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Full Length Article

Head-to-head comparison of four COVID-19 vaccines on platelet activation, coagulation and inflammation. The TREASURE study

Marta Brambilla [a], Paola Canzano [a], Patrizia Della Valle [b], Alessia Becchetti [a], Maria Conti [a], Mariangela Alberti [a], Arianna Galotta [a], Maria Luisa Biondi [a], Paola Adele Lonati [c], Fabrizio Veglia [d], Alice Bonomi [a], Nicola Cosentino [a], Pier Luigi Meroni [c], Gian Vincenzo Zuccotti [e], Armando D'Angelo [b,1], Marina Camera [a,f,*,**,1]

[a] Centro Cardiologico Monzino IRCCS, Milan, Italy
[b] Coagulation Service and Thrombosis Research Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c] Istituto Auxologico Italiano IRCCS, Milan, Italy
[d] Maria Cecilia Hospital, Cotignola, RA, Italy
[e] Department of Pediatrics, Vittore Buzzi Children's Hospital, Università degli Studi di Milano, Milan, Italy
[f] Department of Pharmaceutical Sciences, Università degli Studi di Milano, Milan, Italy

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ABSTRACT

Introduction: Studies exploring alterations in blood coagulation and platelet activation induced by COVID-19 vaccines are not concordant. We aimed to assess the impact of four COVID-19 vaccines on platelet activation, coagulation, and inflammation considering also the immunization dose and the history of SARS-CoV-2 infection.
Methods: TREASURE study enrolled 368 consecutive subjects (161 receiving viral vector vaccines -ChAdOx1-S/Vaxzevria or Janssen- and 207 receiving mRNA vaccines -Comirnaty/Pfizer-BioNTech or Spikevax/Moderna). Blood was collected the day before and 8 ± 2 days after the vaccination. Platelet activation markers (P-selectin, aGPIIbIIIa and Tissue Factor expression; number of platelet-monocyte and -granulocyte aggregates) and microvesicle release were analyzed by flow cytometry. Platelet thrombin generation (TG) capacity was measured using the Calibrated Automated Thrombogram. Plasma coagulation and inflammation markers and immune response were evaluated by ELISA.
Results: Vaccination did not induce platelet activation and microvesicle release. IL-6 and CRP levels (+30%), D-dimer, fibrinogen and F_{1+2} (+13%, +3.7%, +4.3%, respectively) but not TAT levels significantly increased upon immunization with all four vaccines, with no difference among them and between first and second dose. An overall minor post-vaccination reduction of aPC, TM and TFPI, all possibly related to endothelial function, was observed. No anti-PF4 seroconversion was observed.
Conclusion: This study showed that the four COVID-19 vaccines administered to a large population sample induce a transient inflammatory response, with no onset of platelet activation. The minor changes in clotting activation and endothelial function might be potentially involved at a population level in explaining the very rare venous thromboembolic complications of COVID-19 vaccination.

Abbreviations: VITT, vaccine-induced immune thrombotic thrombocytopenia; COVID-19, Coronavirus disease 2019; TF, Tissue Factor; MVs, microvesicles; aGPIIbIIIa, activated glycoprotein IIbIIIa; AnnV, annexinV; PS, phosphatidylserine; CAT, Calibrated Automated Thrombogram; F_{1+2}, prothrombin fragment 1+2; TAT, Thrombin Anti Thrombin; PT, Prothrombin Time; aPTT, Activated Partial Thromboplastin Clotting Time; aPC, activated Protein C; TM, thrombomodulin; TFPI, Tissue Factor Pathway Inhibitor; PAI-1, Plasminogen Activator Inhibitor-1; vWF, von Willebrand Factor; FVIII:C, Factor VIII clotting activity; IL-6, Interleukin-6; CRP, C-Reactive Protein; PRP, platelet rich plasma; PFP, platelet free plasma.

* Corresponding author at: Department of Pharmaceutical Sciences, Università degli Studi di Milano, via Balzaretti, 9, 20133 Milan, Italy.
** Corresponding author at: Centro Cardiologico Monzino IRCCS, Via Parea 4, 20138 Milan, Italy.
E-mail addresses: marina.camera@unimi.it, marina.camera@ccfm.it (M. Camera).
[1] These authors equally contributed.

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1. Introduction

Reports on the vaccine-induced immune thrombotic thrombocytopenia (VITT), the new syndrome characterized by thrombotic events associated mainly, but not exclusively [1], with COVID-19 adenovirus vector-based vaccines [2,3], have led the scientific community to design *ad hoc* studies to assess whether vaccination can lead to hypercoagulability predisposing subjects to thrombosis. Possible alterations of blood coagulation parameters have been recently reported comparing the effect of adenovirus vector- *versus* mRNA-based vaccines [4,5]. One study assessed also the impact on inflammation and endothelial activation [4]. Potential effects of vaccines on platelet activation have been also explored: this issue was pursued with an indirect approach by assessing platelet count, measuring released soluble markers such as P-selectin as well as microvesicle (MVs) production and testing platelet aggregation [4,6,7]. These studies have not however provided concordant results. This may be due to different study designs, timing of blood sampling, and/or use of biomarkers not exclusively platelet-specific; lack of paired analyses and the use of a small sample size should also be considered for some of them [4–7].

With the effort to further gain insights into potential effects of vaccines in inducing an overall platelet prothrombotic phenotype, it is worth considering that the platelet procoagulant potential is supported 1) by exposure of phosphatidylserine, which is essential for the assembly of coagulation factors, 2) by expression of tissue factor (TF) [8], the key activator of the blood coagulation, and 3) by production of TF-positive MVs, this latter event being predictive of adverse cardiovascular outcomes [9]. We have previously provided evidence that SARS-CoV-2 infection leads to a peculiar pattern of platelet activation sustaining the prothrombotic phenotype of COVID-19 patients, characterized by TF expression, formation of TF-expressing platelet-leukocyte aggregates and TF-expressing MVs, rather than by GPIIbIIIa activation supporting classical platelet aggregation [10].

Based on this background, we designed the prospective TREASURE study to assess in a large general population sample whether four different COVID-19 vaccines, two adenovirus vector- and two mRNA-based, can -over a period of 8 ± 2 days after vaccination- 1) sustain platelet activation (direct assessment by whole blood flow cytometry) and promote the onset of a procoagulant platelet phenotype, and 2) affect plasma coagulation, inflammation profiles and immune-response. This comprehensive analysis was performed on the enrolled subjects before and after vaccination, thus allowing pairwise comparisons. We also assessed whether 3) these parameters were differently modulated by the first or second immunization dose, and 4) a history of SARS-CoV-2 infection might influence the haemostatic profile of vaccinated subjects.

2. Material and methods

2.1. Study design and population

The mass COVID-19 vaccination in Italy was launched on December 27, 2020 for Comirnaty Pfizer/BioNTech and on January 7, 2021 for Spikevax Moderna while the two viral vaccines ChAdOx1-S Vaxzevria and Janssen were approved on January 30, 2021 and on March 12, 2021, respectively. The study was addressed to the general population, aged 18–79 years, who was eligible to receive the first or second dose of ChAdOx1-S Vaxzevria or Comirnaty Pfizer/BioNTech or Spikevax Moderna SARS-CoV-2 vaccines or the only dose of Janssen vaccine. All subjects were informed and consented to participate to the study and they were asked to complete a questionnaire to collect medical history and adverse reactions after immunization. Enrollment was from April to July 2021. The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee at Università degli Studi di Milano and Centro Cardiologico Monzino (protocol number: 38/21, 20/04/21).

2.2. Sample collection and preparation

The day before (T0) and 8 ± 2 days after the vaccination (T1), whole blood (WB, 20 ml) was drawn from the antecubital vein with a 19-gauge needle without venous stasis into citrate- (1/10 volume of 0.129 M sodium citrate), K2-EDTA-containing tubes or in tubes without anticoagulant (Vacutainer, Becton Dickinson), discarding the first 4 ml. For analysis of some coagulation factors (F_{1+2}, TAT, aPC) venous blood samples (2.7 ml) were collected in siliconized Vacutainer tubes (Becton Dickinson) containing 0.109 M sodium citrate and 200 mM benzamidine-HCl (20 mM f.c.). For platelet rich plasma (PRP) preparation, 0.129 M citrate anticoagulated WB was centrifuged at 100*g*, for 10 min, brake off, at room temperature (RT). For platelet free plasma (PFP) preparation, WB tubes were centrifuged twice at 2500*g*, for 15 min, at RT according to the ISTH guidelines [11]. PFP aliquots were stored at −80°C until analysis. Serum was obtained from blood collected in tubes without anticoagulant in the presence of a clot activator and centrifuged at 1700*g*, for 10 min at 4°C. Serum aliquots were stored at −80°C until analysis.

For plasma preparation, citrate- and EDTA-anticoagulated WB was centrifuged at 1700*g*, for 10 min at 4°C and plasma aliquots were stored at −80°C until analysis.

2.3. Flow cytometry

Circulating cell-associated tissue factor (TF) expression, platelet activation markers and MV release were analyzed by flow cytometry as previously described [10].

Briefly, platelet surface expression of TF and activation markers [activated glycoprotein IIbIIIa (aGPIIbIIIa) and P-selectin] as well as platelet annexinV (AnnV) binding to phosphatidylserine (PS) were assessed in citrate-anticoagulated WB samples (containing 1×10^6 platelets) under resting conditions. Platelet expression of C4d was assessed as previously described [12] and modified as follows. Briefly, EDTA-WB samples (50 μl) were diluted with DPBS/1% FCS (1:2) and stained with a monoclonal antibody (moAb) against C4d (10 μg/ml) for 30 min at RT, followed by staining with a secondary goat anti-mouse antibody (1:200) at RT in the dark for 30 min. A moAb against the platelet population marker CD41 was used to identify platelets. Leukocyte-platelet aggregates were identified in 100 μl WB as double positive events for leukocyte and platelet population markers ($CD14^{pos}$/$CD41^{pos}$ or $CD66^{pos}$/$CD41^{pos}$ for monocyte-platelet and granulocyte-platelet aggregates, respectively). Fluorochrome-conjugated isotype controls were used in order to quantify the background labeling. A total of 10,000 $CD41^{pos}$ events and 3000 $CD14^{pos}$ events per sample were acquired on a Gallios cytometer (Beckman Coulter). All the data were analyzed with Kaluza analysis software v1.5 (Beckman Coulter) and reported as mean ± standard deviation or median with 25th and 75th percentiles of positive cells as indicated.

For MV characterization, fifty microliters of WB were diluted in 150 μl of 0.22 μm-filtered buffer [Hepes (10 mM), NaCl (140 mM), and $CaCl_2$ (2.5 mM), pH 7.4] containing phe-pro-arg chloromethyl ketone (PPACK, 15 μM) to prevent clot formation. To identify intact MVs, excluding cell debris, samples were incubated with calcein AM (10 μM) at 37°C in the dark for 25 min. Saturating concentrations of moAbs against TF and CD41 were then added for 15 min at RT in the dark. Fluorescence minus one (FMO) control was used for gating correction. Samples were immediately analyzed on Gallios flow cytometer equipped with four solid-state lasers and an enhanced wide forward angle light scatter optimized for MV detection. Flow-check Pro Fluorospheres were daily used to monitor cytometer performance. Megamix-FSC Plus beads (0.5, 0.9, 3 μm) were used to define the analysis gate and BD Trucount tubes™ to have the absolute count of MVs. All samples were processed by the same experienced operator.

2.4. Thrombin generation assay

Platelets (40×10^6), isolated from enrolled subjects before ($n = 35$) and after immunization ($n = 43$), were tested for their capacity to promote thrombin generation using the Calibrated Automated Thrombogram (CAT) assay as previously described [13]. Briefly, isolated cells were resuspended in a MV-free pool plasma prepared from multi-donor WB collected in Corn Trypsin Inhibitors (CTI, 50 μg/ml)-citrate buffer in order to prevent *ex vivo* contact pathway activation. Triplicate samples were incubated for 10 min at 37°C in round-bottom 96-well microtiter plates. In order to make the thrombin generation assay independent from the phospholipid surface of platelets, and dependent only by the endogenous TF, CAT experiments were performed by adding an excess of exogenous phospholipids (4 μM, MP reagent; Diagnostica Stago). To quantify the contribution of TF to thrombin generation, parallel samples were run in the presence of a neutralizing αTF antibody (HTF-1; Thermo Fisher; 8 μg/ml). An irrelevant IgG was used as control. Thrombin generation was started by the addition of a fluorogenic/$CaCl_2$ substrate mixture (FluCa Kit; Diagnostica Stago) and fluorescence was read for 90 min in a Fluoroskan Ascent® reader. Thrombin generation curves were analyzed by dedicated software (Thrombinoscope BV). Lag-Time (min), ETP (Endogenous Thrombin Potential, nM × min), Peak High (nM Thrombin), Time to Peak (min) and Velocity Index (nM/min) were used as main parameters describing thrombin generation.

2.5. Biochemical measurements

Within 2 h from drawing blood samples were centrifugated at 1500*g* for 10 min and analyzed with ACL TOP550 (Instrumentation Laboratory S.p.A). D-dimer testing was performed *via* immuno-turbidometric assay, fibrinogen with Clauss method, PT with RecombiPlasTin 2G, aPTT with SynthAsil reagents. Biochemical tests (Glycaemia, Total Cholesterol, LDL Cholesterol and Triglycerides) were performed on Atellica Solution (Siemens Healthcare Diagnostics).

Activated Protein C (aPC) levels was quantified by a previously described home-made ELISA [14], using the calcium-dependent monoclonal HAPC 1555 characterized by Liaw et al. [15], with minor modifications. Briefly, benzamidine-HCl plasma samples were anticoagulated with hirudin (1.5 μM f.c.) instead of heparin (2 IU/ml, f.c.), and the aPC standard curve ranged from 0 to 20 ng/ml (instead of 0 to 50 ng/ml). The monoclonal antibody HAPC 1555 was a kind gift by Dr. Charles T. Esmon (Oklahoma Medical Research Foundation).

To monitor changes in thrombin generation and neutralization, plasma levels of prothrombin fragment 1+2 (F_{1+2}) and thrombin/antithrombin complex (TAT) were measured by commercial enzyme-linked immunosorbent assay (Enzygnost F_{1+2} and Enzygnost TAT, Siemens Healthcare Diagnostic). Soluble-thrombomodulin (sTM) was measured by commercial ELISA (Quantikine Human Thrombomodulin, R&D Systems).

Plasminogen Activator Inhibitor-1 (PAI-1) antigen, total Tissue Factor Pathway Inhibitor (TFPI) antigen, and anti-heparin-platelet factor 4 (PF4) antibodies were measured in 0.109 M citrated plasma with commercially available ELISA kits (Asserachrom PAI-1, Asserachrom Total TFPI, Asserachrom HPIA, Diagnostica Stago). Samples positive for anti-heparin-PF4 antibodies had absorbance values higher than 0.492. All ELISA readings were in a Multiskan GO (Thermo Fisher Scientific).

FVIII and von Willebrand Factor antigen levels (Liatest VWF:Ag, Diagnostica Stago) were measured in 0.109 M citrated plasma aliquots with an automated coagulometer (STA-R MAX, Diagnostica Stago). FVIII coagulant activity (FVIII:C) was tested in a one-stage aPTT assay (STA C. K. Prest, Diagnostica Stago) using a commercial FVIII immuno-depleted plasma (Immunodef FVIII Diagnostica Stago). Calibration curves were obtained using normal pooled plasma obtained from 70 to 80 apparently healthy volunteers processed, frozen, and stored as previously described [16].

Intra- and inter-assay imprecisions ranged from 2.3% (sTM) to 11.2% (F_{1+2}) and from 1.9% (VWF:Ag) to 14.9% (aPC), respectively.

2.6. Statistical analysis

2.6.1. Sample size

The primary endpoint was the change in platelet activation assessed before and after vaccine administration, based on the expression of the 5 most indicative markers (P-selectin; tissue factor; activated glycoprotein IIbIIIa; platelet-granulocyte and platelet-monocyte aggregates). A total of 368 subjects (207 in one group and 161 in the other) provided a statistical power of 80% to detect as significant ($p < 0.01$) a mean difference between the two treatment groups of at least 0.361 standard deviations. Standard deviation was defined as standard deviation of the within-subject variation of the marker.

2.6.2. Statistical analysis

Continuous variables are expressed as mean and standard deviation (SD) if normally distributed, and as median and interquartile range otherwise; categorical variables are expressed as frequency and percentage. Variables with right skewed distribution were log-transformed before analysis.

Baseline comparisons were performed using one-way ANOVA (four vaccines comparisons) or Student's *t*-test (viral *vs.* mRNA vaccines) for continuous variables, and Pearson's $\chi 2$ test or Fisher's exact test, as appropriate, for categorical variables. Variations between pre- and post-vaccination are expressed as percent change and 95 % confidence interval.

Changes in the variables of interest, measured before and after vaccine administration, were assessed by paired samples *t*-test and were compared between groups by ANOVA or Student's *t*-test for independent samples.

Differences of vaccine effects in the groups were analyzed by General Linear Models, with adjustment for dose and propensity score (computed using a multivariable logistic regression model, to estimate the probability of receiving a vaccine type, giving the following set of variables: age, sex, and comorbidity -diabetes, chronic bronchitis, asthma, hypertension, previous myocardial infarction, previous coronary angioplasty, non-ischemic cardiomyopathy, severe valvular heart disease, hospitalization for heart failure, autoimmune diseases, cancer, dyslipidemia-).

Interaction between vaccine type and dose was also computed and tested by General Linear Models. For all relevant comparisons, in order to account for the large number of tests performed, we applied the Benjamini–Hochberg procedure to control for a false discovery rate (FDR) < 20%. Statistical analyses were performed with SAS Software (version 9.4; SAS Institute Inc.).

3. Results

3.1. Participant characteristics

A total of 368 consecutive subjects were enrolled; 161 subjects received COVID-19 viral vector vaccines (ChAdOx1-S Vaxzevria or Janssen) and 207 subjects were treated with mRNA vaccines (Comirnaty Pfizer/BioNTech or Spikevax Moderna). In particular, 38 (23.6%) and 123 (76.4%) individuals received a first or a second dose of viral vector vaccine, respectively. One hundred sixty-nine (81.6%) and 38 (18.4%) individuals received a first or a second dose of mRNA vaccine, respectively.

Demographic and clinical characteristics of the enrolled subjects, divided by the type of vaccine administered, are summarized in Tables 1 and S1. Subjects who received the viral vaccines were on average 4 years older than those treated with the mRNA vaccine ($p = 0.006$). This difference is due to the guideline of the Italian legislation on the vaccination schedules that were amended during enrollment. Indeed, as of March 8, 2021, the administration of the viral vector vaccine was

Table 1
Demographic variables of the enrolled subjects.

	ChAdOx1-S Vaxzevria	Janssen	Comirnaty Pfizer/ BioNTech	Spikevax Moderna	*p* value (ANOVA)	All	Reference intervals
Number of subjects, n	142	19	169	38		368	
Male, n (%)	47 (33.1)	10 (52.6)	73 (43.2)	17 (44.7)	0.161	147 (40)	–
Age, yrs	49.1 ± 11.7	52.9 ± 7.8	46.1 ± 15.7	43.6 ± 13.0	0.019	47.3 ± 13.8	–
First vaccine dose, n (%)	19 (13)	19 (100)	133 (79)	36 (95)	<0.001	207 (56.3)	
Body Mass Index, kg/m^2	23.4 ± 3.4	24.4 ± 2.8	23.8 ± 4.1	24.1 ± 3.8	0.513	23.7 ± 3.8	[18.5–24.99]
CV risk factors							
Previous myocardial infarction, n (%)	2 (1.4)	0 (0)	2 (1.2)	1 (2.6)	0.732	5 (1.38)	–
Hypertension, n (%)	18 (12.8)	2 (11.1)	18 (10.8)	7 (18.4)	0.623	45 (12.4)	–
Diabetes, n (%)	2 (1.4)	0 (0)	6 (3.6)	1 (2.6)	0.677	9 (2.5)	–
Dislipidemia, n (%)	13 (10.7)	6 (33.3)	17 (11.6)	5 (13.2)	0.056	41 (12.7)	–
Active smoker, n (%)	20 (14.2)	1 (5.6)	30 (18.1)	4 (10.5)	0.172	55 (15.2)	–
Former smoker, n (%)	26 (18.4)	8 (44.4)	37 (22.3)	6 (15.8)	.	77 (21.2)	–
Never smoker, n (%)	95 (67.4)	9 (50)	99 (59.6)	28 (73.7)	.	231 (63.6)	–
Other comorbidities							
Asthma, n (%)	6 (4.3)	1 (5.6)	3 (1.8)	1 (2.6)	0.371	11 (3)	–
Autoimmune diseases, n (%)	7 (5)	0 (0)	11 (6.6)	5 (13.2)	0.255	23 (6.3)	–
Tumors, n (%)	3 (2.1)	1 (5.6)	11 (6.6)	1 (2.6)	0.217	16 (4.4)	–
Pharmacological treatment							
Antiplatelet, n (%)	10 (7.1)	3 (16.7)	8 (4.8)	3 (7.9)	0.209	24 (6.6)	–
Anticoagulant, n (%)	3 (2.1)	0 (0)	2 (1.2)	0 (0)	0.855	5 (1.4)	–
Anti-inflammatory, n (%)	15 (10.6)	2 (11.1)	18 (10.8)	5 (13.2)	0.949	40 (11)	–
Corticoids, n (%)	4 (2.8)	0 (0)	4 (2.4)	1 (2.6)	1.000	9 (2.5)	–
Antihypertensive, n (%)	21 (14.9)	3 (16.7)	18 (10.8)	7 (18.4)	0.464	49 (13.5)	–
Antidiabetic, n (%)	2 (1.4)	0 (0)	4 (2.4)	1 (2.6)	0.751	7 (1.9)	–
Chemotherapy, n (%)	0 (0)	0 (0)	2 (1.2)	1 (2.6)	0.341	3 (0.8)	–
Immunosuppressor, n (%)	0 (0)	0 (0)	1 (0.6)	0 (0)	1.000	1 (0.3)	–
Biochemical parameters							
Glycaemia, mg/dL	91.9 ± 9.4	100.1 ± 11.2	94.7 ± 14	92.5 ± 11.6	0.038	93.8 ± 12.3	[60–99]
Total cholesterol, mg/dL	198.4 ± 34.6	209.4 ± 40.3	193.4 ± 36.4	182.6 ± 27.5	0.047	195.1 ± 35.5	[<200]
LDL cholesterol, mg/dL	131.7 ± 37.6	141.7 ± 35.6	127.2 ± 36.1	116.4 ± 28.9	0.082	128.6 ± 36.3	[<116]
Triglycerides, mg/dL	91 (69.5–121.5)	81 (66–115)	89 (71–120)	77 (56.5–115)	0.494	89 (69;120)	[<150]
Hematological parameters							
Platelet count, 10^3/μl	256.4 ± 56.5	273.2 ± 63.5	238.6 ± 55.7	252.7 ± 67.9	0.011	248.8 ± 58.4	[140–450]
WBC count, 10^3/μl	6.8 ± 1.6	6.9 ± 1.5	6.6 ± 1.4	6.7 ± 1.6	0.875	6.7 ± 1.6	[3.9–10.5]
Neutrophil count, 10^3/μl	3.9 ± 1.3	3.7 ± 1.0	3.7 ± 1.1	3.9 ± 1.3	0.451	3.8 ± 1.2	[1.80–7.70]
Monocyte count, 10^3/μl	0.53 ± 0.14	0.56 ± 0.16	0.52 ± 0.13	0.51 ± 0.09	0.587	0.53 ± 0.13	[0.0–0.8]
Lymphocyte count, 10^3/μl	2.1 ± 0.5	2.4 ± 0.6	2.2 ± 0.6	2.0 ± 0.6	0.064	2.2 ± 0.6	[1.0–4.8]
RBC count, 10^6/μl	4.6 ± 0.5	4.6 ± 0.4	4.7 ± 0.5	4.7 ± 0.5	0.240	4.7 ± 0.5	[4.0–5.75]
Hematocrit, %	40.7 ± 3.4	41.4 ± 3.3	41.3 ± 4.6	40.5 ± 3.7	0.509	41.0 ± 4.1	[39.5–50.5]
Hemoglobin, g/dL	13.7 ± 1.3	14.1 ± 1.1	13.9 ± 1.7	13.7 ± 1.5	0.531	13.7 ± 1.5	[13.5–17.2]
Coagulation parameters							
D-dimer, ng/ml	241.9 (174;341.2)	257.1 (182;367.3)	255.6 (179;349.8)	247.43 (162.9;375.4)	0.950	245.4 (175.6;347.4)	[<500]
Fibrinogen, mg/dL	258.5 (231;309)	303 (254;323)	263 (234;308)	257 (231;324)	0.189	263 (232;310)	[150–450]
F_{1+2}, pM	288 (222;368)	308 (214;392)	288.5 (207.7;382.5)	325.5 (237.2;420)	0.592	293.9 (217.9;385.5)	[115–410]
TAT, ng/ml	4.4 (3.75;5.35)	4.6 (3.6;6)	4.3 (3.7;5.2)	3.6 (3.2;4.2)	0.171	4.4 (3.7;5.3)	[1–10]
PT ratio	0.97 (0.93;1.01)	0.94 (0.92;0.98)	0.97 (0.94;1.01)	0.97 (0.94;1.01)	0.253	0.97 (0.93;1.01)	[0.8–1.1]
aPTT ratio	1.02 (0.96;1.06)	1.02 (0.95;1.07)	1.01 (0.96;1.06)	1 (0.94;1.03)	0.356	1.01 (0.96;1.06)	[0.8–1.2]
aPC, ng/ml	2.5 (1.25;4.4)	4.5 (1.2;6.9)	2 (1.1;3.2)	2.4 (1.5;3.7)	0.059	2.2 (1.1;3.9)	[0.6–4.1]
TM, ng/ml	3.3 (3;3.85)	3.6 (3.2;3.9)	3.6 (3.18;4.3)	3.8 (3.2;4.4)	0.003	3.5 (3.1;4.2)	[2.1–4.6]
TFPI, ng/ml	79.7 (68.2;92)	90 (81.5;100.5)	88 (78;99)	74 (66.5;86.5)	0.020	83 (72;96)	[54–135]
PAI-1, ng/ml	5.1 (2.95;8)	5.5 (4.4;11.8)	4.9 (3.8;8.6)	4.9 (4.1;10.6)	0.422	5.1 (3.3;8.6)	[2–46]
vWF, %	74 (60;90)	71.5 (52;90)	76 (60;94)	63.5 (50.5;82)	0.599	74 (57.5;92)	[47–138]
FVIII:C, %	86 (67;111)	92 (66;135)	85 (63;115.5)	73.5 (61;95)	0.466	85 (63;113)	[65–140]
Inflammatory parameters							
IL-6, pg/mL	1.5 (0.9;2)	1.5 (0.9;1.5)	1.7 (1.3;2.4)	1.5 (1.3;2.2)	0.011	1.5 (1.1;2.2)	[<4.4]
CRP, mg/L	0.25 (0.25;1.5)	1.1 (0.25;2.3)	0.25 (0.25;1.35)	0.25 (0.25;1.3)	0.331	0.25 (0.25;1.4)	[<5]
C4d, % positive platelets	2.01 (0.9;5.59)	2.65 (1.06;6.9)	2.92;(1.06;6.61)	1.88 (0.94;4.61)	0.484	2.46 (1.0;5.45)	
HPIA, O.D.	0.26 (0.16;0.46)	0.31 (0.16;0.6)	0.23 (0.15;0.44)	0.24 (0.14;0.43)	0.609	0.24 (0.15;0.46)	[<0.492]

F_{1+2} = Prothrombin fragment 1+2; TAT = Thrombin Anti Thrombin; PT = Prothrombin Time; aPTT = Activated Partial Thromboplastin Clotting Time; aPC = activated Protein C; TM = Thrombomodulin; TFPI = Tissue Factor Pathway Inhibitor; PAI-1 = Plasminogen Activator Inhibitor-1; vWF = von Willebrand Factor; FVIII: C = Factor VIII clotting activity; IL-6 = Interleukin-6; CRP = C-Reactive Protein. Data are expressed as mean ± standard deviation or median (IQR).

reserved for subjects aged >65 years. For the same reason, considering the time frame of the enrollment, we studied more first doses with mRNA vaccines and more second doses with viral vaccines. Subjects of the Janssen group, besides being the oldest ones, showed also higher levels, although within the normal range, of glycaemia, total cholesterol and platelet count. No further differences were observed in the other hematological variables analyzed. There were no significant differences between the four groups of subjects in terms of gender, BMI, cardiovascular risk factors, and current drug treatment.

The prevalence of adverse events after vaccination was similar in

subjects receiving the viral or mRNA vaccines. Overall, 155 (42%) participants experienced no systemic or local reaction after vaccination, while 213 (58%) reported at least one grade 1/2 adverse event, including fever, chills, fatigue, myalgia, or pain at the injection site that lasted from 24 h to 3 days. No severe adverse event of grade 3 or more was observed [17].

3.2. Blood count indices

Upon vaccination, platelet counts did not change with any type of vaccine (Table 2). Conversely, a small reduction in the number of erythrocytes and leukocytes was observed ($p < 0.001$). This trend was comparable among the four vaccines with the exception of monocyte counts that decreased more in the two adenovirus-based vaccines (−7.5%, $p_{adjusted} = 0.003$, Table S2). The reported changes were observed regardless of first or second vaccine dose; no significant interaction dose × vaccine type was observed for any variable (Table S2).

3.3. Platelet activation markers and procoagulant potential analysis

The effect of vaccines on platelet activation was assessed evaluating the expression of the common platelet activation markers -P-selectin, activated glycoprotein IIbIIIa (aGPIIbIIIa), platelet-leukocyte aggregates and microvesicles (MVs)- as well as of those reflecting the platelet procoagulant capacity -TF and phosphatidylserine (PS).

Pre-immunization levels of these parameters were comparable among groups (Table S3). Vaccination with viral or mRNA vaccines did not change the platelet surface expression of aGPIIbIIIa, P-selectin as well as the number of platelet-monocyte and platelet-granulocyte aggregates (all $p > 0.055$, Fig. 1A–D and Table 3). Similarly, the number of total microvesicles released into the circulation and that of platelet-derived microvesicles was not affected by the type of vaccine administered (Fig. 1E–F). This overall trend was comparable among the four vaccines (Table 3) and was observed regardless of first or second vaccine dose (Table S4).

Analysis of the platelet procoagulant potential showed that TF expression was not substantially modified by the administration of the two vaccine types (Fig. 2A). A mild increase in the number of TF^{pos} platelets (+4.1%; $p = 0.034$) was observed only upon ChAdOx1-S Vaxzevria vaccination. A slight reduction (−5.3%; $p < 0.001$) in the number of PS^{pos} platelets after vaccination with both of them was observed (Fig. 2B and Table 3), independently from the immunization dose (Tables 3 and S4). We then tested the thrombin generation potential on a subset of participants who received ChAdOx1-S Vaxzevria and mRNA vaccines. Results showed a trend toward a faster thrombin generation kinetic in the former (Lag-Time: 22.6 ± 6.5 and 18.4 ± 4.5 min before and after immunization, respectively) without affecting the overall amount of thrombin produced (Fig. 2C–F). The dependence of thrombin generation on TF expression was assessed by the use of a specific neutralizing αTF antibody. The delay exerted by the neutralizing anti-TF antibody was comparable between vaccine types and before and after immunization (ChAdOx: T0 = +3.2 ± 2 min, T1 = +3.6 ± 2.6 min; mRNA: T0 = +3.5 ± 2.7 min, T1 = +3.2 ± 2.4 min; $p < 0.05$ for all comparisons) thus supporting the antigen data reported above. Finally, no significant interaction dose × vaccine type was observed for any variable (Table S4).

3.4. Plasma coagulation markers

We next evaluated the influence of vaccines on the plasma haemostatic balance of the enrolled subjects. Pre-immunization coagulation parameters were all within the reference range with no substantial difference among the four vaccines (Table 1). Participants in the Spikevax Moderna group showed the highest thrombomodulin (TM) and the lowest TFPI levels.

D-dimer, fibrinogen and F_{1+2} levels, but not TAT levels, significantly increased (+13%, +3.7% and +4.3%, respectively) upon immunization with all the four vaccines, with no difference among them or when grouped by vaccine type and between the first and second dose (Tables 4 and S5). A similar but opposite trend was observed for aPC, TM and TFPI levels that were lower in all subjects post vaccination (−8.7%, −3%, −1.8%, respectively; Tables 4 and S5).

No differences in the levels of the inhibitor of the fibrinolytic system PAI-1 as well as in those of vWF and in the FVIII coagulant activity were observed (Table 4). No significant interaction dose × vaccine type was observed for any variable (Table S5).

3.5. Inflammation and immune response

Inflammation is the first immune response generated in subjects receiving vaccination. IL-6 and CRP levels, that were within the reference range at enrollment (Table 1), significantly increased indeed by ~30% when measured after vaccination (Table 5). While such changes were comparable to those observed stratifying data according to the two vaccine types (Table S6), subjects vaccinated with Janssen showed a different trend with a much stronger increase in IL6 (+99.5%) and a decrease (−6.1%) in CRP. Notably, these subjects showed also an opposite change in the percentage of platelets expressing complement C4d being reduced compared to the other groups where it slightly increased (+6.7% in all subjects) post vaccination. No effect of the immunization dose and no significant interaction dose × vaccine type were observed for any variable (Table S6).

At enrollment, anti-PF4/heparin IgG antibodies were detected in 26% (41/160; median OD = 0.606[0.542–0.897]) of the viral vaccine cohort and in 23% (47/205; median OD = 0.661[0.548–0.919]) of the

Table 2
Change (%) in hematological profile.

	ChAdOx1-S Vaxzevria	Janssen	Comirnaty Pfizer/ BioNTech	Spikevax Moderna	p adj (ANOVA)	All subjects (T1-T0) %	Paired *t*-test
	n = 142	*n* = 19	*n* = 169	*n* = 38		*n* = 368	
Platelet count	0 (−1.6;1.6)	0.4 (−4.3;5.1)	0.9 (−0.6;2.5)	−0.1 (−3.4;3.2)	0.858	0.4 (−0.6;1.5)	0.412
WBC count	−4.2 (−7;−1.3)	−3.3 (−10.3;3.6)	−2.6 (−5.6;0.5)	−6.1 (−11.3;−0.9)	0.582	−3.6 (−5.5;−1.7)	<0.001
Neutrophil count	−6.1 (−10.2;−1.9)	−4.6 (−14.2;5)	−4.4 (−8.8;0.1)	−7.6 (−14.5;−0.7)	0.655	−5.4 (−8.1;−2.7)	<0.001
Monocyte count	−6.4 (−10.1;−2.8)	−15.2 (−24.6;−5.8)	−1.2 (−4.5;2)	−2.9 (−9.2;3.4)	0.012	−4.2 (−6.4;−2)	<0.001
Lymphocyte count	−0.2 (−3.1;2.7)	1.7 (−5.3;8.8)	−1.4 (−4.3;1.4)	−5.3 (−11.8;1.1)	0.388	−1.2 (−3;0.7)	0.219
RBC count	−1.7 (−2.5;−1)	−2 (−3.8;−0.2)	−2.1 (−3.1;−1.1)	−2.8 (−4.2;−1.4)	0.963	−2 (−2.6;−1.5)	<0.001
Hematocrit	−2.1 (−2.8;−1.4)	−2.5 (−4.3;−0.7)	−2.3 (−3.4;−1.3)	−2.6 (−4.1;−1.2)	0.961	−2.3 (−2.9;−1.7)	<0.001
Hemoglobin	−1.7 (−2.4;−1)	−2.2 (−4;−0.4)	−2 (−3.1;−0.9)	−2.9 (−4.1;−1.8)	0.873	−2 (−2.6;−1.4)	<0.001

P values are adjusted for immunization dose and propensity score.

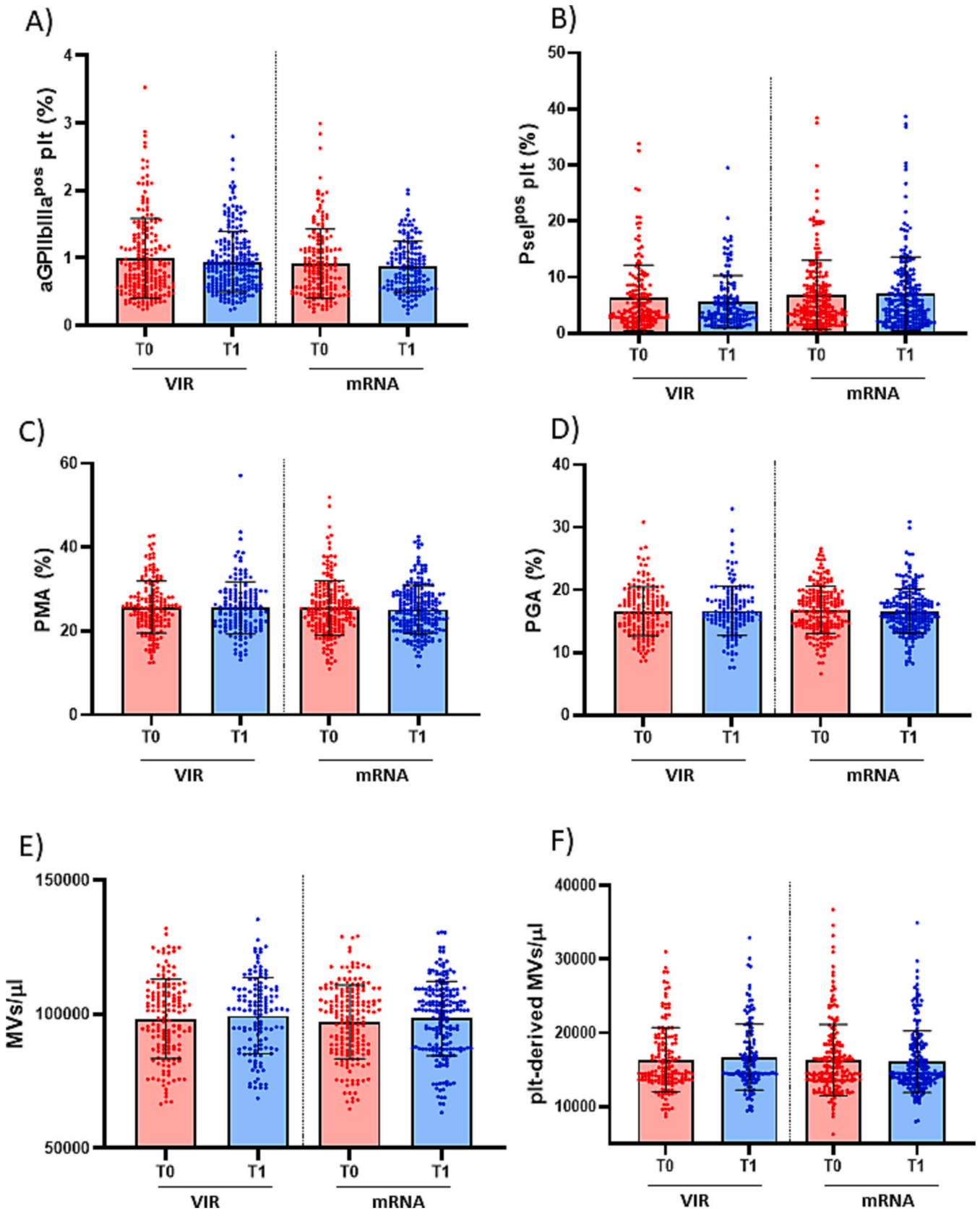

Fig. 1. Assessment of platelet activation before and after vaccination. Platelet aGPIIbIIIa (A) and P-selectin expression (B), platelet-monocyte (PMA; C) and platelet-granulocyte (PGA; D) aggregate formation and the number of total (E) and platelet-derived (F) microvesicles (MVs) were evaluated by flow cytometry in subjects before (T0) and after (T1) receiving COVID-19 viral vector (VIR) or mRNA vaccines. Data expressed as mean ± SD of the percentage of marker positive cells or of the number of MVs were compared by paired sample *t*-test.

Table 3
Change (%) in platelet activation markers.

	ChAdOx1-S Vaxzevria	Janssen	Comirnaty Pfizer/BioNTech	Spikevax Moderna	p adj (ANOVA)	All subjects (T1-T0) %	Paired *t*-test
	n = 142	n = 19	n = 169	n = 38		n = 368	
aGPIIbIIIa	−2.2 (−8.2;3.8)	−13.7 (−30.3;2.8)	−2.8 (−8.9;3.3)	−13.5 (−23.1;−3.9)	0.083	−4.5 (−8.3;−0.7)	0.021
P-selectin	−0.5 (−11.8;12.4)	−23.5 (−47.9;12.3)	0 (−12.4;14.3)	6.6 (−21.3;44.3)	0.491	−0.9 (−8.9;7.8)	0.836
Platelet-monocyte aggregates	−1.4 (−5.8;3.1)	2.4 (−4.4;9.3)	−2.5 (−6.4;1.3)	4.4 (−4.1;12.9)	0.363	−1.1 (−3.8;1.5)	0.393
Platelet-granulocyte aggregates	1.3 (−3.4;6)	−4.9 (−12.5;2.7)	−0.4 (−4;3.2)	−6.3 (−14.9;2.3)	0.479	−0.6 (−3.2;2)	0.640
Tissue factor	4.1 (0.1;8.1)	−8.7 (−24.7;7.2)	−0.3 (−3.9;3.2)	−7 (−16.5;2.5)	0.034	0.1 (−2.5;2.6)	0.959
Phosphatidylserine	−6.1 (−9.9;−2.2)	−8.4 (−22.9;6.1)	−4.9 (−8.4;−1.4)	−3.4 (−10.7;4)	0.963	−5.3 (−7.7;−2.9)	<0.001
Total microvesicles	1 (0.2–1.9)	1 (−0.5–2.5)	−0.8 (−2.5–0.9)	0.9 (−0.1–1.9)	0.302	0.3 (−0.5–1.1)	0.459
Plat-derived microvesicles	0.5 (−2.4–3.3)	−1.8 (−7–3.4)	1.6 (−1–4.2)	0.1 (−5.3–5.5)	0.651	0.8 (−1–2.5)	0.384

P values are adjusted for immunization dose and propensity score.

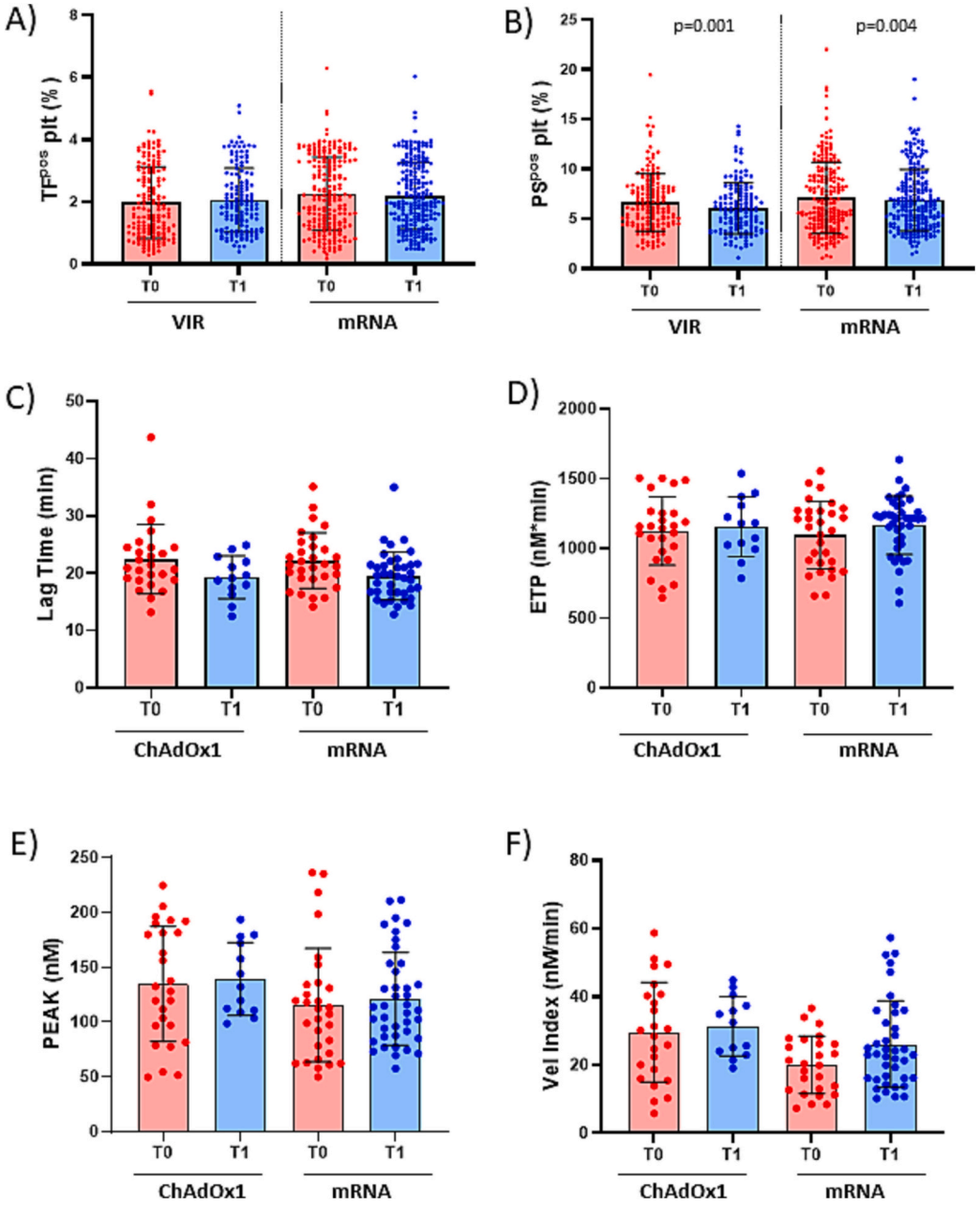

Fig. 2. Evaluation of the platelet procoagulant potential before and after vaccination. Platelet surface expression of Tissue Factor (TF; A) and surface exposure of phosphatidylserine (PS; B) were evaluated by flow cytometry in subjects before (T0) and after (T1) receiving COVID-19 viral vector (VIR) or mRNA vaccines. Data expressed as mean ± SD of the percentage of positive cells were compared by paired sample *t*-test. Platelet-associated thrombin generation (panel C–F) was measured by CAT assay in a subset of participants who received ChAdOx1-S Vaxzevria ($n = 26$ at T0 and $n = 13$ at T1) and mRNA ($n = 30$ at T0 and $n = 39$ at T1) vaccines before and after immunization. Mean value ± SD of Lag-Time (min; C), ETP (Endogenous Thrombin Potential, nM × min; D), Peak High (nM Thrombin; E), and Velocity Index (nM/min; F) were reported in the histogram plots.

Table 4
Change (%) in coagulation parameters.

	ChAdOx1-S Vaxzevria	Janssen	Comirnaty Pfizer/BioNTech	Spikevax Moderna	p adj (ANOVA)	All subjects (T1-T0) %	Paired *t*-test
	n = 142	n = 19	*n* = 169	n = 38		*n* = 368	
D-dimer	10.5 (3.1;18.4)	12 (4.4;20.1)	8.6 (1.9;15.8)	23 (7.4;40.9)	0.352	13 (8.8;17.4)	<0.001
Fibrinogen	3.7 (1;6.4)	−0.6 (−4.6;3.4)	3.8 (1.4;6.3)	5.9 (0.4;11.5)	0.456	3.7 (2.1;5.4)	<0.001
F_{1+2}	7.6 (1.1;14.6)	11.5 (−15.6;47.4)	−1 (−6.7;5)	13.5 (1.6;26.8)	0.140	4.3 (0.2;8.6)	0.041
TAT	0.1 (−7.9;8.7)	−20.4 (−42.7;10.7)	−5.4 (−12.6;2.3)	2.9 (−12.4;20.7)	0.092	−3.8 (−9.2;1.9)	0.183
PT ratio	−0.3 (−1.5;1)	1.1 (−0.9;3.1)	0 (−0.7;0.7)	1.2 (−0.7;3.1)	0.518	0.1 (−0.5;0.7)	0.760
aPTT ratio	−1.1 (−2.7;0.5)	−0.4 (−2.5;1.7)	0.7 (−0.2;1.7)	−1.8 (−4.3;0.8)	0.943	−0.3 (−1.1;0.5)	0.432
aPC	−10 (−17.9;−1.3)	−0.9 (−25.2;31.3)	−5.5 (−12.4;1.9)	−21 (−35.9;−2.6)	0.330	−8.7 (−13.6;−3.5)	0.001
TM	−2.1 (−5.2;0.9)	−4.6 (−8.5;−0.7)	−2.9 (−5.9;0.1)	−5.3 (−10.8;0.1)	0.940	−3 (−4.9;−1.1)	0.002
TFPI	−1.6 (−3.9;0.7)	−0.4 (−6.8;6)	−1.9 (−5.1;1.3)	−7.8 (−20.8;5.1)	0.525	−1.8 (−3.6;−0.1)	0.043
PAI-1	3.1 (−6.2;13.2)	−2.4 (−24.2;25.7)	3.4 (−6.4;14.1)	25.7 (−5.4;67.1)	0.325	5 (−1.7;12)	0.146
vWF	2.4 (−4.7;9.6)	−2 (−18.7;14.7)	4.7 (−1.2;10.6)	−2.8 (−35.5;29.9)	0.699	2.8 (−1.7;7.2)	0.220
FVIII:C	−0.9 (−5.4;3.9)	−5 (−18.1;10.2)	1.5 (−3.1;6.3)	10 (−3.4;25.1)	0.511	1 (−2.1;4.2)	0.532

F_{1+2} = Prothrombin fragment 1+2; TAT = Thrombin Anti Thrombin; PT = Prothrombin Time; aPTT = Activated Partial Thromboplastin Clotting Time; aPC = activated Protein C; TM = Thrombomodulin; TFPI = Tissue Factor Pathway Inhibitor; PAI-1 = Plasminogen Activator Inhibitor-1; vWF = von Willebrand Factor; FVIII:C = Factor VIII clotting activity.
P values are adjusted for immunization dose and propensity score.

Table 5
Change (%) in inflammation parameters.

	ChAdOx1-S Vaxzevria	Janssen	Comirnaty Pfizer/BioNTech	Spikevax Moderna	p adj (ANOVA)	All subjects (T1-T0) %	Paired *t*-test
	n = 142	n = 19	n = 169	*n* = 38		n = 368	
IL-6	38.1 (20.5;58.3)	99.5 (34.8;195.2)	21.4 (9.9;34.2)	28.6 (−2.1;68.7)	0.049	30.1 (20.2;40.9)	<0.001
CRP	24.5 (3.6;49.6)	−6.1 (−35.1;36)	33.6 (14.5;56)	61.4 (15.8;124.9)	0.143	30.2 (17;45)	<0.001
C4d	4.4 (−2.7;11.4)	−12.6 (−35.3;10.1)	10.6 (3.4;17.7)	13 (−10;36.1)	0.131	6.7 (1.8;11.6)	0.008

IL-6 = Interleukin-6; CRP = C-Reactive Protein. P values are adjusted for immunization dose and propensity score.

mRNA group. Stronger positivity (OD > 0.75) was detected in 9% (15/160) and 9% (18/205) of viral vector and mRNA cohort. The median levels of anti-PF4/heparin IgG antibodies of positive subjects remained stable through T1 (viral vector vaccine median OD = 0.601 [0.519–0.891], $p = 0.341$; mRNA vaccine median OD = 0.686 [0.512–0.883], $p = 0.294$).

Of note, in the viral vaccine cohort, 1 (3%) subject became positive after the first dose and 3 (2%) subjects after the second immunization. In mRNA group, 5 (3%) and 0 (0%) subjects became positive after the first and second dose, respectively. All the other subjects with a negative antibody titer remained stable after immunization. Antibody titers did not correlate with platelet count or with the expression of platelet activation markers (data not shown).

3.6. Influence of previous COVID-19 infection

Finally, a sub-analysis of platelet activation, coagulation, inflammation and immune response parameters in relation to previous COVID-19 infection was performed. Overall, 45 subjects, accounting for the 12% of the total population enrolled, reported a previous SARS-CoV-2 infection, documented by the presence of SARS-CoV-2 IgG (35,5 [11,5-78,4] in infected *vs* 1 [1-1] AU/ml in non-infected subjects, respectively; $p < 0.0001$). No differences in the platelet activation, in the coagulation and inflammation parameters as well as in immune response were observed between COVID-19 positive or negative subjects before vaccine administration as well as after immunization with either vaccine types (data not shown).

4. Discussion

TREASURE is a prospective study designed to assess, in a head-to-head comparison, the impact of four COVID-19 vaccines on parameters of platelet activation, coagulation, and inflammation. The study has enrolled the largest number of subjects among those similarly designed to evaluate potential side effects of COVID-19 vaccines. Moreover, all the parameters have been analyzed on the participants before and 8 ± 2 days after vaccine administration; analyses were conducted both on subjects receiving the first dose or the second dose of vaccine to assess whether a "preconditioning" by the first vaccine dose may exacerbate a response after the second dose.

Results of the study show that no platelet activation do occur with any of the administered vaccines and regardless of the immunization dose. Unlike our results, Ostrowsky et al. [4] and Petito et al. [5] both reported increased platelet activation following Vaxzevria vaccination compared to mRNA vaccination by measuring plasma levels of P-selectin. It is worth mentioning, however, that this protein can also be released from extra-platelet sources [18], thus limiting its relevance in this context. In our study, a direct approach to assess whether vaccines could induce platelet activation was used. The expression of platelet activation markers –P-selectin, activated GpIIbIIIa, TF, platelet-granulocyte and platelet-monocyte aggregate formation, total and TF-positive MV formation- was analyzed by flow cytometry on whole blood samples, thus in the absence of any pre-analytical manipulation. Results showed the absence of any change in the classical platelet activation parameters following vaccination, confirming previous data [19–22]. Interestingly a mild, although statistically significant, increase in platelet TF-dependent prothrombotic potential was observed only in ChAdOx1-S Vaxzevria immunized subjects.

In contrast to the platelet activation data, the inflammatory markers CRP and IL-6 significantly increased by about 30%, as expected, with all the vaccines and irrespective of dosing. A lesser, but still significant 7% increase in platelet expression of C4d was also observed. Evidence that transient changes in markers of inflammation may be observed following administration of different vaccines has been previously reported [23,24]. Ostrowsky et al. [4] observed at a median of 11 days (range 8–16 days) after the first dose of either Vaxzevria ($n = 55$) or Comirnaty ($n = 25$) vaccination similar increments in IL-6 and CRP with respect to pre-vaccination levels, together with minor changes in other cytokines, with excess emphasis –in our view- on the negligible differences observed in response to the two vaccines.

We observed a statistically significant 13% and 4% post-vaccination increase in D-dimer and fibrinogen levels, in addition to a 4% increase in F_{1+2} regardless of vaccines and dosing, with no change in PT, aPTT and TAT.

In 72 subjects evaluated before and after an average of 14 days (range 8–23 days) from vaccination, Petito et al. [5] observed a 20% increase in D-dimer with Janssen, but not with Vaxzevria or Comirnaty. The same Authors also reported no change in fibrinogen levels with Janssen, at variance with a 34% and a 22% decrease with Vaxzevria and Comirnaty, respectively. No change was observed with either vaccine in F_{1+2} levels, whereas TAT levels increased by 56% and 45% with Janssen and Comirnaty, but they were unchanged with Vaxzevria. Of note, baseline and post-vaccination F_{1+2} and TAT levels in that study are much higher than those detected in plasma from our subjects, confirming the role of benzamidine HCl in avoiding *in vitro* generation of the two analytes. Furthermore, blood sampling in subjects of Petito et al. [5] occurred at a different timing from vaccination with the three vaccines (from an average of 17 days with Vaxzevria to an average of 11 days with Comirnaty). The Authors, however, made no comment on these results in the discussion, notwithstanding the striking decrease in fibrinogen levels with Vaxzevria and Comirnaty, but not with Janssen.

Campello et al. [7] evaluated whole blood platelet counts, rotational thromboelastometry, impedance aggregometry and thrombin generation pre-vaccination and at an average of 3 ± 2 and 10 ± 2 days after the first or second dose in 122 subjects receiving either Comirnaty or Vaxzevria vaccines. There was no change in thromboelastometry and aggregometry parameters, but a transient 6.8% increase in ETP with a 17.5% decreased response to thrombomodulin after the first dose with the Vaxzevria vaccination only and with both vaccines after the second dose. They also observed a 9% transient reduction in platelet count at three days with Vaxzevria only, and concluded for no clinically meaningful hypercoagulability occurring after either vaccine.

While our findings are quantitatively different from those of Petito et al. [5], we believe that - similarly to the increased thrombin generation observed by Campello et al. [7] - they may result from the established link between inflammation and coagulation [25].

Interestingly, this study shows for the first time an overall minor post-vaccination reduction of key elements involved in the anticoagulant systems such as aPC, TM and TFPI, all possibly related to endothelial function. There was no change in von Willebrand Factor antigen and FVIII levels, which are known to behave as acute phase reactants [26]. No change was also observed in the plasma levels of PAI-1, which is known to be at least partially of endothelial synthesis [27]. If such apparent endothelial stunning is part of the innate immune response or is peculiar of COVID-19 vaccines remains to be determined. Of note, Petito et al. [5] observed a 12% post-vaccination decrease in von Willebrand Factor antigen levels with all vaccines, also pointing to an abnormal endothelial response to vaccination. Ostrowsky et al. [4] observed a clear-cut post-vaccination increase in blood levels of syndecan-1, along with minimal increments in TM, E-selectin, ICAM-1, ICAM-3 and VCAM-1 with all types of vaccines. In 18 participants with a mean age of 35 years, samples collected pre-vaccination, a median of 17 days after the first dose of Comirnaty vaccine, and a median of 9 days after the second dose, showed no evidence of endothelial activation (ICAM, VCAM-1 and P-selectin) or hypercoagulability (PT, aPTT, FVIII, vWF Ag, Clot waveform analysis) [28]. In that study, the statistically significant, but clinically not relevant, post-vaccination increase in ICAM levels was related by the Authors to a local inflammatory immune response to vaccination.

Finally we report the virtual absence of anti-PF4 seroconversion, regardless of the type of vaccine and dosing, in the enrolled population, in agreement with the majority of reports [4,7,22,29–31], with only one exception [32]. This study does not shed any more light on VITT, which requires an idiosyncratic immunological reaction to generate PF4 heparin independent antibodies. However, limited post-vaccination increments in D-dimer and F_{1+2}, along with endothelial stunning may become significant, albeit weakly, at a population level. A pivotal paper published online in November 2022 [33] reported minor, but statistically significant, 12% and 25% higher risks of venous thromboembolism and pulmonary embolism, respectively, following the first dose of either Comirnaty or Vaxzevria compared to the general population. Corresponding risks observed following SARS-Cov-2 infection from September 1st 2020 up to May 2nd 2021 were respectively 61-fold and 49-fold higher; nonetheless, our findings may at least partially explain the rare occurrence of venous thromboembolic complications in subjects with hyper-inflammatory reactions to vaccination.

4.1. Study limitations

Our findings should be interpreted in the context of their limitations. The most relevant one is that, due to the study design, we cannot know whether another vaccine against a different pathogen, such as that for influenza, would have caused the same variations in the parameters considered if analyzed with the same timing as in this study. Second, the platelet associated thrombin generation capacity was assessed only on a subset of participants. Third, two vaccines are over-represented compared to the others in our study. There were only 19 subjects receiving the Janssen vaccine, and such low numerosity might have been causal for the observation of counterintuitive post-vaccination changes observed with this vaccine. Fourth, we did not measure the anti-SARS-CoV-2 antibody formation post-vaccination in our population, and we cannot report on the potential relationship between the minor changes induced by vaccination and the individual immune response. Last but not least, a different time of blood sampling after vaccination might have led to results quantitatively – but hardly qualitatively – different from those of the TREASURE study.

In conclusion, this study shows that the four COVID-19 vaccines - two adenovirus vector-based and two mRNA-based - administered to a large general population sample induce, as expected, a transient inflammatory response, with no onset of the classical platelet activated phenotype (expression of P-selectin, aGPIIbIIIa, platelet-leukocyte aggregates) or thrombocytopenia and minor signs of clotting activation and endothelial stunning, which may however be relevant at a population level.

CRediT authorship contribution statement

MB collect and analyzed the data, and finalized the research; PC and AB performed the flow cytometry experiments; PDV and MCo performed the ELISA experiments; PAL and PLM supervised C4d analysis; MA and GVZ supported subject recruitment; AG and ABo performed the statistical analyses; FV supervised the statistical analyses; MLB and NC provided consultation on the clinical question; ADA wrote the manuscript and supervised ELISA experiments; MC concept and supervised the study, interpreted the data and wrote the manuscript. All Authors contributed to critical revision of the manuscript and provided their final approval for submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data and materials are available from the corresponding authors upon written request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2023.01.015.

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