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Clinical Insights

Thrombosis with Thrombocytopenia Syndrome associated with viral vector COVID-19 vaccines

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On March 11 2021, the European Medical Agency highlighted two relevant issues, after the decision by the Danish Health Authority to temporarily suspend its vaccination campaign with Vaxzevria (the COVID-19 vaccine by AstraZeneca) following the reports of thromboembolic events (TE) associated with the administration of the vaccine: 1) lack of evidence of a cause/effect relationship between the vaccine and TE; 2) the number of reported TE (30 in around 5,000,000 vaccinated people) was no higher than that expected [1]. However, the real essence of the problem emerged about a week later: it was not the number of TE that was of concern, but, rather, their unusual nature: the affected patients were all young (<50 years old) and previously healthy, they displayed widespread thrombi, predominantly in unusual sites, and also had thrombocytopenia [2]. These data suggested that a new syndrome had emerged, which is termed "Thrombosis with Thrombocytopenia Syndrome (TTS)". I think that the two alternative definitions of the syndrome, "vaccine-induced prothrombotic immune thrombocytopenia (VIPIT)" and "vaccine-induced immune thrombotic thrombocytopenia (VITT)", should be dismissed for two reasons: 1) the cause-effect relationship with vaccination has not yet been unequivocally established; 2) the general reference to "vaccine" is not justified, as the syndrome has been observed only in association with 2 particular COVID-19 vaccines.

The association of thrombosis and thrombocytopenia had been previously observed in patients with Heparin-Induced Thrombocytopenia (HIT), an immune prothrombotic disorder caused by antibodies directed against multimolecular complexes of polyanionic heparin and cationic platelet factor 4 (PF4), which activate platelets through their Fc receptor [3]. A combination of thrombocytopenia and thrombosis has also been observed in a similar syndrome affecting patients not treated with heparin, in which the PF4 binding function of heparin is taken over by other polyanions, such as condroitinsulfate, nucleic acids, polyphosphates or bacterial components: this HIT-like syndrome is termed autoimmune HIT [4]. Antibodies against polyanions/PF4 can be assessed by immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA). In case of positivity of these relatively unspecific screening tests, the diagnosis of classical HIT relies on confirmatory tests of activation of normal platelets by patients' sera, which, in the case of classical HIT activate platelets in presence of pharmacological concentrations of heparin (0.2 IU/mL), but not in the absence of heparin or in presence or very high heparin concentrations (100 IU/mL); in contrast, sera of autoimmune HIT may activate platelets also in the absence of heparin [4].

Clinical and laboratory features of TTS

It has recently been demonstrated that TTS is a (sub)type of autoimmune HIT, as it is characterized by positivity of the screening tests for antibodies against polyanions/PF4 [5-8, Scavone M et al. unpublished observations]. It is important to note that only the very unspecific ELISA test gives positive results in TTS, while the CLIA test gives negative results [8]. Sera from TTS patients behave very erratically in the HIT

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confirmatory test of platelet activation, with no typical pathognomonic pattern: therefore, the test is diagnostically useful in TTS only for ruling out classical HIT [5, 8, Scavone M et al. unpublished observations].

About 250 cases of TTS after Vaxzevria vaccination have been recorded as of the first days of April 2021. Although the following figures are approximate, due to difficulties in collecting all cases and reporting all patients' details, they are indicative of the dimensions and characteristics of the syndrome. The F:M ratio is about 2:1, about 50% of patients are younger than 50 years of age, the death rate is about 30%, more than 40% of the thrombotic events are cerebral venous sinus thrombosis (CVST), the time to onset after vaccination is within the 14th day in 70% of cases, with some cases that may develop as late as on day 30.

Table 1 reports some features of the 45 patients with TTS who have been described as of May 6 2021, in published studies plus data from an unpublished study [5-8, Scavone M et al. unpublished observations]. The most common thrombotic event was CVST, the female sex was predominant, the vast majority of patients were young (<50 years), all displayed thrombocytopenia which was severe ($<50 \times 10^9$/L) in most cases and the death rate was 33%. Nine cases of arterial thrombosis and 6 cases of severe bleeding (5 intracranial) were also observed. Overall, only 11 cases of typical venous thromboembolism (deep vein thrombosis [DVT] and pulmonary embolism [PE]) were reported. Of these, 8 were PE with no detectable DVT, 1 was isolated DVT and only 2 were DVT + PE: the much lower frequency of DVT than PE, which is a complication of DVT, is rather surprising. Indeed, although PE may occur in the absence of detectable DVT, this happens in only about 20% of studied patients [9]. Similar findings were reported in patients with COVID-19, which led us to hypothesize that pulmonary occlusions that were interpreted as caused by PE in many COVID-19 patients were actually due to thrombi of the pulmonary vessels [10]. Our hypothesis was later confirmed by post-mortem studies. It is likely that also in TTS many cases of pulmonary vessels occlusions are caused by *in situ* thrombi rather than emboli from peripheral veins. Indeed, one TTS patient with pulmonary occlusion who died was found to have pulmonary thrombi at post-mortem analysis (Table 1). Therefore, TTS seems to be mostly characterized by venous thrombotic events at unusual sites, involving not only cerebral and splanchnic veins, but also pulmonary vessels.

An important question that needs to be addressed is whether or not TTS is associated with Vaxzevria vaccination only or also with other COVD-19 vaccines. As of 12 April 2021 the following cases have been reported to VAERS (Vaccine Adverse Events Reporting System) in USA [11]: 6 cases of CVST and thrombocytopenia after 6860,000 doses of the COVID-19 vaccine by Janssen/Johnson&Johnson's (0.87 cases/1000, 000); zero cases after 97,900,000 doses of the COVID-19 vaccine by Pfizer-BionTech; 3 cases of CVST without thrombocytopenia, and therefore not to be considered cases of TTS, after 84,700,000 doses of the COVID-19 vaccine by Moderna [11]. More recently 6 additional patients with TTS (all with CVST, sometimes associated with thrombosis in other districts) that manifested 6–15 days after vaccination with the COVID-19 Janssen/Johnson&Johnson's vaccine in the USA have been reported together with the initial series of 6 patients [12]. All 12 patients had thrombocytopenia, high D-dimer and low fibrinogen plasma levels, positive ELISA tests anti-plolyanions/heparin and negative confirmatory functional tests for classical HIT [12]. Therefore, it appears that TTS is associated only with vaccination with viral vector vaccines (AstraZeneca and Janssen/Johnson&Johnson's) and not with mRNA vaccines (Pfizer-BionTech and Moderna). What components of the viral vector vaccines could be involved in the pathogenesis of the syndrome is yet unclear.

TTS should be suspected when a patient displays one or more of the following signs and symptoms 4–30 days after vaccination with viral vector COVID-19 vaccines (Table 2): persistent and severe headache, blurred vision, vomiting, seizures, focal neurological deficits, multifocal signs, mental status changes, stupor, coma, severe and persistent abdominal pain, leg pain and/or swelling, dyspnea. The diagnosis of TTS is based on the presence of objectively confirmed thrombotic events, thrombocytopenia (which, however, may be absent is some poatients, especially at the beginning of the clinical manifestation), positivity of the ELISA test for antibodies against PF4/polyanions complexes and, albeit less stringently, negativity of the functional confirmatory tests for

Table 2
Diagnosis and treatment of TTS.

When should TTS be suspected?	When one or more of the following signs and symptoms manifest 4–30 days after the administration of viral vector COVID-19 vaccines: -persistent and severe headache, blurred vision, vomiting, seizures, focal neurological deficits, multifocal signs, mental status changes, stupor, coma - persistent severe abdominal pain - leg pain and/or swelling - dyspnea
Diagnosis of TTS	- Thrombocytopenia (may not be present in some patients) - Thrombotic events (especially in the venous circulation and in unusual sites) - Positivity of the ELISA test for detection of polyanions/PF4 antibodies
Treatment of TTS	Intravenous immunoglobulins (IVIg, 2 gr/Kg body weight over 2–5 days) - Anticoagulation with DTI, DOAC or fondaparinux (avoid heparin and VKA) - Corticosteroids (?) - Plasma exchange for patients who are unresponsive to IVIg - Avoid platelet transfusions

Refer to text for further details.
Abbreviations - TTS: Thrombosis with Thrombocytopenia Syndrome; DTI: Direct Thrombin Inhibitors; DOAC: Direct Oral AntiCoagulants; VKA: Vitamin K Antagonists.

Table 1
Clinical events and characteristics of 45 patients with TTS post-Vaxzevria vaccination, who have been described in published studies and unpublished observations by Scavone M et al. as of May 6, 2021.

Clinical event	Sex		Age		Platelet count				Deaths
	F	M	<50 y	>50 y	$<50 \times 10^9$/L	50–99 $\times 10^9$/L	100–149 $\times 10^9$/L	Unknown	
CVST	23	4	24	3	19	7	0	1	13
Splanchnic vein thrombosis	6	2	6	2	7	1	0	0	1
Pulmonary embolism	4	4	7	1	6	1	0	1	2
Deep vein thrombosis (DVT)	0	1	0	1	0	1	0	0	0
Pulmonary embolism + DVT	2	0	1	1	1	0	0	0	0
Pulmonary artery thrombosis*	1	0	0	1	1	0	0	0	1
Thrombosis in other sites	5	0	3	2	5	0	0	0	0
Arterial thrombosis	7	2	5	4	3	3	3	0	1
Intracranial bleeding	5	0	3	2	3	1	0	1	1
Bleedings in other sites	1	0	0	1	1	0	0	0	0

Data are retrieved from 5 studies reporting on 45 patients with TTS after Vaxzevria vaccination [5- 8 and unpublished observations by Scavone M et al.]. Several patients had more than one clinical event: for this reason, the total numbers of cases are higher than 45. CVST=cerebral vein sinus thrombosis.

classical HIT (Table 2).

The mainstay of TTS treatment is the intravenous infusion of immunoglobulins (IVIg) at high doses (2 gr/Kg body weight over 2 to 5 days) (Table 2). IVIg not only increase the platelet count of TTS patients [6,8, Scavone M et al. unpublished observations], they also normalize the diagnostic tests for the syndrome and markers of platelet activation, suggesting that they contribute importantly in blunting the prothrombotic state of the syndrome. Indeed, in our TTS patients [Scavone M et al. unpublished observations] we found that IVIg infusion normalized the percentage of circulating platelet/monocyte hetero-aggregates, markers of platelet activation that were increased in the circulation of patients at the time of diagnosis. In addition, compared to patients' plasma at the time of diagnosis, post-IVIg patients' plasma failed to increase platelet thrombus formation on collagen-coated surfaces at 950/s shear rate by normal blood, did not induce the *in vitro* formation of platelet/monocyte hetero-aggregates and the binding of annexin V to procoagulant phosphatidylserine exposed to the membrane of activated platelets. In these *in vitro* experiments, we also showed that both aspirin and cangrelor, an antagonist of the platelet ADP receptor $P2Y_{12}$, inhibit platelet activation and potentiation of platelet thrombus formation by patients' plasma.

Anticoagulant treatment should be started as soon as possible in TTS patients, in combination with IVIg. Heparin anticoagulants should be avoided, in analogy with the recommendation for treatment of patients with classical HIT, although heparin is not involved in the pathogenesis of TTS. Vitamin K antagonists should also be avoided. Alternative anticoagulants that should be used include direct thrombin inhibitors (DTI, argatroban and bivalirudin), Direct Oral AntiCoagulants (DOAC) that do not need heparin lead-in (apixaban and rivaroxaban) and fondaparinux. Other treatments may include corticosteroids and plasma exchange, which may be implemented for patients who proved unresponsive to IVIg. Platelet transfusions should be avoided (Table 2).

Implementation of viral vector vaccines in the COVID-19 vaccination campaign

There is limited data on the risk of TTS after the second dose of Vaxzevria to allow any firm conclusion on its implementation in the vaccination strategy. To the best of my knowledge, three cases of thrombosis+thrombocytopenia have been reported as of the end of April 2021 following the second dose, but these have not yet been validated.

What is the risk-to-benefit balance of viral vector COVID-19 vaccines? Considering the extremely low number of TTS cases reported after the Janssen/Johnson&Johnson's vaccine, the question at this stage should actually be posed for vaccination with Vaxzevria only. EMA analyzed the risk/benefit balance, according to different age ranges of the population and three different scenarios of COVID-19 infection rates: high (886/100,000 population), medium (401/100,000) and low (55/100,000) [13]. The number of TTS cases for each age group was balanced against the number of COVID-19 deaths hypothetically saved by vaccination. A clear advantage of vaccination was evident for persons of >40 years of age in the high-risk and medium-risk scenarios, while the advantage in the low-risk scenario was manifest for persons of >60 years of age [13]. However, it is perhaps inappropriate and misleading to compare all cases TTS (which has a death rate of about 30%) with the number of COVID-19 deaths prevented. It is more appropriate to balance all cases of TTS with the number of prevented ICU admissions due to COVID-19: this type of analysis has been done by EMA and by the Winton Centre for Risk and Evidence Communication of the Cambridge University (UK) [14]. The analysis by EMA showed an advantage of vaccination for subjects >20 years of age in the high-risk scenario, >30 years in the medium-risk and >50 years in the low-risk scenarios. The analysis by the Cambridge University, which considered a slightly different prevalence of COVID-19 infection to define the 3 risk scenarios (high, 200/100,000; medium, 60/100,000; low, 20/100,000) showed an advantage of vaccination for subjects >20 years in high-risk scenario and >30 years in both the medium- and the low-risk scenarios.

In conclusion, TTS is a very rare and severe syndrome, with a death rate of about 30%, that is associated with the first administration of viral vector COVID-19 vaccines. TTS manifests between the 4th and the 30th day after vaccination and is characterized by thrombosis in unusual sites, thrombocytopenia and positivity of the ELISA test for antibodies against polyanions/PF4 complexes. No cases have been described after vaccination with mRNA COVID-19 vaccines. Advantages of vaccination with viral vector COVID-19 vaccine certainly outweigh the risks in different age ranges, as a function of the degree of COVID-19 infection exposure. Physicians should suspect TTS when signs and symptoms of CVST, splanchnic vein thrombosis and, less frequently, of thrombotic events in other vascular districts manifest. The mainstay of treatment is the infusion of high-dose IVIg. Heparin containing anticoagulants should be avoided and substituted by DTI, DOAC or fondaparinux.

Declaration of Competing Interest

The author has no conflicts of interest to declare

M.C. is member of the advisory boards of AstraZeneca, Eli-Lilly, Daiichy Sankyo and Novartis Farma.

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