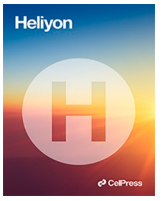
Heliyon 10 (2024) e38336

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Absence of platelet overactivation and thrombosis formation among patients with coronary atherosclerosis disease after vaccination against SARS-CoV-2

Huajie Xu [a,1], Xin Zhao [b,1], Peng Zhang [b,1], Yunjie Zhang [e,1], Qi Zhou [d], Huibin Wu [f], Bing Fan [b,**], Si Zhang [c,***], Hongyi Wu [b,*]

[a] Department of Infectious Disease, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, Shanghai, National Clinical Research Center for Interventional Medicine, China
[c] NHC Key Laboratory of Glycoconjugate Research, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, Shanghai, China
[d] Department of Clinical Medicine, Shanghai Medical College, Fudan University, Shanghai, 200032, China
[e] Department of Biostatistics, School of Public Health, Fudan University, Shanghai, China
[f] Zhongshan Hospital, Fudan University, Shanghai, 200032, China

ARTICLE INFO

Keywords:
Inactivated SARS-CoV-2 vaccine
CAD
Thrombosis
Platelet activation
Prothrombotic antibody

ABSTRACT

Background: Association of Coronavirus disease 2019 vaccines with thrombosis has raised concerns among patients with coronary atherosclerosis disease (CAD).
Objectives: After vaccination against SARS-CoV-2, to detect thrombosis formation in atherosclerosis ApoE$^{-/-}$ mice, and platelet activation, coagulation, the profile of prothrombotic antibodies, and the production of platelet factor 4 (PF4) antibodies in patients with CAD.
Methods: Atherosclerotic ApoE$^{-/-}$ mice were immunized with saline or inactivated SARS-CoV vaccines. We investigated $FeCl_3$-induced thrombus formation *in vivo*, and thrombus formation under flow conditions *ex vivo*. Inpatients undergoing percutaneous coronary intervention (PCI) were consecutively enrolled and defined according to vaccination status. We evaluated coagulation by thrombelastograph (TEG), platelet activation makers by flow cytometry, PF4 antibody and antiphospholipid antibodies by ELISA, and SARS-CoV-2 neutralizing antibody.
Results: In atherosclerotic ApoE$^{-/-}$ mice, $FeCl_3$-induced thrombus formation and thrombus formation under flow conditions were similar between saline-treated and inactivated SARS-CoV-2 vaccines-treated groups. A total of 182 patients undergoing PCI were included in the final analysis, of whom 92 had been vaccinated. Baseline characteristics were well balanced between unvaccinated and vaccinated groups. The expression of PAC-1 and P-selectin, the prevalence of

* Corresponding author. Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, National Clinical Research Center for Interventional Medicine, Shanghai 200032, China.
** Corresponding author. Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, National Clinical Research Center for Interventional Medicine, Shanghai 200032, China.
*** Corresponding author. NHC Key Laboratory of Glycoconjugate Research, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, Shanghai, 200032, China.
E-mail addresses: fan.bing@zs-hospital.sh.cn (B. Fan), zhangsi@fudan.edu.cn (S. Zhang), wu.hongyi@zs-hospital.sh.cn (H. Wu).
[1] These authors contributed equally to the study.

https://doi.org/10.1016/j.heliyon.2024.e38336
Received 22 April 2024; Received in revised form 21 September 2024; Accepted 23 September 2024
Available online 29 September 2024

positivity for PF4 antibodies and antiphospholipid antibodies were similar between these two groups.

Conclusions: Inactivated SARS-CoV-2 vaccines did not potentiate thrombosis formation in atherosclerotic mice. Inactivated SARS-CoV-2 vaccines did not enhance platelet activation, or trigger the production of PF4 and antiphospholipid antibodies in patients with CAD. In light of the observed thrombotic risks associated with adenovirus-based COVID-19 vaccines, inactivated vaccines may offer a potentially safer option for individuals with CAD.

Abbreviations:

ACEI	Angiotensin-converting enzyme inhibitor
ALB	Albumin
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
ApoE	apolipoprotein E
ARB	Angiotensin receptor blocker
AST	Aspartate aminotransferase
ARNI	Angiotensin receptor neprilysin inhibitor
BMI	Body mass index
BUN	Blood urea nitrogen
CAD	Coronary atherosclerosis disease
CCB	Calcium channel blocker
COVID-19	Coronavirus disease 2019
cTnT	Cardiac troponin T
DBIL	Direct bilirubin
ELISA	Enzyme-linked immunosorbent assay
HbAac	Glycosylated hemoglobin Aac
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LVEF	Left ventricular ejection fraction
PCI	Percutaneous coronary intervention
PF4	Platelet factor 4
PPI	Proton pump inhibitor
PRP	Platelet-rich plasma
RBC	Red blood cell
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TBIL	Total bilirubin
TC	total cholesterol
TG	triglyceride
TEG	Thromboelastography
VITT	Vaccine-induced immune thrombotic thrombocytopenia
WBC	white blood cell

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the ongoing coronavirus disease 2019 (COVID-19) global pandemic. Effective vaccines are critical for ending SARS-CoV-2 pandemic and lowering the mortality rate. Several types of vaccines against SARS-CoV-2, based on mRNA, viral vectors, or inactivated viruses are being applied worldwide [1]. However, severe side effects of vaccines may appear in some individuals in mass vaccination programs, especially thrombembolic events.

Over the past months, an increased risk of thrombosis associated with thrombocytopenia, termed vaccine-induced immune thrombotic thrombocytopenia (VITT), characterized by high-titer immunoglobulin G (IgG) class antibodies directed against the cationic platelet chemokine, platelet factor 4 (PF4), has been reported among individuals receiving adenovirus-based vaccines [2,3]. Furthermore, a clinical study indicated that the standardized morbidity ratio for thromboembolic events was 1.97-fold higher among the study population received adenovirus-based vaccines than for the general population [4]. Similarly, increased risks of arterial thromboembolism and ischaemic stroke have been observed after the first dose of the mRNA vaccine [5]. Although the relationship between thrombosis and vaccines is still ambiguous [6,7], highly publicized thrombotic events have raised concerns about the vaccine

safety in the special population with a high risk of thrombosis.

Overwhelming evidence exists that platelet activation and thrombosis play the pivotal roles in coronary atherosclerosis disease (CAD) [8]. Patients with concomitant CAD and COVID-19 have an extremely poor prognosis, with higher mortality (36 %), thromboembolic events (23 %), and septic shock rates (11 %) [9], thus they were prioritized for vaccination against SARS-CoV-2. However, few studies have focused on whether COVID-19 vaccination enhances thrombosis formation, platelet activation or increases the risk of thrombosis or bleeding in CAD patients. Therefore, we conducted this study to observe thrombosis formation in atherosclerotic ApoE$^{-/-}$ mice vaccination with inactivated SARS-CoV-2 vaccines, and assess platelet activity, coagulation, and the profile of prothrombotic antibody in vaccined patients with CAD.

2. Methods

2.1. Atherosclerosis model and vaccination

Animal procedures were approved by the Ethical Committee of Fudan University and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Six-week-old male ApoE homozygous deficient (ApoE$^{-/-}$) mice were obtained from GemPharmatech Company (Nanjing, China). ApoE$^{-/-}$ mice were fed with a high-fat diet containing 1.25 % cholesterol and 20 % fat for 14 weeks. ApoE$^{-/-}$ mice were randomly assigned to receive two intramuscular doses of either 10 μL of inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac) or 10 μL of saline. The immunization schedule consisted of a 21-day interval between doses [10]. After 4 weeks, the subsequent experiments of thrombosis formation were performed. A total of 32 ApoE$^{-/-}$ mice were used in this study. Sixteen ApoE$^{-/-}$ mice received a single injection of the Coronavac SARS-CoV-2 vaccine, while the remaining 16 ApoE$^{-/-}$ mice were administered an equal volume of saline as a control.

2.2. Intravital microscopy of $FeCl_3$-induced thrombosis in mouse mesenteric arteriole

Intravital microscopy of $FeCl_3$-injured thrombus formation in mouse mesenteric arterioles was carried out as described previously [11,12]with minor modification. Briefly, rabbit anti-mouse thrombocyte serum (20 μL) (J1943, Westbury, NY) was intraperitoneally injected into C57BL/6 mice aged 6 weeks for 4 h. Then, if the platelet count is <10 % of the initial count, 150 μL 1000 * 10^{10}/L platelets labeled by calcein (Calcein AM Solution, Sigma-Aldrich) from the immunized ApoE$^{-/-}$ mice with vaccine or saline, would be injected into platelet-depleted mice by the lateral tail vein. Thrombosis was induced by 10 % $FeCl_3$ 5 min later, and recorded with intravital microscopy.

2.3. Thrombus formation under flow conditions ex vivo

The flow chamber assay was prepared as described previously [13]with minor modification. Briefly, Thrombus formation was evaluated in the microfluidic whole-blood perfusion assay on a fibrillar collagen matrix under arterial shear conditions (a shear rate of 1000 s^{-1}) using a Bioflux-200 system (Fluxion, CA). Bioflux plates were coated with fibrillar collagen (40 μg/mL) overnight and blocked with 5 % BSA. Anticoagulated whole blood from immunized ApoE$^{-/-}$ mice were fluorescently tagged with FITC-labeled anti-CD41 antibody for 30 min. After the incubation, blood was then perfused over fibrillar collagen-coated bioflux plates at shear force of 40 dyn/cm2 with a Bioflux-200 system (Fluxion, South San Francisco, CA). The platelets were allowed to adhere to collagen surface for 3 min, and thrombus formation were visualized in real time by Olympus IX73 inverted fluorescence microscope. Images were acquired and the platelet-covered area was measured using Bioflux software (Fluxion, San Francisco, CA, USA).

2.4. Study population and design

This study was an investigator-initiated study, and conducted in accordance with the Declaration of Helsinki, and approved by the Medical Ethical Committee of Zhongshan Hospital affiliated to Fudan University (number: B2021-657R). Informed consent was obtained from all the participants. The potential study population included inpatients admitted to the Department of Dardiology at Zhongshan Hospital with a diagnosis of CAD between August 1 and December 30, 2021. Eligible patients met the following inclusion criteria: 1) age >18 years and less than 80 years, 2) patients with CAD underwent percutaneous coronary intervention (PCI), and 3) treated with dual anti-platelet therapy. Patients were excluded if they met any of the following exclusion criteria: 1) known contraindications to antithrombotic therapy, 2) diagnosed with any malignancies, 3) pregnancy, 4) renal insufficiency (eGFR <30 mL/min/1.73 m^2) or hepatic insufficiency (alanine aminotransferase or aspartate aminotransferase >3 × upper limit of normal), 5) hematologic disorder, 6) left ventricular ejection fraction (LVEF) less than 50 %, or 7) active or known history of SARS-CoV-2 infection. The patients were divided into two groups. Patients completed two doses of inactivated whole-virion SARS-CoV-2 vaccines (CoronaVac) within 2 weeks to 1 months were included into the vaccinated group. According to the guidelines of the National Health Commission of the People's Republic of China, the recommended time interval between the first and second SARS-CoV-2 vaccine doses is 3–8 weeks. Unvaccinated patients were included in the unvaccinated group. The demographics, medical histories, and laboratory results (including platelet parameters and coagulation function) were retrieved from the hospital's electronic health records. Platelet function tests were conducted one month post-vaccination.

2.5. Sample preparation

Human blood was drawn from the antecubital vein and mixed with acid citrate dextrose (85 mM sodium citrate, 71.38 mM citric acid, and 27.78 mM glucose) buffer (6:1 vol/vol). Platelet-rich plasma (PRP), as previously described [13], was filtered through a Sepharose 2B column (Sigma-Aldrich) equilibrated in Tyrode's solution (pH 7.35) to isolate platelets. Platelets can also be separated by centrifuging PRP at 900 g for 10 min and resuspending platelet pellets in Tyrode buffer. Platelet-poor plasma (PPP) was collected and stored at −80 °C before use.

2.6. Flow cytometry analysis

Flow cytometry was performed as previously described [14]. Fluorophore-labeled antibodies were utilized for the detection of P-selectin expression (CD-62P-APC) and the active form of αIIbβ3 integrin (PAC-1-FITC). Resting platelets (1×10^7) were incubated with APC-conjugated anti-CD62P and FITC-conjugated PAC-1 antibodies in the dark at room temperature for 20 min without stirring. CD62P expression and PAC-1 binding were subsequently analyzed using a FACS (FACSCalibur, Becton Dickinson).

2.7. Thromboelastograph testing

A Thromboelastograph (TEG) Hemostasis Analyzer (Haemoscope Corp., Niles, Illinois, USA) was used to measure the dynamic coagulation process. Blood samples for TEG analysis were collected as described previously [15]. The direct parameter measured by this system is the maximum amplitude (MA), which is indicative of the strength of the final clot and is one of the most reliable parameters for determining bleeding and thrombotic risks. For the kaolin channel, 1 mL of whole blood was mixed with 1 % kaolin solution (Haemoscope Corp). Kaolin was used as an acitivator to perform the standard TEG, and the results were analyzed following the manufacturer's instruction. The TEG reference values were as follows: R-Time: 4–8 min; K-Time: 1–4 min; angle α: 47–74°; and maximum amplitude (MA): 54–72 mm, all rounded to the nearest whole number.

2.8. Neutralizing antibody testing

SARS-CoV-2 neutralizing antibody detection kit (Beijing Hotgen Biotech Co., Ltd.) was used to quantitatively detect neutralizing antibodies by magnetic particle chemiluminescence immunoassay according to the manufacturer's instructions [16]. Patient sera were obtained one month post-vaccination. ELISA plates were coated overnight at 4 °C with 0.5 μg/mL of recombinant S proteins (WT, Alpha, Delta, Omicron BA.2, BA.5, BQ.1.1, XBB.1.5, CH.1.1) from ACRO Biosystems (Newark, DE, USA). After blocking with 1 % BSA/PBS, plates were incubated with serially diluted serum samples. Bound antibodies were detected using peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) and visualized with TMB substrate. Absorbance was measured at 450 nm. Diagnostic sensitivity and specificity of the kit were assessed using 40 clinical samples (20 SARS-CoV-2 antibody-positive, 20 SARS-CoV-2 antibody-negative). With only two discrepancies (5 % each) compared to the reference standard, the assay demonstrated a 95 % agreement rate, meeting pre-established acceptability criteria.

2.9. Platelet factor 4 antibody detection

Antibodies to platelet factor 4 (PF4) in complex with poly (vinyl sulfonate) (heparin analogue) in patient plasma were tested using PF4 IgG enzyme-linked immunosorbent assay (ELISA) (Antibodies-online GmbH, Germany) in accordance with the manufacturer's instructions. The cut off value was >0.5 U/mL as determined by the manufacturers.

2.10. Autoantibodies measurement

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using triple tissue cryostat sections (liver-stomach-kidney) and Hep-2 cells as substrate according to the manufacturer's instructions (Euroimmun). The suggested cutoff for ANA is 1:80 according to the international guidelines [17]. Antiphospholipid antibodies, such as aCL IgG, aCL IgM, aCL IgA, anti-β2GPI IgG were measured using the commercial ELISA kit from Inova Diagnostics (Inova Diagnostics, San Diego, USA) according to the manufacturer's instructions. The suggested cutoff for aCL IgG/IgM/IgA is 20 GPL/MPL/APL, for anti-β2GPI IgG is 20 SGU, which was conducted as previously described.

2.11. Statistical analysis

Continuous data were summarized as mean (SD or SEM) or median (interquartile range) depending on the data distribution, and compared using an unpaired Student's *t*-test or Mann-Whitney *U* test, as appropriate. Categorical variables were expressed as numbers and percentages and compared using the Chi-square test or Fisher's exact test. All data were evaluated for normality (Kolmogorov-Smirnov) and subjected to the Bartlett's test for homogeneity of group variances prior to statistical analysis. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using GraphPad Prism (7.0) and STATA/IC 16 (StataCorp LP, College Station, TX, USA).

3. Results

3.1. $FeCl_3$-induced thrombus formation in mouse mesenteric arteriole in vivo

We successfully constructed the atherosclerotic mouse model with ApoE$^{-/-}$ mouse fed with a high-fat diet. To examine the *in vivo* platelet activity after immunization with saline or inactivated SARS-CoV-2 vaccines, we measured mesenteric arteriole thrombosis in WT mice receiving platelets from saline-treated or vaccine-treated ApoE$^{-/-}$ mice. Platelets were separated from saline-treated or vaccine-treated ApoE$^{-/-}$ mice, then transfused into platelet-depleted WT mice. As shown in Fig. 1, mice receiving platelets from vaccine-treated ApoE$^{-/-}$ mice did not exhibited enhanced thrombus formation compared with mice receiving platelets from saline-treated ApoE$^{-/-}$ mice. The mean times to form the first thrombus more than 20 μm were 277.5 s and 288.0 s, and the mean occlusion times were 733.5 s and 801.0 s (n = 10). These results revealed that inactivated SARS-CoV-2 vaccine did not activate platelet activity in atherosclerotic mice *in vivo*.

3.2. Thrombus formation under flow conditions ex vivo

To further evaluate the effect of inactivated SARS-CoV-2 vaccine on *ex vivo* thrombus formation, whole blood from ApoE$^{-/-}$ mice immunization with saline or vaccines was perfused over the collagen surface. As shown in Fig. 2, at all observed time points, whole blood presented the similar thrombus formation area between these two groups (n = 6). Consistent with the above results, inactivated SARS-CoV-2 vaccine did not potentiate thrombus formation under flow conditions *ex vivo*.

3.3. Baseline characteristics of patients

A total of 182 participants were recruited and included in this cross-sectional study between September 1 and December 30, 2021. The study sample included 92 vaccinated and 90 unvaccinated patients. The baseline characteristics of all participants were well balanced and summarized in Table 1. The two groups were similar in age (62 ± 9 vs 61 ± 8, $P = 0.060$), male sex (75.6 % vs 73.9 %, $P = 0.799$) and body mass index (BMI) (24.8 ± 3.7 vs 24.8 ± 3.0, $P = 0.957$). No significant differences were also found for habitual smoking, comorbidities, medications, clinical measurements of hematological parameters, liver function, renal function, lipid profile, glycated hemoglobin A1c (HbA1c), C-reactive protein (CRP), cardiac troponin T (cTnT), N-terminal pro-BNP (NT-proBNP), and left ventricular ejection fraction (LVEF) in two groups (Table 1).

3.4. Platelet parameters

No significant differences were found for platelet parameters, namely platelet count (PLT) (200 ± 52 vs 205 ± 52, $P = 0.097$) (Fig. 3A), mean platelet volume (MPV) (10.70 (10.20–11.30) vs 10.60 (10.10–11.30), $P = 0.970$) (Fig. 3B), platelet crit (PCT) (0.22 ± 0.04 vs 0.22 ± 0.05, $P = 0.087$) (Fig. 3C), platelet larger cell ratio (P-LCR) (30.95 ± 7.51 vs 30.98 ± 7.61, $P = 0.716$) (Fig. 3D), platelet distribution width (PDW) (12.75 ± 2.00 vs 12.70 ± 2.09, $P = 0.947$) (Fig. 3E) between the unvaccinated and vaccinated groups.

3.5. Coagulation functions

No significant differences were found for blood coagulation functions, namely prothrombin time (PT) (11.42 ± 0.54 vs 11.51 ±

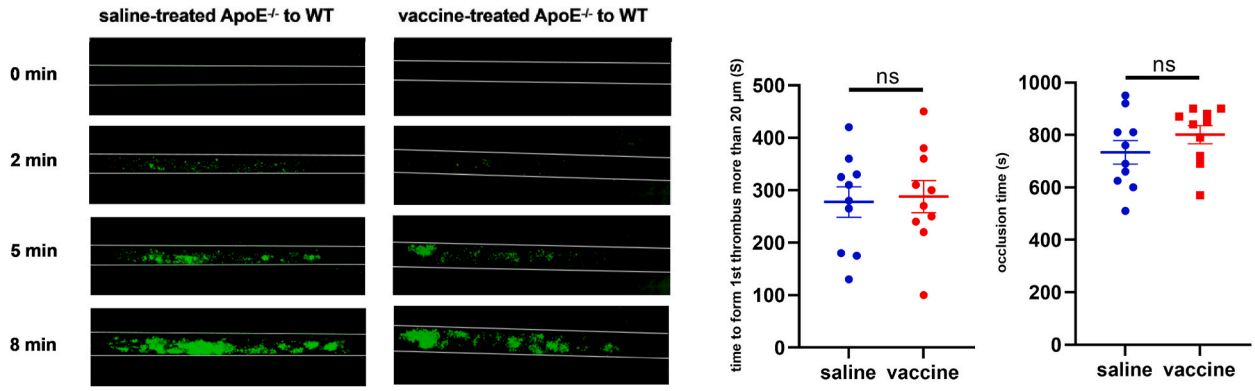


Fig. 1. $FeCl_3$-induced thrombus formation in atherosclerotic ApoE$^{-/-}$ mice with immunized with saline or vaccine. Representative images of thrombus formation at baseline, 2, 5 and 8 min after 10 % $FeCl_3$-induced vascular injury in mouse mesenteric arterioles. Blood flow is from right to left, arterioles measuring ~100 μm diameter were visualized in live mesentery of live mice. $FeCl_3$-induced thrombus formation in mice receiving platelets from vaccine-treated ApoE$^{-/-}$ mice was similar with mice receiving platelets from saline-treated ApoE$^{-/-}$ mice. The time to first thrombus (>20 μm) and occlusion time were determined (n = 10). Data are expressed as mean ± SEM.

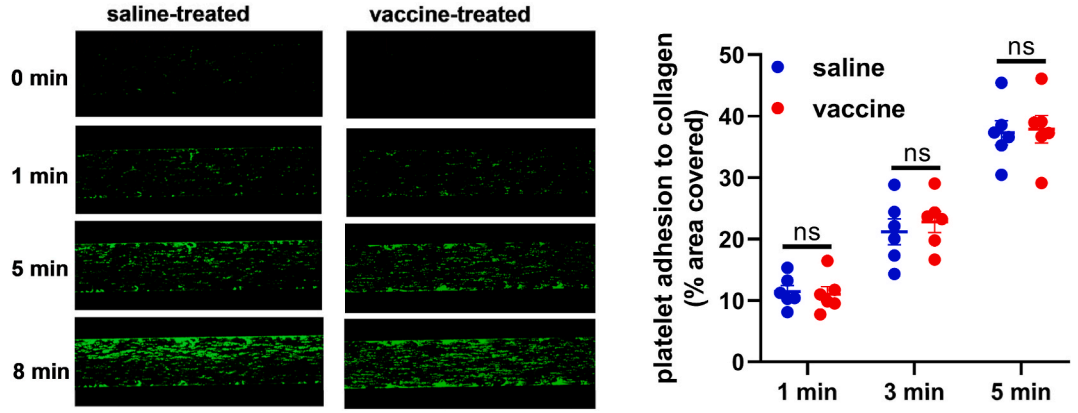


Fig. 2. Thrombosis formation under flow chamber in atherosclerotic ApoE$^{-/-}$ mice. Whole blood from atherosclerotic ApoE$^{-/-}$ mice vaccinated with inactivated SARS-CoV-2 vaccines showed similar thrombus formation over an immobilized collagen surface at a shear rate of 1000 s^{-1} compared with that of ApoE$^{-/-}$ mice immunized with saline (n = 6). The whole blood was tagged by fluorescein isothiocyanate (FITC)-labeled anti-CD41 antibody, and then perfused through fibrillar collagen-coated bioflux plates for 180 s. Representative images of thrombus formation at the indicated time points are presented. Each solid circle represents the thrombus formation area from a single individual. Data are expressed as mean ± SEM.

0.64, $P = 0.447$) (Fig. 4A), activated partial prothrombin time (APTT) (26.04 ± 1.84 vs 25.84 ± 1.68, $P = 0.622$) (Fig. 4B), thrombin time (TT) (16.73 ± 0.85 vs 16.96 ± 0.91, $P = 0.067$) (Fig. 4C), international normalized ratio (INR) (0.98 ± 0.06 vs 0.99 ± 0.07, $P = 0.700$) (Fig. 4D), fibrinogen (FIB) (282.0 (239.0–311.0) vs 289.0 (261.0–333.0), $P = 0.071$) (Fig. 4E), D-dimer (0.28 (0.19–0.47) vs 0.28 (0.20–0.43), $P = 0.911$) (Fig. 4F) between the unvaccinated and vaccinated group.

3.6. Platelet activation marker expression

To evaluate the platelet activation in the two groups, we quantified the expression of the two activation markers on platelet surface. The median fluorescence intensity of resting platelet P-selectin (281 (233.0–409.0) vs 256.0 (238.0–305.5), $P = 0.083$) (Fig. 5A) and PAC-1 (225.0 (156.0–323.0) vs 197.0 (68.25–282.0), $P = 0.070$) (Fig. 5B) detected by flow cytometry did not differ between the unvaccinated and vaccinated groups.

3.7. Thromboelastographic parameters

TEG variables included reaction time (R time), clot formation time (K time), clot formation rate (angle α), and maximal amplitude of clot strength (MA). As shown in Fig. 6, no significant differences in thrombelastographic parameters including coagulation index (0.10 (−0.80, 1.10) vs −0.10 (−0.75, 1.00), $P = 0.639$) (Fig. 6A), R-time (5.43 ± 0.91 vs 5.40 ± 0.86, $P = 0.953$) (Fig. 6B), K-time (1.81 ± 0.54 vs 1.76 ± 0.41, $P = 0.426$) (Fig. 6C), angle α (64.8 ± 5.2 vs 65.3 ± 4.9, $P = 0.486$) (Fig. 6D), and MA (58.32 ± 5.31 vs 57.83 ± 5.59, $P = 0.508$) (Fig. 6E) were found between the unvaccinated and vaccinated groups.

3.82. PF4 antibody and prothrombotic autoantibody prevalence

As shown in Table 2, the prevalence of positivity for antibodies to platelet factor 4–polyanion complexes did not differ between the unvaccinated and vaccinated groups (2.2 % vs 4.4 %, $P = 0.682$). Furthermore, there was no significance in the levels of PF4 antibody between these two groups (0.18 (0.14, 0.25) vs 0.18 (0.15, 0.25), $P = 0.493$), and no strikingly high optical density values—in the range of 0.01–1.89—measured by ELISA. The prevalence of positivity for all five autoantibodies was similar between the two groups: for anticardiolipin antibodies (aCL) IgA (0 vs 0, $P = 1.000$), IgG (0 vs 2.22 %, $P = 1.000$), IgM (3.26 % vs 0, $P = 1.000$), anti-beta-2 glycoprotein I antibodies (aβ2GPI) (5.4 % vs 1.1 %, $P = 0.125$), and antinuclear antibodies (ANA) (12.2 % vs 4.4 %, $P = 0.053$).

3.9. Immunogenicity and adverse reactions after vaccination

None of the enrolled participants reported exposure to known COVID-19 patients, and no serological response to SARS-CoV-2 was detected in the unvaccinated samples. In the vaccinated group, 68.5 % (63/92) of the samples tested positive in the SARS-CoV-2 antibody assay. A total of 40 (43.5 %) patients reported adverse reactions occurring within 7 days after dose 1 and 32 (34.8 %) patients reported adverse reactions occurring within 7 days after dose 2. The most common adverse reactions after dose 1 were injection site pain (10, 10.9 %), followed by muscle pain (5, 5.4 %), fatigue (5, 5.4 %) and headache (3, 3.3 %), and reported adverse reactions after dose 2 were injection site pain (9, 9.8 %), followed by muscle pain (4, 4.3 %), headache (4, 4.4 %) and fatigue (3, 3.3 %). All adverse reactions were mild and limited, and no grade 3 adverse reactions were observed, as shown in Table 3.

Table 1
Characteristics of the patients at baseline. Values presented are mean (SD) or median (interquartile range).

characteristic	Unvaccinated (90)	Vaccinated (92)	P value
Demographics and history			
Age (years)	62 (9)	61 (8)	0.060
Male, n (%)	68 (75.6)	68 (73.9)	0.799
BMI(kg/m^2), mean (SD)	24.8 (3.7)	24.8 (3.0)	0.957
Hypertension, n (%)	51 (56.7)	47 (51.1)	0.450
Dyslipidaemia, n (%)	14 (15.6)	9 (9.8)	0.241
Diabetes mellitus, n (%)	27 (30.0)	24 (26.1)	0.557
Habitual smoker, n (%)	46 (51.1)	51 (55.4)	0.656
Medication			
Statin, n (%)	81 (90.0)	80 (87.0)	0.521
Ezetimibe, n (%)	15 (16.7)	14 (15.2)	0.789
Beta-blockers, n (%)	53 (58.9)	50 (54.4)	0.537
CCB, n (%)	36 (40.0)	29 (31.5)	0.233
ACEI/ARB/ARNI, n (%)	43 (47.8)	40 (43.5)	0.560
PPI, n (%)	51 (56.7)	54 (58.7)	0.782
Nitrates, n (%)	30 (33.3)	34 (37.0)	0.609
Clinical measurements			
RBC (x 10^{12}/L)	4.3 (0.5)	4.4 (0.5)	0.277
Hemoglobin (g/L)	134.3 (14.8)	136.6 (14.1)	0.207
WBC (x 10^9/L)	6.5 (2.0)	6.9 (5.5)	0.548
TBIL (μmol/L)	9.9 (7.8,12.4)	10.5 (8.1,13.7)	0.434
DBIL (μmol/L)	3.1 (2.4, 4.2)	3.1 (2.2, 4.2)	0.515
ALB (g/L)	42.0 (2.6)	42.5 (3.3)	0.458
ALT (U/L)	20.0 (13.0,30.0)	19.5 (12.5,26.5)	0.492
AST (U/L)	20.0 (16.0,23.0)	18.0 (15.0,23.0)	0.267
BUN (mmol/L)	6.1 (1.5)	5.9 (1.4)	0.509
SCr (μmol/L)	79.9 (14.5)	76.8 (16.4)	0.214
BUA (mmol/L)	338.0 (278.0,396.0)	338.0 (278.0,396.0)	0.459
eGFR (mL/min/1.73 m^2)	87.5 (76.0,95.0)	89.0 (83.0,97.0)	0.208
HbA1c (%)	6.4 (1.2)	6.2 (1.2)	0.250
TC (mmol/L)	3.5 (3.0, 4.0)	3.7 (3.2, 4.3)	0.250
TG (mmol/L)	1.2 (0.9, 1.8)	1.4 (1.0, 1.9)	0.266
LDL (mmol/L)	1.7 (1.3, 2.3)	1.8 (1.4, 2.5)	0.399
HDL (mmol/L)	1.1 (0.3)	1.1 (0.3)	0.542
cTnT (μg/L)	0.01 (0.01, 0.02)	0.01 (0.01, 0.01)	0.339
NT-proBNP (pg/ml)	79.05 (45.60,146.0)	79.05 (45.60,146.0)	0.411
LVEF (%)	63.86 (5.15)	63.86 (5.15)	0.912

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ALB, albumin; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; ARNI, angiotensin receptor neprilysin inhibitor; BMI, body mass index; BUN, blood urea nitrogen; CCB, calcium channel blocker; cTnT, cardiac troponin T; DBIL, direct bilirubin; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; PPI, proton pump inhibitor; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; RBC, red blood cell; TBIL, total bilirubin; TC, total cholesterol; TG, triglyceride; WBC, white blood cell.

4. Discussion

To our knowledge, this is the first study to explore the impact of inactivated SARS-CoV-2 vaccines on thrombus formation in atherosclerotic ApoE$^{-/-}$ mice, and platelet functions, thrombotic profile and prothrombotic autoantibody prevalence among patients with CAD receiving PCI. Our study demonstrated that (1) inactivated SARS-CoV-2 vaccines did not potentiate thrombus formation *in vivo* in atherosclerotic ApoE$^{-/-}$ mice; two dose of inactivated SARS-CoV-2 vaccines did not enhance platelet activation or alter platelet parameters among CAD patients receiving PCI; (2) two doses of inactivated SARS-CoV-2 vaccines did not cause hypercoagulability and trigger the production of prothrombotic antibodies among these patients; and (3) no grade 3 adverse reactions after vaccination were recorded.

Since the appearance of studies on possible COVID-19 vaccine-related thrombotic events, questions about potential safety issues of vaccination among patients with CAD have been raised [2–5,18]. However, a recent study conducted in Hong Kong showed no evidence of an increased risk of major adverse cardiovascular events (MACE) after vaccination with mRNA or inactivated vaccine in patients with cardiovascular disease (CVD) [19]. Among individuals aged >60 years, thromboembolism has the highest incidence of any adverse event following CoronaVac vaccination [20]. Indeed, the current findings regarding post-vaccination adverse thrombotic events remains largely inconsistent and limited in scope, especially for the at-risk CAD population.

The mechanisms underlying the pathogenesis of vaccine-related thrombosis and the initial events that trigger platelet activation remain unclear. Ostrowski et al. observed common platelet activation following adenovirus vector-based and mRNA vaccines [21]. Conversely, using mass cytometry, Klug et al. reported that mRNA vaccine does not alter platelet protein expression and reactivity in the healthy individuals [22]. A number of unusual cases of vaccine-induced immune thrombotic thrombocytopenia (VITT) have been observed following adenovirus vector COVID-19 vaccination. These cases typically occur 1–2 weeks post-vaccination and are

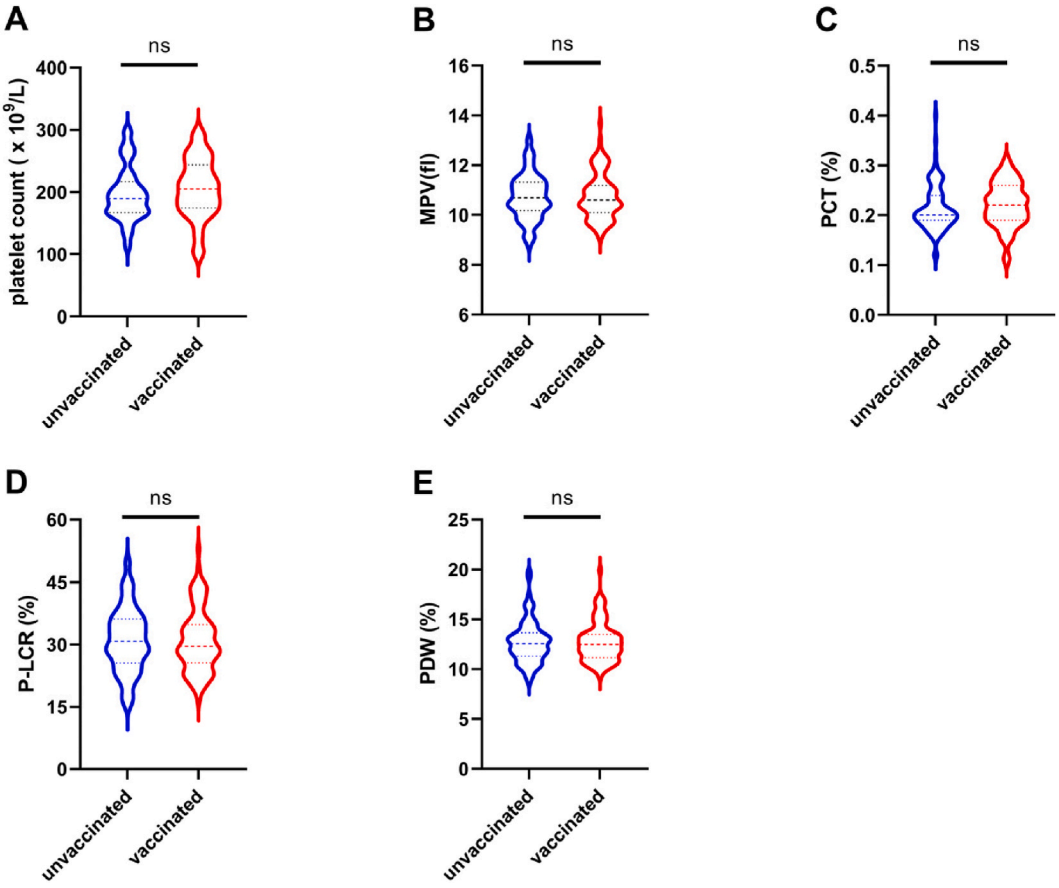


Fig. 3. Platelet parameters in unvaccinated and vaccinated individuals. There was no significant difference in (A) platelet count ($\times 10^9$/L), (B) mean platelet volume (MPV) (fl), (C) platelet crit (PCT) (%), (D) platelet larger cell ratio (P-LCR) (%), (E) platelet distribution width (PDW) (%).

characterized by thrombocytopenia and high titers of immunoglobulin G antibodies targeting platelet factor 4 (PF4) [2,22]. Recent research has demonstrated that PF4 can directly bind to adenoviral vectors, resulting in platelet-adenovirus aggregation, a potential mechanism contributing to VITT [22]. Importantly, no association between inactivated SARS-CoV-2 vaccines and VITT has been established in existing studies. To our knowledge, evidence of inactivated vaccines on platelet activation in patients with CAD remains scarce. Our results showed that inactivated SARS-CoV-2 vaccines did not lead to GPIIb/IIIa activation nor P-selectin expression, which regulate platelet activation. Furthermore, inactivated vaccines did not alter platelet parameters, which might add to the safety profile of patients with CAD receiving PCI.

Adenovirus-based vaccines, such as Vaxzevria (ChadOx1 nCoV-19) and Jcovden (AD26.COV2.S), were among the earliest COVID-19 vaccines approved for use. Extensive research has demonstrated their efficacy in preventing severe COVID-19, hospitalization, and death [23]. Subsequent studies revealed a link between Vaxzevria administration and an increased risk of venous thrombotic events, including cerebral venous thrombosis, splanchnic venous thrombosis, and others. These events were associated with platelet aggregation, thrombocytopenia, and the development of antibodies against PF4 [24–26]. Moreover, cerebral venous thrombosis has also been observed in Jcovden recipients [27,28]. Regarding the possible mechanism of VITT, free DNA in adenovirus vector-based vaccine might induce PF4 antibody production, which in turn could activate platelets and promote immune thrombotic thrombocytopenia, resulting in bleeding or thrombosis [2,3,29]. It has been reported that almost 7 % of vaccinated individuals (both adenovirus vector-based vaccines and mRNA vaccines) had low titers of PF4 antibodies, which were not functionally active [30]. In this study, four cases (4.35 %) were presented with low titers of PF4 antibodies after the administration of the second dose, whereas two cases (2.22 %) without vaccination presented similar titers of PF4 antibodies. A cohort study including health caregivers also reported a low prevalence of PF4 antibodies after COVID-19 vaccination with inactivated vaccines, and none exhibited symptoms of thrombosis [17]. Alternatively, PF4 antibodies may be boosted by COVID-19 vaccine, but they are kept in check by an immune mechanism known as peripheral tolerance, as 0.3%–0.5 % of healthy individuals can harbor PF4 antibodies [31,32].

Although a previous study showed inactivated COVID-19 vaccines did not trigger the production of prothrombotic antibodies in healthy individuals [17], their impact on the at-risk CAD population is still unknown. In our study, the prevalence of antiphospholipid antibodies positivity was similar between the unvaccinated and vaccinated individuals. Consistent with another study on the impact of COVID-19 vaccine on hypercoagulability [33], our findings also indicated that inactivated vaccines did not cause hypercoagulability in

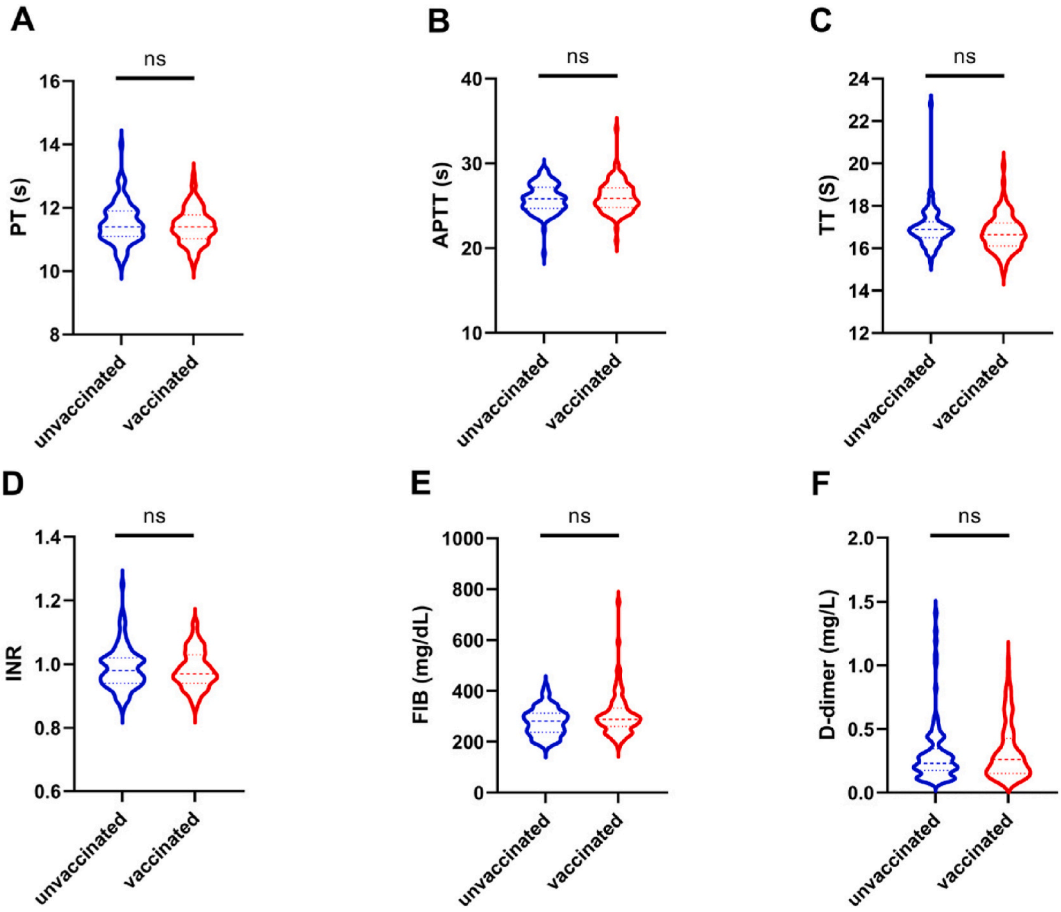


Fig. 4. Coagulation functions in unvaccinated and vaccinated individuals. No significant difference was found in (A) prothrombin time (PT) (s), (B) activated partial prothrombin time (APTT) (s), (C) thrombin time (TT) (s), (D) international normalized ratio (INR), (E) fibrinogen (FIB) (mg/dL), (F) D-dimer (mg/dL).

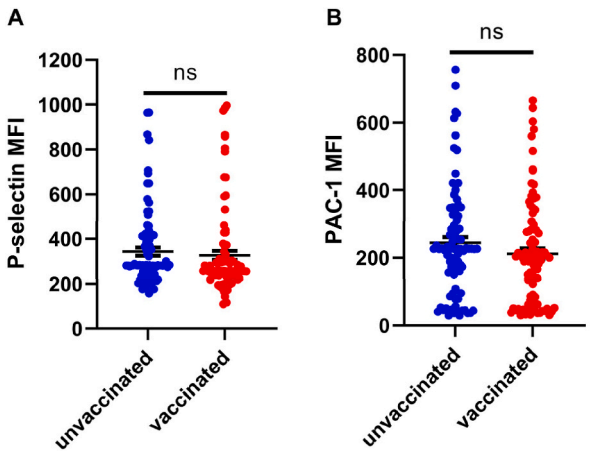


Fig. 5. Platelet activation marker expression in unvaccinated and vaccinated individuals. The median fluorescence intensity of resting platelet (A) P-selectin (281 (233.0–409.0) vs 256.0 (238.0–305.5), P = 0.083) and (B) PAC-1 (225.0 (156.0–323.0) vs 197.0 (68.25–282.0), P = 0.070) were similar between the unvaccinated and vaccinated group.

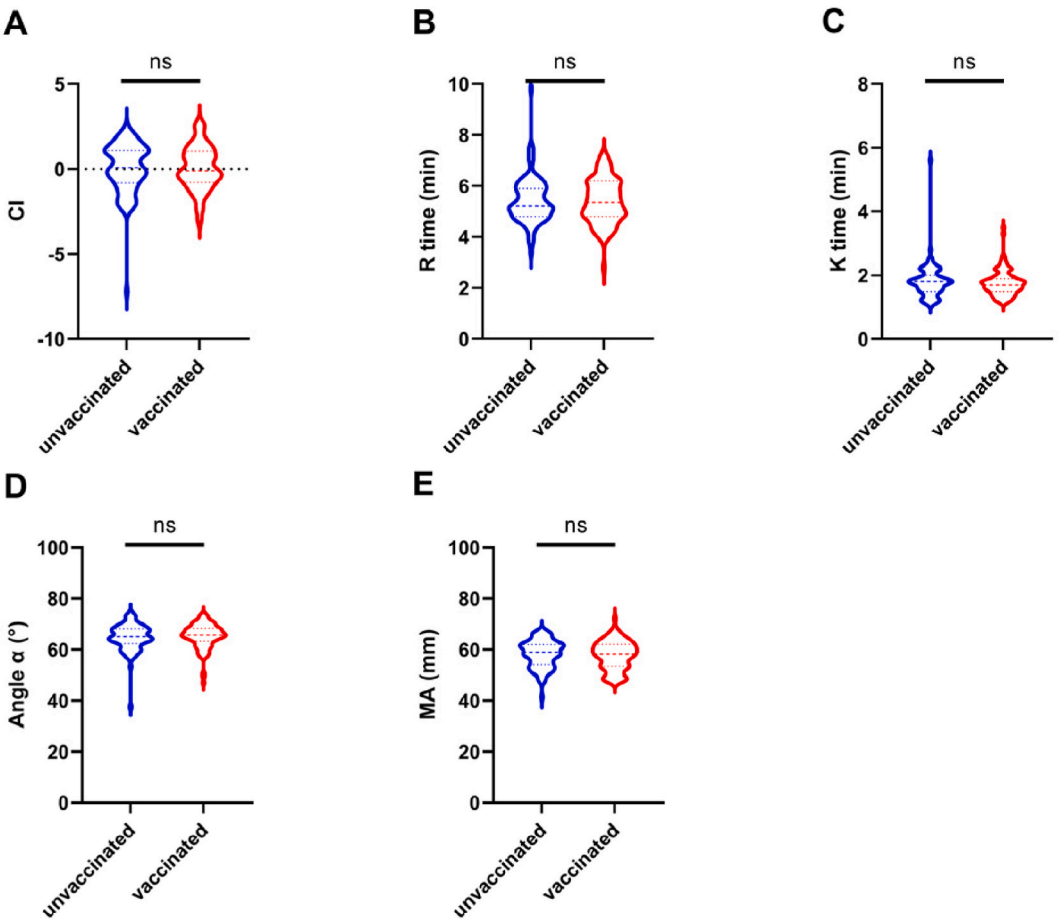


Fig. 6. Thrombelastographic parameters in unvaccinated and vaccinated individuals. It was not significant in (A) coagulation index (CI), (B) R time (min), (C) K time (min), (D) Angel α (°), (E) maximal amplitude (MA) (mm).

Table 2
Prevalence of autoantibodies in serum between unvaccinated and vaccinated individuals.

Autoantibodies	Unvaccinated (n = 90)	Vaccinated (n = 92)	P value
ANA, n (%)	11 (12.22)	4 (4.35)	0.053
aCL IgG, n (%)	2 (2.22)	0	1
aCL IgM, n (%)	0	3 (3.26)	1
aCL IgA, n (%)	0	0	1
aβ2GPIb IgG, n (%)	0	3 (3.26)	1
PF4 antibody, n (%)	2 (2.22)	4 (4.35)	0.682

Manufacturer's cutoff: aCL IgG, IgM, IgA 20 GPL/MPL/APL; ab2GPI IgG 20 SGU; aPF4-heparin complex >0.5.
Abbreviation: aCL, anticardiolipin antibodies; aβ2GPI, anti-beta-2 glycoprotein I antibodies; ANA, antinuclear antibodies; PF4 antibody, platelet factor 4 antibody.

patients with CAD.

Compared with previous studies, the seroconversion rate observed in our cohort following a two-dose vaccination regimen was lower [34]. Our study population predominantly comprised elderly patients with coronary heart disease, contrasting markedly with the younger, healthier participants in other trials. Given the well-established inverse relationship between age and neutralizing antibody levels [35], the diminished immunogenicity in our cohort is likely attributable to advanced age.

Among more than 10 million patients with CAD worldwide [36], thrombotic risk after vaccination against SARS-CoV-2 is a major concern leading to the a low proportion rate of vaccination (47.8 %) [19]. Strategies are needed to eliminate concerns and improve vaccination rates in individuals with CAD. One of the most important strategies could be to provide information about the direct impact of vaccines on platelet activation and thrombosis. Our findings support the safety of inactivated vaccines among individuals with CAD, which might begin to address this research gap and may be helpful in guiding recommendations.

Table 3
Seroconversion rate of SARS-CoV-2 specific antibodies and adverse reactions of vaccinated individuals.

	Dose 1	Dose 2	Total
Seroconversion rate of SARS-CoV-2 specific antibodies	/	63 (68.5)	65 (68.5)
Adverse reactions, n (%)			
Injection site adverse reactions	10 (10.9 %)	9 (9.9 %)	19 (20.9 %)
Fatigue	5 (5.5 %)	3 (3.3 %)	8 (8.8 %)
Muscle pain	5 (5.5 %)	4 (4.4 %)	9 (9.9 %)
Headache	3 (3.3 %)	4 (4.4 %)	7 (7.7 %)
Cough	3 (3.3 %)	2 (2.2 %)	5 (5.5 %)
Appetite impaired	3 (3.3 %)	0	3 (3.3 %)
Fever	2 (2.2 %)	3 (3.3 %)	5 (5.5 %)
Dyspnea	2 (2.2 %)	1 (1.1 %)	4 (4.4 %)
Chill	2 (2.2 %)	1 (1.1 %)	3 (3.3 %)
Diarrhea	2 (2.2 %)	0	2 (2.2 %)
Vomiting	1 (1.1 %)	2 (2.2 %)	3 (3.3 %)
Syncope	1 (1.1 %)	0	1 (1.1 %)
Joint pain	1 (1.1 %)	2 (2.2 %)	3 (3.3 %)
Hypersensitivity	0	1 (1.1 %)	1 (1.1 %)

All adverse reactions were mild and self-limiting, and no grade 3 adverse reactions were recorded.

4.1. Strengths and limitations

This is the first study to explore the effect of inactivated SARS-CoV-2 vaccines on thrombus formation in atherosclerotic ApoE$^{-/-}$mice. We evaluated the impact of inactivated SARS-CoV-2 vaccines on platelet activation, thrombotic profile and prothrombotic autoantibody prevalence among patients with CAD receiving PCI. Our data adds evidence to the safety profile of the inactivated SARS-CoV-2 vaccines in patients with CAD.

The main limitation of this study is that the sample size was relatively small. Thus, data from a larger number of patients with CAD and multiple centers are warranted. Secondly, the findings of this study may not be extrapolated to other vaccines, and further research investigating a variety of vaccines is required.

5. Conclusion

Among CAD patients receiving PCI, inactivated SARS-CoV-2 vaccine did not enhance platelet activation, cause hypercoagulability, or trigger the production of prothrombotic antibodies. In light of the observed thrombotic risks associated with adenovirus-based COVID-19 vaccines, inactivated vaccines may offer a potentially safer option for individuals with CAD.

Ethics approval and consent to participate

Experiments involving human subjects were performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board Fudan University.

Animal experiments were conducted according to the criteria illustrated in the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication 86 − 23 revised 1985).

Sources of funding

This work was supported by grants from the National Natural Science Foundation of China to Hongyi Wu (81,970,298) and Si Zhang (81,770,137), Clinical Research Special Fund of Zhongshan Hospital Fudan University to Hongyi Wu (2020ZSLC57), and 2021 clinical research navigation project of Shanghai Medical College of Fudan University to Hongyi Wu.

Data availability statement

Data sharing with qualified researchers may be considered after submission of a proposal to the corresponding author.

CRediT authorship contribution statement

Huajie Xu: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Xin Zhao:** Validation, Software, Investigation, Formal analysis, Data curation. **Peng Zhang:** Writing – original draft, Software, Investigation, Formal analysis. **Yunjie Zhang:** Writing – original draft, Methodology, Formal analysis. **Qi Zhou:** Investigation. **Huibin Wu:** Investigation. **Bing Fan:** Visualization, Validation, Supervision, Data curation. **Si Zhang:** Writing – review & editing, Visualization, Validation, Resources, Data curation, Conceptualization. **Hongyi Wu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We thank all patients participated in our study. We also thank all members of our team for critical input and suggestions.

References

[1] A. Chattopadhyay, A.A.K. Jailani, B. Mandal, Exigency of plant-based vaccine against COVID-19 emergence as pandemic preparedness, Vaccines (Basel) 11 (8) (2023) 1347.
[2] K.L. Muir, A. Kallam, S.A. Koepsell, K. Gundabolu, Thrombotic thrombocytopenia after Ad26.COV2.S vaccination, N. Engl. J. Med. 384 (2021) 1964–1965.
[3] A. Greinacher, T. Thiele, T.E. Warkentin, K. Weisser, P.A. Kyrle, S. Eichinger, Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination, N. Engl. J. Med. 384 (2021) 2092–2101.
[4] A. Pottegard, L.C. Lund, O. Karlstad, J. Dahl, M. Andersen, J. Hallas, O. Lidegaard, G. Tapia, H.L. Gulseth, P.L. Ruiz, S.V. Watle, A.P. Mikkelsen, L. Pedersen, H. T. Sorensen, R.W. Thomsen, A. Hviid, Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study, Bmj-Brit Med J 373 (2021) n1114.
[5] J. Hippisley-Cox, M. Patone, X.W. Mei, D. Saatci, S. Dixon, K. Khunti, F. Zaccardi, P. Watkinson, M. Shankar-Hari, J. Doidge, D.A. Harrison, S.J. Griffin, A. Sheikh, C. Coupland, Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study, Bmj-Brit Med J 374 (2021) n1931.
[6] C. Pawlowski, J. Rincon-Hekking, S. Awasthi, V. Pandey, P. Lenehan, A.J. Venkatakrishnan, S. Bade, J.C. O'Horo, A. Virk, M.D. Swift, A.W. Williams, G.J. Gores, A.D. Badley, J. Halamka, V. Soundararajan, Cerebral venous sinus thrombosis is not significantly linked to COVID-19 vaccines or non-COVID vaccines in a large multi-state health system, J. Stroke Cerebrovasc 30 (2021) 105923.
[7] N. Uaprasert, K. Panrong, P. Rojnuckarin, T. Chiasakul, Thromboembolic and hemorrhagic risks after vaccination against SARS-CoV-2: a systematic review and meta-analysis of randomized controlled trials, Thromb. J. 19 (2021) 86.
[8] P.A. Gurbel, K.P. Bliden, K.M. Hayes, U. Tantry, Platelet activation in myocardial ischemic syndromes, Expert Rev Cardiovas 2 (2004) 535–545.
[9] R.M. Inciardi, M. Adamo, L. Lupi, D.S. Cani, M. Di Pasquale, D. Tomasoni, L. Italia, G. Zaccone, C. Tedino, D. Fabbricatore, A. Curnis, P. Faggiano, E. Gorga, C. M. Lombardi, G. Milesi, E. Vizzardi, M. Volpini, S. Nodari, C. Specchia, R. Maroldi, M. Bezzi, M. Metra, Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy, Eur. Heart J. 41 (2020) 1821–1829.
[10] J.T. Castro, P. Azevedo, M.J. Fumagalli, N.S. Hojo-Souza, N. Salazar, G.G. Almeida, L.I. Oliveira, L. Faustino, L.R. Antonelli, T.G. Marcal, M. Augusto, B. Valiate, A. Fiorini, B. Rattis, S.G. Ramos, M. Piccin, O.C. Nonato, L. Benevides, R. Magalhaes, B. Cassaro, G. Burle, D. Doro, J. Kalil, E. Durigon, A. Salazar, O. Caballero, H. Santiago, A. Machado, J.S. Silva, F.F. Da, A.P. Fernandes, S.R. Teixeira, R.T. Gazzinelli, Promotion of neutralizing antibody-independent immunity to wild-type and SARS-CoV-2 variants of concern using an RBD-Nucleocapsid fusion protein, Nat. Commun. 13 (2022) 4831.
[11] Y. Chen, W. Fu, Y. Zheng, J. Yang, Y. Liu, Z. Qi, M. Wu, Z. Fan, K. Yin, Y. Chen, W. Gao, Z. Ding, J. Dong, Q. Li, S. Zhang, L. Hu, Galectin 3 enhances platelet aggregation and thrombosis via Dectin-1 activation: a translational study, Eur. Heart J. 43 (2022) 3556–3574.
[12] Z. Qi, L. Hu, J. Zhang, W. Yang, X. Liu, D. Jia, Z. Yao, L. Chang, G. Pan, H. Zhong, X. Luo, K. Yao, A. Sun, J. Qian, Z. Ding, J. Ge, PCSK9 (proprotein convertase subtilisin/kexin 9) enhances platelet activation, thrombosis, and myocardial infarct expansion by binding to platelet CD36, Circulation 143 (2021) 45–61.
[13] S. Zhang, Y. Liu, X. Wang, L. Yang, H. Li, Y. Wang, M. Liu, X. Zhao, Y. Xie, Y. Yang, S. Zhang, Z. Fan, J. Dong, Z. Yuan, Z. Ding, Y. Zhang, L. Hu, SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19, J. Hematol. Oncol. 13 (2020) 120.
[14] Z. Qi, L. Hu, J. Zhang, W. Yang, X. Liu, D. Jia, Z. Yao, L. Chang, G. Pan, H. Zhong, X. Luo, K. Yao, A. Sun, J. Qian, Z. Ding, J. Ge, PCSK9 (proprotein convertase subtilisin/kexin 9) enhances platelet activation, thrombosis, and myocardial infarct expansion by binding to platelet CD36, Circulation 143 (2021) 45–61.
[15] X. Zhao, H. Wu, H. Xu, L. Shen, B. Fan, J. Ge, Association between residual platelet reactivity on clopidogrel treatment and severity of coronary atherosclerosis: intrinsic hypercoagulability as a mediator, Adv. Ther. 36 (2019) 2296–2309.
[16] J. Wang, Z. Hou, J. Liu, Y. Gu, Y. Wu, Z. Chen, J. Ji, S. Diao, Y. Qiu, S. Zou, A. Zhang, N. Zhang, F. Wang, X. Li, Y. Wang, X. Liu, C. Lv, S. Chen, D. Liu, X. Ji, C. Liu, T. Ren, J. Sun, Z. Zhao, F. Wu, F. Li, R. Wang, Y. Yan, S. Zhang, G. Ge, J. Shao, S. Yang, C. Liu, Y. Huang, D. Xu, X. Li, J. Ai, Q. He, M.H. Zheng, L. Zhang, Q. Xie, D.C. Rockey, J.A. Fallowfield, W. Zhang, X. Qi, Safety and immunogenicity of COVID-19 vaccination in patients with non-alcoholic fatty liver disease (CHESS2101): a multicenter study, J. Hepatol. 75 (2021) 439–441.
[17] T. Liu, J. Dai, Z. Yang, X. Yu, Y. Xu, X. Shi, D. Wei, Z. Tang, G. Xu, W. Xu, Y. Liu, C. Shi, Q. Ni, C. Yang, X. Zhang, X. Wang, E. Chen, J. Qu, Inactivated SARS-CoV-2 vaccine does not influence the profile of prothrombotic antibody nor increase the risk of thrombosis in a prospective Chinese cohort, Sci. Bull. 66 (2021) 2312–2319.
[18] C.R. Simpson, T. Shi, E. Vasileiou, S.V. Katikireddi, S. Kerr, E. Moore, C. McCowan, U. Agrawal, S.A. Shah, L.D. Ritchie, J. Murray, J. Pan, D.T. Bradley, S. J. Stock, R. Wood, A. Chuter, J. Beggs, H.R. Stagg, M. Joy, R. Tsang, S. de Lusignan, R. Hobbs, R.A. Lyons, F. Torabi, S. Bedston, M. O'Leary, A. Akbari, J. McMenamin, C. Robertson, A. Sheikh, First-dose ChAdOx1 and BNT162b2 COVID-19 vaccines and thrombocytopenic, thromboembolic and hemorrhagic events in Scotland, Nat. Med. 27 (2021) 1290–1297.
[19] X. Ye, T. Ma, J.E. Blais, V. Yan, W. Kang, C. Chui, F. Lai, X. Li, E. Wan, C. Wong, H.F. Tse, C.W. Siu, I. Wong, E.W. Chan, Association between BNT162b2 or CoronaVac COVID-19 vaccines and major adverse cardiovascular events among individuals with cardiovascular disease, Cardiovasc. Res. (2022).
[20] E. Wan, Y. Wang, C. Chui, A. Mok, W. Xu, V. Yan, F. Lai, X. Li, C. Wong, E. Chan, K.K. Lau, B.J. Cowling, I. Hung, I. Wong, Safety of an inactivated, whole-virion COVID-19 vaccine (CoronaVac) in people aged 60 years or older in Hong Kong: a modified self-controlled case series, Lancet Health Longev 3 (2022) e491–e500.
[21] S.R. Ostrowski, O.S. Sogaard, M. Tolstrup, N.B. Staerke, J. Lundgren, L. Ostergaard, A.M. Hvas, Inflammation and platelet activation after COVID-19 vaccines - possible mechanisms behind vaccine-induced immune thrombocytopenia and thrombosis, Front. Immunol. 12 (2021) 779453.
[22] M. Klug, O. Lazareva, K. Kirmes, M. Rosenbaum, M. Lukas, S. Weidlich, C.D. Spinner, M. von Scheidt, R. Gosetti, J. Baumbach, J. Ruland, G. Condorelli, K. L. Laugwitz, M. List, I. Bernlochner, D. Bongiovanni, Platelet surface protein expression and reactivity upon TRAP stimulation after BNT162b2 vaccination, Thromb. Haemostasis (2021).
[23] L. Cari, M. Naghavi Alhosseini, A. Bergamo, et al., Thrombotic events with or without thrombocytopenia in recipients of adenovirus-based COVID-19 vaccines, Front Cardiovasc Med 9 (2022) 967926, https://doi.org/10.3389/fcvm.2022.967926. Published 2022 Sep. 29.
[24] European Medicines Agency [EMA], AstraZeneca's COVID-19 Vaccine: EMAFinds Possible Link to Very Rare Cases of Unusual Blood Clots with Low Blood Platelets, European Medicines Agency, Amsterdam, 2021.
[25] A. Greinacher, T. Thiele, T.E. Warkentin, K. Weisser, P.A. Kyrle, S. Eichinger, Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination, N. Engl. J. Med. 384 (2021) 2092–2101.
[26] N.H. Schultz, I.H. Sørvoll, A.E. Michelsen, L.A. Munthe, F. Lund-Johansen, M.T. Ahlen, et al., Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination, N. Engl. J. Med. 384 (2021) 2124–2130.
[27] U.S. Food and Drug Administration [FDA], Joint CDC and FDA Statement on Johnson & Johnson COVID-19 Vaccine, U.S. Food and Drug Administration, Silver Spring, MD, 2021.

[28] I. See, J.R. Su, A. Lale, E.J. Woo, A.Y. Guh, T.T. Shimabukuro, et al., US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, JAMA (2021). March 2 to April 21, 2021.
[29] L. Nicolai, A. Leunig, K. Pekayvaz, et al., Thrombocytopenia and splenic platelet-directed immune responses after IV ChAdOx1 nCov-19 administration, Blood, The Journal of the American Society of Hematology 140 (5) (2022) 478–490.
[30] T. Thiele, L. Ulm, S. Holtfreter, L. Schonborn, S.O. Kuhn, C. Scheer, T.E. Warkentin, B.M. Broker, K. Becker, K. Aurich, K. Selleng, N.O. Hubner, A. Greinacher, Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2, Blood 138 (2021) 299–303.
[31] K. Krauel, C. Potschke, C. Weber, W. Kessler, B. Furll, T. Ittermann, S. Maier, S. Hammerschmidt, B.M. Broker, A. Greinacher, Platelet factor 4 binds to bacteria, [corrected] inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia, Blood 117 (2011) 1370–1378.
[32] Y. Zheng, A.W. Wang, M. Yu, A. Padmanabhan, B.E. Tourdot, D.K. Newman, G.C. White, R.H. Aster, R. Wen, D. Wang, B-cell tolerance regulates production of antibodies causing heparin-induced thrombocytopenia, Blood 123 (2014) 931–934.
[33] E. Campello, C. Bulato, C. Simion, L. Spiezia, C.M. Radu, S. Gavasso, F. Sartorello, G. Saggiorato, P. Zerbinati, M. Fadin, D. Tormene, P. Simioni, Assessing clinically meaningful hypercoagulability after COVID-19 vaccination: a longitudinal study, Thromb. Haemostasis (2022).
[34] Q. Zhang, L. Jiao, Q. Chen, et al., COVID-19 antibody responses in individuals with natural immunity and with vaccination-induced immunity: a systematic review and meta-analysis, Syst. Rev. 13 (1) (2024) 189.
[35] X.Y. Zhan, Y. Chen, X. Zhang, et al., Characterization of SARS-CoV-2-specific humoral immunity and associated factors in the healthy population post-vaccination, Vaccine 42 (2) (2024) 175–185, https://doi.org/10.1016/j.vaccine.2023.12.021.
[36] G.A. Roth, G.A. Mensah, C.O. Johnson, G. Addolorato, E. Ammirati, L.M. Baddour, N.C. Barengo, A.Z. Beaton, E.J. Benjamin, C.P. Benziger, A. Bonny, M. Brauer, M. Brodmann, T.J. Cahill, J. Carapetis, A.L. Catapano, S.S. Chugh, L.T. Cooper, J. Coresh, M. Criqui, N. DeCleene, K.A. Eagle, S. Emmons-Bell, V.L. Feigin, J. Fernandez-Sola, G. Fowkes, E. Gakidou, S.M. Grundy, F.J. He, G. Howard, F. Hu, L. Inker, G. Karthikeyan, N. Kassebaum, W. Koroshetz, C. Lavie, D. Lloyd-Jones, H.S. Lu, A. Mirijello, A.M. Temesgen, A. Mokdad, A.E. Moran, P. Muntner, J. Narula, B. Neal, M. Ntsekhe, D.O.G. Moraes, C. Otto, M. Owolabi, M. Pratt, S. Rajagopalan, M. Reitsma, A. Ribeiro, N. Rigotti, A. Rodgers, C. Sable, S. Shakil, K. Sliwa-Hahnle, B. Stark, J. Sundstrom, P. Timpel, I.M. Tleyjeh, M. Valgimigli, T. Vos, P.K. Whelton, M. Yacoub, L. Zuhlke, C. Murray, V. Fuster, Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study, J. Am. Coll. Cardiol. 76 (2020) 2982–3021.