural stroke[2]
Myocardial infarction[3]	Retinal vein occlusion	Spinal stroke[2]
Myocardial necrosis[3]	Retinal vein thrombosis	Stroke in evolution[2]
Papillary muscle infarction[3]	Splenic vein occlusion	Strokectomy[2]
Peripheral artery occlusion	Splenic vein thrombosis	Thrombotic stroke[2]
Peripheral artery thrombosis	Superior sagittal sinus thrombosis	
Peripheral embolism	Superior vena cava occlusion	
Post procedural myocardial infarction[3]	Transverse sinus thrombosis	
Pulmonary artery occlusion	Venous thrombosis	
Pulmonary artery thrombosis		
Renal artery occlusion		
Renal artery thrombosis		
Renal embolism		
Retinal artery embolism		
Retinal artery occlusion		
Retinal artery thrombosis		
Silent myocardial infarction[3]		
Spinal artery embolism		
Spinal artery thrombosis		
Splenic artery thrombosis		
Splenic embolism		
Subclavian artery embolism		
Subclavian artery occlusion		
Subclavian artery thrombosis		
Thromboembolectomy		
Thrombotic microangiopathy		
Thrombotic thrombocytopenic purpura		
Transient ischaemic attack		
Vertebral artery occlusion		
Vertebral artery thrombosis		

Codes: 1 – PT already listed for AESI 'Vaccine associated-disease'; 2 – PT already listed for AESI 'Stroke and other cerebrovascular events, Venous thromboembolism'; 3 – PT already listed for AESI 'Myocardial infarction', no code = selection by PRAC RAP expert

Issues to be addressed in the Monthly Summary Safety Reports:

1. The MAH should discuss possible implications regarding the second dose and commit to timely and closely monitor this issue. Additional information regarding UK cases should be sought, as well as any further information on events reported after the second dose. Liaison with the MHRA should be continued as frequently as possible and necessary.

2. According to the EPAR no formal dose-finding study have been performed. The MAH should comment if the observed thromboembolic adverse reactions potentially could be related to a dosing issue.

3. Non-clinical:In a non-clinical study in monkeys, one animal (26Z) presented with very low (zero) thrombocytes (and other haematological parameters) following vaccination with ChAdOx nCOV-2 vaccine and before viral challenge. The MAH should comment on this finding in relation

to the current issue of rare thrombosis and thrombocytopenia in humans following vaccination with Vaxzevria.

4. PTs related to thrombocytopenia should also include "Platelet count decreased, Heparin-induced thrombocytopenia, Platelet count abnormal, Platelet disorder" in addition to the current list of terms (i.e. immune thrombocytopenia, thrombocytopenia, thrombocytopenic purpura).

5. The list of PTs related to embolic and thrombotic events should include: see list above.

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Annexes

Direct Healthcare Professional Communication (DHPC)

VAXZEVRIA/COVID-19 Vaccine AstraZeneca: link between the vaccine and the occurrence of thrombosis in combination with thrombocytopenia

Dear Healthcare Professional,

AstraZeneca AB in agreement with the European Medicines Agency and the <National Competent Authority > would like to inform you of the following:

Summary

- **A causal relationship between the vaccination with Vaxzevria and the occurrence of thrombosis in combination with thrombocytopenia is considered plausible.**

- **Although such adverse reactions are very rare, they exceeded what would be expected in the general population.**

- **No specific risk factors have been identified at this stage.**

- **Healthcare professionals should be alert to the signs and symptoms of thromboembolism and or thrombocytopenia and inform vaccinees accordingly.**

- **The use of this vaccine should be in accordance with official national recommendations.**

Background on the safety concern

Vaxzevria is indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2, in individuals 18 years of age and older.

A combination of thrombosis and thrombocytopenia, in some cases accompanied by bleeding, has been observed very rarely following vaccination with Vaxzevria. This includes severe cases presenting as venous thrombosis, including unusual sites such as cerebral venous sinus thrombosis, splanchnic vein thrombosis, as well as arterial thrombosis, concomitant with thrombocytopenia. The majority of these cases occurred within the first fourteen days following vaccination and occurred mostly in women under 60 years of age. Some cases had a fatal outcome.

So far, the reported cases occurred after administration of the first dose of Vaxzevria. Experience of exposure to the second dose is still limited.

The PRAC has performed a full investigation including a careful review of EudraVigilance case reports of blood clots and thrombocytopenia in individuals who received the vaccine paying special attention to the information on the sex, age, risk factors, COVID-19 diagnosis (if available), time-to-onset,

outcome, and clinical entity. The investigation has also included a related literature review and an observed to expected analysis conducted with EudraVigilance case reports.

Following input from experts, it is considered that an atypical heparin induced thrombocytopenia (aHIT) like disorder is the most plausible hypothesis given the similarities observed in both the serological profile and clinical presentation of affected patients. It is considered likely that the syndrome, which resembles aHIT, concerns a severe autoantibody against PF4 which exhibits a high binding affinity. It was hypothesised that the antibody itself may change the structure of PF4, similar to what has been shown for aHIT. It was noted that high titres of anti-PF4 antibodies were observed in all patients whose biomaterial was analysed, which contributes to this hypothesis.

A number of studies will be put in place to identify the exact pathophysiological mechanism for the occurrence of these thrombotic events and define the precise magnitude of the risk.

While further evidence is being collected, the PRAC has recommended an update to the product information of Vaxzevria to reflect the current knowledge of the safety issue.
One of these updates is in section 4.8 of the SmPC to reflect thrombocytopenia as an adverse reaction, with a frequency of common, based on data from clinical trials and to include thrombosis in combination with thrombocytopenia with frequency of very rare.

Call for reporting

Healthcare professionals should report any suspected adverse reactions associated with the use of Vaxzevria in accordance with the national spontaneous reporting system *<include the details (e.g. name, postal address, fax number, website address) on how to access the national spontaneous reporting system>*.

Company contact point

<Contact point details for access to further information, including relevant website address(es), telephone numbers and a postal address (company contact point in the concerned EU MS should be included, respectively)>

Yours Faithfully

Medical Director of AstraZeneca AB

Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19
Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously EUDRA-19 Vaccine
AstraZeneca) (Other viral vaccines)
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Communication Plan for Direct Healthcare Professional Communication (DHPC)

DHPC COMMUNICATION PLAN	
Medicinal product(s)/active substance(s)	VAXZEVRIA/COVID-19 Vaccine AstraZeneca suspension for injection (ChAdOx1-S [recombinant])
Marketing authorisation holder(s)	AstraZeneca AB
Safety concern and purpose of the communication	Updated information on the risk of thrombosis in combination with thrombocytopenia.
DHPC recipients	General practitioners and vaccination centres. The target group should be further defined at national level, in agreement with the respective national competent authority.
Member States where the DHPC will be distributed	All EU member states where COVID-19 Vaccine AstraZeneca is marketed.

Timetable	Date
DHPC and communication plan (in English) agreed by PRAC	07/04/2021
DHPC and communication plan (in English) agreed by CHMP	08/04/2021
Submission of translated DHPCs to the national competent authorities for review	09/04/2021
Agreement of translations by national competent authorities	12/04/2021
Dissemination of DHPC	13/04/2021