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Thrombotic events after AstraZeneca vaccine: What if it was related to dysfunctional immune response?

Keywords Thrombosis; Vaccines; COVID-19; Physiopathology; Neutrophil activation

Abbreviations

ACE2	angiotensin-converting enzyme type 2
ADRs	adverse drug reactions
COVID-19	coronavirus disease 2019
EMA	European Medicines Agency
HIT	heparin-induced thrombocytopenia
NET	neutrophil extracellular traps
SARS-COV 2	severe acute respiratory syndrome coronavirus 2
WHO	World Health Organization

As a new wave of the SARS-COV-2 epidemic overwhelms Europe, the vaccine from the Anglo-Swedish laboratory AstraZeneca has come under the spotlight, suspected of having serious thrombotic adverse drug reactions (ADRs) [1]. The first reports of coagulation problems were described in Austria, Italy and the Nordic countries. An unusual number of cerebral venous sinus thrombosis associated with platelet deficiency (thrombocytopenia) and bleeding was reported in Germany. This association was found in 7 cases by 15 March 2021 (date of suspension in Germany) with a temporal association consistent with AstraZeneca vaccination. The 7 affected individuals were women aged 20-50 years; 6 had cerebral venous sinus thrombosis, occurring 4-16 days after vaccination; 3 died. The World Health Organization (WHO) and the European Medicines Agency (EMA) were quick to point out the benefits-risks balance remained in favour of the vaccine, and that there was currently no proven cause-and-effect relationship [1].

In France, over 1,923 million people received the AstraZeneca vaccine between 6 February and 25 March 2021 [2]. A total of 7,439 ADRs were reported of which 91 cases of serious thromboembolic events, including 9 cases of cerebral venous sinus thrombosis, 2 cases of splanchnic venous thrombosis and 1 case of combined stroke/pulmonary embolism/splanchnic venous thrombosis in the context of disseminated intravascular coagulation [2]. These 12 cases of thrombosis of the large veins were atypical in their location (mainly cerebral, but also digestive) and may be associated with thrombocytopenia or coagulation disorders [2]. These cases occurred mostly in women, within a median of 9 days after vaccination in patients mainly under 55 years of age (9 patients vs. 3 over 55 years of age), with no particular history identified to date, apart from oral contraception in 4 cases, associated with a protein C/S deficiency in one fifth and obesity in 1 case. Lymphadenopathy is regularly described. Among them, 4 people died [2]. The very atypical character of these thromboses, their common clinical picture and the homogeneous time of occurrence lead the monitoring committee to confirm the very rare occurrence of this thrombotic risk in people vaccinated with AstraZeneca vaccine [2].

Thrombosis can occur at almost any age depending on comorbidities or genetic factors, in particular sepsis, cancer, prolonged bed rest, active smoking, combined oral contraception, etc. Even if these causes were a priori excluded in these ADRs, the occurrence of thrombosis may be related to one or more of unknown factors. These ADRs may be concomitant with vaccination in the context of a mass campaign during a pandemic. Nevertheless, in order to support the hypothesis of causality between AstraZeneca vaccination and the occurrence of thrombotic events, we propose below a pathophysiological hypothesis involving neutrophils, neutrophil extracellular traps (NET), platelets and innate immunity.

The AstraZeneca vaccine is based on an adenovirus expressing the Spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which allows development of a humoral and cellular immune response against

the virus. During a viral infection, and in particular in the case of SARS-CoV-2, one of the responses of the innate immune system (which is not selective, but can be immediately activated) is the production by neutrophils of a net-like mesh called ''NET'' (neutrophil extracellular traps), the purpose of which is to ''catch'' and ''trap'' circulating viruses. While NET can be useful and effective, multiple studies have shown it can be associated with the occurrence of thrombosis if dysregulated, notably in coronavirus disease 2019 (COVID-19). French reports with thrombotic ADRs showed lymphadenopathy, suggesting an immune reaction. This was also observed in at least 3 Norwegian patients, whose blood parameters suggested an intense immune reaction.

Although not described in the reported cases, we propose the hypothesis that adenovirus injected into the deltoid muscle has passed into the bloodstream, resulting in an innate and adaptive immune response, neutrophil activation and NET release. Thrombus formation would define the severity of thrombosis.

Thrombosis induced by adenoviruses injected in humans is reported in the literature [3,4]. After intravenous injection in mice, adenovirus disappears very rapidly from the bloodstream and is undetectable after 30 minutes. This does not correspond to its elimination but to its entrapment [5].

When large quantities of viral particles are involved, intravenous administration can be lethal to the mouse but also to the non-human primate [6]. If the quantity of virus is too large, various mechanisms of capture by the innate immune system can lead to hepatic capture and an ''inflammatory storm'' with cytokine shock, coagulation cascade activation likely to lead to consumption thrombocytopenia, disseminated intravascular coagulation and multi-visceral damage [5]. Around 50 billion virus particles are needed to achieve this toxic effect in the 20 g mouse (2500 billion virus/kg). In comparison, AstraZeneca's vaccine contains 50 billion adenoviruses (0.7 billion viruses/kg for a 70 kg human, a ratio 35,000 times lower than that of the mouse) [7]. Because the both regimens have very significant differences, this reaction is more probably contributing to a specific and rare mechanism, rather than being totally responsible, by ''lighting the fuse''.

In a recent publication, researchers have shown that the Spike protein of SARS-CoV-2 and coronavirus OC-43 are activators of the complement system and can induce an immune cascade leading to thrombosis [8]. Adenovirus capture by heparan sulphate chains on the surface of endothelial cells and the direct action of complement could be involved in the case of the vaccine. The complement reaction could also be associated with an autoimmune humoral reaction, due to antigenic mimicry between adenoviral epitopes and host epitopes, like in type 2 heparin-induced thrombocytopenia (HIT), where antibodies recognise the heparin-PF4 complex as foreign. These antibodies activate platelets and cause specific thrombocytopenia. This could also be linked to antiphospholipid antibody, which is responsible for primary or secondary antiphospholipid syndrome, and has already been demonstrated for SARS-CoV-2 [9]. The delay in the production of these autoantibodies would explain the occurrence of ADRs within 4-14 days after injection, as in type 2 HIT.

Another factor potentially involved could be the expression of the Spike protein by adenovirus-infected endothelial cells following an accidental intravenous injection of the vaccine. Since Spike binds to angiotensin-converting enzyme type 2 (ACE2), the enzyme could be trapped intracellularly, preventing normal concentrations of ACE2 from being present on the cell surface. This absence or reduction of membrane-bound ACE2 could lead to an increased thrombotic risk [10].

The difference in the vaccinated population between Europe and the UK may also provide additional clues, given the apparent higher prevalence in patients under 55. Mainly people aged 50 and over have been vaccinated in the UK with AstraZeneca vaccine, whereas in Europe it is more likely to be people aged 50 and under, before pharmacovigilance signal in March 2021. This observation could point to a role for the intensity of the vaccine response in the occurrence of these rare forms of thrombosis. Finally, the use of combined oral contraception in women under 50 (4 of 12 cases reported) and/or smoking could be aggravating factors.

In mid-March 2021, the University Medical Centre in Greifswald examined blood samples from seven patients. As in HIT, antibodies directed against a complex of heparin and PF4 are induced, which in turn interact with the CD32 receptor on platelets and activate them, triggering the coagulation cascade that leads to thrombosis.

Thus, one hypothesis linking the vaccine to the occurrence of serious thrombosis could be the passage into bloodstream of adenovirus which, in the presence of factors not fully identified, would generate a discordant immune response with platelet activation, potentially associated with a NET effect or a decrease in the level of ACE2 on the surface of endothelial cells, leading to an excessive thrombotic risk. A simple way to overcome this hypothetical accidental intravascular injection would be to check the absence of blood backflow in the syringe before completing vaccination, a step which is not currently recommended in France.

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Disclosure of interest

The authors declare that they have no competing interest.

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Atypical thrombosis associated with VaxZevria® (AstraZeneca) vaccine: Data from the French Network of Regional Pharmacovigilance Centres

Keywords VaxZevria®; Covid-19 vaccine; Pharmacovigilance; Atypical thrombosis; Thrombopenia; Anti-PF4 antibodies

Abbreviations

ADRs	adverse drug reactions
ANSM	French Medicines Agency
COVID-19	coronavirus disease 2019
CRPV	French Regional Pharmacovigilance Network
CVT	cerebral venous thrombosis
DIC	disseminated intravascular coagulation
EMA	European Medicines Agency
SARS-CoV-2	severe acute respiratory coronavirus 2 syndrome
ST	splanchnic thrombosis
TTS	thrombosis with thrombocytopenia syndrome
VIPIT	vaccine-induced prothrombotic immune thrombocytopenia

Starting in late 2019, the initial cases of a previously unknown form of pneumonia, now referred to as coronavirus disease 2019 (COVID-19), led to a global pandemic. In response, most countries have sought to curb the spread of the virus by imposing periods of lockdown as a function of the national infection rates. By the end of 2020, the advent of vaccines against this severe acute respiratory coronavirus 2 syndrome (SARS-CoV-2) prompted new hope in the global fight against the COVID-19 pandemic. In Europe, mRNA vaccines and adenovirus vector vaccines have received conditional marketing authorizations for active immunization against SARS-CoV-2 in individuals aged 16 and over.

On January 29th, 2021, the European Medicines Agency (EMA) authorized VaxZevria®, the AstraZeneca adenovirus vector vaccine directed against SARS-CoV-2 and in France, the campaign officially started on February 6, 2021.

These new vaccine technologies are now considered to be the best option of countering the COVID-19 pandemic. Given the high level of population likely to be exposed to these drugs, vaccine safety is a critical issue. In order to promptly and accurately identify potential new signal, the French Medicines Agency (ANSM) oversees the assessment of vaccine safety and has initiated a specific strengthened surveillance system for adverse drug reactions (ADRs) related to COVID-19 vaccines in France. This system is based on the collaboration between the Regional Pharmacovigilance Network (CRPV) and the expert council of the specific ANSM/CRPV monitoring committee for vaccines [1].

In this letter, we describe and discuss the VaxZevria® associated-atypical thrombosis specific signal identified by this committee.