Accepted Article

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Abstract

Vaccine campaigns are currently underway worldwide to combat the COVID-19 pandemic. The newly developed vaccines are highly effective with minimal adverse effects. Recently, the AstraZeneca vaccine has raised public alarm with concerns regarding the rare, but serious, development of thrombotic events, now known as vaccine-induced immune thrombotic thrombocytopenia (VITT). These thrombotic events appear similar to heparin-induced thrombocytopenia (HIT), both clinically and pathologically. In this manuscript, the ISTH SSC Subcommittee on Platelet Immunology outlines guidelines on how to recognize, diagnose and manage patients with VITT.

Key Words
COVID-19; AstraZeneca Vaccine; Thrombosis; Thrombocytopenia; Platelet activation
Introduction

The COVID-19 pandemic has resulted in significant morbidity and mortality worldwide.[1, 2] Clinically, critically ill COVID-19 patients develop coagulation abnormalities, leading to significant thrombosis and death.[3-6] Recent studies by Althaus et al., and Nazy et al., indicate that platelet activation by IgG-immune complexes can activate platelets in critically ill COVID-19 patients via platelet FcγRIIa.[7, 8]

COVID-19 vaccination campaigns with several vaccine types are currently underway. Recent reports from Europe have described patients who developed thrombosis and thrombocytopenia 5 – 20 days after administration of SARS-CoV-2 vaccine(s). These include rare thrombotic events such as cerebral sinus vein and splanchnic vein thrombosis (CSVT). This rare syndrome is known as vaccine-induced immune thrombotic thrombocytopenia (VITT).[9, 10] Although data available until now are limited, some clinical and laboratory features seem to be similar to those observed in patients with severe COVID-19 infection and in patients with autoimmune heparin induced thrombocytopenia (aHIT). The SSC Platelet Immunology felt that a brief communication paper could help physicians and laboratory staff to manage these cases.

Possible mechanisms:

Preliminary data suggest that VITT after AstraZeneca Vaccination has some clinical and serological similarities to heparin-induced thrombocytopenia (HIT) with high titre anti-PF4/heparin antibodies that cause platelet activation in functional assays. In addition, mRNA vaccines have been associated with severe thrombocytopenia and bleeding.[11] Early and limited studies have implicated an antibody-mediated platelet activation as the mechanism of the clotting
events. This situation requires immediate clinical recognition followed by confirmatory laboratory
diagnosis, using specialized tests.

**Recommendations for laboratory investigation**

Beside complete blood counting, prothrombin time, activated partial thromboplastin time,
fibrinogen, and D-Dimer, the following actions should be considered:

1. All samples for VITT testing should be collected into serum or plasma tubes based on the
   requirement of the testing facility, prior to the administration of any treatment, especially
   intravenous immunoglobulin (IVIg) and danaparoid. In addition, these samples should be
   conserved for future testing and to be used for improvement of testing and validation of
   newly developed assays.

2. A negative rapid immunoassay (RIA) such as particle centrifugation assay and
   Chemiluminescence Immunoassay (CLIA) may reveal false-negative results. All samples
   for VITT testing should be conserved until modified test methods are published.

3. The clinical picture should guide the management and laboratory investigation:

   a. Thrombocytopenia, bleeding, and normal coagulation parameters (normal aPTT,
      Quick, Fibrinogen and D-Dimer): this may indicate an immunisation associated
      ITP, and depending on bleeding risk, strong consideration is recommended for high
      dose IVIG and no anticoagulation,

         cytometry[15] including the presence of vaccine (where available).

   b. Thrombocytopenia and thrombosis: this may indicate a HIT-like syndrome (VITT),
      and management should be initiated with non-heparin anticoagulation upon
      suspicion, as per HIT syndrome. Testing should follow the algorithm below (where
available): if VITT testing is positive and diagnosis is confirmed, continue non-heparin anticoagulation with consideration for high dose IVIG.

d. Thrombocytopenia without bleeding or thrombosis but abnormal coagulation parameters (at least one of: aPTT, Quick, Fibrinogen and D-Dimer, especially with dynamic change): this may indicate an early VITT syndrome and consideration should be given to initiate thrombo-prophylaxis with non-heparin anticoagulation until the results of the confirmatory testing are available.

c. Thrombocytopenia without bleeding or thrombosis but abnormal coagulation parameters (at least one of: aPTT, Quick, Fibrinogen and D-Dimer, especially with dynamic change): this may indicate an early VITT syndrome and consideration should be given to initiate thrombo-prophylaxis with non-heparin anticoagulation until the results of the confirmatory testing are available.

d. Thrombocytopenia without bleeding or thrombosis and normal coagulation parameters: There is a potential for vaccine associated isolated thrombocytopenia. It is currently unclear whether these cases are induced by the vaccine such as DITP or by primary ITP. Monitor and manage as per local guidelines for thrombocytopenia, based on bleeding risk.

**VITT testing algorithm:**

Testing for VITT should begin with a binding assay (such as ELISA) to identify the presence of anti-PF4/polyanion antibodies.

a. If the binding assay is negative, this patient does not have HIT or a HIT-like VITT.

b. If the binding assay is positive (or not available), the sample should be tested in one or multiple HIT functional assays as available, such as the serotonin release assay (SRA)[16], heparin-induced platelet activation assay (HIPA)[17], platelet aggregation test (PAT)[18], heparin-induced multiple electrode aggregometry (HIMEA)[19], PF4-dependant P-Selectin expression assay (PEA)[20], PF4-
SRA[21], and PF4/heparin-SRA[22]. A positive result strongly suggests this person has VITT in the appropriate clinical context.

**Conclusion and future aspects**

Although VITT is an extremely rare event in the context of COVID-19 specific vaccinations, it can be associated with severe morbidity and mortality. Data are emerging regarding details on the clinical presentation and mechanism(s) leading to the disease, including PF4/heparin associated antibodies and potentially other immune complex related to platelet activation. To ensure these cases are recognized, diagnosed, and properly treated, the recommendations proposed here provide direction to allow clinicians and laboratories to perform initial testing currently known to aid in the diagnosis. It is important to mention that recommendations provided in this letter are made based on expert consensus and limited data on the pathophysiology of VITT. It is almost certain that an update will be needed once more data are available. It is important that samples are conserved for future testing once the pathophysiology of VITT is fully understood and novel assays are developed.

Future directions will focus on correlating clinical presentations with laboratory findings including an international surveillance registry for all VITT due to the various COVID-19 administered vaccines.

**Authors’ contributions**

All the authors designed the study, critically wrote the manuscript, and revised the intellectual content of the manuscript. All authors approved the version of the manuscript.

**Conflict-of-interest disclosure**

The authors declare no competing financial interests.
Figure 1: Recommendations for laboratory diagnosis and patient management following bleeding and/or thrombosis after vaccination with the AstraZeneca Vaccine for COVID-19.

REFERENCES:


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Recent Vaccination within the last 20 days

Laboratory Investigations:
- Platelet count, prothrombin time, partial thromboplastin time, fibrinogen, D-Dimer

<table>
<thead>
<tr>
<th>Low platelet count, Abnormal coagulation, with thrombosis</th>
<th>Low platelet count, Abnormal coagulation, No bleeding or thrombosis</th>
<th>Low platelet count, Normal coagulation parameters, with bleeding</th>
<th>Low platelet count, Normal coagulation parameters, No bleeding or thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VITT-Testing</strong></td>
<td><strong>Blood collection before therapy</strong></td>
<td><strong>Recommended laboratory methods:</strong></td>
<td><strong>Monitoring</strong></td>
</tr>
<tr>
<td><strong>Recommended laboratory methods:</strong></td>
<td></td>
<td>1. MAIPA, MACE, PIBA</td>
<td>Continuous monitoring of clinical and laboratory parameters and manage according to the local guidelines for thrombocytopenia</td>
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<tr>
<td>1. Antigen-binding assay (EUSA) for PF4/heparin antibodies: EUSA testing</td>
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<td>2. If available: with and without vaccine in the tests</td>
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<td>2. NOTE: rapid immunoassay (RiA), Chemiluminescence immunoassay (CLIA) may reveal false-negative results</td>
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<td>3. Functional platelet activation assay (SPA, HIPA, PAT, HTMA, PFA, PF4 SPA, PF4: HIPA and FF4/heparin-SRA) Anti-PF4/heparin-ELISA testing</td>
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<td><strong>Negative</strong></td>
<td><strong>Positive</strong></td>
<td><strong>Negative</strong></td>
<td><strong>Positive</strong></td>
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<tr>
<td>VITT unlikely</td>
<td>VITT likely</td>
<td>Consider other</td>
<td>Consider IVIG</td>
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<tr>
<td>- Rule out false-negative results</td>
<td>- functional platelet activation testing</td>
<td>Diagnoses</td>
<td>Check coagulation during treatment</td>
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<tr>
<td>- Anticoagulation with heparin possible</td>
<td>- Avoid anticoagulation with heparin</td>
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<tr>
<td>- Reevaluation of clinical symptoms</td>
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**Functional platelet activation testing**

**Negative**

- VITT unlikely
- Reevaluation of clinical symptoms

**Positive**

- VITT confirmed
- Non-heparin anticoagulation
- Consider high dose IVIG

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