Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Internal Medicine 105 (2022) 1–7

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Review Article

Potential mechanisms of vaccine-induced thrombosis

Marco Marietta [a,*], Valeria Coluccio [a], Mario Luppi [a,b]

[a] Hematology Unit, Azienda Ospedaliero- Universitaria, Modena, Italy
[b] Department of Medical and Surgical Sciences, Section of Hematology, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Keywords:
COVID-19 vaccines
BNT162b
ChAdOx1 nCoV-19
Ad26.COV2.S
Vaccine- induced immune thrombocytopenia and thrombosis
Autoimmune heparin-induced thrombocytopenia

ABSTRACT

Vaccine-induced immune thrombocytopenia and thrombosis (VITT) is a rare syndrome characterized by high-titer anti-platelet factor 4 (PF4) antibodies, thrombocytopenia and arterial and venous thrombosis in unusual sites, as cerebral venous sinuses and splanchnic veins.

VITT has been described to occur almost exclusively after administration of ChAdOx1 nCoV-19 and Ad26.COV2.S adenovirus vector- based COVID-19 vaccines.

Clinical and laboratory features of VITT resemble those of heparin-induced thrombocytopenia (HIT). It has been hypothesized that negatively charged polyadenylated hexone proteins of the AdV vectors could act as heparin to induce the conformational changes of PF4 molecule that lead to the formation of anti-PF4/polyanion antibodies. The anti-PF4 immune response in VITT is fostered by the presence of a proinflammatory milieu, elicited by some impurities found in ChAdOx1 nCoV-19 vaccine, as well as by soluble spike protein resulting from alternative splice events.

Anti-PF4 antibodies bind PF4, forming immune complexes which activate platelets, monocytes and granulocytes, resulting in the VITT's immunothrombosis.

The reason why only a tiny minority of patents receiving AdV-based COVID-19 vaccines develop VITT is still unknown. It has been hypothesized that individual intrinsic factors, either acquired (i.e., pre-priming of B cells to produce anti-PF4 antibodies by previous contacts with bacteria or viruses) or inherited (i.e., differences in platelet T-cell ubiquitin ligand-2 [TULA-2] expression) can predispose a few subjects to develop VITT.

A better knowledge of the mechanistic basis of VITT is essential to improve the safety and the effectiveness of future vaccines and gene therapies using adenovirus vectors.

1. Background

COVID-19 has so deeply impacted in every aspect of the human existence that most of us in the next years will reconsider its life as "before" and "after" the pandemic. At the time of writing, more than 6 million COVID-19-related deaths have been reported by the World Health Organization (WHO) [1], although the data are likely to be largely underestimated.

Besides its devasting effects on world's health and economy, as well as on the physical and mental wellbeing of billions of people around the world, starting from the healthcare workers, the COVID-19 pandemic left us something good.

Indeed, the development and approval of safe and effective vaccines less than a year after the emergence of a new virus is a stunning scientific achievement, utterly unconceivable just three years ago.

The first COVID-19 vaccine was approved in Europe in December 2020; currently, in the European Community (EC) five vaccines are authorized for use, one has submitted marketing authorization application and three are currently under rolling review (Table 1). Moreover, 157 vaccines against SARS-CoV-2 are in clinical and 198 in pre-clinical development in Europe [2].

In USA, the FDA has approved or authorized for emergency use Comirnaty by Pfizer-BioNTech, Spikevax by Moderna and Janssen's COVID-19 vaccines [3]. Moreover, as of 12 January 2022, three further vaccines have been validated by the WHO Emergency Use Listing process and are currently authorized for the use in countries outside the EC and USA: Sinopharm, Sinovac-CoronaVac and Bharat Biotech BBV152 COVAXIN vaccines [4].

At the end of July 2022, more than 300 COVID-19 vaccine candidates have been developed or are still under development [5].

Many different technology platforms have been used, including messenger RNA (mRNA), viral-vectored, inactivated whole virus,

* Corresponding author.
E-mail address: marco.marietta@unimore.it (M. Marietta).

https://doi.org/10.1016/j.ejim.2022.08.002
Received 27 July 2022; Accepted 4 August 2022
Available online 8 August 2022

Table 1
COVID-19 vaccines authorized or under evaluation in Europe.

Vaccine name	Manufactory	Date of authorizationor application	Mechanism of action
Authorized for use			
Comirnaty BNT162b2	BioNTech and Pfizer	21/12/2020	Single-stranded, 5′-capped messenger RNA produced using a cell-free *in vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2
Spikevax	Moderna	06/01/2021	CX-024414 (single-stranded, 5′-capped messenger RNA (mRNA) produced using a cell-free *in vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2)
Vaxzevria ChAdOx1 nCoV-19	AstraZeneca	29/01/2021	ChAdOx1-SARS-COV-2 (AdV vaccine)
Jcovden Ad26.COV2.S	Janssen	11/03/2021	Adenovirus type 26 encoding the SARS-CoV-2 spike glycoprotein (Ad26.COV2-S) (AdV vaccine)
Nuvaxovid	Novavax	20/12/2021	SARS-CoV-2 recombinant spike protein
Marketing authorization application submitted			
Vidprevtyn	Sanofi Pasteur	30/03/2022	Protein-based vaccine that contains a laboratory-grown version of the spike protein of SARS-CoV-2.
Under rolling review			
Sputnik V, Gam-COVID-Vac	Gamaleya Institute	04/03/2021	Adenovirus type Ad26 and Ad5 encoding the SARS-CoV-2 spike protein; Ad26 is used in the first dose and Ad5 is used in the second to boost the vaccine's effect (AdV vaccine)
COVID-19 Vaccine (Vero Cell) Inactivated	Sinovac	04/05/2021	Inactivated SARS-CoV-2 virus
COVID-19 Vaccine HIPRA (PHH-1 V)	HIPRA Human Health S.L.U.	29/03/2022	Protein-based vaccine that contains two laboratory-grown versions of part of the spike protein of alpha and beta variant

Modif. by Ref. [2].

protein subunit, and plasmid DNA approaches [6]. Moreover, very recently, two further vaccines have been developed that use totally different approaches: a plant-based coronavirus-like particle vaccine, and a receptor-binding domain (RBD)–dimer–based vaccine [7,8].

Worldwide efforts to control the coronavirus SARS-CoV-2 have led to the most rapid and extensive vaccination program ever carried out: at this time, about 12 billion vaccine doses have been administered all over the world [1].

The vaccination of so many people in such a short space of time, so soon after the unparalleled rapid development of the vaccines, has saved a huge number of lives and can be considered as a historic achievement for science and research.

Besides all its benefits, the COVID-19 vaccine, as any other vaccine, entails a very small risk of side effects, including autoimmune responses. Some of these adverse events, although extremely rare, can reach troublesome proportions because of the enormous numbers of individuals exposed to them in a short time frame.

This review is aimed at addressing some relevant issues about the adverse effects of COVID-19 vaccination on the hemostatic system, with a special focus on the thrombotic ones.

2. Vaccines and thrombosis: actual issue or glut of media attention?

The overall incidence of thromboembolic serious adverse events after COVID-19 vaccination is reassuringly low, with an estimated rate of about 7 cases out of one million doses (OMD) reported in the USA [9]. However, such a picture contrasts with a cluster of cases of major thromboembolic events with concurrent thrombocytopenia reported in the UK following vaccination with AstraZeneca COVID-19 vaccine, with an estimated incidence, as of May 2021, of about 13.6 events/OMD after the first dose and 1.8 events/OMD after the subsequent ones [10]. Despite its still low incidence, such a peculiar adverse event displayed a worryingly high fatality rate of 18% with 79 deaths, six of which occurred after the second dose.

The seemingly unexplainable paradox of the co-existence of thrombosis and thrombocytopenia and their devasting impact on the life of affected people [11] resulted very attractive for the media, which soon brought to the spotlight these adverse events also on the wake of a diffuse no-vax feeling.

Moreover, the continuously changing advices about vaccine's safety released by regulatory Agencies further affected vaccine confidence, so many patients who used to trust doctors' recommendation felt reluctant to follow those regarding the vaccines safety, despite the fact that the expected side effects were orders of magnitude much rarer than those of normally prescribed drugs.

On the other hand, the occurrence of thrombosis and thrombocytopenia following vaccination against COVID-19 aroused a massive and unprecedented scientific interested about the relationship of vaccines and thromboembolic events.

Indeed, a PubMed search using the combinations of "vaccines" and "thrombosis "yielded 320 results from 1912 to 2020, as compared to 842 from 2021 to June 2022.

Although a substantial portion of these papers consisted of case reports, narrative reviews or Scientific Societies statements based on expert opinions, a lot of them reported on new experimental data obtained by several research groups all over the world, which allowed a very fast accrual of information about the pathophysiologic mechanisms underlying this new clinical entity.

3. Vaccines and thrombosis or vaccine and thrombosis?

The question is not trivial, as it addresses the core of the problem: does any kind of vaccine increase the risk of venous or arterial thrombosis, or is this adverse event an exclusive prerogative of the COVID-10 ones? And in this instance, do all COVID-19 vaccines carry the same risk of developing thrombosis and thrombocytopenia?

As listed above, prior to the development of anti-SARS-CoV-2 vaccines no increased thromboembolic risk was reported with any vaccination, including that against influenza virus.

Indeed, a population study carried out in Denmark using a self-

controlled case series method found no evidence of increased risk of venous thromboembolism (VTE) in the 10 days following influenza vaccination in adults ≥ 50 years old in 2007–2012, supporting the safety of this annual vaccine campaign [12]. Moreover, another recent paper reported an increased cumulative incidence of thrombotic events at 30 days in subjects vaccinated with COVID-19 vaccines in comparison to patients vaccinated with influenza vaccine (respectively, 12 per 10,000 for COVID-19 group vs. 6 per 10,000 for Influenza group, $P = 0.022$) suggesting that the COVID-19 vaccines could have a weak pro-thrombotic effect [13].

On the other hand, the first reports about the safety of BNT162b2 and ChAdOx1-nCoV-19 AdV vaccines were reassuring, as they demonstrated that systemic and local vaccine-related side effects occurred at lower rates than that reported in phase 3 trials [14].

However, a few months after the starting of the vaccination program in UK and Europe a cluster of uncommon cases of venous thrombosis in unusual sites (i.e., cerebral sinus and splanchnic vein thrombosis) associated with thrombocytopenia and occurring 7–14 days after the vaccination against SARS-CoV-2 with the ChAdOx1-nCoV-19 vaccine raised the attention of the scientific community [15–17].

This led several European countries to decide to suspend its administration or to limit it to subjects over 60 years of age [18]. Meanwhile, the European Medicine Agency's (EMA) Safety Committee (PRAC), after an in-depth review of 62 cases of cerebral venous sinus thrombosis (CVST) and 24 cases of splanchnic vein thrombosis (SVT) reported in the EU drug safety database, concluded on April 7, 2021, that "*unusual blood clots with low blood platelets should be listed as very rare side effects of Vaxzevria*", although "*the overall benefits of the vaccine in preventing COVID-19 outweigh the risks of side effects*." [19].

All these cases shared some uncommon clinical features, including thrombosis at unusual sites, mainly cerebral venous sinuses (CVCT) and splanchnic veens (SVT), but also arterial thrombosis, thrombocytopenia and disproportionately elevated D-dimer levels.

Various names were given to this syndrome, including thrombosis with thrombocytopenia syndrome (TTS) [20], Vaxzevria-associated thrombocytopenia thrombotic syndrome (VATTS) [21], vaccine-induced prothrombotic immune thrombocytopenia (VIPIT) [22] or vaccine- induced immune thrombocytopenia and thrombosis (VITT) [15,23,24]. The latter term has gained widespread use, as it acknowledges the pathogenic similarities of this syndrome with heparin-induced thrombocytopenia (HIT) [25].

Indeed, the earliest papers describing VITT found in affected individuals high titer IgG antibodies directed against platelet factor 4 (PF4), even in the absence of heparin exposition, a picture resembling the already described autoimmune HIT [26].

The presence of these anti-PF4 antibodies in sera of VITT patients can be confirmed by anti-PF4/polyanion enzyme-immunoassays (EIAs) and by PF4-enhanced platelet activation assays using washed platelets [15].

Of note, VITT has been reported almost exclusively after the Astra-Zeneca–Oxford and Johnson & Johnson adenoviral vaccines [27], mostly after the first vaccination, with only two case reports occurring after the Pfizer-BioNTech mRNA vaccine [28,29] and one after the Sinovac one [30]. Moreover, a case exhibiting clinical and laboratory features in line with the VITT diagnosis has been described 10 days after Gardasil 9 vaccination for human papillomavirus (HPV) [31].

However, as fairly recognized by the Authors, it is unclear whether these cases represent "true" VITT or spontaneous HIT, with the recent vaccine as the triggering event. For its part, such a thin distinction has no relevance from a clinical point of view, as the recommended treatment of VITT has been largely borrowed by that of autoimmune HIT [20–24].

A large, well conducted national prospective cohort confirmed that the exposure to first-dose of ChAdOx1 was associated with small increased risks of venous and arterial thromboembolic events in the age groups 16–39 and 40–59 years, whereas no positive associations were seen between BNT162b2 administration and these adverse events in any age group [32].

Of note, both vaccines were associated with a lower overall incidence of VTE as compared to unvaccinated in peopled aged 65 years or more. It is reasonable to assume that in this age group any vaccine provides effective protection from the high thrombotic risk associated with severe forms of COVID-19 disease.

Further record-linkage studies confirmed this finding, pointing out that the increased risk of thrombotic events was mainly due to a higher-than-expected incidence of CVST [33–35] (Table 2).

To sum up, VITT is a very rare event, with an estimated incidence of about 15.8 cases per million after first or unknown dose of ChAdOx1-SARS-COV-2 vaccine and 1.8 cases per million after a second dose [10]. The reported incidence is higher in the younger adult age groups after the first dose as compared to the older groups (21.5 per million doses in subjects aged 18–49 years vs 11.3 per million doses in those aged 50 years and over) [10]. As far as the Ad26.COV2-S vaccine is concerned, the Vaccine Adverse Event Reporting System (VAERS) of the Centers for Disease Control and Prevention reported 45 cases of thrombosis with thrombocytopenia syndrome after 18 million doses, with an estimated incidence of about 2.5 cases per million doses [36,37].

Clinical features of the VITT, very similar to those observed in HIT, and its association with AdV vaccines set the stage for the ensuing studies aimed at unraveling the pathophysiological mechanisms underlying this syndrome.

4. Potential mechanisms of vaccine-induced thrombosis and thrombocytopenia

As reported above, very early after the first cases on VITT, three different groups reported the presence of high-titer antibodies to PF4 in affected patients, although they had never been exposed to heparin. The same groups did not find anti-PF4 antibodies in people vaccinated against SARS-CoV2 not displaying the clinical features of VITT [15–17]. Moreover, aggregation of healthy donor platelets by patient sera was demonstrated in the presence of buffer or ChAdOx1 vaccine and was also suppressed by heparin [38], suggesting that the adenoviral (AdV) vectors could be guilty of this atypical immunological response.

However, which could be the pathophysiological link between AdV vectors and the development of anti-PF4-antibodies?

The most credited mechanisms involve the electronegative surface charge of the AdV hexon.

Indeed, platelet factor 4 (PF4 and CXCL4) is a strongly positively charged tetramer released by activated platelets with a high binding affinity for heparin and other glycosaminoglycans (GAGs). Negatively charged heparin binds to PF4 and promotes PF4 aggregation, forming ultra large and antigenic PF4–heparin complexes, which become "neo-antigens" and induce the formation of antibodies against them in a small, but non-negligible percentage of patients exposed to heparin. In autoimmune HIT, the same conformational change of PF4 monomers can be induced by some pre-existing high-avidity anti-PF4 antibodies [26].

Circulating PF4–heparin antibody complexes can bind to the Fc receptors on platelets, monocytes and neutrophils. The activation of platelets and neutrophils by HIT antibodies can activate the vascular endothelium, which switches toward a pro-thrombotic phenotype, leading a minority of patients to develop devasting arterial and venous thrombosis.

In VITT, it has been hypothesized that following microvascular damage during vaccine administration, trace amounts of 50 billion virus particles in each dose come into contact with blood, bringing AdV DNA and polyadenylated hexone proteins the AdV vectors in contact with PF4. Either component of AdV could replace heparin as a scaffold of negative charges leading to the conformational changes of PF4 molecule already described in HIT and to the formation of anti-PF4/polyanion antibodies [39–42]. These VITT antibodies bind to PF4 epitopes which overlap with the binding site of heparin but differ from those recognized by anti-PF4 antibodies seen in HIT [43].

Table 2
Risk of venous thromboembolic events in individuals vaccinated with ChAdOx1 and BNT162b2.

Cerebral venous sinus thrombosis							
		ChAdOx1			**BNT162b2**		
Age (years)	**Days from vaccination**	**Risk 100.000 person/years**	**Adjusted RI***	**P**	**Risk 100.000 person/years**	**Adjusted RI (95% CI)**	**p**
	4–13	30.78	**16.3 (9.9–27)**	**<0.0001**	2.68	NA	NA
15–39	**14–27**	11.25	**6.1 (3.0–2.5)**	**<0.0001**	3.96	1.9 (0.5–8.0)	0.36
	+28	12.34	6.6 (3.5–2.5)	**<0.0001**	2.98	1.6 (0.6–4.5)	0.38
	4–13	6.77	**2.7 (1.6–4.6)**	**0.0032**	2.34	0.8 (0.2–3.4)	0.79
40–64	**14–27**	**7.05**	**2.8 (1.7–4.7)**	**0.0001**	2.46	0.9 (0.3–2.9)	0.86
	+28	3.78	1.4 (0.7.2.7)	0.32	1.96	0.7 (0.3–1.7)	0.44
	4–13	1.37	0.4 (0.1–1.7)	0.22	3.89	1.3 (0.4–3.4)	0.57
≥65	14–27	6.42	1.9 (0.9–4.0)	0.10	4.04	1.3 (0.5–3.0)	0.59
	+28	1.89	0.5 (0.2–1.3)	0.15	2.04	0.6 (0.3–1.4)	0.25
***Compared to baseline risk in unvaccinated people (100.000 person/years): 1.89 for 15–39 years; 2.42 for 40–64 years; 2.46 for ≥65 years.**							
Other venous thromboses							
		ChAdOx1			**BNT162b2**		
Age (years)	**Days from vaccination**	**Risk 100.000 person/years**	**Adjusted RI***	**P**	**Risk 100.000 person/years**	**Adjusted RI**	**p**
	4–13	80.03	**2.2 (1.7–3.0)**	**<0.0001**	50.96	1.2 (0.7–1.8)	0.55
15–39	**14–27**	77.48	**2.3 (1.8–3.0)**	**<0.0001**	45.50	1.0 (0.7–1.6)	0.90
	+28	52.21	**1.9 (1.1–1.9)**	**0.016**	40.30	1.0 (0.8–1.4)	0.82
	4–13	140.71	**1.3 (1.1–1.4)**	**<0.0001**	142.56	1.0 (0.9–1.2)	0.87
40–64	**14–27**	125.48	**1.3 (1.1–1.4)**	**<0.0001**	153.04	1.1 (1.0–1.3)	0.11
	+28	134.49	**1.2 (1.1–1.4)**	**0.0018**	111.15	1.0 (0.9–1.1)	0.78
	4–13	346.37	**0.9 (0.8–1.0)**	**0.012**	303.22	**0.7 (0.7–0.8)**	**<0.0001**
≥65	**14–27**	325.45	**0.8 (0.7–0.9)**	**<0.0001**	300.18	**0.7 (0.6–0.8)**	**<0.0001**
	+28	280.66	**0.8 (0.7–0.8)**	**<0.0001**	300.11	**0.7 (0.6–0.7)**	**<0.0001**

*Compared to baseline risk in unvaccinated people (100.000 person/years): 27.71 for 15–39 years; 109.13 for 40–64 years; 314.46 for ≥65 years.
Adapted from Ref. [33].

Following the binding of anti-PF4 antibodies, PF4 tetramers cluster and form immune complexes, which in turn cause Fcγ receptor IIa-dependent platelet activation, monocytic activation, release of procoagulant platelet microparticles (MPs) and production of neutrophil extracellular traps (NETs). The resulting immunothrombosis drives the clinical features of VITT [41].

Of note, the Ad26 and Ad5 AdV vectors used in the Ad26.COV2.S and Sputnik V COVID-19 vaccines have lower negative surface charges compared to ChAdOx1. This finding is consistent with the lower incidence of VITT observed in recipients of these vaccines [44].

The induction of the anti-PF4 immune response requires a proinflammatory milieu, which can be elicited by several vaccine components, such as human cell line proteins, free virus proteins, EDTA and AdV genetic material. Relevant to this, a higher proportion of host-cell proteins, active proteases and unassembled hexon proteins has been found in the ChAdOx1 nCoV-19 vaccine as compared to the Ad26.COV2.S [45]. This finding suggests that a different intensity of the inflammatory response elicited by different AdV vaccines can play a role in the production of the functionally active PF4 antibodies involved in the development of VITT.

Another postulated mechanism for the development of VITT takes into account the availability of soluble spike protein variants resulting from alternative splice events following administration of the ChAdOx1 vaccine [46]. Soluble spike protein variants can bind to ACE2-expressing endothelial cells, thus triggering the immune-mediated endothelial cell damage and subsequent thrombosis. Moreover, the spike protein can act as superantigen, thus eliciting a polyclonal activation. This, together with the high immunogenicity of PF4-adenovirus complexes, may facilitate the induction of PF4-specific antibodies [47].

Moreover, besides their potential to elicit anti-PFA antibodies, AdV vaccines induce a more pronounced increase in thrombin generation, inflammatory (i.e., TNF-a, IL-1b and IL-8) and platelet activation (i.e., TGF-b and CD40L) markers compared to the mRNA ones [48].

We can conclude that AdV vectors, and peculiarly the ChAdOx1 one, can induce, in a very small faction of recipients, an immunopathological response leading to the production of high-titer anti-PFA antibodies that trigger the thrombotic phenomena characterizing the VITT.

According to these models, mRNA vaccines do not develop VITT, as they do not contain the polyanionic molecules involved in the starting of this adverse event.

5. Natural history of anti-PF4 antibodies in VITT

Although most countries precautionally decided to stop the use of ChAdOx1 and Ad26.COV2.S vaccines, and to avoid re-exposure to an AdV vaccine of patients experiencing a VITT, the natural history of anti-PF4 antibodies in VITT has some relevance to learn more about this intriguing immunologic disease.

It has been demonstrated that the temporal decline of anti-PF4 IgG antibodies in VITT is similar to the antibody dynamics seen in HIT, although the VITT ones tend to persist for a considerably longer time [49,50]. Schönborn et al., in a cohort of 65 VITT patients prospectively followed after VITT, found that the platelet-activation assay became negative in 73.8% of patients within a median follow-up of 25 weeks, with a median time to a negative test result of 15.5 weeks (range, 5–28 weeks). However, seroreversion to a negative EIA optical density (OD) result (i.e., < 0.5 OD units) was seen in only 14 patients, although OD values decreased from median 3.12 to 1.52 ($P < 0.0001$). Five (7.5%) patients showed persistent platelet-activating antibodies and high EIA ODs for > 11 weeks [51].

Of note, 29 patients received a second vaccine shot with an mRNA vaccine; twenty-two of them still received therapeutic dose and one prophylactic dose anticoagulation. None of them developed either symptomatic new thrombotic events or recurrence of platelet-activating antibodies regardless of the results of the EIA test, thus demonstrating the safety of mRNA vaccines also in these patients.

Relevant to the laboratory issues on the diagnosis of VITT, Craven et al., in a large cohort of 148 VITT patients from United Kingdom, reported substantial differences between two different ELISA tests in terms of time to normalization of anti-PF4 antibodies [52]. They found a median duration of positivity of the PF4 assay of 87 days, with 72% of patients remaining positive after a median duration of follow up of 105 days. However, 51% of patients diagnosed by the Stago assay had a persistently positive anti-PF4/polyanion levels 100 days post diagnosis, whilst 94% of patients monitored using the Immucor assay remained positive. This substantial difference is likely to be mainly due to the

different technical characteristics of either test, although a little contribute by some difference in the clinical and demographic characteristics of the two populations cannot be excluded.

From a practical point of view, this means that clinicians have to be aware of the technical features of the PF4 enzyme-linked immunosorbent assay (ELISA) test available at their own institution, to ensure an appropriate clinical interpretation of results in the context of a suspected or confirmed VITT. In this study, despite the persistence of PF4 antibodies in about three quarter of population, the rate of relapse was low (12.6%), and only in one case it was associated with extension of their thrombosis.

To sum up, anti-PF4 IgG antibodies measured by ELISA are usually detectable for longer times than platelet-activating antibodies in functional assays, and the duration of detectability is highly assay-dependent. Many factors can account for the variability in duration of the anti-PF4 antibodies between patients, including the titer of antibodies produced at the time of acute VITT and the individual persistence of anti-PF4 antibody-producing B-cells.

Nevertheless, neither subsequent vaccination with an mRNA vaccine nor re-exposure to adenoviral vector-based vaccines in a small subset of VITT patients have been associated with adverse reactions [49].

6. The core question: why me?

If the AdV vectors are the culprits of VITT, why this syndrome is so rare, as it develops in a few cases out of millions of subjects receiving an AdV-based vaccine?

This question, very compelling for both affected patients and researchers, still remains unanswered.

Anti-PF4/polyanion antibodies were detected by a PF4 IgG ELISA immunoassay only in 1.2% of Norwegian health care workers vaccinated with the first dose of ChAdOx1-SARS-COV-2, and none of them developed either thrombocytopenia or VITT [53].

Moreover, a post-hoc analysis of sera from subjects recruited in a phase 3 trial of ChAdOx1-SARS-COV-2 vaccine found no increased rate of detection of anti-PF4 IgG post- vaccination compared to placebo during the period of highest TTS risk. Indeed, 19/1727 (1.1%, ChAdOx1) vs 7/857 (0.8%, placebo) participants were anti-PF4-IgG-negative at baseline, but had moderate Day-15 levels ($P = 0.676$) and 0/35 and 1/20 (5.0%) had moderate levels at baseline but high Day-15 levels [54]. None of the participants to the trial experienced VITT following administration of vaccine or placebo.

Thiele et al. found a slightly greater proportion of anti-PF4 antibodies in health care workers who received either ChAdOx1 nCoV-19 or BNT162b2 vaccine [55]. In total, 6.8% of participants tested positive for anti-PF4/polyanion antibodies postvaccination (BNT162b2: 5.6%; ChAdOx1 nCoV-19: 8.0%). However, optical densities were mostly low (between 0.5 and 1.0 units; reference range, < 0.50), and none of the PF4/polyanion EIA+ samples induced platelet activation in the presence of PF4. The Authors concluded that positive PF4/polyanion EIAs can occur after COVID-19 vaccination with both mRNA- and AdV-based vaccines, but most of these antibodies have no clinical relevance.

On the other hand, the same question can be asked regarding the development of HIT: why only a small proportion of people exposed to heparin develops this adverse event?

It has been proposed that HIT may occur in susceptible individuals immunologically primed to produce PF4 antibodies, possibly as the result of exposures to other environmental factors (for example, bacterial infection) that produce the same antigen as that produced by the heparin/platelet 4 complexes [56].

A similar individual predisposition could be involved also in the development of VITT, and the hypothesis of a previous priming by bacterial infections fits well with the peculiar sites of thrombosis in VITT, i.e. cerebral venous sinuses and splanchnic veins. These venous territories share the common feature of draining the nasal sinus and intestines, thus allowing access of microbiotal and viral products.

The presence of high titer anti-PF4 autoantibodies in these tissue specific sites of susceptibility, coupled with the antigenic stimulus provided by AdV vectors, may start the sequence of events that lead to immunothrombosis and VITT.

The hypothesis that VITT patients may carry a subset of B-cells already primed to produce anti-PF4 antibodies is coherent with the kinetics of this disease. Indeed, the typical time window of 5–20 days between the vaccine administration and the onset of symptoms in VITT, strongly recall the temporary pattern of a secondary, rather than primary, immune response. The inflammatory response associated to vaccine inoculation could provide an important co-signal that stimulates antibody production by preformed B-cells capable of producing anti-PF4 antibodies, as occurs in the pathogenesis of classic HIT [42,57,58].

A genetic individual susceptibility can also be involved. Indeed, it has been demonstrated that platelet T-cell ubiquitin ligand-2 (TULA-2) is a negative regulator of FcgRIIA-mediated signaling in platelets. Platelets with low levels of TULA-2 strongly respond to immune complexes, whereas high levels of TULA-2 protect from platelet aggregation in response to immune complex binding to FcgRIIA. Unidentified single nucleotide polymorphisms, epigenetic changes and protein turnover may affect the level of TULA-2 within individuals, thus contributing to their susceptibility to HIT and possibly to VITT [59].

Finally, an impaired NET degradation in VITT patients has been found, similar to that observed in patients affected by systemic lupus erithematosus. It can be speculated that individual endogenous mechanisms of NET regulation exist, which can elicit or aggravate autoimmunological mechanisms and induce a vicious cascade via inflammatory pathways and complement activation leading to immunothrombosis [60].

However, despite this huge amount of data about the pathophysiological mechanisms behind the VITT, summarized in Table 3, we are currently unable to predict which patient is at risk of developing VITT (or HIT, of course).

The main measure so far adopted to minimize the risk of VITT (i.e., to simply avoid the use of AdV-based vaccines) is agreeable, since it has been taken according to the precautionary principle in an emergency setting, but nevertheless it poses some compelling questions in terms of global health [61].

7. Concluding remarks

An ancient popular saying warns about the risk of "*throwing out the baby with the bathwater*".

This also applies to the issue of AdV vectors-based COVID-19 vaccines.

The COVID-19 pandemic strongly reminded us that in a global world

Table 3
Proposed pathophysiological mechanisms of VITT.

Step	Involved mechanism	Refs.
Conformational changes of PF 4	Polyanionic AdV hexon proteins	[38–42]
	Process-related impurities	[42,44]
	Soluble spike proteins variants	[46,47]
Development of anti-PF4 antibodies	Proinflammatory milieu	[42,48]
	Marginal zone B cells (individually preprimed?)	[39,42]
PF4 tetramers clustering and immune complexes formation	Fcγ- IIa receptor -dependent platelet activation	[38–42]
	Monocytic activation	
	Release of procoagulant platelet microparticles (MPs)	
	Production of neutrophil extracellular traps (NETs)	
VITT	High-titer anti-PF4	[15–17]
	Thrombosis in unusual sites	[20–24]
	Thrombocytopenia	

Adapted from Ref. [42].

'nobody is safe until everybody is safe'. The hope of ending COVID-19 pandemic relies in the possibility to ensure a safe and effective vaccination also in countries where the cold chain delivery required for mRNA vaccines is unfeasible. A better knowledge of the mechanistic basis of VITT is therefore essential to develop safe, VITT-resistant AdV-based vaccines for rollout in such populations.

Moreover, the information achieved on this field can substantially contribute to improve the safety and the effectiveness of future vaccines and gene therapies that will use adenovirus as vectors, because of the many advantages provided by these platforms.

Last, but not least, the history of VITT teaches us to trust the pharmacovigilance systems operating in high-income countries, such as USA, UK and Europe.

Indeed, besides some initial problems in collecting reliable data and in effectively conveying them to the general population, the involved regulatory Agencies have been able to promptly detect even very small signals of an increased risk of adverse events in recipients of some vaccines, and to take timely measures to tackle this issue.

More broadly, despite an anti-scientific feeling sometimes underscored by the media during the several waves of the disease, the history of COVID teaches us to trust science and the scientific method, which allowed to save a huge number of lives by an unparalleled rapid development of effective and very safe vaccines.

Declaration of Competing Interest

None.

References

[1] World Health Organization COVID-19 Dashboard, https://covid19.who.int/;2022 [accessed July 25, 2022].

[2] European Medicine Agency COVID-19 Vaccines, https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines/;2022 [accessed July 25, 2022].

[3] U.S Food and Drug Administration. COVID-19 Vaccines Authorized for Emergency Use or FDA-Approved, https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines#authorized-vaccines/;2022 [accessed July 25, 2022].

[4] World Health Organization. Coronavirus disease (COVID-19): Vaccines, https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-(covid-19)-vaccines/;2022 [accessed July 25, 2022].

[5] London School of Hygiene and Tropical Medicine. COVID-19 vaccine tracker, https://www.lshtm.ac.uk/research/centres/vaccine-centre/covid-19/;2022 [accessed July 25, 2022].

[6] Nohynek H, Wilder-Smith A. Does the world still need new COVID-19 vaccines? N Engl J Med 2022;386(22):2140–2. https://doi.org/10.1056/NEJMe2204695.

[7] Hager KJ, Pérez Marc G, Gobeil P, Diaz RS, Heizer G, Llapur C, et al. Efficacy and safety of a recombinant plant-based adjuvanted COVID-19 vaccine. N Engl J Med 2022;386:2084–96. https://doi.org/10.1056/NEJMoa2201300.

[8] Dai L, Gao L, Tao L, Hadinegoro SR, Erkin M, Ying Z, et al. Efficacy and safety of the RBD-dimer-based COVID-19 vaccine ZF2001 in adults. N Engl J Med 2022;386:2097–111. https://doi.org/10.1056/NEJMoa2202261.

[9] Center for Disease Control and Prevention. The Vaccine Adverse Events Reporting System (VAERS), https://covid.cdc.gov/covid-data-tracker/#vaccinations_vacc-total-admin-rate-total/;2022 [accessed April 20, 2022].

[10] UK Government. Medicines and Healthcare products Regulatory Agency. Coronavirus vaccine-weekly summary of Yellow Card reporting. Updated 22 July 2022, https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting/;2022 [accessed July 25, 2022].

[11] Smith S, Chandler C, Brereton A. Life-changing consequences of vaccine-induced immune-mediated thrombosis with thrombocytopenia. Br J Haematol 2022;197:121–3. https://doi.org/10.1111/bjh.18005.

[12] Vickers ER, McClure DL, Naleway AL, Jacobsen SJ, Klein NP, Glanz JM, et al. Risk of venous thromboembolism following influenza vaccination in adults aged 50 years and older in the vaccine safety datalink. Vaccine 2017;35:5872–7. https://doi.org/10.1016/j.vaccine.2017.08.086.

[13] Vallone MG, Falcón AL, Castro HM, Ferraris A, Cantarella RF, Staneloni MI, et al. Thrombotic events following COVID-19 vaccines compared to influenza vaccines. Eur J Intern Med 2022;99:82–8. https://doi.org/10.1016/j.ejim.2022.03.002.

[14] Menni C, Klaser K, May A, Polidori L, Capdevila J, Louca P, et al. Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID symptom study app in the UK: a prospective observational study. Lancet Infect Dis 2021;21:939–49. https://doi.org/10.1016/S1473-3099(21)00224-3.

[15] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2092–101. https://doi.org/10.1056/NEJMoa2104840.

[16] Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2124–30. https://doi.org/10.1056/NEJMoa2104882.

[17] Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2202–11. https://doi.org/10.1056/NEJMoa2105385.

[18] Wise J. COVID-19: European countries suspend use of Oxford-AstraZeneca vaccine after reports of blood clots. BMJ 2021;372:n699. https://doi.org/10.1136/bmj.n699.

[19] European Medicine Agency news. AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets, https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood/;2021 [accessed June 5, 2022].

[20] World Health Organization. Guidance for clinical case management of thrombosis with thrombocytopenia syndrome (TTS) following vaccination to prevent coronavirus disease (COVID-19), https://apps.who.int/iris/bitstream/handle/10665/342999/WHO-2019-nCoV-TTS-2021.1-eng.pdf/;2021 [accessed June 5, 2022].

[21] Gresele P, Marietta M, Ageno W, Marcucci R, Contino L, Donadini MP, et al. Management of cerebral and splanchnic vein thrombosis associated with thrombocytopenia in subjects previously vaccinated with Vaxzevria (AstraZeneca): a position statement from the Italian society for the study of haemostasis and thrombosis (SISET). Blood Transfus 2021;19:281–3. https://doi.org/10.2450/2021.0117-21.

[22] Oldenburg J, Klamroth R, Langer F, Albisetti M, von Auer C, Ay C, et al. Diagnosis and management of vaccine-related thrombosis following AstraZeneca COVID-19 vaccination: guidance statement from the GTH. Hamostaseologie 2021;41:184–9. https://doi.org/10.1055/a-1469-7481.

[23] Makris M, Pavord S, Lester W, Scully M, Hunt B. Vaccine-induced immune thrombocytopenia and thrombosis (VITT). Res Pract Thromb Haemost 2021;5:e12529. https://doi.org/10.1002/rth2.12529.

[24] Klok FA, Pai M, Huisman MV, Makris M. Vaccine-induced immune thrombotic thrombocytopenia. Lancet Haematol 2021;9:73–80. https://doi.org/10.1016/S2352-3026(21)00306-9.

[25] Marcucci R, Berteotti M, Gori AM, Giusti B, Rogolino AA, Sticchi E, et al. Heparin induced thrombocytopenia: position paper from the Italian society on thrombosis and haemostasis (SISET). Blood Transfus 2021;19:14–23. https://doi.org/10.2450/2020.0248-20.

[26] Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost 2017;15:2099–114. https://doi.org/10.1111/jth.13813.

[27] See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA 2021;325:2448–56. https://doi.org/10.1001/jama.2021.7517.

[28] Sangli S, Virani A, Cheronis N, Vannatter B, Minich C, Noronha S, et al. Thrombosis with thrombocytopenia after the messenger RNA-1273 vaccine. Ann Intern Med 2021;174:1480–2. https://doi.org/10.7326/L21-0244.

[29] Pishko AM, Cuker A. Thrombosis after vaccination with messenger RNA-1273: is this vaccine-induced thrombosis and thrombocytopenia or thrombosis with thrombocytopenia syndrome? Ann Intern Med 2021;174:1468–9. https://doi.org/10.7326/M21-2680.

[30] Hosseinzadeh R, Barary M, Mehdinezhad H, Sio TT, Langer F, Khosravi S. Thrombotic thrombocytopenia after Sinopharm BBIBP-CorV COVID-19 vaccination. Res Pract Thromb Haemost 2022;6:e12750. https://doi.org/10.1002/rth2.12750.

[31] Johansen S, Laegreid IJ, Ernstsen SL, Azrakhsh NA, Kittang AO, Lindås R, et al. Thrombosis and thrombocytopenia after HPV vaccination. J Thromb Haemost 2022;20:700–4. https://doi.org/10.1111/jth.15604.

[32] Simpson CR, Shi T, Vasileiou E, Katikireddi SV, Kerr S, Moore E, et al. First-dose ChAdOx1 and BNT162b2 COVID-19 vaccines and thrombocytopenic, thromboembolic and hemorrhagic events in Scotland. Nat Med 2021;27:1290–7. https://doi.org/10.1038/s41591-021-01408-4.

[33] Andrews NJ, Stowe J, Ramsay ME, Miller E. Risk of venous thrombotic events and thrombocytopenia in sequential time periods after ChAdOx1 and BNT162b2 COVID-19 vaccines: a national cohort study in England. Lancet Reg Health Eur 2022;13:100260. https://doi.org/10.1016/j.lanepe.2021.100260.

[34] Whiteley WN, Ip S, Cooper JA, Bolton T, Keene S, Walker V, Denholm R, et al. Association of COVID-19 vaccines ChAdOx1 and BNT162b2 with major venous, arterial, or thrombocytopenic events: a population-based cohort study of 46 million adults in England. PLoS Med 2022;19:e1003926. https://doi.org/10.1371/journal.pmed.1003926.

[35] Kerr S, Joy M, Torabi F, Bedston S, Akbari A, Agrawal U, et al. First dose ChAdOx1 and BNT162b2 COVID-19 vaccinations, and cerebral venous sinus thrombosis: a pooled self-controlled case series study of 11.6 million individuals in England, Scotland, and Wales. PLoS Med 2022;19:e1003927. https://doi.org/10.1371/journal.pmed.1003927.

[36] Centers for Disease Control and Prevention. Vaccine Adverse Event Reporting System (VAERS), https://wonder.cdc.gov/controller/datarequest/D8;jsessionid=AC239F4CF07390490F345CAC38C5/;2022 [accessed on June 30, 2022].

[37] Centers for Disease Control and Prevention. COVID Data Tracker, https://covid.cdc.gov/covid-data-tracker/#vaccinations_vacc-total-admin-rate-total/;2022 [accessed on June 30, 2022].
[38] Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccination. Blood 2021;138:350–3. https://doi.org/10.1182/blood.2021011958.
[39] McGonagle D, De Marco G, Bridgewood C. Mechanisms of immunothrombosis in vaccine-induced thrombotic thrombocytopenia (VITT) compared to natural SARS-CoV-2 infection. J Autoimmun 2021;121:102662. https://doi.org/10.1016/j.jaut.2021.102662.
[40] Iba T, Levy JH. Thrombosis and thrombocytopenia in COVID-19 and after COVID-19 vaccination. Trends Cardiovasc Med 2022;S1050-1738(22). https://doi.org/10.1016/j.tcm.2022.02.008.00038-X.
[41] Toh CH, Wang G, Parker AL. The aetiopathogenesis of vaccine-induced immune thrombotic thrombocytopenia. Clin Med 2022;22(2):140–4. https://doi.org/10.7861/clinmed.2022-0006.
[42] Greinacher A, Schönborn L, Siegerist F, Steil L, Palankar R, Handtke S, et al. Pathogenesis of vaccine-induced immune thrombotic thrombocytopenia (VITT). Semin Hematol 2022;59:97–107. https://doi.org/10.1053/j.seminhematol.2022.02.004.
[43] Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia. Nature 2021;596:565–9. https://doi.org/10.1038/s41586-021-03744-4.
[44] Ledford H. COVID vaccines and blood clots: what researchers know so far. Nature 2021;596:479–81. https://doi.org/10.1038/d41586-021-02291-2.
[45] Michalik S, Siegerist F, Palankar R, Franzke K, Schindler M, Reder A, et al. Comparative analysis of ChAdOx1nCoV-19 and Ad26.COV2.S SARS-CoV-2 vector vaccines. Haematologica 2022;107:947–57. https://doi.org/10.3324/haematol.2021.280154.
[46] Kowarz E, Krutzke L, Külp M, Streb P, Larghero P, Reis J, et al. Vaccine-induced COVID-19 mimicry syndrome. eLife 2022;11:e74974. https://doi.org/10.7554/eLife.74974.
[47] Kircheis R. Coagulopathies after vaccination against SARS-CoV-2 may be derived from a combined effect of SARS-CoV-2 spike protein and adenovirus vector-triggered signaling pathways. Int J Mol Sci 2021;22:10791. https://doi.org/10.3390/ijms22191079.
[48] Ostrowski SR, Søgaard OS, Tolstrup M, Stærke NB, Lundgren J, Østergaard L, Hvas AM. Inflammation and platelet activation after COVID-19 vaccines -possible mechanisms behind vaccine-induced immune thrombocytopenia and thrombosis. Front Immunol 2021;12:779453. https://doi.org/10.3389/fimmu.2021.779453.
[49] Schönborn L, Greinacher A. Longitudinal aspects of VITT. Semin Hematol 2022;59:108–14. https://doi.org/10.1053/j.seminhematol.2022.03.001.
[50] Schönborn L, Thiele T, Kaderali L, Greinacher A. Decline in pathogenic antibodies over time in VITT. N Engl J Med 2021;385:1815–6. https://doi.org/10.1056/NEJMc2112760.
[51] Schönborn L, Thiele T, Kaderali L, Günther A, Hoffmann T, Seck SE, et al. Most anti-PF4 antibodies in vaccine-induced immune thrombotic thrombocytopenia are transient. Blood 2022;139:1903–7. https://doi.org/10.1182/blood.2021014214.
[52] Craven B, Lester W, Boyce S, Thomas W, Kanny A, Davies C, et al. Natural history of PF4 antibodies in vaccine-induced immune thrombocytopenia and thrombosis. Blood 2022;139:2553–60. https://doi.org/10.1182/blood.2021014684.
[53] Sørvoll IH, Horvei KD, Ernstsen SL, Laegreid IJ, Lund S, Grønli RH, et al. An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination. J Thromb Haemost 2021:191813–8. https://doi.org/10.1111/jth.15352.
[54] Cohen TS, Kelly EJ, Nylander S, Bansal H, Jepson BM, Bhuyan P, et al. Serum levels of anti-PF4 IgG after AZD1222 (ChAdOx1 nCoV-19) vaccination. Sci Rep 2022;12:7961. https://doi.org/10.1038/s41598-022-11623-9.
[55] Thiele T, Ulm L, Holtfreter S, Schönborn L, Kuhn SO, Scheer C, et al. Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2. Blood 2021;138:299–303. https://doi.org/10.1182/blood.2021012217.
[56] Greinacher A, Holtfreter B, Krauel K, Gätke T, Weber C, Ittermann T, et al. Association of natural anti-platelet factor 4/heparin antibodies with periodontal disease. Blood 2011;118:1395–401. https://doi.org/10.1182/blood-2011-03-342857.
[57] Zheng Y, Wang AW, Yu M, Padmanabhan A, Tourdot BE, Newman DK, et al. B-cell tolerance regulates production of anti- bodies causing heparin-induced thrombocytopenia. Blood 2014;123:931–4. https://doi.org/10.1182/blood-2013-11-540781.
[58] Jaax ME, Krauel K, Marschall T, Brandt S, Gansler J, Fürll B, et al. Complex formation with nucleic acids and aptamers alters the antigenic properties of platelet factor 4. Blood 2013;122:272–81. https://doi.org/10.1182/blood-2013-01-478966.
[59] Zhou Y, Abraham S, Andre P, Edelstein LC, Shaw CA, Dangelmaier CA, et al. Anti-miR-148a regulates platelet FcγRIIA signaling and decreases thrombosis *in vivo* in mice. Blood 2015;126:2871–81. https://doi.org/10.1182/blood-2015-02-631135.
[60] de Buhr N, Baumann T, Werlein C, Fingerhut L, Imker R, Meurer M, et al. Insights into immunothrombotic mechanisms in acute stroke due to vaccine-induced immune thrombotic thrombocytopenia. Front Immunol 2022;13:879157. https://doi.org/10.3389/fimmu.2022.879157.
[61] Lobello PA, Squizzato A, Picozzi M. The precautionary principle in the COVID-19 vaccination campaign: the complicated relationship between the scientific community, medicines regulatory agencies and citizens. Eur J Intern Med 2022;101:118–9. https://doi.org/10.1016/j.ejim.2022.03.011.