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Efficacy and Mechanism Evaluation

Synopsis

Understanding mechanisms of thrombosis and thrombocytopenia with adenoviral SARS-CoV-2 vaccines: a comprehensive synopsis

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Published July 2025
DOI: 10.3310/FFSS9010
Volume 12 • Issue 7

Abstract

Background: Thrombosis with thrombocytopenia syndrome is a rare condition known to occur spontaneously or after heparin use. With the advent of COVID-19 vaccines during the pandemic, thrombosis with thrombocytopenia syndrome cases emerged post administration of adenoviral vaccines, termed vaccine-induced immune thrombosis and thrombocytopenia. In response, the thrombosis with thrombocytopenia syndrome consortium was formed to deepen our understanding of this syndrome post vaccination.

Methods: The consortium employed a comprehensive approach across five work packages. This included designing cohort studies covering the entire English population and analysing local linked regional data sets to detect thrombosis with thrombocytopenia syndrome occurrences in real time. Various patient and healthy control specimens, including those from vaccinated individuals, underwent testing for antiplatelet factor 4 antibodies using three different assays. Patients who developed vaccine-induced immune thrombosis and thrombocytopenia after the AstraZeneca (AZD1222) COVID-19 vaccine underwent whole-genome and ribonucleic acid sequencing to identify genetic susceptibility factors. Multiple studies were conducted to investigate the mechanism of antiplatelet factor 4 antibody formation, including assessments of adenoviral vector structure and binding to platelet factor 4. Detailed studies were also conducted to understand the immune response to vaccines, the role of immune complexes involving platelet factor 4 and their effects on proinflammatory cytokines, neutrophil extracellular traps and platelets in the pathogenesis of the syndrome.

Results: Cohort studies revealed a higher risk of arterial and venous thromboses after COVID-19 infection compared to vaccination. Specifically, regarding vaccines, the risk of thrombosis and/or thrombocytopenia was higher after the first dose of the AZD1222 vaccine but not with subsequent doses of. Regional linked data indicated that real-time ascertainment of diseases across multiple acute hospital sites' secure data environments is not yet feasible at scale. The overall background seroprevalence of antiplatelet factor 4 antibodies was low in healthy individuals, vaccinated individuals and those infected with COVID-19. Whole-genome sequencing did not identify significant variants predisposing to vaccine-induced immune thrombosis and thrombocytopenia, with ongoing work on ribonucleic acid sequencing. An electrostatic interaction between the hexon hypervariable regions of the ChAdOx1 capsid and platelet factor 4 was suggested as a possible mechanism for antiplatelet factor 4 antibody development. Strong immune response drove the formation of neutrophil extracellular traps, significant inflammatory responses and clot formation in distant organs. Platelet activation post immune complex formation against platelet factor 4 was dependent on FcγRIIa but independent of complement, also occurring through binding with c-Mpl. T-cell reactivity against the AZD1222 vaccine indicates potential cross-reactivity with prevalent human adenoviruses.
Conclusions: The consortium's comprehensive work has uncovered new potential mechanisms of vaccine-induced immune thrombosis and thrombocytopenia and identified novel biomarkers and therapeutic strategies for further development and validation. This is crucial, as the combination of thrombosis and thrombocytopenia, alongside antiplatelet factor 4 antibodies, can occur without exposure to heparin or adenovirus vaccines.
Future considerations: Recommendations include the development of a national reference laboratory and registry for diagnosis and further study of thrombosis with thrombocytopenia syndrome; future vaccine development using the adenoviral vector platform to focus on the reduction of the electrostatic interaction between viral hexons and platelet factor 4; international genomics collaboration; and studies focused on understanding the symptoms suffered by patients as well as strategies to ameliorate them.
Limitations: Direct identification of vaccine-induced immune thrombosis and thrombocytopenia patients was hindered by poor recording. The rarity of vaccine-induced immune thrombosis and thrombocytopenia limited the number of patients recruited for genomic and mechanistic studies.
Funding: This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme as award number NIHR135073.
A plain language summary of this synopsis is available on the NIHR Journals Library Website https://doi.org/10.3310/FFSS9010.

Introduction

Rationale for research and background

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the subsequent COVID-19 pandemic have presented a multitude of challenges. To date, the death toll globally from the pandemic exceeds 7 million.[1] COVID-19 infection has been associated not only with respiratory manifestations but also with complications affecting other systems, including long COVID, which has added to the considerable morbidity associated with the emergence of SARS-CoV-2.[2]

A manifestation of COVID-19 infection was thrombotic events, occurring during or soon after infection.[3–5] With the authorisation and mass deployment of vaccines from December 2020, reports of thrombosis temporally related to the administration of COVID-19 vaccines began to appear.[6–8] While the benefits of COVID-19 vaccines in preventing severe illness and death are well established,[9] concerns regarding rare but serious side effects caused a lot of concern among the public. Furthermore, given that there is a background incidence of thrombotic events in the population, and COVID-19 infection itself is also associated with thrombosis,[3] the determination of whether the vaccines also caused thrombosis became quite complex. Therefore, understanding the nature and extent of the thrombotic risks of COVID-19 and its vaccines as well as the pathophysiological mechanisms underlying these is crucial for ensuring public trust in vaccination programmes and tailoring strategies for individual patient care in the future.

Thrombosis with thrombocytopenia syndrome in COVID-19

COVID-19 is known to be associated with excessive inflammation and thrombosis.[3] The latter occurs in up to 60% of severely ill patients treated on intensive care units.[3] Markers of thrombosis (such as D-dimer) and inflammatory markers (such as C-reactive protein) are independently associated with an increased risk of respiratory failure and death from COVID-19 as well as with an increased risk of thrombosis.[3–5] Both venous and arterial macrothrombosis are common in patients with acute COVID-19 infection, with the former being more common.[10,11] Venous and arterial microthrombi and endothelial cell damage are frequently present at autopsy from those who have died of COVID-19.[12–14]

These microthrombi are likely to be a result of disseminated intravascular coagulation (DIC) or thrombotic microangiopathy (TMA). As well as thrombotic markers, markers of consumptive coagulopathy and DIC, such as low fibrinogen and high fibrin degradation products, predict non-survival in acute COVID-19 infection.[15] Low platelet counts also independently predict poor survival.[16] The pathophysiology behind thrombocytopenia in COVID is generally felt to be due to DIC. However, other causes have been postulated to be antiphospholipid syndrome,[17] immune thrombocytopenia,[18] hemophagocytic lymphohistiocytosis,[19,20] heparin-induced thrombocytopenia (HIT)[21,22] and pseudothrombocytopenia caused by antibody-mediated platelet clumping.[23]

Patients present with infection and high D-dimers and normal fibrinogen levels, but then these abruptly drop 7–10 days after admission – this is an independent predictor of mortality.[12,15] Levels of these markers are consistent with a change from a hypo- to a hyperfibrinolytic DIC.[24] Indeed, as well as thrombosis, bleeding has been noted in autopsy samples from patients who have died from COVID-19. While the role for thromboprophylaxis with low-dose anticoagulants, such as low-molecular-weight heparin, is well established,[25] neither therapeutic anticoagulation nor antiplatelet medications improve clinical outcomes, such as survival or reduction of organ support over prophylactic anticoagulation when treating patients with acute COVID-19.[26,27]

Thrombosis with thrombocytopenia syndrome following vaccination for COVID-19 with adenoviral vector vaccines

In February and March 2021, rare cases of patients with life-threatening thrombosis and thrombocytopenia began to emerge in the UK and other European countries.[6–8] This was recognised to be linked to the first injection of the adenoviral-based SARS-CoV-2 vaccine produced by AstraZeneca in association with the University of Oxford (AZD1222). This syndrome was later shown to be also associated with injection of the adenoviral-based SARS-CoV-2 vaccine (Ad26.COV2.S) produced by Johnson & Johnson (Brunswick, NJ, USA).[28] The formation of antibodies to the chemokine platelet factor 4 (PF4), as detected by enzyme-linked immunosorbent assay (ELISA), was found to be a hallmark of the syndrome, being adopted early on as being an essential component of the case definition.[6–8] The clinical symptoms and timing of onset (5–30 days after a trigger) bore a strong resemblance to heparin-induced thrombocytopenia with thrombosis (HITT), which is known to be caused by immune complexes to PF4 bound to heparin, but with the distinction that patients had not been exposed to heparin and that the thrombosis occurred in unusual places, including the cerebral venous sinus. This was later recognised as a single condition and has been named vaccine-induced immune thrombosis and thrombocytopenia (VITT). The molecular trigger for the thrombosis in both HIT and VITT is the activation of the low-affinity immunoglobulin gamma Fc region receptor II-a (FcγRIIa) on platelets and neutrophils leading to platelet aggregation and release of neutrophil extracellular traps (NETs), respectively, and formation of occlusive thrombi.[29,30] The mortality associated with VITT initially approached 50%.[31] Thanks to increasing awareness of the condition following widespread dissemination of information, more recent case series show a reduction in mortality to 5%.[32] However, those who survive can be left with significant neurocognitive disability.

It was unclear whether these PF4 antibodies were pre-existing or occurring de novo in patients with VITT as a result of adenovirus vaccine exposure with aberrant antivector immunity. Reported background rates of PF4 antibodies in different populations vary widely and are thought to be partly dependent on the laboratory testing method used. Large studies and meta-analyses have reported background rates of 1.0–6.6%.[33,34] Many of these study participants only had weakly positive antibody titres, however, and the rates of highly positive PF4 antibodies were only 0.3%.[33]

Thrombosis with thrombocytopenia syndrome following vaccination for COVID-19 with messenger ribonucleic acid-based vaccines

In addition to thrombosis with thrombocytopenia syndrome (TTS) post adenoviral vector vaccines, the separate incidences of thrombosis and thrombocytopenia post any COVID vaccine has been studied to a limited degree. In a study using routinely collected healthcare data from European and North American databases, there was a trend towards a lower risk of TTS following BNT162b2 [Pfizer-BioNTech (Pfizer Inc., New York, NY, USA) COVID-19 vaccine] than with Ad26.COV2.S.[35] However, this is difficult to contextualise, as it was not compared to the risk of TTS following COVID infection.

Population-level identification of patients with thrombosis with thrombocytopenia syndrome

National Health Service data, including disease diagnoses, are often used for large-scale cohort studies. However, there are delays between data being provided to NHS England and provision of data to researchers; this lag-time makes analyses in emergencies difficult. Data from NHS England (and similarly NHS Scotland) have the advantage of relatively easy access to whole population data; however, there are limitations, including (1) limited disease

phenotyping with uncertain accuracy; (2) delays between collection and provision of data; and (3) lack of data availability until hospital admission. Hospital electronic health record systems contain detailed information about diseases, including text and image data generated by clinicians, laboratory and radiology systems. Rapid access to accurately coded clinical data, using widely adopted systems like systematized nomenclature of medicine clinical terms (SNOMED CT), could enable the timely identification of both new and existing diseases on a larger scale and at a lower cost compared to traditional methods, such as clinical reporting systems, which depend on clinician time and motivation.

Long COVID is possibly associated with thrombosis

More recently, heterogeneous long-term sequelae following COVID-19 infection (termed 'long COVID') have begun to emerge.[36] This condition shows striking similarity to chronic conditions known to be associated with viral infections such as myalgic encephalomyelitis/chronic fatigue syndrome.[36] Patients who were more severely ill with COVID-19 infection are more likely to develop long COVID.[36] Studies from South Africa have claimed that the severity of long COVID symptoms correlates with the presence and number of heterogeneous protein aggregates in the blood which have been termed 'microclots'.[37] The clinical significance of these microclots is still hotly debated. It is forming the basis of trials of anticoagulant and antiplatelet therapy in patients with long COVID.[38,39] However, it is clear that vaccination against COVID-19 reduces the incidence of long COVID as well as reducing the rate and severity of COVID-19 infection itself.[36,40,41]

Objectives

Given the uncertainties around causality and pathogenesis of thrombosis together with thrombocytopenia occurring in association with the adenoviral vaccines, the TTS consortium was established to understand more about the incidence, pathophysiology and risk factors for developing thrombosis and thrombocytopenia after COVID-19 vaccination. Integral to this was also developing a greater understanding of the incidence of thrombosis and thrombocytopenia after COVID-19 infection itself and how this is influenced by vaccination. The main aim of the work was to understand why a very small number of those vaccinated against COVID-19 develop blood clotting disorders and to understand the changes in the body that lead to the unique combination of blood clots and low platelet count seen in TTS. To achieve this, the work was split into five work packages with specific aims and objectives of:

Aim 1. To evaluate SARS-CoV-2 vaccine safety using real-world epidemiology data (work package 1)

- Establish background rates of thrombotic, thrombocytopenia and combined disorders in general population.
- Evaluate association between SARS-CoV-2 infection and thrombotic disorders (venous and arterial, including rare disorders such as intracranial venous thrombosis, portal vein thrombosis and splanchnic/mesenteric vein thrombosis) with/without thrombocytopenia.
- Evaluate the association between SARS-CoV-2 vaccination (stratified by which vaccine and first/second dose) and thrombotic disorders with/without thrombocytopenia (as above).

Aim 2. To understand the prevalence of anti-platelet factor 4 antibody positivity (work package 2)

- Measure the background rate of PF4 antibody in healthy, unvaccinated individuals.
- Measure the prevalence of anti-PF4 antibodies in vaccinated individuals and after COVID-19 infection.
- Compare the utility of different anti-PF4 assays.

Aim 3. To investigate the genomics of thrombosis and thrombocytopenia related to COVID-19 and SARS-CoV-2 vaccines [work package 3: fully funded by Genomics England]

- Determine any genomic factors influencing the risk of developing severe COVID-19 with clotting disorders.
- Investigate genomic factors influencing the risk of developing thrombotic thrombocytopenia associated with the vaccines.

Aim 4. To understand the relationship between the immune response initiated by COVID-19, COVID-19 vaccines and haemostatic dysfunction (work package 4)

- Establish the role of immune complexes in thrombotic thrombocytopenia and characterise binding interfaces to inform vaccine design.
- Investigate the role and mechanisms of NETs in thrombotic thrombocytopenia.

- Investigate the responses of T cells and memory B cells to different vaccines and vectors.
- Evaluate tissue immune cells and endothelial cell activation in response to vaccines and vectors.
- Develop clinical immunoassays for autoantibodies, immune complexes and their correlation to thrombosis and thrombocytopenia.

Aim 5. To develop in vitro platelet models to understand mechanisms of vaccine-associated adverse events, and identify potential therapeutic interventions (work package 5)

- Investigate platelet activation by patient sera and inhibition by antiplatelet and other agents.
- Understand the mechanism of platelet activation by patient sera.
- Investigate the variation in responsiveness of donor platelets to patient sera.
- Investigate biomarkers of platelet activation.
- Analyse the histology of thrombi in post-mortem tissue.
- Investigate the effect of adenoviruses on macrophages and endothelial cells and their interaction with platelets.

Methods

A summary of the methods for each work package is provided below. More detailed information can be found in the papers published by each work package.

Work package 1

Incidence and associated adjusted hazard ratios (aHRs) of arterial and venous thromboses and thrombocytopenia were estimated by comparing people before (or without) vaccination or infection with people afterwards. Large-scale, linked health system data were used. This was provided by NHS England and primary care data controllers through the cardiovascular disease-COVID-UK consortium (supported by the British Heart Foundation Data Science Centre) and accessed through the NHS England Trusted Research Environment.[42]

Cohorts with the entire adult (≥ 18 years) English and Welsh population registered with a general practitioner (GP) on 1 January 2020 (45.7 million individuals) were designed by linking NHS hospital admissions (Hospital Episode Statistics and Secondary Uses Service data from 1997 onwards), COVID-19 laboratory testing data, COVID-19 vaccination data (NHS England Immunisation Management System), national community drug dispensing data (NHS Business Services and Administration dispensed medicines from 2018) and death registrations. aHRs associated with vaccination and infection were calculated. Self-controlled case series (SCCS) of all cases of acute myocardial infarction (MI) and myocarditis from 1 January 2020 were also created with the same data to estimate aHRs for associations between COVID-19 vaccinations and these events.

A collaborative group was convened across five regional acute hospital-based secure data environments (SDEs) to conduct analyses using local data: Barts Health NHS Trust (North East London), Combined Intelligence for Population Health Action (CIPHA) (North West England), iCARE/WSIC (North West London), PIONEER Data Hub (Birmingham) and DataLoch (South East Scotland).

Further methodology, including details on handling of potential confounders and sensitivity analyses, can be found in Ip *et al.*[43] and Whiteley *et al.*[44]

Work package 2

Source of specimens

Specimens from patients with VITT were identified from 33 different hospital trusts; adult patients from England (age range 21–75) with suspected VITT were identified by the Expert Haematology Panel between March and June 2021, and serum samples were sent to the Virus Reference Department at Colindale of the UK Health Security Agency (UKHSA), formerly Public Health England (PHE) for COVID serology testing as part of the early investigation of suspected patients.[45] These were compared with three panels of non-VITT controls. To assess the background prevalence of anti-PF4 among healthy unvaccinated individuals without exposure to SARS-CoV-2 infection, residual sera from participating laboratories collected in 2019 as part of the routine PHE sero-epidemiology programme, and therefore before the COVID pandemic and COVID vaccination programme, were selected (pre-COVID healthy individuals). This sero-epidemiology programme has been collecting anonymised residual serum from NHS and public health laboratories across England since 1986–7 and has primarily been used to monitor the impact of the national vaccination programme by assessing age-specific immunity in the population.

The second non-VITT panel were blood donor samples from individuals with evidence of prior COVID infection, but who were not vaccinated. These were identified through the testing of donor samples collected prior to the roll-out of the COVID vaccination programme. From the start of the pandemic, approximately 2000 geographically distributed samples had been collected

each week and tested for SARS-CoV-2 nucleoprotein (*N*) antibody using the Roche assay (Elecsys Anti-SARS-CoV-2 total antibody assay, Roche Diagnostics, Basel, Switzerland),[46] to monitor population exposure by age and region over time. Over 1800 samples from the pre-vaccine period that tested positive for *N* antibody, distributed across age groups and by ethnicity, were selected for testing in this study.

The third non-VITT panel was COVID vaccinated controls. Postvaccination serum samples were obtained by UKHSA in collaboration with the Royal College of General Practitioners (RCGP) Research and Surveillance Centre (RSC) sentinel network of GP practices across England. As part of the UKHSA COVID sero-surveillance programme, an additional sample is collected from patients attending primary care for routine blood testing and can be linked to clinical (including details and timing of COVID vaccinations) and relevant demographic details.[47] The majority of samples collected through this network were from individuals who had received only AstraZeneca (AZD1222) vaccine or Pfizer-BioNTech (BNT162b2) vaccine with a smaller number who had received AstraZeneca vaccine followed by either Pfizer or Moderna messenger ribonucleic acid (mRNA) vaccine.

Assays

LIFECODES PF4 immunoglobulin G (IgG) (Immucor, GTI diagnostics, Waukesha, WI, USA) assays were purchased from the manufacturer, and testing was performed on all samples used according to the manufacturer's instructions. Results were considered positive if the optical density (OD) was ≥ 0.400, as recommended by the manufacturer. In addition, a strongly positive cut-off of OD ≥ 1.0 was used for further analyses.

A subset of approximately 200 control samples were tested using two alternative PF4 assays: Zymutest heparin-induced antibodies (HIA), (Cat. no. RK040A, Quadratech, United Kingdom) and Asserachrom (heparin-induced platelet-activation assay) HPIA-IgG (Cat. no. 00624, Stago, Germany). A third assay, Aeskulisa HiT IgG (Cat. no. 3290, Aesku, Australia) was planned to be tested but was unavailable at the time of testing. These alternative assays are designed for the detection of HIT, and specifically have heparin on the solid phase of the plate. Results for the alternative assays were interpreted according to the manufacturers' instructions. For Zymutest HIA, an OD of ≤ 0.30 and > 0.50 was considered negative and positive, respectively. In addition, an OD between > 0.30 and ≤ 0.50 was considered weakly positive. For Asserachrom HPIA-IgG, an OD of < 0.20 and ≥ 0.20 was considered negative and positive, respectively. Approximately 100 control samples were also retested with Immucor to understand intra-assay consistency. The reported sensitivity and specificity of the Immucor, Zymutest and Asserachrom for HIT are 99.6% and 89.9%; 99.2% and 85.8%; and 72% and 93.8%, respectively.[48]

Samples from patients with possible VITT were also tested for Roche *N* antibodies, as a marker of SARS-CoV-2 infection, and for spike protein antibodies, which could be infection- or vaccine-derived (Elecsys Anti-SARS-CoV-2 spike total antibody assay, Roche Diagnostics: positive ≥ 0.8 arbitrary units/ml) to assess vaccine response. The COVID vaccinated controls were also tested for Roche *N* antibody.[49]

Statistical analysis

For each panel, the seroprevalence (the proportion of samples tested positive) of PF4 IgG was determined using the Immucor assay at the two different cut-offs (≥ 0.4 and ≥ 1.0). The distribution of the Immucor was visualised using violin plots, and the geometric mean ODs [and 95% confidence interval (CI)] were estimated for each panel and compared using the Wilcoxon test. Subgroup analysis of the Immucor anti-PF4 IgG seroprevalence and OD in COVID vaccinated controls and suspected VITT was undertaken at various intervals between sampling and vaccination. The Immucor results were compared with the alternative assays (Zymutest and Asserachrom), and a Spearman test of correlation undertaken on the ODs from the three assays.

Work package 3

All cases and controls were consented for longitudinal life course follow-up, including baseline data and all NHS Digital Data. Participants with TTS were enrolled via NHS clinicians. As TTS may potentially be a rare disease, the Genomics England (GEL) consent was utilised and the Real-time Assessment of Community Transmission (REACT) consent was adapted to allow inclusion as controls. GEL or the REACT study oversaw consent, bio-sampling, nucleic acid extraction and quality assurance.

From across the UK NHS, we identified 102 cases, where accompanying deoxyribonucleic acid (DNA) was available, with 91 definitely matching TTS criteria (defined as unusual clot formation post AZD1222 vaccination and PF4 antibody positive). We used a super control design by including 500 REACT controls who were age, gender, ethnicity and vaccine status matched. A super control ratio of 5 : 1 was used, meaning we have 500 controls who have whole-genome sequencing (WGS), total ribonucleic acid (RNA) sequencing and deep immune cell repertoire sequencing.

100,000 Genomes Project and mild and asymptomatic controls

There were 44,000 100KGP (100,000 Genomes Project) controls with WGS available as population health controls and 14,000 mild or asymptomatic controls. This scale of controls was needed to evaluate rare variants using gene burden testing.

Genomics and transcriptomics quality assurance analysis

After DNA and RNA extraction at UK Biocentre and at Affinity Laboratories followed by standardised quality assurance, samples were transferred to Illumina Granta Park for WGS via the GEL Sequencing and Bioinformatics pipeline. WGS (30x read depth) was undertaken with alignment and variant calling using Dynamic Read Analysis for GENomics (DRAGEN) software (version 3.2.22) for both cases and controls. Quality assurance of all WGS was performed using the GEL pipeline. The WGS variant calling files are available in the GEL Cloud Research Environment.

After RNA extraction, 85 cases and 500 controls underwent total RNA sequencing at Illumina Cambridge. Deep immune cell repertoire sequencing of the T- and B-cell receptor regions was undertaken in Illumina Foster City.

Quality assurance

Sequencing data quality control: all genome sequencing data were required to meet more than 85×10^{-9} bases with Q ≥ 30, and at least 95% of the autosomal genome covered at 15 × or higher calculated from reads with mapping quality > 10.

Whole-genome sequencing alignment and variant calling: all sequencing data alignment and variant calling were performed with GEL pipeline 2.0, which used DRAGEN software (version 3.2.22). Alignment was performed to genome reference GRCh38, including decoy contigs and alternative haplotypes.

Aggregation: this was conducted separately for the 100KGP controls (X Ten) and the VITT and mild control samples analysed (mild cohort, cancer-realigned 100KGP) and those analysed with the Illumina North Star Version 4 pipeline (100KGP).

Genome-wide association study (GWAS) analyses variant quality control: we restricted all GWAS analyses to common variants applying the following filters using PLINK software (version 1.9): minor allele frequency (MAF) > 0 in both cases and controls, MAF > 0.5% and minor allele count > 20, missingness 0.1% in both aggregates.

Model: we used a two-step logistic mixed-model regression approach as implemented in Scalable and Accurate Implementation of Generalized mixed-model (SAIGE; version 0.44.5); The SAIGE Development Team; 2024; http://www.sagemath.org) for single-variant association analyses. We used the high-quality common variant sites for fitting the null model and sex, age, age2, age-by-sex and 20 principal components (PCs) as covariates in step 1. The PCs were computed separately by predicted genetic ancestry.

Multiple testing correction: we calculated the *p-value* significance threshold by estimating the effective number of tests with the Bonferroni-corrected *p-value* threshold as 0.05 divided by the number of linkage disequilibrium (LD)-pruned variants tested setting genome-wide significance *p-value* at 2.2×10^{-8}.

Functional annotation of credible sets: we annotated all variants included in each credible set identified by SusieR using the online Variant Effect Predictor, version 104 (https://www.ensembl.org/vep) and selected the worst consequence across GENCODE basic transcripts.

Transcriptome-wide association analysis: to infer the effect of genetically determined variation in gene expression on disease susceptibility, we performed a transcriptome-wide association study (TWAS) using gene expression data (GTEx version 8; https://www.gtexportal.org/home/) in the MetaXcan (https://github.com/hakyimlab/MetaXcan) framework and the GTEx version 8 expression quantitative trait loci and splicing quantitative trait loci MASHR-M models available for download in http://predictdb.org/. We focused the TWASs on whole blood with the S-PrediXcan function and applied Bonferroni correction to the results to choose significant genes and introns for each analysis.

Enrichment analysis: enrichment analysis was performed to identify ontologies in which discovery genes were over-represented in transcriptome-wide association analysis. This generated a *p-value* and false-discovery rate (FDR) for over-representation of genes within each of the ontologies.

Human leukocyte antigen (HLA) imputation and association analysis: HLA types were imputed at two-field (four-digit) resolution for all samples within the aggregate for the following seven loci: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1 and HLA-DPB1, using the HIBAG package in R15 (https://github.com/zhengxwen/HIBAG).

Gene burden testing: to assess the contribution of rare variants to TTS, we performed gene-based analysis using Optimal Sequence Kernel Association Test (SKAT-O) in SAIGE-GENE for which the genome sequencing data was processed with the same alignment and variant calling pipeline. We tested the burden of rare (MAF < 0.5%) variants considering the predicted variant consequence type and assessed the burden using a strict definition for damaging variants (high-confidence putative loss-of-function variants).

Work package 4

Sources of samples

Vaccine-naive peripheral blood mononuclear cells (PBMCs) were accessed through the Liverpool healthy donor biobank. Additional information on sample sources can be found in Abrams *et al.*[50]

As for samples in work package 2, vector immunity work samples from VITT patients (*n* = 64) were obtained from the Virus Reference Department at Colindale of the UKHSA, formerly PHE. The samples were de-identified and supplied with no additional information, so it is unclear when in the illness samples were taken and what the age and gender of the patients were. All, however, were in receipt of the AZD1222 vaccine, although the interval between vaccination and clinical presentation for this group varied between 5 and 30 days. Controls collected by the University College London (UCL) lab consisted of serum obtained from healthy individuals vaccinated with AZD1222 taken 4 weeks after the first vaccine dose (*n* = 16) as well as samples taken 4 weeks after healthy recipients received a first dose of Pfizer (*n* = 10) or Johnson & Johnson (*n* = 10) SARS-CoV-2 vaccines. These control cohorts are described in detail elsewhere.[51]

Reagents and assays

Information on reagents used can be found in Baker *et al.*,[52] Abrams *et al.*[50] and Gardner *et al.*[53] Methods for solving the structure of ChAdOx1, predicting PF4 binding, obtaining full kinetic binding affinities between ChAdOx1/Ad26 and PF4, performing surface plasmon resonance (SPR), NETosis assays, measurement of proinflammatory cytokines, coagulation and markers of T-cell and endothelial activation as well as immunofluorescent staining of histological samples can be found in the *Report Supplementary Material 1*. Methods for measuring T-cell stimulation following AZD1222, ChAdOx1-spike protein, Ad26 and BNT162b2 exposure are detailed in Ewer *et al.*[54] and Gardner *et al.*[53]

Antivector immunity studies were performed using a protein microarray containing adenovirus proteins that was developed specifically for this purpose by Antigen Discovery Inc. (Irvine, CA, USA). This incorporated proteins from synthesised chimpanzee adenovirus Y25 (*n* = 31) and human adenovirus 26 (Ad26, *n* = 22) genes as well as cloned Ad4 and Ad5 genes (*n* = 43 and 39, respectively), Ad40 and Ad41 libraries (*n* = 36 and 34, respectively) and SARS-CoV-2 spike and *N* (a total of 207 proteins).

Work package 5

Sources of samples

Details of sample sources and ethical approval for sample collection can be found in Smith *et al.*,[55] Montague *et al.*[56] and Buka *et al.*[57] All patients presenting with VITT at University Hospitals Birmingham NHS Foundation Trust who survived to discharge were recruited. Blood samples were collected from 11 patients with VITT at or close to the time of diagnosis. In addition, follow-up samples from the seven surviving patients were taken over a period of 18 months with samples donated every 4–6 weeks. Informed consent was provided by the patients or next of kin in those who lacked capacity. All AZD1222-vaccinated control samples were from 4 to 30 days following first dose of vaccine. All studies were performed in line with the Declaration of Helsinki.

Reagents and assays

Information on reagents used can also be found in Smith *et al.*,[55] Montague *et al.*[56] and Buka *et al.*[57] Details of methods for light transmission aggregometry and flow cytometry assays can be found in the *Report Supplementary Material 1* and in Smith *et al.*[55] and Montague *et al.*[56] Details of platelet receptor quantification can be found in Montague *et al.*[56] Details of PF4 and anti-PF4 antibody measurement can be found in the *Report Supplementary Material 1*. Details of methods for Western blotting to look at tyrosine phosphorylation upon platelet activation with serum, purified antibody and PF4 can be found in Buka *et al.*[57] Details on purification of IgG antibodies from patients with VITT can be found in Buka *et al.*[57]

Results summary

Work package	Key result	Reference
1	Association between first dose (but not second dose) AZD1222 and CVST and/or thrombocytopenia confirmed at population level (aHR 2–5.9)	Whiteley *et al.* 2022[43]

Work package	Key result	Reference
1	It is not yet feasible to identify VITT in real time at a regional level using existing systems of linked health data	HDR-UK[58]
2	Prevalence of anti-PF4 antibodies in the (pre-vaccinated) general population was low, and this was not increased post AZD1222/BNT162b2 in those who did not develop VITT	Detailed in this report
2	The Immucor ELISA was more sensitive at detecting serum anti-PF4 antibodies than both the Zymutest (Quadratech) and Asserachrom (Stago) ELISAs	Detailed in this report
3	GWAS did not find any genetic variants that were associated with the development of VITT	Detailed in this report
4	The levels of antiadenoviral antibodies in patients with VITT were no higher than in healthy individuals exposed to AZD1222	Detailed in this report
4	T-cell responses following exposure to AZD1222 were unexpectedly strong, even in pre-pandemic vaccine-naive individuals	Gardner *et al.*[53]
4	The hexon hypervariable regions (HVRs) of ChAdOx1 were identified as binding with high affinity to PF4. This was much less marked with the HVRs of Ad26	Baker *et al.*[52]
4	Serum from patients with VITT strongly activated NETs and degree of NETosis predicts mortality	Abrams *et al.*[50]
5	AZD1222 did not activate, but serum from patients with VITT strongly activated platelets via FcγRIIa and could be blocked by intravenous immunoglobulin (IVIg) and antiplatelet drugs	Smith *et al.*[55]
5	Platelet activating anti-PF4 antibodies in patients with VITT persisted for many months	Montague *et al.*[56]
5	There was significant variation in platelet reactivity to VITT serum between different healthy individuals	Montague *et al.*[56]
5	PF4 itself activated platelets via the thrombopoietin receptor c-MPL. This was blocked by Janus kinase 2 (JAK2) inhibitors	Buka *et al.*[57]

Ad, adenovirus.

Work package 1

Evaluating SARS-CoV-2 vaccine safety using population-level linked data

Firstly, the association between COVID-19 vaccines and thrombocytopenia, venous and arterial thromboses in different time periods after vaccination for different vaccine products was estimated. VITT could not be directly measured because this was not reliably recorded. Data were analysed very early in the pandemic (before VITT was widely known) and more recently.[43,44] Soon after first AZD1222 vaccine, the incidence of cerebral venous sinus thrombosis (CVST) was higher than before or without vaccine (aHR 2.27, 95% CI 1.33 to 3.88) in people 18–40 years in our earlier analysis and in the whole population in our later analysis (aHR 5.92, 95% CI 4.07 to 8.63). There was no increased risk of CVST after second or subsequent vaccinations with AZD1222, or with any dose for other vaccine brands. The incidence of thrombocytopenia was also higher after first dose of AZD1222 (aHR 2.27, 95% CI 1.33 to 3.88), with a similar estimate in the later analysis (aHR 2.07, 95% CI 1.67 to 2.58), but not generally after receiving other types of COVID vaccines. However, the incidence of both common arterial and venous thromboses was lower after all types of vaccination than before or without vaccination.

Secondly, the association between COVID-19 infection and risk of venous and arterial thromboses was evaluated.[59] There was a markedly higher aHR in the week following infection for arterial [21.7 (95% CI 21.0 to 22.4)] thrombosis. This persisted but fell to an aHR of 1.34 (95% CI 1.21 to 1.48) in weeks 27–49. For venous thromboses, these same aHRs were 33.2 (95% CI 31.3 to 35.2) up to 1 week, and 1.80 (95% CI 1.50 to 2.17) in weeks 27–49 following infection. The aHRs were higher for hospitalised infection, and for people of black or Asian ethnicity. The estimated whole-population increases in risk of arterial thromboses and venous thromboembolism (VTE) 49 weeks after COVID-19 diagnosis were 0.5% and 0.25%, respectively, corresponding to 7200 and 3500 additional events, respectively, after 1.4 million COVID-19 diagnoses.

Thirdly, because COVID-19 infection was known to worsen maternal outcomes, these analyses were repeated in pregnant women.[60] The incidence of common (preterm, gestational hypertensive, gestational diabetes, small for gestational age) and rare (pre-eclampsia, venous thrombotic events) adverse pregnancy outcomes were higher after COVID-19 infection, compared with before or without COVID-19. These risks attenuated with time after COVID-19 diagnosis, after the vaccination roll-out, and in

the first and second trimester of pregnancy. No evidence of higher risks of adverse outcomes following any vaccine type was found, regardless of timing of administration during pregnancy.

These analyses were also repeated in children and young people under the age of 18. COVID-19 infection was associated with higher risks of VTE (aHR 2.84, 95% CI 2.33 to 3.46), thrombocytopenia (2.08, 1.47 to 2.94), myocarditis/pericarditis (1.95, 1.35 to 2.81) and inflammatory conditions (7.51, 6.02 to 9.35) in the first 4 weeks following infection. We examined inflammatory conditions in children to cover the condition 'Multisystem inflammatory syndrome in children (MIS-C)', a complication of COVID-19 infection,[61] which at the beginning of the period had no specific code. These aHRs remained elevated for a further 6 months. In children/young people, a higher incidence of myocarditis/pericarditis in the 4 weeks following the first BNT162b2 vaccine was found (1.84, 1.25 to 2.72).

The results from the cohort studies and SCCS were directly compared, and only very small differences in the magnitude and direction of the association were found between the SCCS- and cohort-estimated associations for COVID-19 vaccination and subsequent acute MI and myocarditis (statistical analysis not performed). Used in conjunction, these two analysis methodologies provide triangulated, convergent evidence.

Evaluating SARS-CoV-2 vaccine safety using regional linked health data

To try and identify VITT in real time but on a large scale, regional linked health data were examined to define the population of interest as people arriving in the emergency department from 5 to 30 days after vaccination between 8 December 2020 and 1 May 2022. Classification of VITT cases has been previously defined.[45] The term 'algorithmically definite' VITT was used for patients who matched all criteria for definite VITT (*Table 1*). The term 'clinically confirmed' VITT was used for those algorithmically identified patients when additional information was obtained from direct clinician review of the clinical notes; VITT was then defined as definite, probable or possible (see *Table 1*). It was not possible to aggregate results between local health systems, because the available data sources and time periods of analyses were different.

Data provision delays led to the exclusion of some data sources. From sources covering a population of approximately 8.5 million people, three clinically confirmed cases of VITT were identified using a combined SNOMED and laboratory data algorithm. The same three cases were also detected using an *International Statistical Classification of Diseases and Related Health Problems*, Tenth Revision (ICD-10) code-only algorithm, simulating data typically available in national NHS data sets. An algorithmic definition for 'definite, probable, or possible VITT', based on near real-time data from point-of-care hospital coding (e.g. SNOMED emergency care data set ± SNOMED in hospitals, laboratory and vaccination data), demonstrated either modest precision (in Barts Health) or excellent precision (in WSIC/iCARE) in detecting clinician diagnoses of 'definite, probable, or possible VITT'. Although applied on a relatively small scale across four SDEs, this shows that for certain conditions, like rare VITT, it is possible to generate automated alerts for potential cases, enabling timely clinical follow-up that might otherwise be delayed or missed when relying solely on national data.

Work package 2

Measurement of the background rates of platelet factor 4 antibodies in healthy, unvaccinated individuals as well as in vaccinated people and those following infection with COVID-19

The results of testing the four different panels with the *Immucor* anti-PF4 antibodies are shown in *Table 2* and *Figure 1*. The background seroprevalence in pre-COVID healthy individuals, using the manufacturer's cut-off of ≥ 0.4, was 3%. Using the higher cut-off of > 1.0, the seroprevalence was only 0.2% (see *Table 2*). Compared to pre-COVID healthy individuals, the seroprevalence among blood donors with prior COVID infection was slightly higher (3.7% and 0.4% using the ≥ 0.4 and ≥ 1.0 cut-offs), with no difference in mean OD (see *Table 2* and *Figure 1*). COVID vaccinated controls who had received only AZD1222 or BNT162b2 vaccines had lower seroprevalence of anti-PF4 (1.1% to 1.3% using the ≥ 0.4 cut-off and 0.3% using the ≥ 1.0 cut-off) and lower mean OD when compared with pre-COVID healthy individuals and COVID-positive blood donors.

The seroprevalence of anti-PF4 in each of the different panels of non-VITT controls was much lower than in suspected VITT cases (1.1% to 3.7% or 0.0% to 0.4%, compared to 66.2% or 62.7% using the cut-off of ≥ 0.4 and ≥ 1.0, respectively). Suspected VITT cases also had a substantially higher anti-PF4 IgG OD (0.75) compared to the non-VITT controls (0.09 to 0.15).

Date of vaccination was available for 56 (39%) suspected VITT cases, with 43 (77%) and 13 (23%) having samples collected within 0–29 days and 30–59 days, respectively (see *Table 2*). The importance of this distinction is that in the overwhelming majority of patients, VITT occurred within 30 days of vaccination. The seroprevalence for

This synopsis should be referenced as follows:
Nicolson PLR, Abrams ST, Amirthalingam G, Brown K, Buka RJ, Caulfield MJ, *et al.* Understanding mechanisms of thrombosis and thrombocytopenia with adenoviral SARS-CoV-2 vaccines: a comprehensive synopsis. *Efficacy Mech Eval* 2025;**12**(7). https://doi.org/10.3310/FFSS9010

TABLE 1 Algorithmic definitions of VITT

	Categorisation of VITT							
	Definite	Probable		Possible			Unlikely	
		Definition (1)[a]	Definition (2)	Definition (1)	Definition (2)	Definition (3)	Definition (1)	Definition (2)
Onset time 5–30 days	Yes	Yes or no	Yes	Yes or no	Yes or no	Yes	Yes	Yes
Thrombosis	Yes	Yes or no	Yes	Yes or no	Yes or no	Yes	No	Yes
Platelet count < 150×10^9/l	Yes	Yes or no	Yes	Yes or no	Yes or no	Yes	Yes	No
D-dimer level (mg/l)	> 4000	> 4000	2000–4000/ unknown	2000–4000/unknown	4000	< 2000	< 2000	< 2000
Anti-PF4 antibodies	Yes	Yes or no	Yes	Yes or no	Yes or no	Yes or no	Yes or no	Yes or no
Conditions to be met for definition	All met	D-dimer > 4000 AND total yes= 3	D-dimer 2000–4000 AND total yes= 4	D-dimer 2000–4000 AND total yes= 2 or 3	D-dimer > 4000 AND total yes= 2	D-dimer < 2000 AND total yes= 4 or 3 with no-anti-PF4 antibodies		

a Algorithmic definitions used to classify people with VITT: definition 1 used SNOMED and laboratory data, definition 2 used ICD-10 and laboratory data, while definition 3 used ICD-10 codes only.

TABLE 2 Seroprevalence of Immucor anti-PF4 IgG and geometric mean OD in the serum panels

		Immucor cut-off ≥ 0.4		Immucor cut-off ≥ 1.0		
Panel	*N*	Number positive	Proportion positive (95% CI)	Number positive	Proportion positive (95% CI)	Geometric mean OD (95% CI)
Pre-COVID healthy individuals	1997	52	2.60% (1.95% to 3.40%)	3	0.15% (0.03% to 0.44%)	0.148 (0.145 to 0.151)
COVID-positive blood donors	1889	70	3.71% (2.90% to 4.66%)	7	0.37% (0.15% to 0.76%)	0.145 (0.142 to 0.149)
COVID vaccinated controls (AstraZeneca AZD1222 only)	1385	25	1.81% (1.17% to 2.65%)	4	0.29% (0.08% to 0.74%)	0.101 (0.099 to 0.104)
COVID vaccinated controls (Pfizer-BioNTech only)	718	9	1.25% (0.57% to 2.37%)	2	0.28% (0.03% to 1.00%)	0.102 (0.098 to 0.105)
COVID vaccinated controls (AstraZeneca and Pfizer-BioNTech/Moderna)	88	1	1.14% (0.03% to 6.17%)	0	0.00% (0.00% to 4.11%)	0.088 (0.081 to 0.096)
Suspected VITT	142	94	66.20% (57.79% to 73.91%)	89	62.68% (54.17% to 70.64%)	0.748 (0.582 to 0.962)

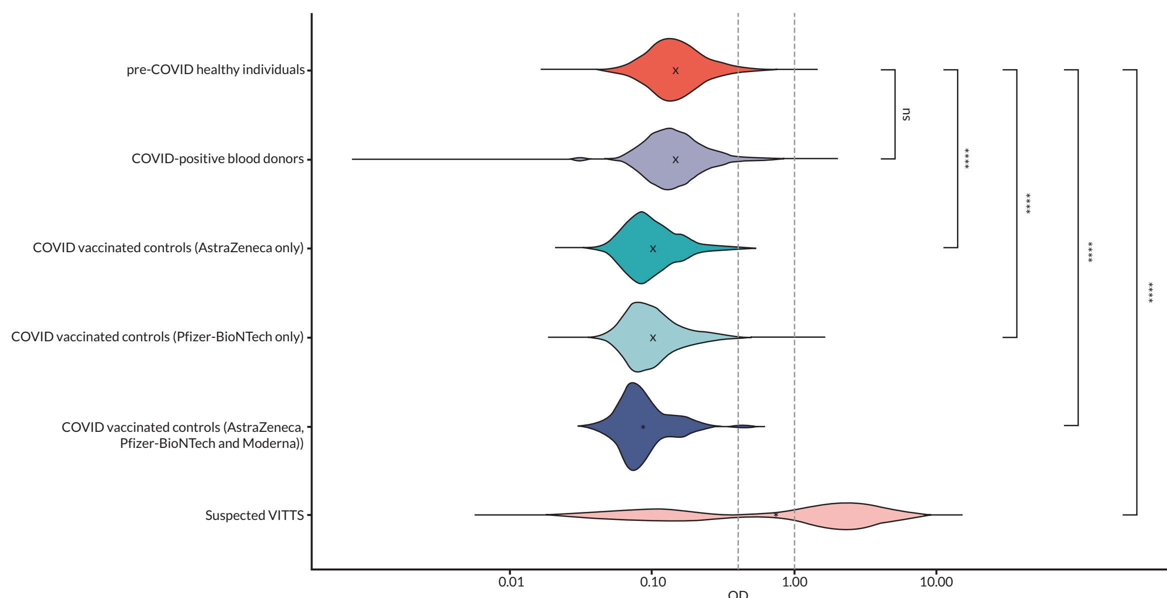

FIGURE 1 Distribution of Immucor anti-PF4 IgG OD by panel. OD in log10 scale. The two dashed lines indicate the cut-offs of 0.40 and 1.00. Geometric mean (cross) and its 95% CI (error bars) are shown within each violin plot. The statistical significance of each panel compared to pre-COVID healthy individuals was compared using the Wilcoxon test. NS, not significant; ****$p < 0.0001$.

cases with samples collected between 0 and 29 days post vaccination was 86% and 83% at the cut-offs of ≥ 0.4 and ≥ 1.0, a higher proportion than in those with samples collected between 30 and 59 days (69%). Geometric mean OD for samples collected between 0 and 29 days was 1.58, nearly twice as high as samples collected between 30 and 59 days (0.85). A lower anti-PF4 IgG level was not observed in COVID vaccinated controls tested more than 2 months after vaccination (data not shown). Indeed, controls who had samples collected between 60 and 89 days after receiving only AZD1222 or BNT162b2 had the highest seroprevalence.

A subset of 245 samples were tested with Zymutest and Asserachrom (*Table 3*). Anti-PF4 seroprevalence was comparable in this subset [18.8% for Immucor (≥ 1.0 cut-off), 17.6% for Zymutest (positive only) and 20.4% for Asserachrom], when only (strong) positive results were considered. However, anti-PF4 seroprevalence was substantially higher on Immucor if weakly positive results were also classified positive (Immucor: 57.1% and Zymutest: 23.6%). Among samples that were negative by Immucor, a high proportion were also negative on Zymutest and Asserachrom (91% and 92%, respectively). Only around two-thirds of samples identified positive by Immucor at the higher cut-off (≥ 1.0) were also positive using Zymutest (67%) and Asserachrom (61%). Weakly positive samples identified by Immucor (cut-off ≥ 0.4 and < 1.0) were largely discordant on the other two assays, with > 80% of these samples testing negative by Zymutest and Asserachrom (see *Table 3*). The Immucor assay had a lower correlation coefficient with Zymutest (0.54; $p < 0.001$ Spearman correlation test) and Asserachrom (0.52; $p < 0.001$) than the correlation between the two alternative assays (0.66; $p < 0.001$). For a small subset of 121 samples tested twice by Immucor, the correlation between first and second tests was 0.75 ($p < 0.001$).

Work package 3

Patients and controls included in the genomic studies

The demographics of patients with VITT ($n = 91$) and controls ($n = 21,122$) included in the genomic studies are

TABLE 3 Comparison of Immucor anti-PF4 IgG ELISA tests with Zymutest (Quadratech) and Asserachrom (Stago) on subsets of non-VITTS controls ($n = 245$)

	Zymutest (Quadratech)			Asserachrom (Stago)	
Immucor	Negative	Weakly positive	Positive	Negative	Positive
Negative ($n = 105$)	96 (91%)	5 (5%)	4 (4%)	97 (92%)	8 (8%)
Weakly positive ($n = 94$)	83 (88%)	3 (3%)	8 (9%)	80 (85%)	14 (15%)
Positive ($n = 46$)	8 (17%)	7 (15%)	31 (67%)	18 (39%)	28 (61%)

TABLE 4 Demographics of cases and super controls

Characteristic	Cases (*N* = 91)	Controls (*N* = 21,122)
Female sex	47% (43)	55% (11,674)
Age (mean) (SD)	48 years old (± 14)	54 years old (± 16)
European	95% (86)	82% (17,407)
South Asian	4% (4)	6% (1332)
African	0% (0)	3% (700)
East Asian	0% (0)	1% (296)
American	0% (0)	1% (158)
Unassigned	1% (1)	6% (1229)

SD, standard deviation.

shown in *Table 4*. There were two control groups – one from REACT, which is age, gender, ethnicity and COVID vaccine matched. The second controls are unrelated genomes from the 100KGP, which contains other ethnicities.

Genome-wide association of common variants

There were no common variants that achieved genome-wide significance thresholds (denoted in *Figure 2* by the red serrated line) when compared with the REACT controls. Genome-wide signals were also not identified when cases were compared with the 100,000 genomes controls.

Gene burden testing of rare variants

No rare variants that met genome-wide significance from gene burden testing denoted by the red serrated line (*Figure 3*).

Transcriptome-wide analysis and immune repertoire sequencing analysis

These are both currently underway.

Work package 4

Evaluation of the antivector immunity seen in patients with VITT and otherwise healthy individuals following adenoviral vector SARS-CoV-2 vaccination

The serum from patients with VITT (*n* = 64) and healthy controls within 30 days following vaccination with first dose of AZD1222 (*n* = 16), Ad26.COV2.S (*n* = 10) and BNT162b2 (*n* = 10) were studied using the Antigen Discovery Inc. protein microarray of 207 adenoviral proteins as described above. The global view of the antibody reactivity is shown in *Figure 4*. One hundred

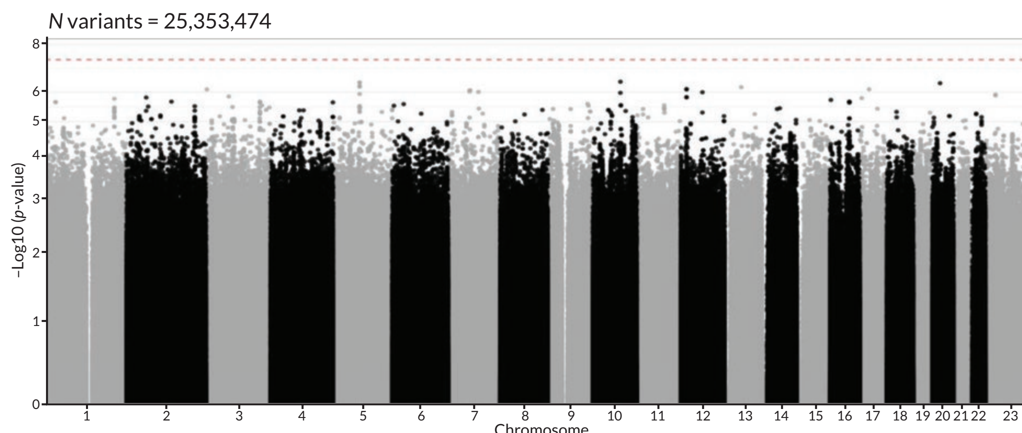

FIGURE 2 Manhattan plot of VITT cases vs. controls from the REACT study.

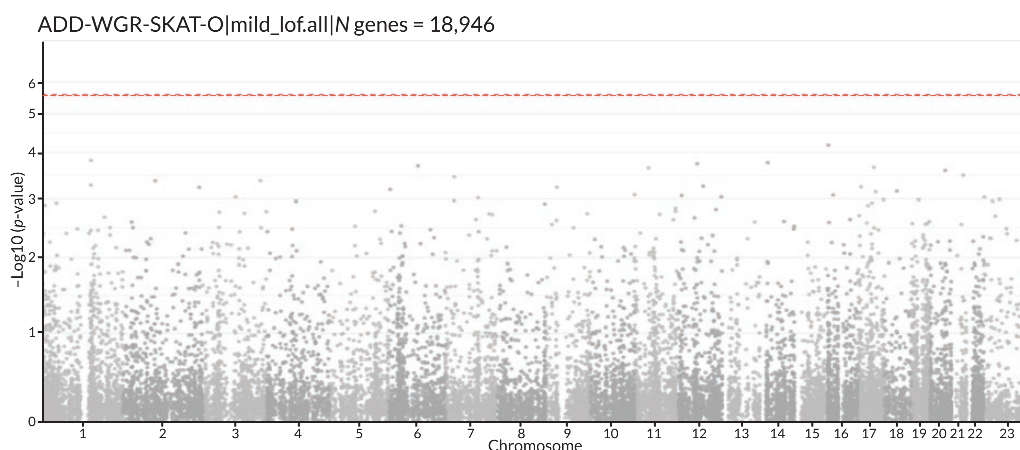

FIGURE 3 Manhattan plot showing no genome-wide significant rare variants.

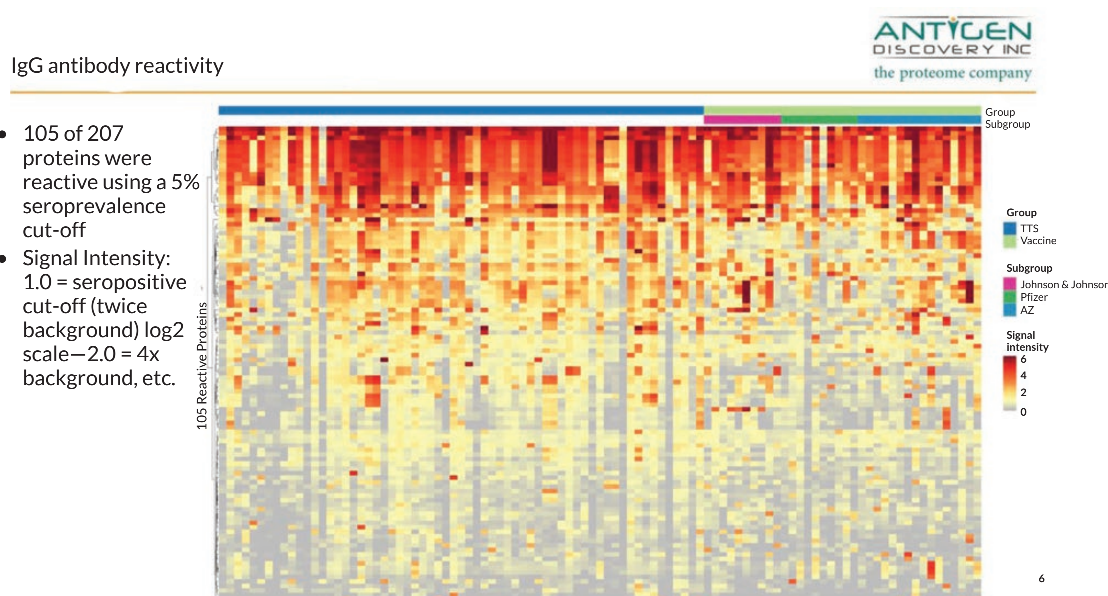

FIGURE 4 Heatmap of IgG antibody reactivity with the adenovirus protein microarray for the entire TTS/VITT and control cohorts.

and five of the 207 proteins were reactive using a 5% seroprevalence cut-off.

A univariate analysis of antiprotein antibody levels in the VITT patients compared to all the vaccinated controls combined was then undertaken. Responses to six adenovirus antigens were higher in the VITT group than the vaccinated controls grouped together, but none achieved statistical significance after correction for the FDR (*Figure 5a*). Five antiprotein antibody levels in VITT patients were higher than in controls who received Ad26.COV2.S; the greatest difference was seen in the responses to the chimpanzee adenovirus Y25-derived gp17 protein (*Figure 5b*). Unsurprisingly, several antiadenoviral protein antibodies were significantly higher in the VITT patients compared to BNT162b2 vaccinees (*Figure 5c*). Interestingly, antibody responses to the SARS-CoV-2 nucleocapsid (*N*) antigen are significantly higher in the TTS/VITT group than in the Pfizer control group (see *Figure 5c*). There is no suggestion that the TTS/VITT group were infected at the time of clinical presentation, and this finding is not replicated in the other analyses undertaken. Importantly, there was no difference in antiprotein antibody levels in VITT patients compared to AZD1222 vaccine recipients (*Figure 5d*), suggesting that adenovirus responses following AZD1222 are similar in those who develop VITT compared to those who do not.

Responses of T cells and memory B cells to different vaccines and vectors

T-cell assays performed in pre-pandemic vaccine-naive healthy donors revealed widespread lymphocyte stimulation after exposure to AZD1222 (95%), ChAdOx1-spike (90%) and Ad26.COV2.S, but not on exposure to

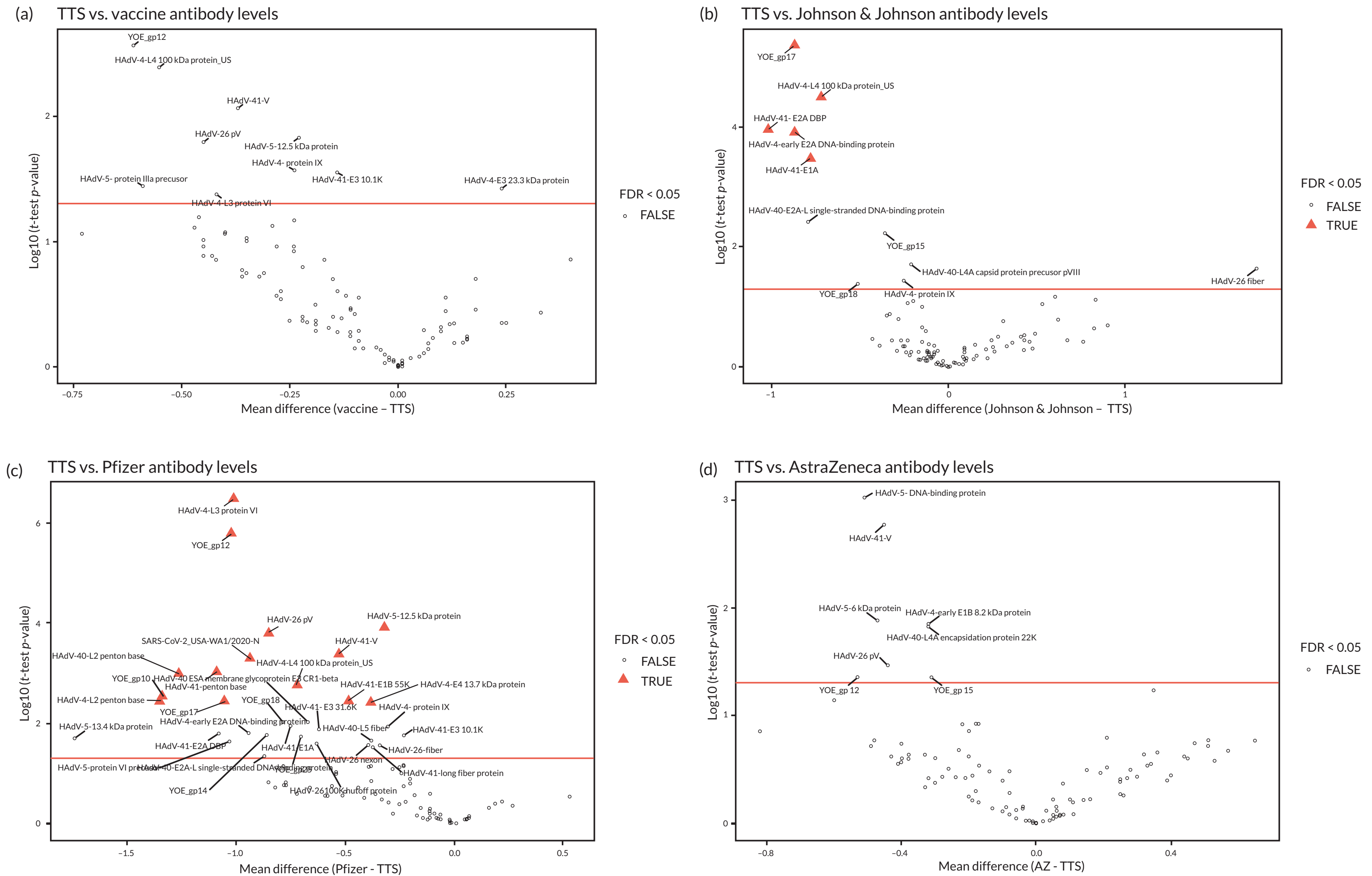

FIGURE 5 Differences in antiprotein antibody levels between patients with VITT and healthy recipients of Ad26.COV2.S, BNT162b2 and AZD1222. Volcano plots showing levels of antiprotein antibodies in patients with VITT compared to (a) pooled recipients of Ad26.COV2.S, BNT162b2 and AZD1222 (n = 36), (b) recipients of Ad26.COV2.S (n = 10), (c) recipients of BNT162b2 (n = 10) and (d) recipients of AZD1222 (n = 16). Univariate. x-axis = mean difference: right of 0.00 represents higher antiprotein antibody levels in the control vaccinees and left of 0.00 represents higher antiprotein antibody levels in the VITT patients. Antigens are labelled by name if they have an unadjusted *p-value* below 0.05, and those that remain significant after correction for the FDR, if any, are shown in red triangles.

the BNT162b2. The findings have defined unexpected, cross-reactive $CD4^+$ $CD45RO^+$ memory T-cell responses to AZD1222 in healthy, vaccine-naive PBMC samples collected prior to the COVID-19 pandemic. Although previous exposure to chimpanzee adenoviruses has been reported in certain demographics, seroprevalence is low and inconsistent with the high level of responses observed in these vaccine-naive donors.[62] Consequently, this suggests that immune stimulation after AZD1222 exposure may arise from pre-existing immunity to prevalent human adenoviruses with shared homology between T-cell epitopes. When studying T-cell responses to AZD1222 in a cohort of patients with VITT, no differences in T-cell stimulation or cytokine activity were observed. This was due to the strong, proliferative lymphocyte responses seen within the unexposed healthy donor cohort, and therefore it was not possible to distinguish immunoreactivity between subject groups.

Role and mechanisms of immune complexes in thrombotic thrombocytopenia

The structure of the ChAdOx1 viral vector, which forms the basis for AZD1222, was solved to a high resolution by cyro-electron microscopy. Using this structure, we have highlighted the high electronegativity of the ChAdOx1 capsid, largely due to the hexon hypervariable regions (HVRs). We also simulated and predicted how PF4 can bind with high affinity at the apex between the ChAdOx1 hexon proteins using highly pure caesium chloride gradient preparations of ChAdOx1 (KD 616 nm) and commercial AZD1222 vaccine (KD 514 nm).[52] Commercial vaccine samples and ChAdOx1 demonstrated similar affinities to PF4, which indicates that the association between PF4 and the vaccine was an interaction between the virus and PF4, rather than any cell-line-derived proteins in the vaccine following manufacture.[63] PF4 is held in a preferred orientation by the electrostatic interaction between the highly positively charged PF4 tetrameric protein and the negatively charged HVRs. We have subsequently confirmed this interaction between ChAdOx1 and PF4, obtained full kinetic binding affinities, and shown that the less negatively charged HVRs of Ad26 that form the basis of the Janssen vaccine (Ad26.COV2.S), likely results in VITT being less commonly observed in patients receiving Ad26.COV2.S compared to AZD1222. SPR analysis using immobilised purified hexon protein from ChAdOx1 has confirmed the greater affinity for PF4 than that observed for both purified ChAdOx1 and AZD1222.

The role and mechanisms of neutrophil extracellular traps in thrombosis with thrombocytopenia syndrome

In work to explore why, how and what happens when NETs form, we found that NETs were directly induced by serum from patients with VITT but not in serum from pre- and post-AZD1222 controls.[50] Unlike previous publications on NETs in VITT, we showed that platelets were not necessary for NETs formation. The driver for NETs formation was the strong immune responses in patients with VITT compared to pre- (n = 9) and post-AZD1222 (n = 22) controls. This was characterised by significant elevations in proinflammatory cytokines (including IL-6 and IL-8) and T-helper-1 and -2 cell activation (including IFNγ and IL-13). Markers of systemic endothelial activation (including ICAM-1 and E-selectin) and coagulation activation (including D-dimer) in the circulation were also significantly elevated. About 70% (n = 15/22) of patients met the International Society for Thrombosis and Haemostasis criteria for DIC despite negligible changes in the prothrombin time. To further confirm that patients with VITT had systemic coagulation activation and clot formation in distant organs other than the reported CVSTs, we examined for fibrin(ogen) deposition within organs. Immunofluorescent staining of post-mortem kidney samples showed clear evidence of fibrin entwined with NETs in these tissues.

Enhanced NETs formation and lymphopenia combined with high circulating lactate dehydrogenase (LDH) and histone levels demonstrate systemic immune cell injury or death. Lymphopenia and elevated circulating histones independently predicted 28-day mortality in VITT patients. Systemic cell injury or death, along with immune-mediated inflammation and coagulation activation, including NETosis, are key pathophysiological drivers and clinically relevant in severe VITT. The work has also identified targets for therapeutic advancement, including targeting immune cell activation (e.g. IL-6 and IL-8) and death/NETosis (e.g. histones and NETs).

Work package 5

Platelet activation in vaccine-induced immune thrombosis and thrombocytopenia is mediated through FcɣRIIa and c-Mpl

The first goal was to establish whether platelet activation in VITT was mediated by a common mechanism and to identify reagents that could inhibit activation. We showed that platelets were not activated following exposure to AZD1222 but that activation was mediated by immune complexes against PF4 that was critically dependent on FcɣRIIa but independent of complement.[55] In addition, we demonstrated that platelet activation could be blocked by classical antiplatelets (aspirin and $P2Y_{12}$ receptor antagonists), by drugs used to treat blood cancers, such as inhibitors of the Bruton tyrosine kinase (BTK), and by intravenous immunoglobulin (IVIg).[55] Treatment with IVIg or with plasma exchange also reduced or abrogated

platelet activation ex vivo.[55] In addition, in a limited number of patients due to the rarity of the condition, we went on to show that the activating anti-PF4 antibodies could be detected in patients for up to 7 months, thereby placing them at further risk of thrombosis and justifying continued treatment with anticoagulants.[56]

As part of this research, we studied the reasons for the variation in response of healthy individuals to diagnostic and follow-up serum from the patients, as this has implications for the susceptibility to VITT. We demonstrated, as is the case in HITT, that only a proportion of donors were responsive to VITT serum/plasma but that this was not linked to the level of expression of the low-affinity Fc receptor, FcγRIIa, on platelets.[56] We have developed a tetravalent ligand based on the cross-linking of nanobodies to FcγRIIa and used this to identify individuals who showed high sensitivity to ligands for the Fc receptor.[56,64] This is the first ligand of known stoichiometry for FcγRIIa.

Further delving into possible explanations for the differential sensitivity of healthy individuals to FcγRIIa stimuli led to the discovery that platelet activation by VITT sera is supported by the binding of PF4 to the receptor for the cytokine thrombopoietin, c-Mpl.[57] This PF4 c-Mpl interaction was not hitherto known. This interaction not only generates intracellular signals that synergise with those from the FcγRIIa to mediate platelet activation but also increases the strength of binding of the antibodies to FcγRIIa as a result of avidity. Blocking downstream signalling of c-Mpl using the Janus kinase 2 (JAK2) inhibitor ruxolitinib blocked PF4-mediated and VITT serum-mediated platelet activation.[57]

Discussion

The TTS consortium was established at the height of the pandemic when the vaccines were first deployed, and an unusual vaccine-associated syndrome characterised by thrombosis occurring together with thrombocytopenia began to be reported. Although a funding decision was made soon after application, the start of the work was delayed because of contractual issues. By that time, the numbers of reports had virtually disappeared as a result of regulatory actions worldwide which limited the use of the AZD1222 and Ad26.COV2.S vaccines. However, many questions remained, including the need to understand the mechanisms (which would help in determining biological plausibility), identify individual susceptibility factors, learn lessons for the future in terms of early detection, biomarker identification and potential treatment strategies. It is also important to remember that adenovirus-based vaccines are still being developed for other pathogens, and this remains an important vaccine platform because of its lower cost, the ability to upscale manufacturing and the reduced need for cold-chain distribution and storage.[65]

The collective findings have pieced together clinically identifiable and pathogenic pathways in VITT with identified translatable outputs, for example, new adenoviral-based vaccine design, clinically relevant biomarkers and diagnostic profiles as well as potential therapeutic targets.[66] The insight leads us to speculate that in certain individuals, the adenoviral vaccine is misrecognised as a severe viral infection and a misguided antiviral immune response is triggered. This unmasking of an innate danger response causes significant lymphocyte death and release of damage-associated molecular patterns to invoke systemic cell damage with immunothrombotic complications that involve widespread NETosis. More recently, there have been cases reported of platelet-activating, VITT-like, anti-PF4 antibodies without preceding treatment with heparin or adenovirus vector-based vaccines. These are associated with a poor prognosis and are characterised by thrombosis and thrombocytopenia.[67] Therefore, quick recognition and understanding of the pathophysiology of severe TTS will continue to be important.

Evaluating SARS-CoV-2 vaccine safety using population-level linked data

Reports of TTS associated with the AZD1222 vaccine were first received by the yellow card spontaneous reporting scheme. Spontaneous reporting systems are a cornerstone of pharmacovigilance and have been used to identify many safety issues.[68] However, such schemes suffer from limitations of under-reporting and cannot be used to ascribe causality. As such, the yellow card system represents a hypothesis generating tool which needs to be followed up by formal pharmacoepidemiological studies. In this consortium, we used a cohort design, the advantages of this approach being the familiarity of the method by the community, and the ability to calculate longer-term aHRs. However, the disadvantages are that creating the cohort, coding covariates and performing analyses in a population of this size (~57 million people, with ~42 million adults) are time and computationally intensive. Other groups used an alternative, but complementary approach, the SCCS.[69] The advantages of this approach – which examines only cases – is that it needs less computational resource, and it adjusts for all non-time varying confounders. However, the SCCS is less familiar, and its assumptions that the event rates are constant over time, and the inability to account for time-varying confounders are limitations. Furthermore, given the rapidity with which the pandemic evolved, which included the emergence of new viral variants,

and the development of non-vaccine therapeutics, the SCCS may not take into account other factors that can potentially modify the risk of the adverse events (AEs). In our analysis, the availability of primary care records meant we could better ascertain some thrombotic events (but not all), adjust for a wide range of potential confounders and define a very large and representative population.

Our findings show that there was a higher risk of thrombotic events associated with adenoviral vaccines but not with the mRNA vaccines. However, the risk of these events was higher with COVID-19 infection compared with the vaccines, a finding also seen in SCCS analysis.[69] A limitation of the analysis was that we could not identify cases who had both thrombosis and thrombocytopenia after vaccination because it was poorly recorded (including a lack of laboratory data to confirm a decrease in platelet levels). The higher aHR of CVST in our later analysis could have been partly due to heightened awareness with time and the increase in CT venography targeted at people post AZD1222.[70] The incidence of common arterial and venous thromboses being lower after all types of vaccination may be due to a 'healthy vaccinee' bias, in that vaccination may have reduced the risk of arterial and venous thromboses by reducing the risk of COVID-19 infection, or its severity.

Evaluating SARS-CoV-2 vaccine safety using regional linked health data

We also evaluated whether regional linked health data could be used in near real time to identify vaccine-related AEs. Our assessment was that near real-time ascertainment of many diseases and events of public health importance using data from across multiple acute hospital sites' SDEs are not yet feasible at scale, although it may be possible in some digitally mature regional SDEs and as new initiatives develop, for example, NHS England subnational SDEs.[71] This work resulted in some key recommendations which are being fed into the NHS England and UK-wide regional SDE planning and development.

To enable near real time, scalable analyses of linked health data across local health systems of current NHS priorities for both current NHS priorities, and in preparations for any future pandemic, we recommend the following actions:

- Enable improved clinical coding to identify important diseases with greater fidelity. We recommend identifying and addressing the barriers to using the NHS-mandated SNOMED coding system much more widely at the point of care.[72]
- Regional SDEs should have seamless and streamlined access to the data arising from or pertinent to their local health systems that are collated and curated nationally.
- A centralised governance and data access approval process implemented across regional SDEs, modelled on current ethics approvals, with time targets from application to approval, similar to the Integrated Research Application System.
- Leverage regional expertise embedded within each local health and care systems – for example, build relationships between population health groups, data groups, research and standardise ways of working.

To continue to enable large-scale analyses of linked health data at whole population scale from different sources, for current NHS priorities and in preparation for any future pandemic, we recommend the following actions:

- To ensure rapid availability of the entire coded primary care record (rather than a subset of codes) linked to other national health data sources in secure environment(s) with whole population coverage.
- To ensure nationally agreed open code list repositories [such as opencodelists, and the Health Data Research UK (HDR-UK) phenotype library are sustained and appropriately resourced].[42,73]

The immunopathogenesis of vaccine-induced immune thrombosis and thrombocytopenia

A key feature in the pathogenesis of VITT is the development of high-titre platelet-activating anti-PF4 antibodies.[74] Anti-PF4 antibodies have also been detected in HIT, and as with VITT, they appear 5–10 days after exposure, and they cause FcγRIIa (CD32a) receptor-mediated activation of platelets.

Our study confirmed the low seroprevalence of PF4 antibodies in different populations *without* VITT, as measured by the Immucor PF4 assay, with positivity between 1% and 4%, similar to the background prevalence reported in the literature.[33,75] VITT patients who were positive for anti-PF4 tended to have much higher ODs, suggesting that a more appropriate cut-off for the Immucor assay in relation to risk of developing VITT would be > 1.0. Using this more stringent cut-off, only 0.34% or below of the control populations had detectable PF4 antibody. In individuals without VITT, neither COVID infection nor vaccination with either AZD1222 or BNT162b2 led to an increase in PF4 antibodies, suggesting that high levels of PF4 antibodies could be used as a reliable marker for VITT. In our study, both seroprevalence and anti-PF4 antibody levels of VITT cases were lower in those sampled more than 30 days after vaccination. This is consistent with an association between high antibody levels and clinical

symptoms, as most case reports of VITT have an onset of symptoms within 30 days of vaccination. Commercial anti-PF4 antibody assays have different substrates on the solid surface of the ELISA plate, and could therefore give different results when used as a diagnostic marker of VITT. Our results suggest that the Immucor assay had good specificity and was more sensitive than the two alternative assays in detecting samples with high PF4 antibody levels.

The finding of high-titre anti-PF4 antibodies in VITT is therefore consistent with a secondary immune response. In VITT, the antibodies are directed against PF4 alone, while in HIT, they are directed against PF4-heparin complexes. The neoepitopes created by binding of either heparin or vaccine components differ with antiheparin PF4 antibodies binding to two different sites in the polar region of PF4, while in VITT, binding occurred on eight amino acids on PF4, overlapping with the heparin binding sites.[76] Binding of the antibodies in VITT is also stronger than in HIT. Importantly, in VITT, the anti-PF4 antibodies do not cross-react with SARS-CoV-2 spike protein.[77]

How does the administration of an adenoviral vector vaccine induce anti-PF4 antibodies in VITT patients? While we did not find any suggestion that aberrant antiadenovirus antibody responses contributed to the development of pathogenic anti-PF4 antibodies, a key finding from our consortium was the structural work that identified the binding sites of PF4 with the ChAdOx1 hexon.[63] We have shown that PF4 can form stable complexes with clinically relevant adenoviruses through electrostatic interaction. We were able to highlight the remarkable electronegativity of the ChAdOx1 capsid, largely due to the highly negatively charged hexon HVRs and simulated and predicted how the positively charged chemokine PF4 can bind with high affinity at the apex between the ChAdOx1 hexon proteins. The conformational change in PF4 induced by this electrostatic binding can lead to neoepitope generation and the development of anti-PF4 antibodies. Further important findings from this work included: (1) binding affinity was lower with the less negatively charged HVRs of adenovirus 26 (Ad26) that form the basis of the Janssen vaccine (Ad26.COV2.S) than with AZD1222, consistent with the fact that VITT was less common with the former than with the latter vaccine; and (2) commercial vaccine samples and ChAdOx1 demonstrated similar affinities to PF4, which indicates that the association between PF4 and the vaccine is an interaction between the virus and PF4, rather than any cell-line-derived proteins in the vaccine following manufacture, a hypothesis which was put forward to explain VITT occurrence with AZD1222.[63] The lack of proteomics access and inability to successfully immobilise the viral vector impacted our attempts to identify factors in healthy and VITT patient serum that bind to ChAdOx1. We did, however, identify that antiadenoviral antibody levels in patients with VITT were no different to those vaccinated with AZD1222 who did not develop VITT. Under the terms of agreement, we were unable to perform site-directed mutagenesis on the AZD1222 or Ad26 platforms. Instead, we created Ad5-based vectors containing the ChAdOx1 hexon protein, and performed site-directed modifications. However, the resultant particles were not compatible with successful virus assembly.

The ChAdOx1 vector is derived from the chimpanzee adenovirus which has been modified to neutralise its replication potential. This vector was chosen because it has been shown that there was limited pre-existing immunity to the chimpanzee adenovirus ChAdY25, from which ChAdOx1 is derived, which would therefore result in better vaccine efficacy.[54,62] A study has shown that clinically relevant neutralising antibody titres against ChAdY25 were identified in 0% and 9% of 100 UK and 57 Gambian adults, respectively.[62] This is in contrast to the human adenovirus (HAd) serotype 5 (Had5), where pre-existing immunity is widespread.[78] The low pre-existing seroprevalence of neutralising antibodies to ChAdOx1 contrasts with our finding of T-cell reactivity. Using PBMCs stored prior to the pandemic, we were able to study T-cell responses across a large number of samples without prior SARS-CoV-2 or vaccine exposure. This showed that $CD4^+$ $CD45RO^+$ memory T-cell responses to AZD1222 nCoV-19 were detected using pre-pandemic healthy donor PBMC samples, suggesting that T-cell adenoviral cross-reactivity, unlike antibody cross-reactivity, is likely to be highly prevalent in the UK population. A limitation of our work is that we were unable to incorporate various prevalent human adenoviruses within the T-cell assays conducted in healthy donor studies which would have enabled comparative analyses of the stimulatory effects induced by ChAdOx1 and other human adenovirus. This would have provided insights regarding the cross-reactivity encountered with ChAdOx1 and shed light on the likely adenoviral vector antigens.

Nevertheless, the finding of cross-reactive T cells is highly relevant not only with respect to the development of VITT but also more widely to the occurrence of thrombotic thrombocytopenia in people who have had neither heparin nor adenoviral vaccine exposure. A recent case series described nine patients who had either arterial or venous thrombosis, together with thrombocytopenia, greatly elevated D-dimer levels, and positive tests for anti-PF4 antibodies, but had no history of exposure to heparin or vaccine.[67] One patient had proven previous adenovirus infection.

Thrombosis, thrombocytopenia and inflammation after vaccination in VITT patients

In patients who develop platelet activating anti-PF4 antibodies, a constellation of events occur, which result in thrombosis in multiple vessels and thrombocytopenia. Systemic inflammation, platelet activation, innate immune activation and hypercoagulability with a DIC that is atypical from those in sepsis are important manifestations of the disease in patients with VITT. The DIC seems to be driven by histone-dependent alternative prothrombinase, which does not alter the prothrombin time unlike in sepsis.[79] A key finding was that the development of NETs, that is, NETosis, was directly induced by serum from patients with VITT without the requirement of platelets, the driver for this being the strong immune responses in patients with VITT. This was accompanied by a systemic inflammatory state characterised by elevation in proinflammatory cytokines, T-helper-1 and -2 cell activation, systemic endothelial activation and coagulation activation, together with clot formation in distant organs. Both lymphopenia and circulating histone levels independently predicted 28-day mortality in patients with VITT and may serve as future biomarkers for poor outcome. The work has also identified targets for therapeutic advancement, including targeting immune cell activation (e.g. IL-6 and IL-8) and death/NETosis (e.g. histones and NETs). The key strengths of the work were access to matched pre- and post-AZD1222 vaccine controls, along with availability of post-mortem tissue samples from VITT patients. A limitation was the low case numbers due to the rarity of VITT. However, in the context of VITT this is a substantial cohort. In addition, these patients were all hospitalised and 12/22 died of VITT. Therefore, there is a selection bias towards severe VITT rather than milder cases and will provide a limited ethnicity mix that limits generalisability to other populations. Ideally, the inclusion of an animal model could have enhanced the causative-effect relationship between cellular death and severe VITT and allowed for the screening of potential novel therapeutic interventions.

Platelet activation is an important factor in the pathogenesis of VITT. This is critically dependent on activation of FcγRIIa by immune complexes against PF4, but there was differential sensitivity to FcγRIIa in healthy individuals after incubation with sera from VITT patients. A novel finding which may partly explain the differential sensitivity is that platelet activation was also due to binding of PF4 to the receptor for the cytokine thrombopoietin, c-Mpl. This interaction generates intracellular signals that synergise with those from the FcγRIIa to mediate platelet activation. Our work also highlighted potential therapeutic avenues to be explored in the future: the JAK2 inhibitor ruxolitinib blocked downstream signalling of c-Mpl, while FcγRIIa-mediated platelet activation was blocked by antiplatelets (aspirin and $P2Y_{12}$ receptor antagonists), IVIg (which was used extensively in the treatment of patients with VITT) and by the BTK inhibitor, ibrutinib.[55] Interestingly, a recent case report described a woman who first presented in 2007 with venous thromboembolism, which recurred in 2009, after which she had multiple venous and arterial thromboses together with thrombocytopenia and positive anti-PF4 antibodies.[80] Her clinical course was punctuated with multiple relapses, and in December 2022, she was started on ibrutinib, which stabilised her condition.

Individual susceptibility to vaccine-induced immune thrombosis and thrombocytopenia

Vaccine-induced immune thrombosis and thrombocytopenia is a rare phenomenon, estimated to occur in about 1 in 100,000 vaccinated individuals.[74] By contrast, the AZD1222 vaccine has been used extensively worldwide and has been estimated to have saved 6.3 million lives in the first year of the vaccine roll-out.[81] Therefore, other susceptibility factors are likely to be important. In order to investigate, we undertook WGS in 91 patients with VITT and compared them with over 20,000 super controls. We failed to show any genome-wide significant hits after analysis for both common variants, and gene burden testing for rare variants. A limitation here is the sample size, which although significant (n = 91 cases) in the context of VITT, was inadequate in defining clear predisposing loci, despite the use of a large number of controls. VITT represents a pharmacogenetic phenotype, and previous studies have shown that pharmacogenetic variants have larger effect sizes than genetic variants associated with complex traits.[82] Unfortunately, this does not seem to apply to VITT, and hence it will be important in the future to increase sample size by collaborating with other groups who have recruited patients with VITT. We have identified specific T- and B-cell receptor clonal patterns that were present only in cases, but this finding needs validation, work which is currently underway.

Engagement with partners and stakeholders

Despite being formed during a pandemic with several lockdowns, the consortium worked well together. The consortium had monthly update meetings, attended by patient representatives. In addition, regular meetings were held between the Chief Investigator and National Institute for Health and Care Research (NIHR), Department of Health and Social Care and the vaccines taskforce. Data were also discussed during bi-weekly update meetings between work packages 4 and 5; these teams also

shared knowledge/experience and reagents [including PF4 (Toh group)]. We also engaged with other groups performing vaccine-related epidemiology in the UK. The BHF Data Science Centre had an active patient and public involvement (PPI) group. A number of key findings were presented at British Society for Immunology Congress 2022 (7 December 2022), a TTS consortium patient and public involvement and engagement (PPIE) event on World Thrombosis Day (12 May 2023) and recordings were made available on the Thrombosis UK and HaemSTAR websites.

Individual training and capacity-strengthening activities

Researchers from work packages 4 and 5 visited each other's laboratories and shared various techniques (e.g. NETosis and endothelial activation assay and platelet isolation/activation) and protocols. Throughout this project, each of the contributing institutions trained and supported multiple postdoctoral researchers in statistical epidemiology and laboratory techniques. In addition, BHF project grants were awarded to two early career researchers in work package 5, one on the molecular basis of variation in response to platelet activation by FcγRIIa (£236,000), and another on identification and function of the PF4 receptor on platelets (£100,000).

Patient and public involvement

The aim of PPI in the TTS consortium was twofold: first, to involve patients in the generation of research questions, interpretation of results and help guide further work to be performed; and second, to create plain language summaries of results to be used to feedback results to the patient groups rather than waiting until the end of the grant to perform this step.

Patients and public were not involved in the initial development of the projects within the work packages. This is because the work was developed at pace, and the patients who had developed TTS had been through a traumatic experience and were at the early stages of recovery. However, patients and their families were involved in the project steering committee. A lead role was performed by the spouse of a TTS sufferer. This person was identified as suitable by communications with her spouse's treating consultant and by the fact of her prominent role in the vaccine injured and bereaved (VIB) patient advocacy group (www.vibuk.co.uk). This person attended the consortium steering committee meetings and, importantly, was involved in interpretation of results across the work packages. Our aim was that she would be able to help shape the next steps of each of the five work packages as the project progressed. We attempted to embed a PPI representative in each of the work packages, but further TTS sufferers did not feel able to participate due to fatigue and neurocognitive effects, and members of their families were too burdened with carer duties to be able to commit their time.

Thrombosis UK is the main thrombosis patient charity in the UK. It is dedicated to raising awareness and improving care for patients with blood clots. They rapidly adopted the role as the main patient advocacy charity for people suffering from VITT and, among other things, funded psychology input for those patients struggling to come to terms with their condition, its causes and long-term sequelae. A key stakeholder from Thrombosis UK was part of the project steering committee.

Through HDR-UK, researchers in work package 1 held a PPIE workshop to understand public perspectives in making regional, linked health data available for research use, to support high-priority research, such as COVID-19 vaccine safety. Lay summaries of publications were distributed to TTS sufferers via the VIB UK Group, other related websites and news sites.

A PPI representative attended the regular consortium meetings and listened to presentations of results and participated in discussions about their meaning. The large scale of the consortium's work meant that these presentations and discussions were high-volume and wide-ranging. These factors limited the PPI representative's opportunity to meaningfully contribute during these meetings. A member of the panel would debrief with her afterwards, give lay explanations of the results discussed and take feedback on how the work could be made more relevant for patients.

Patient and public involvement and engagement events were held on 26 April 2022, 5 October 2022 and 12 May 2023. These were organised initially via the West Midlands NIHR Local Clinical Research Network and subsequently through Thrombosis UK. These took the form of 2-hour online video meetings, where research developments were presented in bite-sized chunks in lay language. They were followed by an opportunity for discussion between the audience, speakers and panel members. The meeting format was adjusted following participant feedback after each session. Later sessions included highly relevant research/clinical work from outside the consortium such as that performed by Paul Bennett and the VITT Expert Haematology Panel.[45,83] Ongoing TTS sufferer fatigue prevented participants' travel for in-person meetings and limited their concentration during the events.

Meetings were recorded and made available to the wider community via the Thrombosis UK YouTube channel (YouTube, LLC, San Bruno, CA, USA) (www.youtube.com/@thrombosisuk180). Themes from discussion were fed back to the TTS consortium steering committee for consideration of incorporation into future work. As well as presenting the results of the work to patients directly, some lay summaries were distributed to patients via VIB, and were posted onto the website of HaemSTAR, the UK haematology research network (www.HaemSTAR.org/lay-summaries). Blog posts (https://substack.com/@richardbuka) and podcasts (https://soundcloud.com/dontjustread) were also prepared for patients and their families.

We worked hard to ensure appropriate PPIE in the planning, delivery and feedback of the consortium's work. There was significant engagement with dissemination of results and feedback to the PPIE group. Because of significant issues associated with the pandemic, such as limitations to group gatherings, and the difficulties affected patients and families faced because of their health, it was not possible to increase their involvement beyond what was ultimately achieved. It was very helpful, however, to keep researchers grounded by having to regularly justify and present their work to the patient group at the engagement events.

In conclusion, the extent of PPI on this project was less than had been planned. This was largely due to a combination of patient fatigue and the large consortium being spread across multiple institutions throughout the UK. The effects of a patient's chronic illness meant that fewer than anticipated were able to participate. Carer responsibilities also meant fewer family members of affected individuals were able to engage. The wide geographical distribution of the consortium members necessitated online meetings, and the lack of in-person interactions prevented relationship development. In addition, the PPI group struggled to provide meaningful input on the work packages focusing on COVID-related TTS. The majority of the PPI group were made up of VIB people. COVID-related TTS does not have a specific patient advocacy group, and the patients are not looked after by members of the consortium. Thus, this group was not accessible. This limited the patient involvement with work packages 1 and 2. Nevertheless, work package 1 did still manage to hold a PPIE workshop that influenced their priority setting.

Equality, diversity and inclusion

Initial data suggested that VITT preferentially affected white people.[31] There was concern initially that this was due to reporting-biases or due to poorer uptake of vaccine in ethnic minority groups.[84] VITT antibodies have been identified as being mono- and oligo-clonal and indeed, in unrelated patients, have been shown to have stereotypic amino acid sequences encoded for by alleles of the IGLV3-21 gene that are commonest in those of European white heritage.[85,86] Data from the US commercial and Medicare claims databases have shown that TTS after Ad26.COV2.S affecting common sites was higher among males and increased with age.[87] In adults above the age of 65 years, the highest rates were found among non-Hispanic black adults. For unusual site TTS, incidence rates were highest among non-Hispanic American Indian/Alaska native adults. A recent evaluation of 32 cases of VITT from Brazil, China, India, Islamic Republic of Iran, Mexico, Pakistan and Turkey showed that anti-PF4 antibodies were less likely to be measured compared to high-income countries (HIC), but clinical manifestations and treatment with IVIg were similar between low middle-income countries (LMIC) and HICs.[88] Interestingly, however, in hospital, mortality was lower in LMICs when compared to HICs. It does seem that VITT is less common in non-European populations, despite extensive use of adenoviral vector vaccines. However, the possibility of underascertainment of cases in these countries cannot be excluded. Although our work concentrated on a predominantly European ancestry population, we feel that our results, and potential future diagnostic and treatment strategies, will be relevant for all ethnic groups.

Impact and learning

The consortium enabled the development of cross-disciplinary collaborations which did not exist before and will be continued in terms of joint work and future grant funding applications. For instance, Toh and Parker have already published a position paper on the aetiopathogenesis of VITT, and their groups will continue to collaborate to improve the identification and understanding of anti-PF4 immunothrombosis.[89] ChAdOx1 can infect human endothelial cells through the Coxsackie and Adenovirus Receptor, and we believe that this process involves a high-affinity second receptor. Collaborations have also been developed with the University of Liverpool Shared Research Facility to characterise T-cell responses using Cytometry by Time-of-Flight (CyTOF), which will help to understand the nature of vaccine-induced T-cell activation and provide confirmation of a true memory T-cell response.

An important learning from the work of the consortium is that there is a need for better data linkages which can help in detecting and evaluating rare syndromes such as VITT in near real time. This work has resulted in some key

recommendations which have been fed into NHS England and UK-wide regional SDE planning and development. In addition, further funding from the Data and Connectivity National Core Study enabled two further 'driver' use cases to be conducted generating further learnings on the use of cross-regional-level linked data.[58,90]

The activities also enabled networking with the Medicines and Healthcare products Regulatory Agency (MHRA), the manufacturers of the vaccines (including AstraZeneca and Janssen), the European Medicines Agency, and academic investigators in Europe, USA and other countries. As an example, work is continuing with labs around Europe to identify which serum proteins from healthy and VITT patients bind to ChAdOx1 (Grants: EU MSCA mobility fund and INVECTA). In addition, the academic collaborations outside the consortium will allow an increase in sample size, important particularly for the genomic studies. The potential longer-term impact of this study may involve collaborative efforts with vaccine developers to assess the impact of adenoviral cross-reactivity on vaccine effectiveness. A long-term aim would be to assess the serotype of individuals for pre-existing immunity to human adenoviruses and comparatively evaluate both the incidence of adverse reactions and vaccine efficacy.

A long-term legacy of this project is the biobank of rare, precious VITT samples that were donated by patients, which will help future discoveries especially because of the reports of TTS which are not caused by either heparin or vaccines (so-called autoimmune TTS).[67] There is now increasing evidence that TTS existed pre-pandemic and has been documented post pandemic following adenovirus infections.[67,91]

The new understanding has led to the proposal a new model of coagulation – termed the convergent model of coagulation, this highlights how the unified host response to injury converges coagulation with innate immune activation and inflammation.[79] It addresses the shortcomings of previous models of coagulation, for example, cascade and cell-based models, and highlights the key junctional points for potential therapeutic development. Another important finding is that TTS may not be solely mediated through FcɣRIIa, which has previously been widely accepted, but that PF4 itself can activate platelets through the c-Mpl receptor in VITT.[57]

The deep understanding of the pathophysiology in VITT will also benefit future use of adeno-associated viral vectors in a variety of gene therapy programmes.

The TTS consortium has helped in establishing the careers of many early career researchers. Most of the work packages involved early career postdoctoral researchers who have received training in new techniques and had the benefit of presenting to the whole consortium at our monthly meeting. Sam Montague (University of Birmingham) has been awarded a BHF project grant (PG/23/11230) to study the variability of healthy volunteer platelet responses to FcɣRIIa stimuli.[92] This will aid in future diagnostic tests for and potentially in identifying people at higher risk of TTS. In addition, Richard Buka (University of Birmingham) has gone onto to further funding on a dedicated BHF scholarship to study the role of PF4 in TTS and other thrombo-inflammatory disorders.

Implications for decision-makers

There are several implications of our work for decision-makers in government (UK and internationally), funders and vaccine developers. These are as follows:

- TTS is a wider syndrome and has many similarities with other conditions, such as HITT, autoimmune TTS, catastrophic antiphospholipid syndrome (CAPS), DIC and TMAs associated with a plethora of underlying conditions, such as adenocarcinoma and pancreatitis. Further work in these areas will be important to develop and validate new biomarkers (some of which have been highlighted by our work), and new therapeutics (some potential avenues have been suggested by our work).
- Data availability and connectivity is crucial in detecting, evaluating, monitoring and determining causality of unusual syndromes such as VITT. This ideally needs to happen in near real time but will only be possible through improved clinical coding and data linkages, seamless and streamlined access to data, faster data access approval processes, and leveraging of regional expertise. More details on our recommendations can be found above.
- Vaccines based on ChAdOx1, and other adenovirus vectors, are being developed against viral haemorrhagic fevers, Nipah virus, human immunodeficiency virus and hepatitis B.[93] The many potential advantages of this vector have been outlined above. Clearly the administration of vaccines is critically dependent on the benefit-risk of using a vaccine to prevent potentially fatal infections. However, the fear of the occurrence of VITT has been one of the factors which has fuelled vaccine hesitancy.[94] It is, therefore, important to consider how the risks of VITT can be mitigated by further understanding the mechanisms and whether modification of the vector is necessary and/or feasible.

Future research recommendations

From the work of this consortium and from that of others such as Greinacher and Warkentin, it is clear that anti-PF4-mediated TTS is something that has been present prior to the COVID-19 pandemic and will continue to occur.[67,80] The unknown pathological triggers and rarity make it difficult to study. There are similarities to other rare and devastating thrombotic thrombocytopenic syndromes, such as CAPS and malignancy-associated DIC with thrombosis. Together, these provide a strong rationale for a linked clinical and laboratory registry to further study the pathophysiology, treatments and outcomes of these rare conditions. The establishment of a national diagnostic laboratory needs to be considered. A panel of clinical tests, which are normally sent to many different laboratories (including NHS Blood and Transplant in Bristol, various immunophenotyping laboratories, UCL hospital and Sheffield Teaching Hospitals coagulation laboratory), could instead be performed in one place, streamlining the diagnostic process for clinicians and giving access to expert advice about diagnosis and treatment strategies for these complex and high-risk patients. Patients whose samples are sent to this laboratory should be given the option to consent to their clinical data and unused samples to be used in further research into TTS.

There is a need for additional studies to define the mechanisms of VITT – this should include further delineation of the electrostatic interaction between the vector and PF4 (and whether these can be prevented), and the subsequent downstream events that lead to systemic inflammation and thrombosis. This is important to identify and validate biomarkers and test potential therapeutic strategies such as the use of ibrutinib with the aim of identifying as early as possible the occurrence of this syndrome and intervening to reduce mortality and short- and long-term morbidity. Whether prior sensitisation with adenovirus infections primes the immune system in susceptible individuals to mount an aberrant immune response needs to be part of the evaluation of the mechanisms underpinning VITT.

Given the rarity of VITT, international collaborations are going to be necessary to undertake better-powered genomic studies to identify any genetic susceptibility factors. We are aware of at least three other groups (with whom we are in contact) that have recruited patients (albeit with different diagnostic criteria) and have undertaken different genomic approaches [whole genomic sequencing (as we have done), exome sequencing and genome-wide single nucleotide polymorphism typing].

We were not funded to evaluate the spectrum of morbidities suffered by patients who developed VITT. The feedback we have received from the PPIE group is that those who have been affected have been left with considerable morbidity, which has been life-changing not only for the patients but also for their families. As well as helping to prevent other people developing this devastating condition in the future, their priority is understanding more about how to ameliorate their symptoms. We recommend specific funding streams are established to encourage further research in this area, specifically to define the complexity of symptoms being suffered by these patients, and develop better treatment pathways to alleviate the suffering and improve their quality of life. This would also benefit patients who suffer non-TTS-related CVST.

Conclusions

Vaccine-induced immune thrombosis and thrombocytopenia is a complex multisystem syndrome precipitated in susceptible individuals by the administration of adenoviral vaccines. This leads to the development of anti-PF4 antibodies followed by a concatenation of events that results in systemic inflammation, thrombosis, thrombocytopenia, haemorrhage, and injury to multiple organ systems, which in the most severe cases, or where diagnosis and treatment is delayed, death. The key findings from our consortium include a delineation of the possible mechanism of the electrostatic interaction between the negatively charged adenoviral vector and the highly cationic PF4 protein, platelet activation is not only dependent on FcɣRIIa but also on the c-Mpl receptor, NETosis is accompanied by a systemic inflammatory state characterised by elevation in proinflammatory cytokines, systemic endothelial activation and coagulation activation, together with clot formation in distant organs. The work has identified potential biomarkers that could be used for diagnosis and prognosis, and potential therapeutic strategies to treat patients with VITT.

Another important finding was that by using pre-pandemic samples we were able to show that T-cell adenoviral cross-reactivity seems to be prevalent in the UK population, unlike antibody cross-reactivity. Although this does not seem to have hampered vaccine efficacy responses, it provides a possible pathway for pre-sensitisation in susceptible individuals that leads to the aberrant immune seen in VITT. Of importance here is that TTS, in the absence of either heparin or vaccine, has been reported before the pandemic and more recently post pandemic, suggesting the intriguing possibility that it is triggered by adenoviral infection

(or by other unknown pathogens infecting the host in isolation or in combination with the adenovirus). Adenovirus infection is common, and although it is asymptomatic in many cases, it can occasionally result in serious illness. To date, more than 50 serotypes and > 100 genotypes of the adenovirus have been identified, and the seroprevalence of neutralizing antibodies during the convalescent period is reported to be approximately 30–60%.[95] This is an important area for further study (as mentioned above) but also highlights the fact that given the rarity of VITT, affected patients have an unidentified susceptibility factor. Our studies used a WGS approach to evaluate both common and rare variants, but failed to identify any signals suggesting that the study was underpowered with a lower effect size than seen with other pharmacogenomic phenotypes. Further collaborative work across international boundaries will be important to increase the sample size.

Availability of data in real time is important in identifying and monitoring complex adverse effects such as VITT. We have identified the need to improve data sources and linkages to allow near real-time detection of complex events beyond VITT, both within and out with public health emergencies.

Finally, although VITT has caused a lot of concern, it is important to emphasise that the overall benefits of COVID-19 vaccination far outweigh the risks. This is further exemplified in our most recent analysis using the OpenSAFELY software platform, which has shown that people who have had COVID-19 before or without being vaccinated are at higher risk of cardiovascular events for at least 2 years, the risk being greatest in weeks 1–4 after infection, while COVID-19 vaccination reduces the risks of cardiovascular events after COVID-19 infection.[92]

Additional information

CRediT contribution statement

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Acknowledgements

Importantly, the authors wish to thank all the patients who generously donated samples, and all participants who donated their time as part of the PPIE group. We would also like to thank (a) the MHRA for their support, and in particular Alicia Ptaszynska-Neophytou for her administrative support; (b) all the staff of the RCGP RSC and SEU for sample collection and processing and staff in the Virus Reference Department for completing the serology testing. Patients at Oxford-RCGP RSC practices who do not opt out of data sharing and who consented to additional blood samples being taken for serology, and the RSC member practices who share data and for sampling; (c) Lara Edwards at HDR-UK, and Prof Beverley Hunt at Thrombosis UK for their support; and (d) Margaret Goodall (deceased) at the University of Birmingham for her expertise. The consortium was funded by the Department of Health and Social Care (DHSC) and supported by the National Institute for Health and Care Research (NIHR) (NIHR135073). Funding was also provided by the Liverpool University Hospitals NHS Foundation Trust (LUHFT) and the UKHSA – an executive agency of DHSC – for collection/collation of specimens and senior staff oversight. The consortium also received support from the BHF Data Sciences Centre, GEL, Illumina incorporated and Barts Charity. The salary of SPW is covered by the British Heart Foundation (Chapter03/003).

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Patient data statement

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone (#datasaveslives). There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that they are stored and used responsibly. Everyone should be able to find out about how patient data are used. You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation

Data-sharing statement

The data for whole human genome sequencing are available via GEL. Data for the population level data analysis from England used in the study are available in the NHS England SDE and SAIL Databank at Swansea University, because restrictions apply, they are not publicly available. Those wishing to gain access to the data should contact bhfdsc@hdruk.ac.uk and https://saildatabank.com/contact/. Data used for the regional level data analysis are available in regional and NHS trust-level SDEs. Because restrictions apply they are not currently publicly available. Access to data for accredited researchers for CIPHA data is currently restricted to local regional accredited researchers only. Access to data for accredited researchers for Imperial College Healthcare NHS Trust (iCARE) data should contact Imperial.DataAccessRequest@nhs.net, for access to DataLoch (NHS Lothian) data via the DataLoch website https://dataloch.org/connect-with-us and for access to Barts Health NHS Trust contact bartshealth.researchdatarequest@nhs.net. Data on other aspects that support the findings of the consortium are available from the authors of the different papers upon reasonable request.

Ethics statement

The research studies undertaken by the consortium were covered by a number of ethics committee approvals. In studies led by University of Birmingham, patient blood collection was approved under research ethics 15/NW/0079, amendment 3 (27/2/15). Ethical approval for collecting blood from healthy volunteers was granted by Birmingham University Internal

Ethical Review Committee (ERN_11- 0175) and for AZD1222 vaccinated controls from the COCO study, approved under research ethics 20/HRA/1817 (22/5/20). In Liverpool, VITT patients were retrospectively included in this study from across multiple hospitals in England, United Kingdom through shared protocol and ethics: [Research Ethics Committee (REC) Ref: 20/EE/0035 (23/4/20), 15/NW/0079 (27/2/15) and 18/NW/0187 (23/4/18)]. DIC patients with a diagnosis of bacterial sepsis were recruited from consecutive general adult intensive care unit (ICU) patients admitted at the Royal Liverpool University Hospital, UK between June 2013 and Jan 2014 [REC Ref: 13/NW/0089 (10/4/13)]. Written informed consent or assent from next of kin was obtained for all patients within this study. AZD1222 vaccine controls [REC Ref: 16/NW/0170 (8/3/16)], and healthy controls were recruited following written, informed consent and ethical approval was obtained from the Liverpool research ethics committee. Vaccine-naive PBMCs were accessed through the Liverpool healthy donor biobank which contains 1200+ cryopreserved and HLA typed PBMC samples collected prior to the COVID-19 pandemic – this was approved by Liverpool REC. For the population level data analysis approval was obtained from the Newcastle and North Tyneside REC [20/NE/0161 (27/5/20)], the NHS Digital Data Access Request Service (DARS-NIC 381078-Y9C5K) and the British Heart Foundation Data Science Centre Cardiovascular Disease-COVID-UK Approvals and Oversight Board. For regional level data analysis, the study was classified as a service evaluation with individual patient level data accessed, linked and analysed within each participating organisation with approval of local Research Ethics Committees and Caldicott Guardians at each site. We sought permission from relevant NHS data controllers for analysis of pseudo-anonymised data in SDEs. For the UKHSA, all data were collected within statutory approvals granted for infectious disease surveillance and control. The collection of seroprevalence samples is approved by the PHE Caldicott Guardian under Regulation 3 (Health Protection) of The Health Service (Control of Patient Information) Regulations 2002. Specific ethical approval is not required for this surveillance work. National Research Ethics Service approval for the seroepidemiological surveillance of the National Immunisation programme of England and Wales, REC number 05/Q0505/45, was granted by the Joint University College London/University College London Hospital Committees on the Ethics of Human Research. For REACT, ethics approval was granted by South Central-Berkshire B REC (IRAS ID: 283787). The GEL ethics approval was from the East of England – Cambridge South REC, Ref 14/EE/1112 (20/2/15).

Information governance statement

The University of Liverpool adheres to the UK Data Protection Act (2018) and the General Data Protection Regulation (EU GDPR) 2016/679 when it comes to managing personal information. As per the Data Protection legislation, the University of Liverpool serves as the Data Controller. For detailed information on how personal data is handled, including guidance on exercising individual rights and contacting the Data Protection Officer, please refer to this link (legal@liverpool.ac.uk).

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page at https://doi.org/10.3310/FFSS9010.

Primary conflicts of interest: Phillip LR Nicolson and Richard J Buka have received unrelated research funding from AstraZeneca as well as an educational grant from Sobi. Phillip LR Nicolson has received honoraria from AstraZeneca, Chugai, Grifols, Sobi and Takeda. Phillip LR Nicolson and Steve P Watson have received research funding from Rigel, Novartis, Principia Biopharma and Sanofi and have a US Patent application (US20240207277A1). Gayatri Amirthalingam, Simon T Abrams, Kevin Brown, David Goldblatt, Charotte Lovatt, Samantha J Montague, Mary E Ramsay, Jonathan AC Sterne, Cathie LM Sudlow, Cheng Hock Toh, Guozheng Wang and Angela M Wood have no interests to disclose. Richard J Buka has been funded through the British Heart Foundation Accelerator (AA/18/2/34218). Mark J Caulfield reports income from being a non-executive director of the Barts Health Board and unpaid role as the non-executive director of the MedCity board. British Heart Foundation as part of the British Heart Foundation Data Science Centre led by HDR-UK (British Heart Foundation grant number SP/19/3/34678). Data and Connectivity National Core Study led by HDR-UK in partnership with the Office for National Statistics and funded by UK Research and Innovation (grant reference MC_PC_20058). Longitudinal Health and Wellbeing COVID-19 National Core Study, which was established by the UK Chief Scientific Officer in October 2020 and funded by UK Research and Innovation (grant references MC_PC_20030 and MC_PC_20059). Alan Parker is founder and Chief Scientific Officer of Trocept Therapeutics. Sue Pavord reports royalty payments from Cambridge University Press, advisory board membership for Sobi and Janssen, honoraria from Sobi, Amgen, Janssen Novartis and Pharmacosmos, educational grants from Octapharma, Pharmacosmos, LFB and Haemonetics, payment for expert testimony from Butcher & Barlow and shares in Ely Lilly. Steve P Watson holds a BHF Chair (Chapter03/003). William Whiteley reports grants from Eisai and advisory board membership of Viatris. The Immunisation and Vaccine Preventable Diseases Division of the UKHSA has provided vaccine manufacturers (including Pfizer) with post-marketing surveillance reports on pneumococcal and meningococcal infection which the companies are required to submit to the UK Licensing authority in compliance with their Risk Management Strategy. A cost recovery charge is made for these reports. Munir Pirmohamed has received partnership funding for the following: MRC Clinical Pharmacology Training Scheme (co-funded by MRC and Roche, UCB, Eli Lilly and Novartis). He has developed an HLA genotyping panel with MC Diagnostics but does not benefit financially from this. Munir Pirmohamed and Dean J Naisbitt are

part of the IMI Consortium ARDAT (www.ardat.org). None of the funding Munir Pirmohamed received is related to the current paper. Munir Pirmohamed is also Chair of the Commission on Human Medicines.

Department of Health and Social Care disclaimer

This publication presents independent research commissioned by the National Institute for Health and Care Research (NIHR). The views and opinions expressed by authors in this publication are those of the authors and do not necessarily reflect those of the NHS, the NIHR, MRC, NIHR Coordinating Centre, the Efficacy and Mechanism Evaluation programme or the Department of Health and Social Care.

This synopsis was published based on current knowledge at the time and date of publication. NIHR is committed to being inclusive and will continually monitor best practice and guidance in relation to terminology and language to ensure that we remain relevant to our stakeholders.

Funding

This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme as award number NIHR135073.

Award publications

This synopsis provided an overview of the research award Understanding Mechanisms of Thrombosis and Thrombocytopenia in COVID-19 and with SARS-CoV-2 Vaccines. Other articles published as part of this thread are:

Whiteley WN, Ip S, Cooper JA, Bolton T, Keene S, Walker V, *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

Baker AT, Boyd RJ, Sarkar D, Teijeira-Crespo A, Chan CK, Bates E, *et al*. ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome. *Sci Adv* 2021;**7**:eabl8213. https://doi.org/10.1126/sciadv.abl8213

Buka RJ, Montague SJ, Moran LA, Martin EM, Slater A, Watson SP, *et al*. PF4 activates the c-Mpl–Jak2 pathway in platelets. *Blood* 2024;**143**:64–9. https://doi.org/10.1182/blood.2023020872

For more information about this research please view the award page (www.fundingawards.nihr.ac.uk/award/NIHR135073).

Additional outputs

Smith CW, Montague SJ, Kardeby C, Di Y, Lowe GC, Lester WA, *et al*. Anti-platelet drugs block platelet activation by vaccine-induced immune thrombocytopenia and thrombosis patient serum. *Blood* 2021;**138**:2733–40.

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About this synopsis

The contractual start date for this research was in July 2021. This synopsis began editorial review in April 2024 and was accepted for publication in February 2025. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The Efficacy and Mechanism Evaluation editors and publisher have tried to ensure the accuracy of the authors' article and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this synopsis.

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List of supplementary material

Report Supplementary Material 1
Supplementary materials

Supplementary material can be found on the NIHR Journals Library report page (https://doi.org/10.3310/FFSS901).

Supplementary material has been provided by the authors to support the report and any files provided at submission will have been seen by peer reviewers, but not extensively reviewed. Any supplementary material provided at a later stage in the process may not have been peer reviewed.

Glossary

Adenoviral vector vaccines A type of vaccine that uses a modified adenovirus to deliver genetic material from a pathogen (such as SARS-CoV-2) to stimulate an immune response without causing disease.

AZD1222 The vaccine developed by AstraZeneca and the University of Oxford, based on a chimpanzee adenoviral vector, used for COVID-19 vaccination.

ChAdOx1 The chimpanzee adenovirus-based viral vector used in the AstraZeneca vaccine, modified to carry the genetic code for the SARS-CoV-2 spike protein.

C-reactive protein A protein produced by the liver in response to inflammation. Elevated C-reactive protein levels can indicate infection or inflammation.

Cryoelectron microscopy A high-resolution imaging technique used to study the structure of proteins and viruses.

D-dimer A fibrin degradation product present in the blood after a clot dissolves. Elevated D-dimer levels indicate abnormal clotting activity, as seen in vaccine-induced immune thrombosis and thrombocytopenia.

FcγRIIa A receptor on immune cells and platelets that mediates immune complex-induced platelet activation.

Gene burden testing A genomic analysis technique that assesses the collective impact of rare genetic variants on disease susceptibility.

Genomic variants Differences in DNA sequence between individuals that may increase or decrease the risk of certain diseases.

Heparin-induced thrombocytopenia A rare immune-mediated condition triggered by heparin, leading to low platelet counts and thrombosis.

Immune complex A structure formed when antibodies bind to antigens, which can activate the immune system. In vaccine-induced immune thrombosis and thrombocytopenia, immune complexes form with PF4, leading to platelet activation and clotting.

Immunoassay A laboratory test that uses antibodies to detect the presence and quantity of specific proteins.

Long COVID A condition characterised by persistent symptoms following recovery from acute COVID-19 infection. Long COVID may involve complications like thrombosis.

NETosis A form of cell death in which neutrophils release extracellular traps (NETs) composed of DNA and proteins, contributing to clot formation in diseases like vaccine-induced immune thrombosis and thrombocytopenia.

Neutrophil extracellular traps Web-like structures released by neutrophils during NETosis that trap pathogens. In vaccine-induced immune thrombosis and thrombocytopenia, NETs contribute to excessive clot formation.

Platelet factor 4 A protein released by platelets during blood clotting.

Platelets Small blood cells that play a critical role in clotting. In vaccine-induced immune thrombosis and thrombocytopenia, platelets become activated abnormally due to immune complexes involving PF4.

Seroprevalence The proportion of a population that tests positive for antibodies against a particular antigen, indicating previous infection or vaccination.

Thrombocytopenia A condition characterised by abnormally low platelet counts, which increases the risk of bleeding. In vaccine-induced immune thrombosis and thrombocytopenia, thrombocytopenia is associated with clotting complications.

Thrombosis The formation of blood clots in blood vessels, which can cause blockages. Thrombosis can occur in unusual sites in vaccine-induced immune thrombosis and thrombocytopenia, such as the cerebral venous sinuses.

Thrombosis with thrombocytopenia syndrome A condition characterised by blood clot formation (thrombosis) and low platelet counts (thrombocytopenia), which can be triggered by certain adenoviral COVID-19 vaccines.

Transcriptomics The study of all RNA transcripts in a cell or tissue, used to understand gene expression patterns in diseases like vaccine-induced immune thrombosis and thrombocytopenia.

Vaccine-induced immune thrombosis and thrombocytopenia A rare and serious condition triggered by adenoviral COVID-19 vaccines, characterised by blood clots and low platelet counts, often associated with antibodies against PF4.

Whole-genome sequencing A technique for determining the complete DNA sequence of an organism's genome, used to identify genetic factors related to diseases such as vaccine-induced immune thrombosis and thrombocytopenia.

List of abbreviations

100KGP	100,000 Genomes Project
AE	adverse event
AZD1222	AstraZeneca COVID-19 vaccine (also known as Vaxzevria)
BNT162B2	Pfizer-BioNTech COVID-19 vaccine
BTK	Bruton tyrosine kinase
CAPS	catastrophic antiphospholipid syndrome
CIPHA	Combined Intelligence for Population Health Action
CVST	cerebral venous sinus thrombosis
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FCΓRIIA	low-affinity immunoglobulin gamma Fc region receptor II-a
FDR	false-discovery rate
GEL	Genomics England
GP	general practitioner
GWAS	genome-wide association study
HDR-UK	Health Data Research UK
HIC	high-income countries
HIT	heparin-induced thrombocytopenia
HITT	heparin-induced thrombocytopenia with thrombosis
HLA	human leukocyte antigen

HVR	hexon hypervariable region
ICD-10	*International Statistical Classification of Diseases and Related Health Problems*, Tenth Revision
IGG	immunoglobulin G
IL-6	interleukin 6
IVIG	intravenous immunoglobulin
JAK2	Janus kinase 2, a protein involved in blood cell signalling
LMIC	low middle-income countries
MAF	minor allele frequency
MHRA	Medicines and Healthcare products Regulatory Agency
MI	myocardial infarction
MRNA	messenger ribonucleic acid
NETS	neutrophil extracellular traps
OD	optical density
PBMC	peripheral blood mononuclear cell
PF4	platelet factor 4
PHE	Public Health England
PLINK	a whole-genome association analysis toolset
PPI	patient and public involvement
PPIE	patient and public involvement and engagement
RCGP	Royal College of General Practitioners
REACT	Real-time Assessment of Community Transmission
RNA	ribonucleic acid
RSC	Research and Surveillance Centre
SAIGE	Scalable and Accurate Implementation of Generalized mixed model
SARS-COV-2	severe acute respiratory syndrome coronavirus 2
SCCS	self-controlled case series
SDE	secure data environment
SNOMED CT	systematized nomenclature of medicine clinical terms
SPR	surface plasmon resonance
TMA	thrombotic microangiopathy
TTS	thrombosis with thrombocytopenia syndrome
TWAS	transcriptome-wide association study
UCL	University College London
UKHSA	UK Health Security Agency
VIB	vaccine injured and bereaved
VITT	vaccine-induced immune thrombosis and thrombocytopenia
WGS	whole-genome sequencing

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