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BRIEF REPORT

# Damage-associated cellular markers in the clinical and pathogenic profile of vaccine-induced immune thrombotic thrombocytopenia

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## Abstract

**Background:** Adenoviral vector-based COVID-19 vaccine-induced immune thrombotic thrombocytopenia (VITT) is rare but carries significant risks of mortality and long-term morbidity. The underlying pathophysiology of severe disease is still not fully understood. The objectives were to explore the pathophysiological profile and examine for clinically informative biomarkers in patients with severe VITT.

**Methods:** Twenty-two hospitalized patients with VITT, 9 pre- and 21 post-ChAdOx1 vaccine controls, were recruited across England, United Kingdom. Admission blood samples were analyzed for cytokine profiles, cell death markers (lactate dehydrogenase and circulating histones), neutrophil extracellular traps, and coagulation parameters. Tissue specimens from deceased patients were analyzed.

**Results:** There were strong immune responses characterized by significant elevations in proinflammatory cytokines and T helper 1 and 2 cell activation in patients with VITT. Markers of systemic endothelial activation and coagulation activation in both circulation and organ sections were also significantly elevated. About 70% ($n$ = 15/22) of patients met the International Society for Thrombosis and Haemostasis criteria for disseminated intravascular coagulation despite negligible changes in the prothrombin time. The increased neutrophil extracellular trap formation, in conjunction with marked lymphopenia, elevated lactate dehydrogenase, and circulating histone levels, indicates systemic immune cell injury or death. Both lymphopenia and circulating histone levels independently predicted 28-day mortality in patients with VITT.

**Conclusion:** The coupling of systemic cell damage and death with strong immune-inflammatory and coagulant responses are pathophysiologically dominant and clinically relevant in severe VITT.

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**KEYWORDS**
circulating histones, disseminated intravascular coagulation (DIC), lactate dehydrogenase (LDH), neutrophil extracellular traps (NETs), vaccine-induced immune thrombocytopaenia and thrombosis (VITT)

## 1 | INTRODUCTION

Vaccination against COVID-19 reduces COVID-19-related hospital admissions and mortality [1]. However, between February and March 2021, cases of thrombocytopenia with unusual thrombosis developed after vaccination with COVID-19 adenoviral vector vaccines [2]. The emergence of this syndrome, termed vaccine-induced immune thrombotic thrombocytopenia (VITT), was unexpected and catastrophic. Although rare, with an incidence estimated to be 10 cases per million following initial vaccination, VITT carries significant risks of morbidity and mortality [3,4].

VITT is suspected when symptoms arise 5-30 days after vaccination with the presence of thrombosis, thrombocytopenia, significantly raised D-dimer, and a positive antiplatelet factor 4 (PF4) on ELISA [3]. VITT shares similarities with heparin-induced thrombocytopenia (HIT). Both result from PF4 and anti-PF4 immune complexes. In HIT, heparin binds to PF4, altering its structure and causing an immunogenic neoepitope [5–9]. In VITT, antibodies against PF4 are independent of heparin [10]. Anti-PF4 complexes from patients with VITT induce thrombosis *in vitro* and *in vivo* through binding the Fc gamma receptor II (FcγRII) [10] and inducing platelet activation and neutrophil extracellular traps (NETs) [11] in a similar way to HIT [12]. However, many patients with VITT have a severe and rapidly fulminant disease process that is difficult to explain simply by anti-PF4 complex-induced platelet activation, NETosis, and thrombosis. We, therefore, undertook a systematic investigation of patients with VITT to further understand its pathophysiology.

## 2 | METHODS

### 2.1 | Study design

Twenty-two patients with VITT 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, 15/NW/0079, and 18/NW/0187) on fulfilling these diagnostic criteria [3]: (1) onset of symptoms 5 to 30 days after COVID-19 vaccination, (2) presence of anti-PF4 antibodies (PF4 immunoglobulin (Ig)G, Immucor), (3) thrombosis, (4) thrombocytopaenia ($<150 \times 10^9$/L) and (5) elevated D-dimer levels (>4000 fibrinogen equivalent units). Paired ChAdOx1 vaccine controls were also recruited (9 pre- and 21 post-vaccine) (REC Ref: 07/H1009/64). Clinical data were recorded, which included age, sex assigned at birth, days since vaccination with ChAdOx1nCoV-19, and site of thrombosis. Tests performed in local laboratories included the lymphocyte count, platelet count, prothrombin time (PT), fibrinogen, D-dimer, and lactate dehydrogenase (LDH) with normal ranges determined locally and performance standardized through UK National External Quality Assessment Service. Disseminated intravascular coagulation (DIC) scoring was performed using the International Society for Thrombosis and Haemostasis (ISTH) criteria [13]. For comparison, 15 patients with DIC with a diagnosis of bacterial sepsis were recruited from consecutive general adult intensive care unit patients admitted at the Royal Liverpool University Hospital, UK, between June 2013 and January 2014 (REC Ref: 13/NW/0089). Samples at the time of DIC diagnosis were compared to patients with VITT. Written informed consent or assent from next of kin was obtained for all patients within this study.

### 2.2 | Cytokine quantification

Twenty cytokines, chemokines, and cell adhesion activation markers (cytokines: granulocyte-macrophage colony-stimulating factor, interferon (IFN)α, IFNγ, interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17a, and tumor necrosis factor α; chemokines: IP-10, MCP-1, MIP1α, and MIP1β; adhesion activation markers: ICAM-1, E-selectin, and P-selectin) were measured by MultiPlex (Thermo Fisher Scientific) in serum of patients with VITT at diagnosis and pre-and post-ChAdOx1 vaccine controls, using a Bio-Plex 100 instrument according to manufacturers' instructions.

### 2.3 | Circulating histones

Circulating histones were quantified in the serum of patients with VITT at presentation and pre-and post-ChAdOx1 vaccine controls, as described previously [14].

### 2.4 | NETs assay

NETs formation was investigated by incubating serum from patients or pre-and post-ChAdOx1 vaccine controls (1:5 dilution) with

**TABLE** Comparison of factors between survivors and nonsurvivors in patients with vaccine-induced immune thrombotic thrombocytopenia on hospital admission.

| Clinical characteristics | Total, median [IQR][a] | Survivors, median [IQR][a] | Nonsurvivors, median [IQR][a] | *P* value[b] | AUC [95% CI] | *P* value[c] | Crude odds ratio [95% CI] | *P* value[d] |
|---|---|---|---|---|---|---|---|---|
| Total number (*N*) | 22 | 10 | 12 | | | | | |
| **Demographics** | | | | | | | | |
| Age (years) | 39.5 [29.0, 47.3] | 45.0 [29.3, 47.3] | 32.0 [27.0, 48.0] | .597 | 0.433 [0.174, 0.693] | .598 | 0.972 [0.901, 1.049] | .468 |
| Male, *n* (%) | 10 (45.5) | 4.0 (40.0) | 6.0 (50.0) | .691 | - | - | 1.500 [0.275, 8.189] | .640 |
| White ethnicity, *n* (%) | 21 (95.5) | 10 (100.0) | 11 (91.7) | 1.000 | - | - | - | - |
| Days since vaccination with ChAdOx1 nCoV-19 | 12.0 [10.0, 17.0] | 11.5 [10.0, 20.0] | 13.0 [10.1, 17.0] | .923 | 0.513 [0.248, 0.777] | .921 | 0.960 [0.799, 1.153] | .662 |
| Site of thrombosis, CVST, *n* (%) | 18 (81.8) | 7 (70) | 11 (91.7) | .293 | - | - | 4.714 [0.405, 54.826] | .215 |
| **Coagulation and thrombosis** | | | | | | | | |
| Platelets ($\times 10^9$/L) | 36.0 [19.3, 62.3] | 55.5 [23.8, 79.5] | 31.0 [12.8, 46.8] | .080 | 0.279 [0.057, 0.501] | .081 | 0.969 [0.934, 1.004] | .086 |
| Fibrinogen (g/L), | 1.4 [0.9, 2.1] | 1.8 [1.0, 3.3] | 1.2 [0.7, 1.9] | .210 | 0.342 [0.105, 0.579] | .210 | 0.656 [0.308, 1.399] | .275 |
| D-dimer (ng/mL) | 22 700 [10 610.0, 63 671.0] | 20 000 [6421.0, 30 812.5] | 30 350.0 [12 060.5, 77 868.5] | .155 | 0.685 [0.453, 0.917] | .155 | 1.000 [1.000, 1.000] | .140 |
| Prothrombin time (sec) | 14.2 [12.4, 16.2] | 13.8 [12.4, 16.8] | 14.6 [12.2, 15.9] | .845 | 0.471 [0.179, 0.764] | .845 | 0.959 [0.823, 1.118] | .593 |
| DIC, *n* (%) | 15 (68.2) | 4 (40.0) | 11 (91.7) | .020 | - | - | 16.500 [1.487, 183.070] | .022 |
| **Cell damage** | | | | | | | | |
| Lymphocyte count ($\times 10^9$/L) | 0.6 [0.4, 1.0] | 1.0 [0.6, 1.6] | 0.4 [0.3, 0.7] | .005 | 0.136 [0.000, 0.293] | .005 | 0.004 [0.000, 0.559] | .028 |
| LDH (U/L) | 278.9 [173.8, 582.4] | 184.3 [118.9, 254.0] | 443.5 [249.8, 832.2] | .013 | 0.824 [0.644, 1.000] | .013 | 1.007 [1.000, 1.015] | .050 |
| Circulating histones (μg/mL) | 18.7 [2.9, 52.0] | 3.3 [0.5, 4.7] | 50.0 [33.0, 75.1] | <.001 | 0.925 [0.813, 1.000] | .001 | 1.093 [1.021, 1.171] | .011 |
| **Immunothrombosis** | | | | | | | | |
| IL-6 (pg/mL) | 37.1 [12.9, 432.8] | 24.4 [13.2, 392.3] | 79.7 [12.6, 1,065.8] | .575 | 0.571 [0.320, 0.822] | .575 | 1.000 [1.000, 1.001] | .702 |
| IL-8 (pg/mL) | 77.3 [0.0, 416.9] | 0.0 [0.0, 416.9] | 168.0 [0.0, 509.3] | .332 | 0.617 [0.375, 0.859] | .356 | 1.001 [0.999, 1.003] | .292 |
| **Endothelial activation** | | | | | | | | |
| ICAM-1 (ng/mL) | 241.7 [197.2, 306.8] | 249.3 [195.2, 309.4] | 241.2 [188.0, 280.0] | .598 | 0.433 [0.186, 0.681] | .598 | 1.000 [1.000, 1.000] | .355 |
| E-selectin (ng/mL) | 116.0 [97.0, 148.8] | 125.1 [107.9, 161.4] | 110.0 [84.4, 114.9] | .262 | 0.358 [0.121, 0.596] | .262 | 1.000 [1.000, 1.000] | .334 |

(Continues)

TABLE (Continued)

| Clinical characteristics | Total, median [IQR][a] | Survivors, median [IQR][a] | Nonsurvivors, median [IQR][a] | *P* value[b] | AUC [95% CI] | *P* value[c] | Crude odds ratio [95% CI] | *P* value[d] |
|---|---|---|---|---|---|---|---|---|
| **Adaptive immunity** | | | | | | | | |
| IFNγ (pg/mL) | 14.2 [9.8, 17.5] | 16.5 [12.2, 29.2] | 11.7 [7.4, 15.3] | .029 | 0.225 [0.027, 0.423] | .030 | 0.855 [0.717, 1.021] | .083 |
| IL-13 (pg/mL) | 4.5 [3.2, 6.1] | 5.2 [4.2, 8.2] | 3.9 [2.4, 5.2] | .059 | 0.258 [0.049, 0.467] | .056 | 0.769 [0.515, 1.148] | .199 |

AUC, area under the receiver operating characteristic curve; DIC, disseminated intravascular coagulation; CVST, cerebral venous sinus; IFN, interferon; IL, interleukin; LDH, lactate dehydrogenase.

[a] Unless otherwise specified.

[b] *P* value for comparisons between survivors and nonsurvivors. Performed using the Kruskall–Wallis test for continuous variables.

[c] *P* value for mortality using AUC curve analysis.

[d] *P* value for mortality using univariate regression analysis. Breakdown of patients with single (*N* = 18: pulmonary embolism [PE], *n* = 1; CVST, *n* = 16; stroke, *n* = 1) and multiple (*N* = 3: PE and deep vein thrombosis, *n* = 1; PV (pulmonay vein) and CVST, *n* = 1; PE and CVST, *n* = 1) sites of thrombosis. For 1 patient, the site of thrombosis was not described.

heterologous neutrophils ($2 \times 10^5$) from healthy volunteers. Propidium iodide stained extracellular DNA was visualized and quantified by immunofluorescence microscopy (Olympus; × 20 magnification), as previously described [15].

### 2.5 | Immunofluorescence staining on tissue sections

NETs and fibrin deposition were determined in formalin-fixed paraffin-embedded samples of kidney and liver (4 μm in thickness) from fatal VITT cases (*n* = 10) by a multiplex Tyramide Signal Amplification system (Opal-TSA, Akoya Biosciences), using rabbit anti-histone citrulline H3 (ab5103, Abcam), anti-neutrophil elastase (LS Bio, LS-B4244), and rabbit anti-fibrin beta-chain (clone 8E5). Samples were visualized using species-specific horseradish peroxidase conjugated secondary antibodies (ImPRESS horseradish peroxidase IgG Polymer Detection Kit, Vector Laboratories) and fluorophore-labeled tyramide (Opal, 10 minutes room temperature). (Supplementary Methods for a detailed description).

### 2.6 | Statistical analysis

The Kruskall–Wallis test was used to compare continuous variables, presented as median (IQR), and the Fisher's exact/chi-squared test was used to compare categorical variables, presented as counts (percentage). Circulating histone levels were analyzed as continuous variables or categorized based on a previously determined threshold for cytotoxicity (30 μg/mL) [16,17]. The Mann–Whitney U-test was used to compare categorical to continuous clinical variables. Correlation analysis was performed using Spearman's rank correlation coefficient. Receiver operating characteristic (ROC) curve analysis and univariate and multivariate regression (adjusted for age and sex) assessed diagnostic parameters in predicting 28-day mortality. Kaplan–Meier survival curve analysis was performed to analyze the probability of mortality over time. Statistical tests were performed on SPSS (IBM, version 26). A 2-tailed *P* value of <.05 was considered significant.

## 3 | RESULTS AND DISCUSSION

Twenty-two patients with VITT were studied (Table): the median age was 39.5 years (IQR, 29.0, 47.3), 10 patients were male (45.5%), and 21 were of White ethnicity (95.5%). The median onset of symptoms was 12.0 days (IQR, 10.0-17.0) after ChAdOx1nCoV-19 vaccination. Patients presented with thrombosis, which was primarily in the cerebral venous sinus (81.8%), thrombocytopenia ($<150 \times 10^9$/L platelets), and circulating anti-PF4 antibodies. Twelve died within 28 days (54.5%).

Patients with VITT had significant elevations in circulating proinflammatory markers compared with pre-and post-ChAdOx1 controls (Figure 1A, B and Supplementary Table S1), which included IL-6 ($P < .001$) and IL-8 ($P = .004$). They also had elevated circulating markers of endothelial activation (Figure 1C, D and Supplementary Table S1), including ICAM-1 ($P < .001$) and E-selectin ($P < .001$). To assess immune cell responses in VITT, T helper 1 and 2 activation markers (Figure 1E, F and Supplementary Table S1) were quantified. Specifically, IFNγ ($P = .015$) and IL-13 ($P < .001$) were elevated compared with pre-and post-ChAdOx1 controls.

Seventy-five percent (*n* = 16/22) of patients with VITT presented with lymphopenia (lymphocyte count $<1 \times 10^9$/L), suggesting lymphocyte loss. To determine whether patients with VITT had systemic cellular death, circulating LDH, a routine clinical marker of cell injury or death, was measured. LDH was significantly elevated (Figure 2A) in these patients compared with pre-and post-ChAdOx1 controls ($P = .033$), suggesting cell damage or cell death occurred in VITT. Histones are a major damage-associated molecular pattern (DAMP) released into the circulation from injured or dying cells that can further potentiate systemic cell death [14]. In patients with VITT, histone levels were significantly elevated ($P = .001$; Figure 2B). Levels strongly correlated with LDH concentrations (R = 0.854) to consolidate the finding that VITT is not just a disease characterized

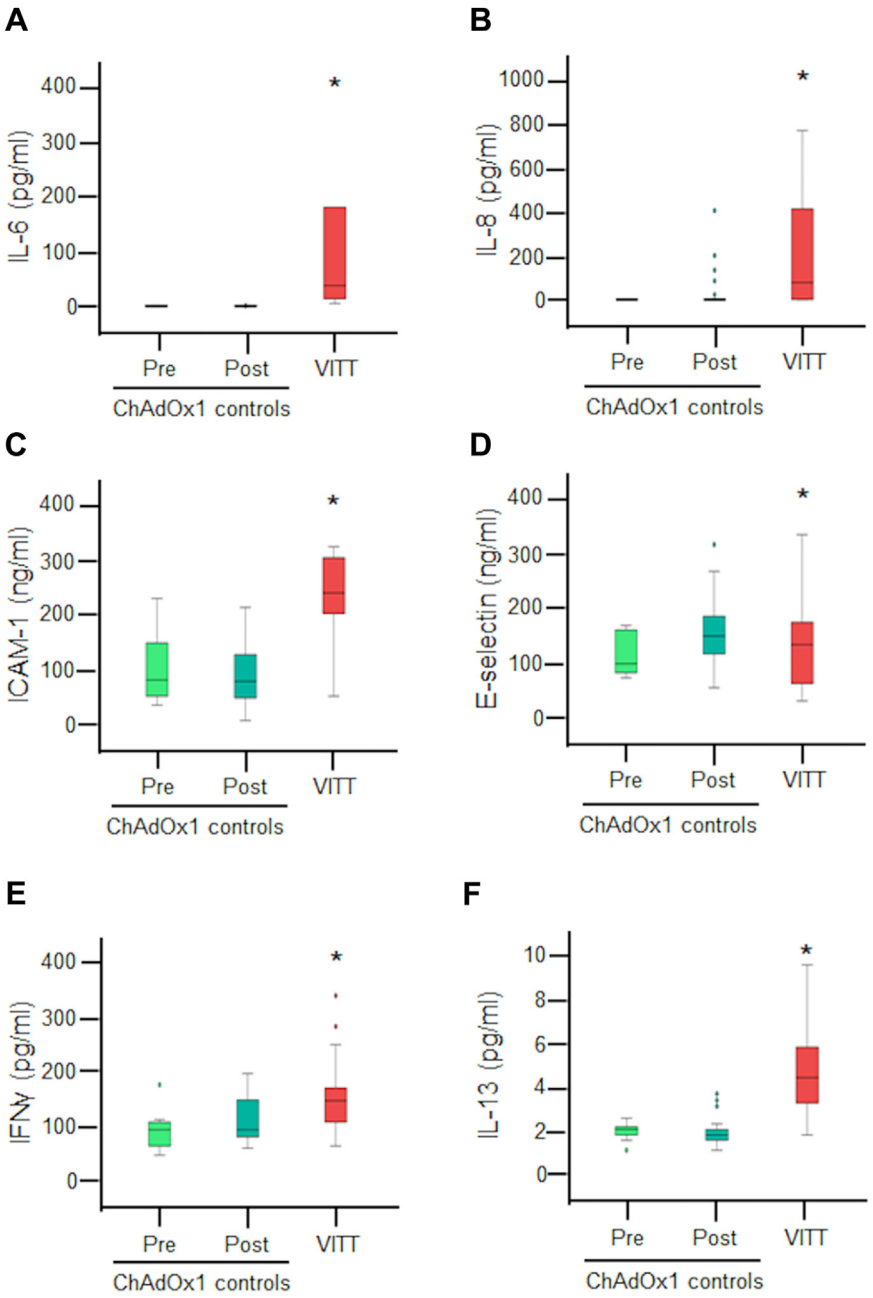


**FIGURE 1** Systemic inflammation and cellular activation in patients with vaccine-induced immune thrombotic thrombocytopenia (VITT). Proinflammatory cytokines interleukin (IL)-6 (A) and IL-8 (B) are elevated in patients with VITT ($n$ = 22) compared with pre- ($n$ = 9) and post- ($n$ = 21) ChAdOx1 controls. Circulating markers of endothelial activation, ICAM-1 (C) and E-selectin (D), are elevated in patients with VITT ($n$ = 22) serum compared with pre- ($n$ = 9) and post- ($n$ = 21) ChAdOx1 controls. T helper 1 (interferon [IFN]γ) (E) and T helper 2 (IL-13) (F) cell responses are increased in patients with VITT ($n$ = 22) compared with pre- ($n$ = 9) and post- ($n$ = 21) ChAdOx1 controls. Mann–Whitney U-test; *$P < .05$ compared with ChAdOx1 controls.

by thrombosis and thrombocytopenia driven by PF4-anti-PF4 complexes but one that is associated with systemic cell damage or cell death.

The ability of PF4-anti-PF4-adenoviral vector complexes to stimulate NETs formation and platelet activation via FcγRII [18–22] has been described, along with NETs formation in patients with VITT [11]. In this study, serum from patients with VITT was examined for NETs formation using our established *ex vivo* assay [15]. We found that NETs were directly induced by serum from all patients with VITT (Figure 2C) but not in serum from pre-and post-ChAdOx1 vaccine controls (Figure 2D). These data confirm strong NETs-forming potential in VITT, but our assay shows that the inclusion of platelets is not necessary for NETs formation. Immunofluorescence staining demonstrated NETs deposition in the vessels of liver and kidney tissue from fatal VITT cases (Figure 2E) beyond that reported within large vessel thromboses.

Another key finding is the presence of systemic inflammation, hypercoagulability, and innate immune activation. The convergence of these processes is archetypal of immunothrombosis [23], which was evident in severe COVID-19, where the role of extracellular histones was demonstrated [24]. Circulating histones directly drive these 3 facets of immunothrombosis by triggering the release of proinflammatory cytokines [14], platelet activation [17], and thrombin generation [25], as well as inducing NETosis [14]. Vaccine-induced

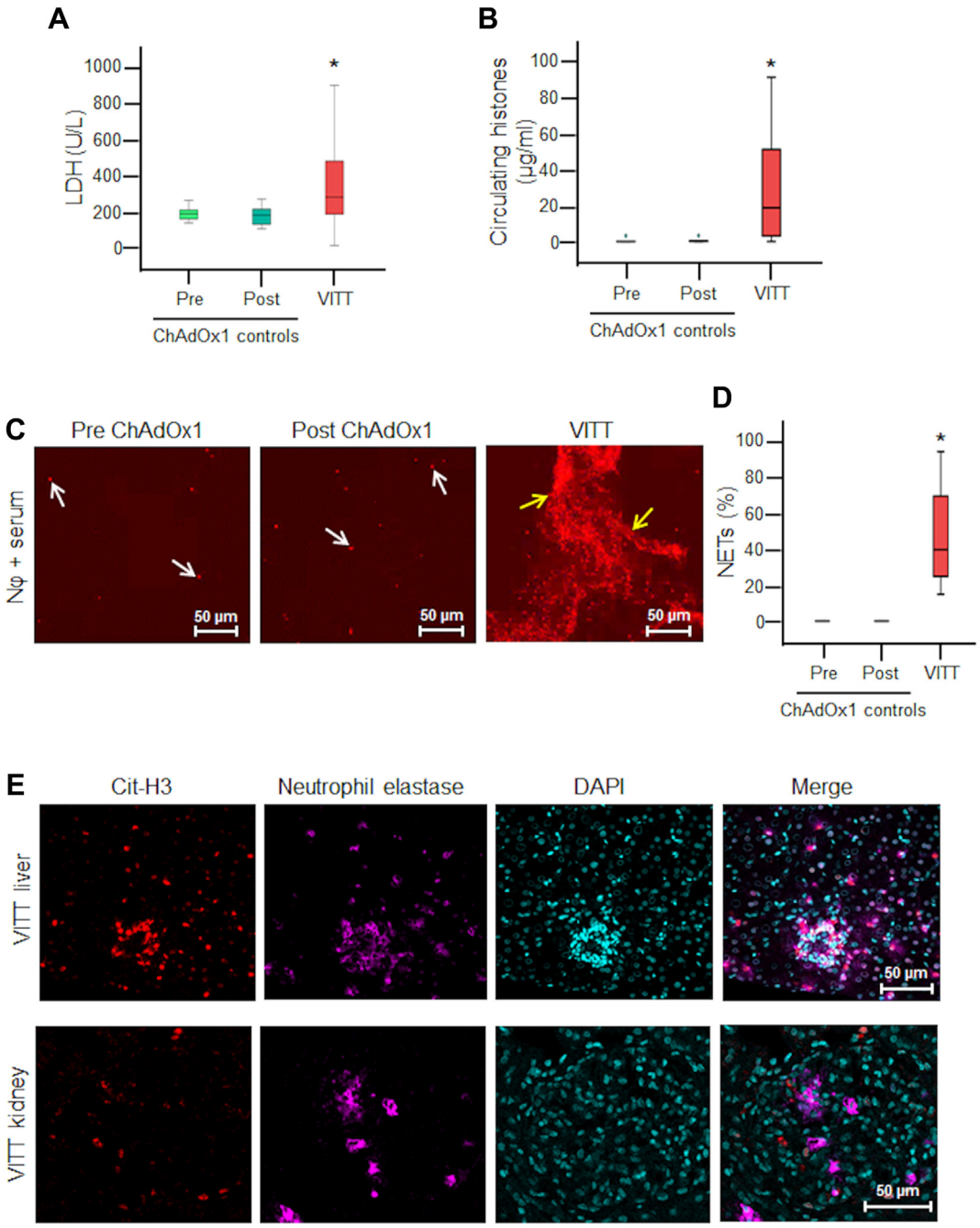


**FIGURE 2** Cell death and NETosis are elevated in patients with vaccine-induced immune thrombotic thrombocytopenia (VITT). Quantification of lactate dehydrogenase (LDH) (A) and histone (B) levels in pre- ($n$ = 9) and post- ($n$ = 21) ChAdOx1 controls and patients with VITT ($n$ = 22). Both LDH and circulating histone levels were higher in patients with VITT than pre-and post-ChAdOx1 controls. (C) Neutrophil extracellular traps (NETs) can be directly induced by serum from patients with VITT (right panel; $n$ = 22) following incubation with normal healthy human neutrophils (Nϕ) but not from pre- ($n$ = 9; left panel) and post-ChAdOx1 controls ($n$ = 21; upper middle panel) for 4 hours and stained with propidium iodide for extracellular DNA. Typical images are presented. (D) Quantitative analysis demonstrated that the percentage of neutrophil forming NETs (NETs %) was elevated by serum of patients with VITT compared with pre-and post-ChAdOx1 controls (bottom right panel). Mann–Whitney U-test; *$P$ < .05 compared with ChAdOx1 controls. (E) Representative immunofluorescent staining of postmortem liver (upper panels) and kidney (lower panels) tissue from patients with VITT ($n$ = 10) demonstrating NETs (Cit-H3, Opal570 pink; Neutrophil elastase, Opal690 red; and DAPI, blue) formation within these tissues.

inflammation may also be involved, and cytokine release, eg, IL-8, could also induce NETosis [15,26]. Furthermore, DAMPs released from NETosis can potentiate a vicious cycle to enhance inflammation and coagulation.

The third key finding is the coagulopathy of VITT. In this study, circulating histones were strongly associated with thrombocytopaenia (histones vs platelet count; R = −0.854), reduced fibrinogen (histones vs fibrinogen; R = −0.469), and elevated D-dimer (histones vs D-dimer;

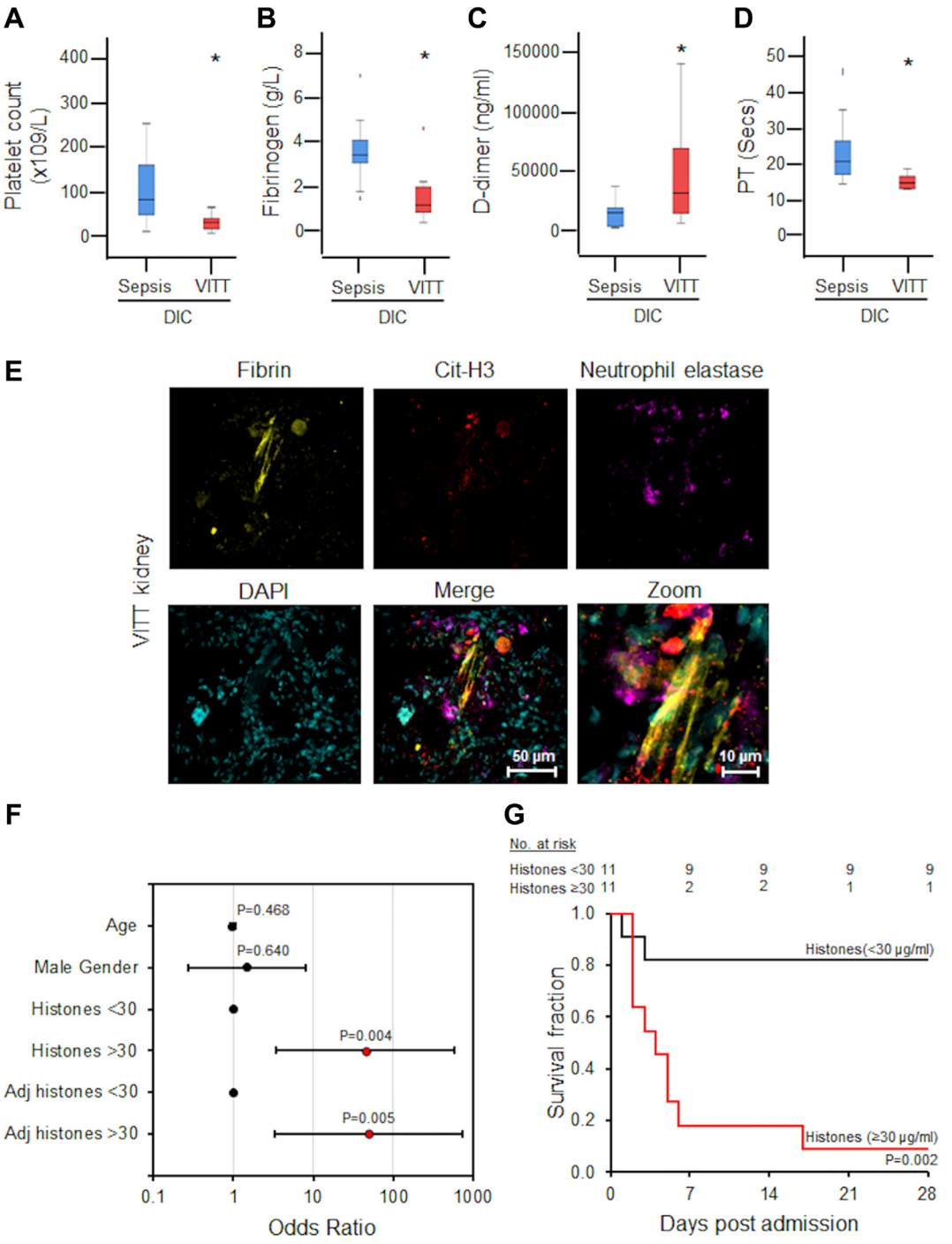


**FIGURE 3** Patients with vaccine-induced immune thrombotic thrombocytopenia (VITT) have atypical disseminated intravascular coagulation (DIC), and cell death is associated with 28-day mortality in VITT. Platelet counts (A), fibrinogen (B), and D-dimer (C) concentrations and prothrombin time (PT) (D) were compared between DIC ($n$ = 15) from patients with sepsis from intensive care unit recruited between 2013 and 2014 and VITT patients with DIC ($n$ = 15). (E) Representative immunofluorescent staining of postmortem tissue from patients with VITT ($n$ = 10) demonstrating fibrin (Opal 520; yellow) deposition and NETs (Cit-H3, Opal570 pink; Neutrophil elastase, Opal690 red; and DAPI, blue) formation within the kidney. (F) Multivariate analysis of crude and adjusted odds ratios (with patients adjusted for age and sex). Circulating histone levels >30 µg/mL were independently associated with 28-day mortality. (G) Kaplan–Meier survival curve for the probability of mortality during 28 days, stratified by circulating histone levels (<30 µg/mL vs ≥30 µg/mL).

R = 0.420) levels. In this cohort, 15/22 (68.2%) of patients with VITT met the ISTH DIC criteria (score ≥5), but compared to a cohort of bacterial sepsis intensive care unit patients diagnosed with DIC (*n* = 15), VITT patients with with DIC (*n* = 15) had significantly greater reductions in platelets (Figure 3A; *P* = .001) and fibrinogen (Figure 3B; *P* < .001) with higher D-dimer levels (Figure 3C; *P* = .02). DIC has been reported in severe HIT [27], and it is not surprising that these severely ill patients with VITT also developed this complication. However, the PT in the DIC of VITT was not significantly affected (*P* < .001, Figure 3D). The reasons are not clear but might link more to NETs and histones as compared to a primarily monocyte tissue factor-driven process in bacterial sepsis [28]. Histones in free or NETs-tethered form can directly bind prothrombin and factor Xa to facilitate FXa cleavage of prothrombin into thrombin without requiring phospholipid surfaces or FVa, as in the classical prothrombinase reaction [25]. This alternative prothrombinase reaction, which contributes to DIC in an *in vivo* model [25], is also independent of tissue factor activation and would not affect the PT. To further confirm whether patients with VITT had systemic coagulation activation and clot formation in distant organs other than the reported cerebral venous sinus, we examined for fibrin(ogen) deposition within organs. Immunofluorescent staining of postmortem kidney samples showed clear evidence of fibrin entwined with NETs in these tissues (Figure 3E).

The Table shows the comparison in demographics and clinical laboratory markers between VITT survivors (*n* = 10) and nonsurvivors (*n* = 12). Markers associated with cellular damage and death were highly associated with 28-day mortality in patients with VITT. Compared with survivors, nonsurvivors had reduced lymphocyte counts (*P* = .005), elevated LDH (*P* = .013), and histone levels (*P* < .0001).

Univariate analysis for lymphocyte counts (odds ratio [OR], 0.004; 95% CI, 0.000-0.559; *P* = .028) demonstrated that a low lymphocyte count was associated with mortality (ROC AUC = 0.136; 95% CI, 0.000-0.293; *P* = .005). Similarly, univariate analysis using continuous circulating histones demonstrated that rising levels were associated with mortality (OR, 1.093; 95% CI, 1.021-1.171; *P* = .011) (ROC AUC = 0.925; 95% CI, 0.813-0.1.000; *P* = .001). Using categorical data with stratification based on a ≥30 μg/mL cytotoxic threshold [17], similar results were obtained (OR, 45.000; 95% CI, 3.465-58.339; *P* = .004), patients exceeding the threshold (*n* = 11) had significantly (*P* = .002) higher mortality (*n* = 10/11; 90.1%) compared with patients below (*n* = 2/11; 18.2%). Multivariate analysis, after adjustment for age and sex, demonstrated that both lymphocyte count (OR, 0.004; 95% CI, 0.00-0.682; *P* = .035) and histone levels, treated as either continuous (OR, 1.097; 95% CI, 1.023-1.177; *P* = .009) or categorical variables (OR, 49.047; 95% CI, 3.233-744.049; *P* = .005) (Figure 3F), were independent predictors of mortality. The Kaplan–Meier survival curve demonstrated a significant increase in the probability of 28-day mortality in patients with ≥30 μg/mL circulating histones compared with patients with <30 μg/mL histones (Figure 3G; *P* = .002).

These findings highlight an array of complex pathways in VITT from which damage-associated cell markers, eg, lymphopenia and circulating histones, have emerged as independent predictors of mortality. This insight leads us to speculate that in certain individuals, the adenoviral vaccine is misrecognized 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 DAMPs to invoke systemic cell damage with immunothrombotic complications that involve widespread NETosis.

The limitation of this study is the low case numbers due to its rarity. These patients were all hospitalized, and 12/22 died of VITT. Therefore, there is a selection bias toward severe VITT rather than milder cases. Nonetheless, it is these severe cases that enable us to understand the pathways that cause mortality and identify clinically relevant biomarkers, which can help us develop better management and treatment strategies. A deep understanding of the pathophysiology of VITT will help vaccine development against future emerging infectious diseases, especially as adenoviral-based vaccines have many practical advantages in a global health emergency [22]. It will also benefit future use of adenoviral vectors in various gene therapy programs [29]. More recently, there has also been an increasing recognition of VITT-like, platelet-activating anti-PF4 antibodies in the absence of preceding exposure to heparin or adenovirus vector-based vaccines being associated with a severe prothrombotic disorder characterized by thrombocytopenia [30]. Therefore, understanding the pathophysiology of severe VITT will continue to have important implications.

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## AUTHOR CONTRIBUTIONS

S.T.A., M.D., R.J.S., J.S, and J.Y analyzed clinical samples and performed *ex vivo* experiments. S.T.A. and R.J.S. analyzed the clinical data. L.T. provided pre- and post-vaccine control samples. P.L.R.N provided access to VITT patient samples; C.J. and D.M. performed fluorescent staining of tissue sections and analyzed the images under the supervision of C.M. S.T.A., J.S., G.W., and C-H.T. wrote, edited, and reviewed the manuscript and figures. G.W. and C-H.T. supervised the work.

## DECLARATION OF COMPETING INTERESTS

All authors have no conflicts of interest to declare.

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## SUPPLEMENTARY MATERIAL

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