Review

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Review and evolution of guidelines for diagnosis of COVID-19 vaccine induced thrombotic thrombocytopenia (VITT)

Abstract: Coronavirus disease 2019 (COVID-19) is a life-threatening infectious disease caused by Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2). In response to the still ongoing pandemic outbreak, a number of COVID-19 vaccines have been quickly developed and deployed. Although minor adverse events, either local (e.g., soreness, itch, redness) or systematic (fever, malaise, headache, etc.), are not uncommon following any COVID-19 vaccination, one rare vaccine-associated event can cause fatal consequences due to development of antibodies against platelet factor 4 (PF4), which trigger platelet activation, aggregation, and possible resultant thrombosis, often at unusual vascular sites. Termed thrombosis with thrombocytopenia syndrome (TTS) by reporting government agencies, the term vaccine-induced (immune) thrombotic thrombocytopenia (VITT) is more widely adopted by workers in the field. In response to increasing reports of VITT, several expert groups have formulated guidelines for diagnosis and/or management of VITT. Herein, we review some key guidelines related to

diagnosis of VITT, and also provide some commentary on their development and evolution.

Introduction

Coronavirus disease 2019 (COVID-19) is a life-threatening infectious disease caused by Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), which has now become a pandemic. At time of writing, there have been over 240 million recorded infections worldwide, with nearly five million attributable deaths [1]. A large number of COVID-19 vaccines have been quickly developed as a response, with many also now deployed throughout the world to minimize the risk of severe COVID-19 and death, and also to prevent SARS-CoV-2 infection, including those caused by new "variants of concern". Of interest, a total of "330 vaccine candidates are in various stages of development, with 102 in clinical research, including 30 in Phase I trials, 30 in Phase I–II trials, 25 in Phase III trials, and eight in Phase IV development" [2]. At time of writing, over 20 vaccines have been authorized by at least one national regulatory authority for public use: one DNA vaccine (ZyCoV-D), two RNA vaccines (Pfizer–BioNTech and Moderna), 10 conventional inactivated vaccines (BBIBP-CorV, Chinese Academy of Medical Sciences, CoronaVac, Covaxin, CoviVac, COVIran Barekat, FAKHRAVAC, Minhai-Kangtai, QazVac, and WIBP-CorV), five viral vector vaccines (Sputnik Light, Sputnik V, Oxford–AstraZeneca, Convidecia, and Janssen), and five protein subunit vaccines (Abdala, EpiVacCorona, MVC-COV1901, Soberana 02, and ZF2001) [2, 3].

Minor adverse effects are an expected consequence of any vaccination program. For COVID-19 vaccines, these include soreness, redness, rash, and inflammation at the injection site. Other common side effects include fever, fatigue, headache, myalgia (muscle pain), and arthralgia

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(joint pain), which generally resolve within a few days [2, 3]. A less-frequent side effect (that generally occurs in less than one in 1,000 people) is hypersensitivity (allergy) to one or more vaccine ingredients, which in some rare cases may cause anaphylaxis. An ever-rarer side effect (that generally occurs in less than one in 50,000 people), now recognized to occur following immunization with adenovirus-based vaccines, has been termed thrombosis with thrombocytopenia syndrome (TTS), for TTS, by government reporting agencies, including the Centers for Disease Control (and Prevention) (CDC) in the US [4], the European Medicines Agency (EMA) [5], and the Therapeutic Goods Administration (TGA) in Australia [6].

However, the term vaccine-induced (immune) thrombotic thrombocytopenia (VITT) is more widely adopted by workers in the field [7], and will generally be used in this review, which is aimed to review the key guidelines related to diagnosis of this condition, and also provide some commentary on their development and evolution.

A brief early history of VITT identification

VITT was first reported in a non-reviewed preprint [8], by a German group of researchers led by Andreas Greinacher, and which was later used as the basis for a formal peer reviewed publication in the New England Journal of Medicine (NEJM) [9]. The German researchers originally called the syndrome VIPIT, for vaccine-induced prothrombotic immune thrombocytopenia [8], but later used the term VITT [9], as did two other European groups publishing also at that time in the NEJM, one from Norway [10], and the other from the United Kingdom [11]. All three publications reported on case series, respectively 11, 5, and 23 cases, and all associated to use of the adenovirus-based AstraZeneca (AZ) vaccine (alternatively called ChAdOx1 nCoV-19, AZD1222, Vaxzevria). Separate cases were later described in the USA, as associated to use of the adenovirus-based Janssen (Johnson & Johnson) vaccine (JJ; alternatively known as Ad26.COV2.S or JNJ-78436735) [12, 13]. A subsequent review by one of us detailed some findings from the composite of approximately 100 cases of VITT published in the literature by the end of May, 2021 [7], with all cases associated with either the adenovirus-based AZ or JJ vaccines. Despite availability of other adenovirus-based vaccines [2, 3], the authors remain unaware of any substantiated reports of VITT/TTS by any other adenovirus-based COVID-19 vaccine, which may be due to lower worldwide usage of other formulations. Whether this actually reflects an absence of events or an absence of reporting is also unknown. We are aware, however, of a single case of potential VITT associated with use of the Moderna (mRNA-1273) vaccine [14], with this publication accompanied by an editorial [15]. Although this report fits a potential VITT/TTS case definition, and was positive for anti-PF4 antibodies, the test was performed after initiation of heparin treatment, so that such case might alternatively reflect a heparin-related anti-PF4 antibody, or even might be co-incidental and reflect a kind of "spontaneous HIT syndrome". In summation, only the use of AZ and JJ COVID-19 vaccines have consistently led to reports of vaccine associated VITT/TTS.

Common to patient presentation in all case series, was thromboses, usually at atypical sites, and for laboratory testing, positivity for the presence of platelet factor 4 (PF4) antibodies detected by enzyme linked immunosorbent assays (ELISA) [7]. Of interest, these assays are normally used to identify PF4/heparin (PF4/H) antibodies present in a potentially "related" condition called heparin induced thrombocytopenia (HIT) [16]. Of additional interest, other rapid assays normally used to identify HIT PF4/H antibodies [16–18] were usually negative in VITT [7]. Thus, although the VITT syndrome has been identified to be mechanistically similar to HIT, the antibodies did not appear to be the same as those seen in HIT. More specifically, VITT antibodies were found to bind some surface PF4 amino acids, specifically located within the heparin binding site, but differing from the protein moiety recognized by anti-PF4 antibodies which develop in HIT patients [19]. The consequent pathogenetic process of VITT is therefore partially different from HIT, in that immune complexes independent of heparin are generated in patients with VITT; these then promote cross-linking of FcγRIIa receptors on platelet surface, which is then followed by platelet activation and aggregation [19].

Differences have also been observed in functional assays looking for platelet activation and aggregation [7]. For example, inclusion of heparin in standard serotonin release assays (SRAs) is used to identify heparin-associated platelet activation and aggregation in HIT, but inclusion of heparin in SRA testing of VITT patients appeared to more often inhibit (rather than augment) platelet activation and aggregation [7, 9, 20], according to the aforementioned differing molecular target(s). This therefore offered a potential for differentiation of HIT vs. VITT [7].

On the other hand, it is also important to recognize the potential for false negative functional assays in VITT/TTS investigations, should blood be collected after initiation of IVIG, this being a recognized and important early treatment in course of VITT/TTS, to both inhibit platelet

activation and help return platelet counts to "normal" baseline values [21].

Irrespective of the above considerations, given the potentially serious consequences seen in a proportion of patients with VITT/TTS, several expert groups were quick to provide guidance on both the diagnosis and management of VITT; however, the current review details the key guidelines related to diagnosis of VITT.

Guidelines from Europe

Considering that VITT was first described in a German cases series, followed quickly by cases from Norway and the UK, it is fitting to begin with a review and comparison of diagnostic guidelines across Europe. One of the first "expert group" guidelines to appear was from the Society for Thrombosis and Haemostasis Research (GTH [Gesellschaft für Thrombose- und Hämostaseforschung]), a nonprofit, interdisciplinary German-Austrian-Swiss scientific society with the aim of promoting the scientific interests of blood coagulation research [22]. This publication included an algorithm that provided guidance for both identification and management of VITT, that appears to be partially modeled on that published by the German group in NEJM [8]. The portion related to the GTH diagnostic pathway is summarized in Figure 1, but their algorithm had the primary aim to provide appropriate management according to the diagnostic pathway findings. The key features are: exposure to the AZ vaccine (the only adenovirus-based vaccine available in the GTH countries) 4–16 days earlier, followed by evidence of thrombosis and thrombocytopenia; a "HIT screening test for PF4/H antibodies" would then be performed (with the following qualification: "Based on preliminary observations, the HYPHEN BioMed ZYMUTEST and the Immucor GTI Diagnostics enzyme immunoassays appear to have appropriate sensitivity for all pathophysiologically relevant antibodies"). If the test is negative, this essentially "excluded" VITT (or HIT), and thus treatment with heparin would be possible. If the test is positive this essentially 'infers' VITT (or HIT), and thus further "discriminatory" functional testing would be required. At first, 'standard' functional platelet activation assays normally used for HIT (i.e., with inclusion of heparin to identify augmented platelet activation and aggregation) is performed. If these "standard" assays are positive for PF4/H antibody-mediated platelet activation and aggregation, the patient is identified as having HIT, exits the diagnostic pathway and is treated accordingly. If the patient is found to be negative for PF4/H antibody-mediated platelet activation and aggregation by standard

assays, then a modified heparin induced platelet aggregation (HIPA) assay is performed, according to guidance from the Greinacher laboratory. A positive finding in the modified HIPA assay would identify VITT, and the patient managed according to a VITT management plan. An alternative negative finding in the modified HIPA assay would exclude VITT, and thus heparin treatment would instead be possible. Also notable within the guideline is a requirement to identify the presence of thrombocytopenia and/or thrombosis at the start of the process. It is important to consider this guidance within a historical context, since more recent evidence suggests that not all VITT cases will necessarily initially present with either thrombocytopenia or thrombosis (discussed later). Also, D-dimer testing did not feature in the algorithm, although this testing was recommended in the text of the manuscript. Nevertheless, the test for "PF4/H" antibodies was the key discriminator for the presence of VITT (or HIT), and a negative result provided an exit point for treatment. Again, this should be considered within a historical context, and it is now known that there is a small possibility of false negative PF4/H ELISA test results [7]. But perhaps most striking in the GTH guideline is the requirement for functional testing to assess platelet activation after a positive PF4/H ELISA assay result.
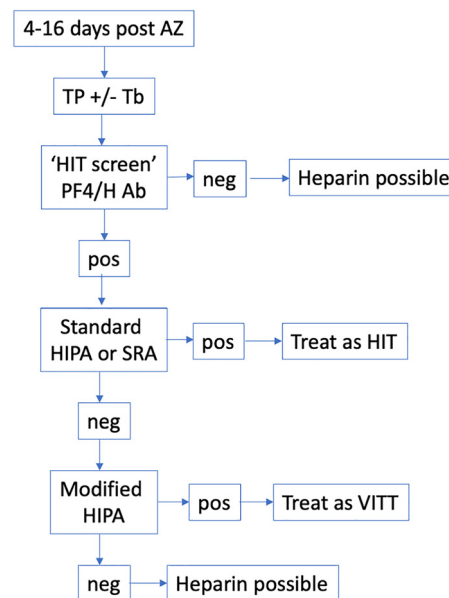
Figure 1: The VITT diagnostic pathway proposed by the Society for Thrombosis and Haemostasis Research (GTH) [22].
Ab, antibody; AZ, AstraZeneca (COVID-19 vaccine); H, heparin; HIPA, heparin induced platelet aggregation; HIT, heparin induced thrombocytopenia (with thrombosis); neg, negative; PF4, platelet factor 4; pos, positive; SRA, serotonin release assay; TP, thrombocytopenia; Tb, thrombosis; VITT, vaccine induced thrombotic thrombocytopenia.

The UK group also published an algorithm in their NEJM paper [11], as partly summarized in Figure 2 for the diagnostic pathway. Again, this was based on their experience with AZ vaccination. The post vaccination timeframe was longer than that of the GTH guideline, and also advocated early involvement of a hematologist. Again, presence of thrombosis and/or thrombocytopenia created the entry point for the pathway. A recommendation for routine coagulation tests, comprising prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and D-dimer provided the deflection point for likelihood of VITT (D-dimer >4,000 fibrinogen equivalent units (FEU), low or normal fibrinogen, and no alternate diagnosis) or not VITT (D-dimer <2,000 FEU, normal PT/APTT/fibrinogen). This was sufficient to initiate differential treatment, at which time a "HIT ELISA" would be performed on suspected VITT cases. A negative result would trigger a review of diagnosis and treatment, or consideration of alternative investigations, including functional tests. A positive test result would justify continued treatment as VITT.

These initial UK guidelines no doubt provided the basis for further guidance by means of "living documentation" (meaning regularly updated) by the UK Expert Haematology Panel (EHP) focused on VITT [23]. Although the main aim of this expert guidance is on management of patients with VITT, there is a summary of VITT case definitions, which derives from another publication in NEJM [24], and as summarized in Table 1, as well as an investigative algorithm, which is similar to that shown in Figure 2. Also provided on the guidance is advice about ELISA testing, storing samples, sending samples for whole genome testing, presumably to help identify any potential genetic predeterminants for VITT development, and guidance about future vaccination.

Additional guidance is available from the National Institute for Health and Care Excellence (NICE), whose role is "to improve outcomes for people using the NHS and other public health and social care services" [25]. However, the guidelines, published on July 29th, and available online [26], are noted to be adapted from the UK EHP's guidance.

The Italian Society for the Study of Haemostasis and Thrombosis (SISET) has produced guidance for the management of cerebral and splanchnic vein thrombosis (CSVT or SVT) associated with thrombocytopenia in people previously vaccinated with AZ or other adenovirus-based vaccines [27]. This includes an algorithm that identifies possible VITT with symptoms within 30 days of vaccination, recommending to
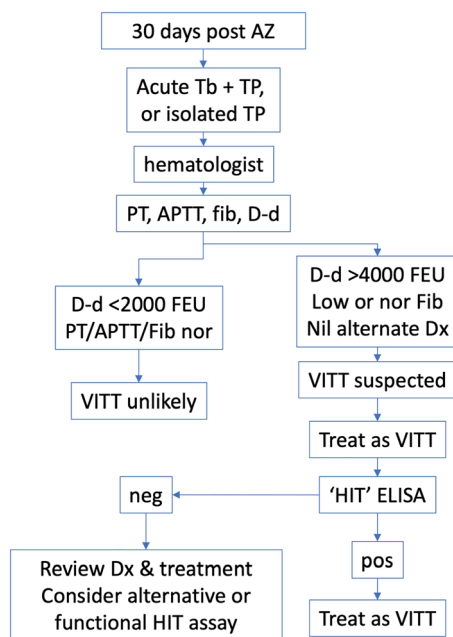
Figure 2: The VITT diagnostic pathway proposed by the UK group based on their case series [23].
APTT, activated partial thromboplastin time; AZ, AstraZeneca (COVID-19 vaccine); D-d, D-dimer; Dx, diagnosis; fib, fibrinogen; HIT, heparin induced thrombocytopenia (with thrombosis); neg, negative; pos, positive; PT, prothrombin time; TP, thrombocytopenia; Tb, thrombosis; VITT, vaccine induced thrombotic thrombocytopenia.

**Table 1:** VITT Case definition criteria from the UK EHP.

VITT case definition criteria	1. Onset of symptoms 5–30 post COVID-19 vaccine (or up to 42 days if isolated DVT/PE) 2. Presence of thrombosis 3. Thrombocytopenia (platelet count <150 × 10⁹/L) 4. D dimer >4,000 µg/mL (FEU) 5. Positive anti-PF4 antibody ELISA assay
Definite VITT	Meets all five criteria
Probable VITT	D dimer >4,000 µg/mL FEU but one criterion not fulfilled (timing, thrombosis, thrombocytopenia, anti-PF4 antibodies) or D dimer unknown or 2,000–4,000 µg/mL FEU with all other criteria present
Possible VITT	D dimer unknown or 2,000–4,000 µg/mL FEU with one other criterion not fulfilled, or two other criteria not fulfilled (timing, thrombosis, thrombocytopenia, anti-PF4 antibodies)
VITT Unlikely	Platelet count <150 × 10⁹/L without thrombosis with D dimer <2,000 µg/mL FEU, or thrombosis with platelet count >150 × 10⁹/L and D dimer <2,000 µg/mL FEU, regardless of anti-PF4 antibody result, and/or alternative diagnosis more likely

DVT, deep vein thrombosis; PE, pulmonary embolism; FEU, fibrinogen equivalent units; PF4, platelet factor 4; EHP, Expert Haematology Panel.

perform testing for platelet count, international normalized ratio (INR), APTT, fibrinogen, D-dimer, an anti-PF4 assay, and HIPA if positive. Subsequent treatment is then dependent on the platelet count, as well as on results of the anti-PF4 assay.

The Spanish Federation of Medical and Scientific Associations (FACME) has also recently published some guidelines for diagnosis and treatment of VITT manifesting as cerebral venous sinus thrombosis (CVST) [28], with the diagnostic pathway summarized in Figure 3. Apart from identification of CVST within three weeks of vaccination with adenovirus-based vaccines, the presence of thrombocytopenia (or interestingly, a 50% decrease in baseline platelet count) provides the entry point in the pathway, with suggestion for standard management but also continued monitoring of clinical signs and also blood counts for those without. For those with thrombocytopenia (or 50% decrease from a recently obtained previous value), a PF4 antibody test was recommended, and for positive cases to be managed as VITT. For PF4 antibody negative cases, D-dimer testing was recommended, and if value was >4× the upper limit of normal (ULN), these cases should still be managed as VITT. If D-dimer was <4× ULN, but no alternative explanation is available for concomitant thrombocytopenia, then these cases should also still be managed as VITT. Nonetheless, when alternative explanations are available for thrombocytopenia, then the case could be managed as per standard process, but with continued monitoring of clinical signs and blood counts.
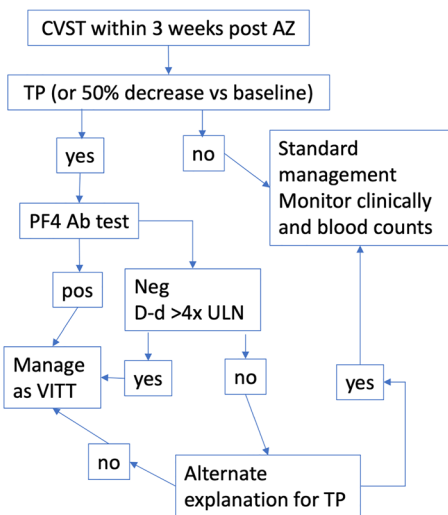
Figure 3: The VITT diagnostic pathway proposed by the Spanish Federation of Medical and Scientific Associations (FACME) [28]. Ab, antibody; AZ, AstraZeneca (COVID-19 vaccine); neg, negative; PF4, platelet factor 4; pos, positive; TP, thrombocytopenia; ULN, upper limit of normal; VITT, vaccine induced thrombotic thrombocytopenia.

Guidelines from North America

As noted, VITT can also occur subsequent to the JJ vaccine [7, 12, 13]. One living document outlining both the identification and treatment of VITT is provided as an online resource from the American Society of Hematology [29], although they preferentially use the term TTS. Although an algorithm is not provided, like the UK EHP, the ASH guidance provides advice on "definitive diagnosis" of TTS, as well as its potential exclusion, as summarized in Table 2. Given this is a living document, there is recognition in the most recent version of the guidance that a normal platelet count may not exclude VITT/TTS, since this may represent an early stage of the disease. A separate online resource is available from a Canadian group [30]. The guidance in relation to testing is summarized in Figure 4. The document has a May 7, 2021, publication date, and starts with

Table 2: TTS Case definition criteria from the American Society of Hematology.

Definitive diagnosis of TTS (must meet all five criteria)	1. COVID vaccine 4–42 days prior to symptom onset (a)
	2. Any venous or arterial thrombosis (often cerebral or abdominal)
	3. Thrombocytopenia (platelet count <150 × 10^9/L) (b)
	4. Positive PF4 "HIT" (heparin-induced thrombocytopenia) ELISA
	5. Markedly elevated D-dimer (>4 times upper limit of normal)
	a) "To date, TTS appears far more likely following AstraZeneca/Johnson and Johnson adenoviral vaccines than Moderna/Pfizer mRNA vaccines"
	b) "A patient who presents with thrombosis and a normal platelet count post-vaccination might be in an early stage of TTS. Continued assessment for development of thrombocytopenia/TTS is required"
Initial laboratory work-up:	1. CBC with platelet count* and peripheral smear
	2. PF4-ELISA (HIT assay); draw blood prior to any therapies
	3. Fibrinogen and D-dimer
	(*a normal platelet count is less concerning for TTS)
TTS is excluded if:	PF4 ELISA is negative and there is no thrombocytopenia
Likely ITP if:	Thrombocytopenia but no thrombosis and negative PF4 ELISA

CBC, complete blood count; HIT, heparin induced thrombocytopenia; ITP, immune thrombocytopenia purpura; PF4, platelet factor 4; TTS, thrombosis with thrombocytopenia syndrome.

assessment of patient symptoms reflective of potential thrombosis or bleeding, between 4 and 28 days after vaccination, with complete blood count (CBC) being the initial recommended blood test. Thrombocytopenia (i.e., platelet count $<150 \times 10^9$/L) represents the deflection point between further testing or not; if present, D-dimer, a blood film, and diagnostic imaging based on clinical suspicion, is then recommended. The composite of these then decides the further progression, which includes "HIT ELISA testing" in cases "presumptive of VITT".

Another source of information for those wishing to learn about VITT/TTS is an online resource written by key North American researchers Warkentin and Cuker [31]. Again, this document is regularly reviewed and updated.

Guidelines from Australia (and New Zealand)

Diagnostic and management guidelines for VITT from Australia and New Zealand have been prepared by a group of experts on behalf of the Thrombosis and Haemostasis Society of Australia and New Zealand (THANZ). The main document is another "living" document, regularly updated, initially with weekly meetings of the THANZ vaccine thrombocytopenia advisory working group, comprising some 25 members, including two of the authors of this review. Available online [32], the resource was first provided

Figure 4: The VITT diagnostic pathway proposed by the Ontario COVID-19 Science Advisory Table [30].
CBC, complete blood count; Tb, thrombosis; TP, thrombocytopenia; VITT, vaccine induced thrombotic thrombocytopenia.

in April, 2021, and last updated in mid-August. Some key elements of the guidance, and some sample Australian cases of VITT have been recently published [21, 33]. It can be noted that AZ has been the only adenovirus-based vaccine used in Australia so far. No adenovirus-based vaccine is used in New Zealand; however, THANZ membership inclusive of New Zealand participation was identified as important since there is potential for cross travel of AZ vaccinated individuals from Australia and abroad, who may have also received adenovirus-based vaccines. A summary of the diagnostic pathway for VITT is shown in Figure 5. Recognizing this is a recently updated version, some key points can be noted, as informed based on local Australian cases: (a) extension of vaccine exposure out to 42 days; (b) recognition that thrombocytopenia may not be evident in VITT, either because of early stage disease or high baseline pre-vaccination platelet counts, not always available in VITT investigations; (c) inclusion of high D-dimers ($>5\times$ ULN) as a marker of VITT; (d) a different pathway of testing for PF4 antibodies/functional testing based on presence/absence of thrombosis – thus, (i) no thrombosis would lead to PF4 ELISA testing, but functional testing would only progress in ELISA positive cases, (ii) presence of thrombosis would lead to concurrent anti-PF4 ELISA and functional testing. This differential was mostly aimed at managing the resource intensive functional testing to those most warranting testing based on management considerations. Additional guidance on management according to findings is also provided [21, 32]. From a laboratory testing approach, the THANZ strategy represents perhaps the most comprehensive and nationally coordinated approach, reflective of strong local research presence and includes functional testing by several tests, namely SRA, by a novel flow cytometry-based method, and by whole blood Multiplate testing. In addition to guidance prepared for Hematologists, several THANZ members have also helped prepare a number of other guidance documents, including a general VITT Multidisciplinary Guideline for Doctors [34]. Similar to the UK EHP, the THANZ also has criteria for diagnosis/exclusion of VITT, with the current scheme summarized in Table 3. Again, this classification is reflective of the Australian VITT cohort, currently in excess of 100 cases. Comprehensive data on these VITT cases from Australia is currently being prepared for publication.
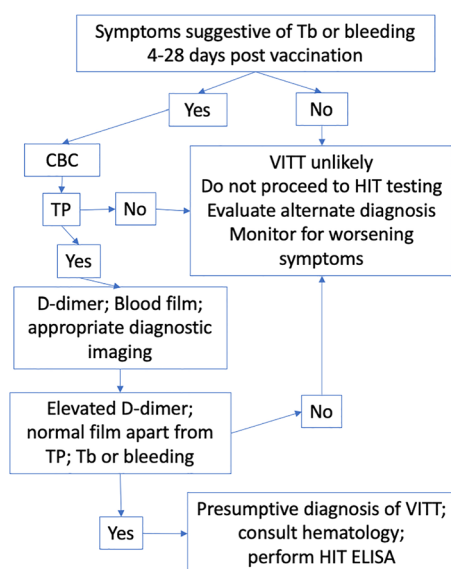
Guidelines from the ISTH

As the premier body representing international work in the field of thrombosis and hemostasis, the International
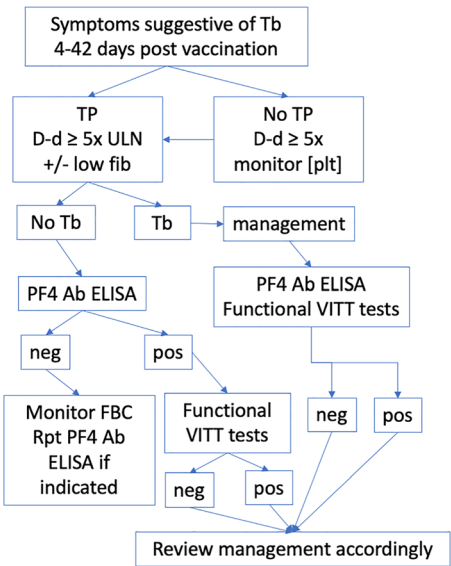
Figure 5: The VITT diagnostic pathway proposed by THANZ based on the Australian case series [21].
Ab, antibody; D-d, D-dimer; ELISA, enzyme linked immunosorbent assay; FBC, full blood count; fib, fibrinogen; neg, negative; PF4, platelet factor 4; pos, positive; TP, thrombocytopenia; Tb, thrombosis; VITT, vaccine induced thrombotic thrombocytopenia.

Society on Thrombosis and Haemostasis (ISTH) has also prepared guidance on VITT identification and management, inclusive of a flow chart [35]. The diagnostic pathway is summarized in Figure 6. It also needs to be recognized that the flow chart was produced quite early in the VITT story, and so is due for revision. At current understanding of VITT, 28 days post-vaccination may be too restrictive to permit exclusion of VITT. Also, thrombosis is not always apparent on first presentation, and may arise subsequently. Third, thrombocytopenia is not always present on first presentation, and may or may not arise subsequently. Finally, there is a small incidence of false ELISA negative in some cases of VITT, which would require either testing with another ELISA assay, or by functional assay(s), to truly exclude VITT.

Discussion

In this review, we have identified and detailed several diagnostic guidelines for VITT/TTS. It can be noted that all the guidelines differ in some aspects of the suggested approach. This may then determine different rates of VITT detection or exclusion, and indeed the potential identification of different VITT cohorts. In the early VITT publications, most patients presented with unusual site thromboses, including CVST or SVT, but this may in part

Table 3: VITT Case classification criteria from THANZ.

Classification prior to specific VITT testing	
Suspected	VITT is suspected if (1) the platelet count is <150×10^9/L AND either (2) D-dimers are elevated (5× ULN) OR (3) fibrinogen is reduced
Probable	VITT is probable if there is evidence of thrombosis in suspected VITT
Possible	VITT is possible if there is no evidence of thrombosis in suspected VITT
Less likely	VITT is less likely if the platelet count is >150×10^9/L, but D-dimers are elevated or fibrinogen is reduced
Much less likely	VITT is much less likely if repeated platelet counts are >150×10^9/L, D-dimers are not elevated AND fibrinogen is normal
Classification following specific VITT testing	
Confirmed	VITT is *confirmed* by positive "VITT" ELISA, AND positive "VITT" functional testing in cases of suspected VITT
Strongly supported	VITT is *strongly supported* by strong positive "VITT" ELISA testing, or by "VITT" functional testing, in cases of suspected VITT
Possible	VITT remains *possible* in suspected VITT whose "VITT" ELISA is positive, but functional "VITT" testing is negative
Unsupported	VITT is *unsupported* by negative "VITT" ELISA, and negative functional "VITT" testing, in cases of suspected VITT
Unclassified	1. Serious thrombosis (likely VITT) with negative ELISA and functional testing 2. Normal platelet count, without raised D-dimer, but thrombosis within time frame: ELISA and functional positive

ELIZA, enzyme linked immunosorbent assay; ULN, upper limit of normal; "VITT" ELISA represents a platelet factor 4 (PF4) ELISA; "VITT" functional testing infers one or more of: "VITT" modified serotonin release assay (SRA), flow cytometry platelet activation assay, Multiplate whole blood aggregation assay.

reflect ascertainment bias. Thus, thrombosis in VITT can also occur in standard sites, including deep vein thrombosis (DVT) and pulmonary embolism (PE) [24, 36]. That lack of initial detection of these cases could simply have reflected case ascertainment bias is now recognized [37], given DVT and PE are otherwise common thrombosis presentations outside of the VITT setting and may not have been considered as a protentional consequence of vaccination. The initial and subsequent laboratory testing recommendations also differ between guidelines. To some extent, such differences can also reflect the timing of release of the guidelines, with later documents reflecting improved knowledge about VITT. Let us take, for example, the presence of thrombocytopenia. In early guidelines, absence of thrombocytopenia was taken to mean an absence of VITT. This was based on the clear reported
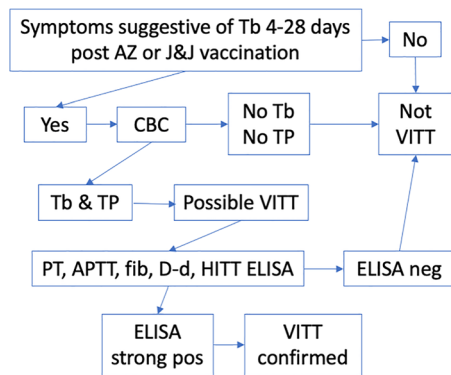
Figure 6: The VITT diagnostic pathway proposed by ISTH [35]. AZ, AstraZenica vaccine; D-d, D-dimer; ELISA, enzyme linked immunosorbent assay; CBC, complete blood count; fib, fibrinogen; neg, negative; HITT, heparin induced thrombocytopenia with thrombosis; J&J, Johnson & Johnson vaccine; pos, positive; TP, thrombocytopenia; Tb, thrombosis; VITT, vaccine induced thrombotic thrombocytopenia.

findings of thrombocytopenia in all cases of reported VITT to date at that time [7]. To some extent, this may also reflect case ascertainment bias. We know from studies on HIT, that not all cases of HIT present with thrombocytopenia, such as for example in patients with a baseline high platelet count (i.e., over 300–400 × 10^9/L). Indeed, built into the 4T HIT probability score, instead of thrombocytopenia, a 50% drop in platelet count can also be used to infer on the likelihood of HIT. For a patient with high baseline count (e.g., >350 × 10^9/L), a drop of 50% can still reflect a platelet count above 150 × 10^9/L. However, in VITT investigations, being community-based cohorts of otherwise generally healthy individuals, a pre-vaccination platelet count is rarely available. Thus, a lack of thrombocytopenia should not be used to exclude VITT, and patients with other signs pointing to VITT should have their platelet counts monitored for subsequent reduction.

The other main initial marker of suspected VITT is the D-dimer level, which have generally been >5× ULN. To our minds, a highly raised D-dimer may reflect a more consistent finding in VITT that thrombocytopenia. Only some of the guidelines place sufficient emphasis on the importance of D-dimer in the initial triaging of cases for investigation. Indeed, a patient presenting with thrombocytopenia but normal or only mildly elevated D-dimer (i.e., <2× ULN) is more likely to have immune thrombocytopenic purpura (ITP), than VITT.

Although fibrinogen testing is useful, it has a less important role in triaging patient investigation than either platelet count or D-dimer. Historically, some 70% of VITT patients seem to have reduced levels of fibrinogen, probably due to its consumption within the thrombotic process

[7]. Nonetheless, fibrinogen monitoring is important also for assessing bleeding risk, particularly if also associated with severe thrombocytopenia. Monitoring of fibrinogen, along with D-dimer and platelet count, may also help identify improvements due to treatment (e.g., IVIG and anticoagulation) [38].

The testing of anti-PF4 antibodies by ELISA is crucial to a correct diagnosis or exclusion of VITT, but again is not fool-proof. It is interesting that some of the guidelines do not specify that anti-PF4 antibodies should be evaluated by ELISA, and not by say other rapid anti-PF4 antibodies normally used to investigate HIT, and which tend to be negative in VITT [7]. Also, some guidelines actually mention "HIT ELISA" instead of anti-PF4-ELISA, which may confuse some non-experts. It has also been shown that anti-PF4 antibody ELISA assays modified for VITT testing will show increased sensitivity and specificity for VITT [7].

It is also interesting that not all guidelines provide strong support for functional VITT testing. Strong support for specific functional VITT testing, including VITT modified assays, is provided by the Greinacher group [9], as well as the associated GTH guidelines [22], and the THANZ guidelines [21, 32]. The lack of consensus to date in this aspect is at least partially attributable to the fact that positivity in functional tests like SRA seems to be influenced by certain important technical aspects, such as the addition of FP4 to the test reagents with the purpose of enhancing the diagnostic sensitivity [7]. Also, functional assays are not as widely available as anti-PF4 ELISA assays, and are more complex to perform. In summation, we would note here that the situation is complex. One the one hand, a background rate of anti-PF4 antibodies (albeit generally weak) of up to 5% can be observed in otherwise healthy individuals according to the studies of Thiele et al. [39] & Sørvoll et al. [40]. Co-incidental (mild) thrombocytopenia and thrombosis may also arise in otherwise healthy individuals unrelated to vaccine use. Thrombocytopenia with normal or only mildly elevated D-dimer may instead be pointing to ITP. Naturally, the composite of all three events (thrombocytopenia, thrombosis, highly increased D-dimer values) post-adenovirus based COVID-19 vaccination, in the right timeframe, together with positive anti-PF4 ELISA, does strongly suggest VITT, and may be sufficient for clinical diagnosis, especially if functional testing is not available. On the other hand, functional testing provides yet additional evidence, and is perhaps the most definitive test for VITT from a pathophysiological perspective. Two of the authors are members of the THANZ Advisory Group mentioned in this paper, and we are of course influenced by our own Australian experience, which has actually evaluated a number of different functional assays [21].

In terms of post vaccination timeframe for VITT, the early reports of VITT within 5–16 days [9], 7–10 days [10], 6–24 days [11], biased to presentations within the early cases series, have continued to expand, given presentations outside those timeframes. The latest iteration of the THANZ guidelines indicate a timeframe of 4–42 days [27, 29] based on presentations of Australian VITT cases, and the latest EHP guidance indicates that "cases usually present 5–30 days after vaccination" but "that DVT and PE can present up to 42 days after vaccination" and that DVT probably "develops subclinically between days 5–30" [19].

Conclusions

As the specific knowledge on the pathogenesis, clinical and laboratory presentation of VITT grows, the availability, applicability and completeness of diagnostic guidelines will expectedly evolve in parallel, though the accuracy will remain limited, due to the variegated, often subtle presentations of VITT. Notably, the frequency of both vaccine-associated thrombocytopenia and vaccine-associated ITP has been assessed in a large Scottish population-based study, which included people who received both AstraZeneca and Pfizer vaccines. This study revealed that the risk of developing ITP was reported at around 1.13 per 100,000 recipients of AZ adenovirus-based vaccine, especially between 1 and 3 weeks post vaccination, and without any further evidence of thrombosis [41]. Similarly, although it is now already ascertained that anti-PF4 antibodies develop in a significant proportion of adenovirus-based COVID-19 vaccine recipients [39, 40, 42], they are very rarely associated with thrombotic episodes and, importantly, their levels also seem to correlate with those of anti-SARS-CoV-2 neutralizing antibodies [42], perhaps suggesting that baseline anti-SARS-CoV-2 positive individuals should preferably be administered with non-adenovirus-based formulations of COVID-19 vaccines. Therefore, an accurate diagnosis can only be reached when multiple abnormalities are integrated, to define a highly suggestive clinical and laboratory picture. What can hence be summarized so far from the existing documents is that diagnostic algorithms should encompass (at least) a complete blood cell count for obtaining the platelet number, along with assessment of plasma fibrinogen and D-dimer, eventually followed by anti-PF4-based ELISA, and perhaps reserving functional platelet assays to diagnostically challenging cases [21, 32, 43], or for research investigation.

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