Changes in haemostasis and inflammatory markers after mRNA BNT162b2 and vector Ad26.CoV2.S SARS-CoV-2 vaccination

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Changes in haemostasis and inflammatory markers after mRNA BNT162b2 and vector Ad26.CoV2.S SARS-CoV-2 vaccination

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ABSTRACT

Introduction: Reported thromboembolic events after SARS-CoV-2 vaccinations are still raising concerns, predominantly in non-scientific population. The aim of our study was to investigate the differences between haemostasis and inflammatory markers in the subjects vaccinated with mRNA BNT162b2 and vector Ad26.CoV2.S vaccine.

Materials and methods: The study included 87 subjects vaccinated with mRNA BNT162b2 and 84 with Ad26.CoV2.S vaccine. All the laboratory parameters (TAT, F 1 + 2, IL-6, CRP, big endothelin-1, platelets, fibrinogen, D-dimers, VWF activity) were investigated for the mRNA vaccine at five (before the first dose, 7 and 14 days after the first and second vaccine dose), and three time points (before the first dose, 7 and 14 days after) for the vector vaccine, respectively. All the markers were measured by well-established laboratory methods.

Results: Our results have shown statistically higher CRP levels in the vector group 7 days after vaccination ($P = 0.014$). Furthermore, study has revealed statistically significant rise in D-dimers ($P = 0.004$) between tested time points in both vaccine groups but without clinical repercussions.

Conclusion: Although statistically significant changes in haemostasis markers have been obtained, they remained clinically irrelevant. Thus, our study implicates that there is no plausible scientific evidence of a significant disruption in the coagulation and inflammatory processes after vaccination with BNT162b2 mRNA and Ad26.CoV2.S vector SARS-CoV-2 vaccines.

What is known on this topic

- COVID-19 is a highly procoagulant infectious disease
- SARS-CoV-2 vaccines provide a strong protection from severe forms of COVID-19
- thromboembolic events connected to SARS-CoV-2 vaccination have been observed
- Vaccine-induced immune thrombotic thrombocytopenia is caused by PF4 antibodies

What does the paper add

- First real-life comparison of a mRNA BNT162b2 and vector Ad26.CoV2.S. vaccine
- Change in CRP after vaccination in the vector vaccine group was noted
- Subtle rise in D-dimers was observed in both vaccine groups after vaccination
- All the laboratory changes remained clinically irrelevant

1. Introduction

For the last three years COVID-19 disease has become a worldwide healthcare and socioeconomic issue. Coronavirus (SARS-CoV-2), emerging in Asia in 2019, has spread worldwide, causing burning problems for countries, regardless of their developmental status. As a result of strenuous effort to control the pandemic crisis, vaccination was introduced as a secure and highly effective instrument against the

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disease. More than 13 billion doses have been utilised worldwide [1].

Soon after the vaccine release individual cases of thromboembolic events have been assigned to the vaccination [2–4].

Lately, a new prothrombotic syndrome has been described, named vaccine-induced immune thrombotic thrombocytopenia (VITT) with clinical features of heparin-induced thrombocytopenia (HIT) [5]. The pathologic process of VITT usually starts 5–10 days post-vaccination and becomes clinically evident between 5 and 30 days post-vaccination [6].

Greinacher et al. presented a 2-step mechanism in which vaccine particles form neoantigen with platelet factor 4 (PF4) and trigger a B-cell response leading to the production of high-avidity anti-PF4 antibodies. Consequently, activation of the platelets and neutrophils results in a hypercoagulable state and thrombosis [7].

The question is, are some people genetically predisposed to produce antibodies to a specific stimulus like vaccination since anti-PF4 antibodies can be found in a healthy population who does not develop thrombosis [8].

The effects of other vaccines on the hematologic parameters and the immune system markers have been recognized in the past. Immune mediated thrombocytopenia has been observed after measles-mumps-rubella immunisation in the paediatric population [9]. Increased systemic levels of interleukin-6 have been described after diphtheria toxoid vaccination, Bacillus Calmette-Guérin, foot and mouth disease and influenza vaccination [10–13].

The possible mechanism by which SARS-CoV-2 vaccine could potentially trigger the coagulation cascade in the susceptible subjects is not well understood. On the other hand, the procoagulant nature of the COVID-19 has been well documented by now [14]. Also, the cytokine storm, a hallmark of severe COVID-19 disease is characterised by a marked elevation of the proinflammatory parameters (especially interleukin-6), endothelial damage, and haemostasis imbalance [15].

The connection between inflammation and thrombosis was evident before the COVID-19 era. Formation of the atherosclerotic plaque due to the lipid storage and inflammatory processes in the endothelial wall will eventually lead to the acute coronary syndrome (ACS) [16]. Acute infections, such as community acquired pneumonia can elicit systemic inflammatory response and platelet activation and thus increase the incidence of ACS [17]. Enhanced systemic inflammation induced by a major surgical procedure promotes atherosclerotic plaque instability which can cause serious cardiovascular events [18].

During the COVID-19 pandemic, various markers of haemostasis and inflammation have been studied in order to predict the disease trajectory and hopefully lead to a therapeutical intervention.

Beside D-dimers, prothrombin fragments 1 + 2 (F 1 + 2) and thrombin-antithrombin complexes (TAT) have been studied as a more specific markers of thrombosis and predictors of a more severe COVID-19 illness [19–21]. Von Willebrand factor (VWF), has been found to be vastly elevated in the circumstances of endothelial damage which is a hallmark of moderate to severe forms of the disease [22,23]. The higher levels of VWF were found to be more pronounced in nonsurvivors versus survivors of severe COVID-19 disease which reflects the deleterious effects of SARS-CoV-2 virus on the vasculature [24].

The role of big endothelin-1 (big ET-1), a potent vasoconstrictor released from the endothelial cells, in predicting the severity of viral pneumonia was discovered before the COVID-19 era [25]. With the emergence of a new pandemic disease, big ET-1 has been once again studied as a potential marker of endothelial injury. Abraham et al. demonstrated a positive correlation between an ET-1 rise and the mortality in COVID-19 patients [26].

C-reactive protein (CRP) has been widely used as a reliable inflammatory marker, but during COVID-19 crisis efforts have been made to find a more specific prognostic marker of inflammation for clinical use. Interleukin-6 (IL-6) has shown such accuracy in the depiction of severe forms of illness that it was introduced in the intensive care unit setting as a reliable predictor of both mortality and therapeutic response [27].

In the light of the emerging scientific evidence of the potential effect of the SARS-CoV-2 vaccination on the haemostasis balance, the primary aim of our study is to investigate differences between various haemostasis and inflammatory markers in the subjects vaccinated with two different SARS-CoV-2 vaccines; mRNA BNT162b2 and vector Ad26.CoV2.S vaccine.

2. Materials and methods

2.1. Subjects

This prospective observational clinical study included 171 adult subjects who were vaccinated with mRNA BNT162b2 (Pfizer, BioNTech, Germany) vaccine and recombinant vector Ad26.CoV2.S (Janssen, Belgium) vaccine from April to August 2021 at the University Hospital Centre Sestre milosrdnice and Medical Centre Centar, Zagreb, Croatia, according to the vaccination program issued by the Ministry of Healthcare of the Republic of Croatia.

Of 171 subjects who were recruited consecutively, 87 of them (51 %), median age of 35 years (range 20–62 years), were vaccinated with mRNA vaccine and 84 subjects (49 %), median age 44 years (range 19–66 years), received recombinant vector vaccine.

The main exclusion criteria were active malignant disease, recent major surgery (one month before vaccination), thromboembolic incident 3 months prior to the vaccination, dialysis, peripheral artery disease, diabetes requiring insulin therapy, active immunologic disorder, pregnancy, puerperium, oral contraceptive therapy, anticoagulant therapy, use of nonsteroid antirheumatic therapy, immune thrombocytopenia and haemophilia. Subjects with acute infection were excluded by the vaccination criteria.

The study was performed according to the guiding principles of the Declaration of Helsinki and was approved by the University Hospital Centre and Medical Centre's Ethic committees. All subjects gave their informed written consent.

2.2. Samples

Blood samples were collected between 7 a.m. and 9 a.m. from each subject in the BNT162b2 group at 5 different time points: before 1st vaccination (within 24 h), 7 and 14 days after 1st, 7 and 14 days after 2nd vaccination. The period between two mRNA vaccinations was 21 days. In case of the Ad26.CoV2·S vaccine, blood was drawn at 3 different time points: before vaccination (within 24 h), 7 and 14 days after. The blood was drawn in the morning, after an overnight fast.

At each time point total of 15 mL of venous blood was collected in Vaccuette® test tubes (Greiner Bio-One, Kremsmünster, Austria): one with K_2EDTA anticoagulant (full blood count), two with 3.2 % sodium citrate (fibrinogen, D-dimers, TAT, F 1 + 2, VWF:Ac, big endothelin-1) and one without anticoagulant (CRP, IL-6, PF4 antibodies). Tubes without anticoagulant and with 3.2 % sodium citrate were centrifuged for 10 min on 1800 $\times g$ within 3 h after venepuncture and processed at the University Department of Chemistry, Sestre milosrdnice University Hospital Centre. Since coagulation analysis were postponed, 3.2 % sodium citrate samples were centrifuged twice (1800 $\times g$ for 10 min) to obtain platelet poor plasma ($<10 \times 10^9$/L).

Laboratory investigation of full blood count and CRP were done immediately, while the rest of the laboratory parameters were measured from the specimens of the serum and plasma stored at −80 °C for 8 months. For each subject seven aliquots (4 plasma and 3 serum) were stored.

3. Methods

Full blood count was analysed on Sysmex XN1000 (Sysmex Europe SE, Norderstedt, Germany). Fibrinogen and D-dimers were measured on BCSXP analyser (Siemens Healthineers, Marburg, Germany). Mutifibren® U was used for measurement of fibrinogen concentration.

Concentration of the D-dimers was measured using Innovance® D-dimers with the respect to the original manufacturer protocols (Siemens Healthineers, Marburg, Germany). On the same analyser, VWF activity was determined using Innovance VWF:Ac reagent (Siemens Healthineers, Marburg, Germany).

Prothrombin fragments 1 + 2 were done using Enzygnost® F 1 + 2 (monoclonal) (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) ELISA method following manufacturer instructions. Measuring range of the test is 20–1200 pmol/L. According to the manufacturer, total precision of the test is 6.8–11.8 % CV, while the coefficients of variation are 3.6–5.5 % (inter-assay CV) and 4.4–11.2 % (inter-assay CV) for the concentration range from 40 to 700 pmol/L.

Enzygnost® TAT micro (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) is an ELISA method used for measurement of TAT complexes. Test was used according to the manufacturer instructions and the measurement range is declared from 2 to 60 μg/L. Intra-assay CV is 4–6 % and inter-assay CV is 6–9 %.

We measured CRP concentrations on the Architect c8000 analyser (Abbott Diagnostics, Abbott Park, IL) using particle enhanced immunoturbidimetric assay provided by the manufacturer where CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies.

The Roche Elecsys IL-6 assay was a non-competitive (sandwich) electrochemiluminescent immunoassay performed on Roche Cobas analyser e801 (Roche Diagnostics GmbH, Mannheim, Germany). The assay has a claimed measuring range of 1.5–5000 pg/mL, a limit of quantitation of 2.5 pg/mL, an inter-assay precision (CV) of 17.4 % (at 1.82 pg/mL) and 2.0 % (at 4461 pg/mL).

The plasma big ET-1 level was measured using a highly sensitive and specific commercial sandwich enzyme immunoassay (BI-20082H, Biomedica, Wien, Austria). The calculated overall intra-assay precision (CV) of the big ET-1 immunoassay is ≤5 %. Biotinylated anti-big ET-1 antibodies were raised with 100 % reactivity to big ET-1 and <1 % reactivity to ET-1.

Anti-platelet factor 4 antibodies (anti-PF4) was tested using LIFECODES PF4 IgG (Immucor Medizinische Diagnostik GmbH, Dreieich, Germany), ELISA assay for qualitative screening of heparin associated IgG antibodies. The manufacturer declared for the assay coefficient of variation ≤10 % for OD values from 0.090 to 2.701 (negative to highly positive).

The samples were analysed in batches. All time points for each subject were analysed simultaneously and with the same lot of reagents. This applies for every analysed marker in the study.

3.1. Statistical analysis

Distribution of the data was tested by using Kolmogorov-Smirnov test. Results that were not normally distributed are presented as median and interquartile range (IQR). Age is presented as median and range (minimum and maximum). Categorical data are presented as counts and ratios. Non-parametric Mann-Whitney test was used to test the difference between mRNA BNT162b2 and Ad26.CoV2.S vaccine groups. Differences between results obtained at different time points for each tested parameter in the same vaccine group were tested using Friedman test. The value of $P < 0.050$ was considered statistically significant. The data collected by this study was processed by the computer programme MedCalc® Statistical Software version 20.010 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2021) and SPSS Statistics for Windows, Version 23.0 (Released 2015. IBM. Armonk, NY: IBM Corp.).

4. Results

Fiftyone women (59 %) were vaccinated with the mRNA BNT162b2 vaccine, while 40 (48 %) women had received vector Ad26.CoV2.S vaccine. There was a statistically significant higher number of subjects vaccinated with mRNA BNT162b2 who had SARS-CoV-2 infection than those vaccinated with Ad26.CoV2.S (72 % vs 54 %; $P = 0.022$). The rest of the clinical characteristics did not differ between studied groups. Characteristics of the subjects are presented in Table 1.

In Table 2 are presented results of the tested parameters between subjects vaccinated with mRNA BNT162b2 and Ad26.CoV2.S vaccine. Statistically significant differences between studied groups were found for TAT levels for all three comparable time points ($P < 0.001$). Difference is also shown for CRP at the second time point ($P = 0.014$). Further, statistically significant differences were found between vaccinated groups for F 1 + 2 and big endothelin-1 at the baseline and 14 days after 1st vaccination. The results have shown statistically significant differences for TAT ($P = 0.011$); big endothelin-1 ($P < 0.001$); fibrinogen ($P = 0.003$); D-dimers ($P = 0.004$) and VWF:Ac ($P < 0.001$) in mRNA BNT162b2 vaccinated subjects. In the group of Ad26.CoV2.S vaccinated subjects differences between three time points were demonstrated for CRP ($P = 0.006$); D-dimers ($P = 0.004$); VWF:Ac ($P < 0.001$); and platelets ($P = 0.021$) (Table 2).

Since almost 1/5 of all subjects included in the study had arterial hypertension (AH), we have tested the difference of the studied parameters between subjects with and without AH.

For mRNA BNT162b2 vaccinated subjects, results have shown statistically significant differences between subjects with and without AH for F 1 + 2 at the baseline ($P < 0.001$); 7 days after 1st vaccination ($P = 0.011$); 7 days after 2nd vaccination ($P = 0.012$) and 14 days after 2nd vaccination ($P < 0.001$). In the same vaccine group of subjects, D-dimers were statistically different between the subjects with and without AH at baseline, 7 and 14 days after 1st vaccination (Table 3). When tested differences between the results at different time points after vaccination in subgroups according to AH, the results have shown statistically significant differences for big endothelin-1 ($P = 0.004$); fibrinogen ($P = 0.030$); D-dimers ($P = 0.010$) and VWF:Ac ($P = 0.002$) in group of subjects without AH. Further, in group of subjects with AH results have revealed differences only for IL-6 ($P = 0.008$) and VWF:Ac ($P = 0.033$) (Table 3).

In the group of Ad26.CoV2.S vaccinated subjects, results have shown statistically significant differences in subgroup of subjects with AH for CRP ($P = 0.019$); fibrinogen ($P = 0.021$); D-dimers ($P = 0.042$) and VWF:Ac ($P = 0.023$), whereas in the subgroup without AH only VWF:Ac ($P = 0.001$) and platelets ($P = 0.010$) have shown statistically significant difference (Table 4).

Table 1
Demographic characteristics of 171 subjects after vaccination divided according to the vaccine type.

	mRNA BNT162b2 ($n = 87$)	Ad26.CoV2.S ($n = 84$)	P
Age, median (years) (Min-max years)	35 (20–62)	44 (19–66)	0.085
Sex, female [n(%)]	51 (59)	40 (48)	0.149
Body mass index (BMI) (kg/m^2) [Median (IQR)]	25.2 (22.9–28.6)	25.3 (22.6–28.7)	0.731
SARS-CoV-2 infection, yes [n(%)]	56 (72)	41 (54)	0.022
SARS-CoV-2 infection before vaccination, yes [n(%)]	11 (22)	6 (21)	0.953
SARS-CoV-2 infection after vaccination, yes [n(%)]	36 (63)	21 (51)	0.241
SARS-CoV-2 infection before and after vaccination, yes [n(%)]	7 (12)	10 (24)	0.123
Arterial hypertension, yes [n(%)]	17 (22)	12 (16)	0.381
Chronic illness, yes [n(%)][a]	8 (10)	11 (15)	0.408

Data on SARS-CoV-2 infection was collected for 78 subjects vaccinated with mRNA BNT162b2 vaccine and 76 subjects vaccinated with Ad26.CoV2.S vaccine.

[a] Chronic illnesses in the mRNA group: rheumatoid arthritis (n = 2), hyperthyroidism (n = 3), hypothyroidism (n = 3); chronic illnesses in the vector vaccine group; diabetes type 2 (n = 3), epilepsy (n = 1), psoriasis (n = 1), asthma (n = 2), hyperthyroidism (n = 2), hyperlipidaemia (n = 2).

Table 2
Proinflammatory and haemostatic marker differences between mRNA BNT162b2 and Ad26.CoV2.S and between different time points after vaccination in studied groups.

	mRNA BNT162b2	Ad26.CoV2.S	P*
CRP (mg/L)			
Before vaccination	0.9 (0.40–2.20)	1.2 (0.50–2.20)	0.176
7 days after 1st dose	0.9 (0.60–2.00)	1.4 (0.70–2.60)	0.014
14 days after 1st dose	0.9 (0.40–1.80)	1.1 (0.63–2.38)	0.067
7 days after 2nd dose	1.0 (0.60–1.90)	/	
14 days after 2nd dose	0.8 (0.40–1.90)	/	
P#	0.109	0.006	
Interleukin - 6 (IL-6) (pg/mL)			
before vaccination	1.5 (1.50–2.05)	1.5 (1.50–2.61)	0.104
7 days after 1st dose	1.5 (1.50–2.03)	1.6 (1.50–2.26)	0.133
14 days after 1st dose	1.5 (1.50–2.07)	1.6 (1.50–2.36)	0.367
7 days after 2nd dose	1.5 (1.50–2.09)	/	
14 days after 2nd dose	1.5 (1.50–1.86)	/	
P#	0.622	0.790	
Thrombin-antithrombin complexes (TAT) (μg/L)			
Before vaccination	2.6 (2.00–3.30)	3.2 (2.56–4.94)	<0.001
7 days after 1st dose	2.6 (2.00–3.10)	3.4 (2.66–4.55)	<0.001
14 days after 1st dose	2.7 (2.00–3.40)	3.1 (2.66–4.14)	<0.001
7 days after 2nd dose	2.7 (2.00–3.50)	/	
14 days after 2nd dose	3.0 (2.00–4.30)	/	
P#	0.011	0.752	
Prothrombin fragment F 1 + 2 (F 1 + 2) (pmol/L)			
Before vaccination	180.4 (145.40–245.50)	216.1 (172.00–330.09)	0.012
7 days after 1st dose	185.7 (143.90–258.60)	192.5 (156.15–258.54)	0.440
14 days after 1st dose	168.3 (137.10–219.70)	191.3 (146.99–283.62)	0.032
7 days after 2nd dose	180.2 (146.30–231.60)	/	
14 days after 2nd dose	167.5 (143.10–233.60)	/	
P#	0.111	0.123	
Big endothelin-1 (big ET-1) (pg/mL)			
Before vaccination	1.4 (1.00–1.80)	1.7 (1.31–1.89)	0.001
7 days after 1st dose	1.6 (1.20–1.90)	1.7 (1.29–2.12)	0.077
14 days after 1st dose	1.5 (1.20–1.90)	1.7 (1.39–2.20)	0.009
7 days after 2nd dose	1.6 (1.30–2.00)	/	
14 days after 2nd dose	1.6 (1.20–1.90)	/	
P#	<0.001	0.403	
Fibrinogen (g/L)			
Before vaccination	2.9 (2.5–3.4)	2.9 (2.5–3.5)	0.913
7 days after 1st dose	3.0 (2.5–3.5)	3.1 (2.6–3.6)	0.394
14 days after 1st dose	2.9 (2.5–3.5)	3.0 (2.6–3.5)	0.574
7 days after 2nd dose	2.9 (2.5–3.6)	/	
14 days after 2nd dose	2.8 (2.4–3.4)	/	
P#	0.003	0.052	
D-dimers (mg/L FEU)			
Before vaccination	0.26 (0.19–0.35)	0.29 (0.22–0.41)	0.086
7 days after 1st dose	0.28 (0.20–0.42)	0.31 (0.23–0.49)	0.110
14 days after 1st dose	0.27 (0.20–0.41)	0.31 (0.23–0.46)	0.066
7 days after 2nd dose	0.28 (0.20–0.42)	/	
14 days after 2nd dose	0.29 (0.22–0.43)	/	
P#	0.004	0.004	
VWF activity (VWF:AC) (%)			
Before vaccination	121 (84–139)	130 (95–147)	0.146
7 days after 1st dose	118 (88–145)	131 (102–150)	0.112
14 days after 1st dose	107 (85–142)	125 (90–144)	0.355
7 days after 2nd dose	118 (85–141)	/	
14 days after 2nd dose	111 (79–138)	/	
P#	<0.001	<0.001	
Platelets (10^9/L)			
Before vaccination	258 (221−301)	262 (216–292)	0.501
7 days after 1st dose	254 (223−300)	255 (210–291)	0.449
14 days after 1st dose	257 (221–297)	258 (222–305)	0.781
7 days after 2nd dose	259 (217–294)	/	
14 days after 2nd dose	259 (218–296)	/	
P#	0.812	0.021	

P* - differences between vaccine groups; P# - differences between different time points after vaccination. The results are expressed as medians and interquartile ranges.

Antibodies to PF4 were found to be negative (data not shown) in all the tested subjects and the test is excluded from further statistical analysis.

5. Discussion

The aim of our study was to detect the possible differences in the coagulation parameters and proinflammatory markers between subjects vaccinated with two different SARS-CoV-2 vaccines; mRNA vaccine BNT162b2 (Pfizer, BioNTech, Germany) and recombinant vector vaccine Ad26.CoV2.S (Janssen, Belgium).

Comparing the laboratory results of the subjects between the two vaccine groups, F1 + 2, TAT, big ET-1 and CRP showed significant statistical difference and were higher in the vector vaccine group.

F1 + 2 and TAT are terminal markers of the coagulation cascade and big ET-1 is a potent vasoconstrictor released during endothelial injury. However, based on our results, it would be bold to presume that the vector vaccine has more thrombogenic potential or causes a more prominent endothelial injury, due to the higher baseline levels of F1 + 2, TAT and big ET-1 (prior to the vaccination) of these particular parameters in the vector group.

Subjects in the vector vaccine group had higher CRP levels 7 days after vaccination. As stated by the manufacturer, mild reactions to the vaccination are possible and can be expected, especially in the younger population [28]. It is thought that adenovirus vaccines produce larger immune stimuli than the mRNA vaccines.

When comparing laboratory parameters by time points in each vaccine group several findings do stand out. In the both vaccine groups, D-dimer levels showed statistically higher difference after vaccination ($P = 0.004$).

Our results are not in line with the studies of Hasan et al. and Peyvandi et al. who found no changes in D-dimers in the subjects after vaccination with BNT162b2 vaccine. However, these studies included less subjects and fewer time points (Hasan et al.; 50 subjects/3 time points, Peyvandi et al.; 30 subjects/4 time points) compared to ours [29,30].

To the best of our knowledge, there are no similar studies that provide information about D-dimer levels after Janssen vaccination. Having in mind that both Ad26.CoV2.S and ChAdOx1-S are adenovirus

Table 3
Haemostatic and proinflammatory marker differences between subjects with and without arterial hypertension in mRNA BNT162b2 vaccinated patients.

	mRNA BNT162b2		P*
	without AH** (n = 70)	With AH (n = 17)	
CRP (mg/L)			
Before vaccination	1.0 (0.3–2.3)	1.0 (0.6–1.9)	0.538
7 days after 1st dose	0.9 (0.5–1.9)	1.1 (0.8–2.4)	0.456
14 days after 1st dose	0.9 (0.4–1.9)	1.0 (0.6–2.0)	0.669
7 days after 2nd dose	0.9 (0.6–1.9)	1.6 (0.8–3.6)	0.119
14 days after 2nd dose	0.8 (0.4–1.9)	1.0 (0.6–2.2)	0.282
P#	0.083	0.769	
Interleukin - 6 (IL-6) (pg/mL)			
Before vaccination	1.5 (1.50–2.22)	1.5 (1.50–1.80)	0.541
7 days after 1st dose	1.5 (1.50–2.21)	1.5 (1.50–2.21)	0.854
14 days after 1st dose	1.5 (1.50–2.20)	1.7 (1.50–2.22)	0.358
7 days after 2nd dose	1.5 (1.50–2.20)	1.7 (1.50–2.21)	0.147
14 days after 2nd dose	1.5 (1.50–2.21)	1.5 (1.50–1.70)	0.398
P#	0.741	0.008	
Thrombin-antithrombin complexes (TAT) (μg/L)			
Before vaccination	2.5 (2.00–3.30)	2.9 (2.10–3.10)	0.521
7 days after 1st dose	2.6 (2.00–3.20)	3.0 (2.10–3.20)	0.531
14 days after 1st dose	2.7 (2.00–3.30)	2.8 (2.10–3.60)	0.578
7 days after 2nd dose	2.7 (2.00–3.20)	2.7 (2.10–3.30)	0.936
14 days after 2nd dose	2.8 (2.00–3.90)	3.0 (2.30–4.40)	0.357
P#	0.564	0.351	
Prothrombin fragment 1 + 2 (F 1 + 2) (pmol/L)			
Before vaccination	175.2 (143.10–209.50)	274.4 (183.50–298.20)	<0.001
7 days after 1st dose	179.7 (142.70–251.90)	214.4 (185.60–360.00)	0.011
14 days after 1st dose	163.8 (138.00–202.50)	216.3 (147.20–326.20)	0.045
7 days after 2nd dose	173.7 (143.70–211.30)	232.9 (176.80–318.60)	0.012
14 days after 2nd dose	160.0 (140.00–200.80)	258.0 (185.80–319.20)	<0.001
P#	0.503	0.262	
Big endothelin 1 (ET-1) (pg/mL)			
Before vaccination	1.4 (1.00–1.70)	1.5 (1.10–1.90)	0.445
7 days after 1st dose	1.6 (1.20–1.90)	1.5 (1.00–1.70)	0.247
14 days after 1st dose	1.5 (1.30–1.90)	1.7 (0.80–1.90)	0.863
7 days after 2nd dose	1.6 (1.30–2.00)	1.7 (1.20–2.10)	0.957
14 days after 2nd dose	1.6 (1.20–2.00)	1.5 (1.10–1.80)	0.291
P#	0.004	0.411	
Fibrinogen (g/L)			
Before vaccination	2.9 (2.5–3.5)	3.1 (2.6–3.4)	0.806
7 days after 1st dose	2.9 (2.5–3.5)	3.0 (2.6–3.5)	0.531
14 days after 1st dose	2.9 (2.5–3.4)	3.1 (2.5–3.5)	0.616
7 days after 2nd dose	2.9 (2.5–3.6)	3.0 (2.6–3.6)	0.702
14 days after 2nd dose	2.8 (2.5–3.4)	2.8 (2.4–3.5)	0.877
P#	0.030	0.601	
D-dimers (mg/L FEU)			
Before vaccination	0.24 (0.20–0.30)	0.33 (0.20–0.40)	0.048
7 days after 1st dose	0.25 (0.20–0.40)	0.41 (0.30–0.60)	0.011
14 days after 1st dose	0.25 (0.20–0.40)	0.41 (0.30–0.60)	0.005
7 days after 2nd dose	0.27 (0.20–0.40)	0.34 (0.30–0.50)	0.140
14 days after 2nd dose	0.28 (0.20–0.40)	0.33 (0.30–0.50)	0.130
P#	0.010	0.072	
VWF activity (VWF:Ac) (%)			
Before vaccination	123 (87–139)	124 (86–150)	0.825
7 days after 1st dose	117 (89–143)	131 (82–161)	0.583
14 days after 1st dose	107 (85–142)	130 (84–148)	0.551
7 days after 2nd dose	120 (86–143)	134 (84–144)	0.933
14 days after 2nd dose	113 (83–137)	111 (73–142)	0.866
P#	0.002	0.033	
Platelets (10^9/L)			
Before vaccination	261 (218–313)	261 (213–284)	0.672
7 days after 1st dose	254 (221–304)	269 (203–297)	0.981
14 days after 1st dose	257 (220−310)	255 (198–295)	0.404
7 days after 2nd dose	258 (213−301)	251 (202–284)	0.474
14 days after 2nd dose	258 (216–303)	258 (216–289)	0.721
P#	0.722	0.521	

P* - differences between vaccine groups; P# - differences between different time points after vaccination. AH** arterial hypertension. The results are expressed as medians and interquartile ranges.

vaccines, we used information from the trials with ChAdOx1-S vaccine.

De Laat et al. reported changes in D-dimer levels 32 days on average after 2nd vaccination with the vector ChAdOx1-S vaccine in 9 % of total of 631 subjects. Although the number of subjects was significant, D-dimers were obtained only at one-time point after vaccination [31].

Ostrowski et al. found higher D-dimers median 11 days post vaccination with ChAdOx1-S vaccine in comparison with control group while the subjects in BNT162b group did not differ from controls [32]. However, the number of participants was rather small; adenovirus group had 55 subjects and mRNA group total of 25 subjects composed of two vaccine subgroups (Pfizer and Moderna). Also, there was only one-time point after vaccination.

In our study none of the participants experienced thromboembolic event. A slight increase in D-dimer levels can be explained by the mild proinflammatory reaction to the vaccination.

In the mRNA group, apart from the D-dimer levels, a rise in TAT ($P = 0.011$), fibrinogen ($P = 0.003$) and big endothelin-1 ($P < 0.001$) was noted after vaccination.

Ostrowski et al. found a rise in fibrinogen levels median 11 days after mRNA (Pfizer, Moderna) vaccination in 25 subjects which is in line with our results (the biggest rise was noted 7 days after the 1st vaccination) [32]. TAT, in our study, was the highest 14 days after the 2nd vaccination. The subtle changes in all three coagulation parameters could be explained by a mild proinflammatory reaction to the vaccination.

An expected rise in the VWF activity was not observed, rather statistically significant decline was noted ($P < 0.001$). Peyvandi et al. found no difference in VWF antigen in 4 time points [30]. VWF half-life is 8–20 h so both studies could have missed a potential rise considering the time span between the venepunctures.

In our study, a rise in big ET-1 was noted after mRNA vaccination. To the best of our knowledge there is no study of big ET-1 post COVID-19 vaccination so the data from studies investigating other markers of a potential endothelial injury was used.

Terentes-Printzios et al. postulated that an increase in hsCRP levels

Table 4
Haemostatic and proinflammatory marker differences between subjects with and without arterial hypertension in Ad26.CoV2.S vaccinated patients.

	Ad26.CoV2.S		P*
	without AH** ($n = 72$)	with AH ($n = 12$)	
CRP (mg/L)			
Before vaccination	1.3 (0.5–2.2)	0.9 (0.5–2.8)	0.914
7 days after 1st dose	1.5 (0.7–2.5)	1.4 (0.9–4.1)	0.520
14 days after 1st dose	1.1 (0.7–2.0)	1.6 (0.6–2.6)	0.510
P#	0.169	0.019	
Interleukin - 6 (IL-6) (pg/mL)			
Before vaccination	1.5 (1.50–2.70)	1.6 (1.50–2.60)	0.907
7 days after 1st dose	1.6 (1.50–2.30)	1.7 (1.50–2.70)	0.850
14 days after 1st dose	1.5 (1.50–2.30)	1.8 (1.50–2.40)	0.823
P#	0.551	0.562	
Thrombin-antithrombin complexes (TAT) (μg/L)			
Before vaccination	3.2 (2.50–5.20)	3.3 (2.60–3.80)	0.697
7 days after 1st dose	3.2 (2.60–4.40)	4.0 (2.60–5.80)	0.389
14 days after 1st dose	3.0 (2.60–4.00)	4.6 (2.70–6.40)	0.079
P#	0.343	0.052	
Prothrombin fragment 1 + 2 (F 1 + 2) (pmol/L)			
Before vaccination	194.2 (158.90–291.20)	301.3 (201.10–640.50)	0.094
7 days after 1st dose	188.2 (145.80–248.80)	254.1 (147.50–336.40)	0.129
14 days after 1st dose	189.8 (141.50–259.70)	289.3 (160.40–332.90)	0.120
P#	0.142	>0.999	
Big endothelin 1 (ET-1) (pg/mL)			
Before vaccination	1.6 (1.30–1.90)	1.8 (1.70–2.30)	0.038
7 days after 1st dose	1.5 (1.30–2.00)	1.8 (1.60–2.10)	0.159
14 days after 1st dose	1.8 (1.40–2.10)	2.2 (1.40–2.60)	0.071
P#	0.061	0.923	
Fibrinogen (g/L)			
Before vaccination	2.8 (2.4–3.5)	3.1 (2.6–3.5)	0.783
7 days after 1st dose	3.1 (2.6–3.6)	3.4 (2.8–3.6)	0.478
14 days after 1st dose	2.9 (2.5–3.5)	3.1 (2.6–3.6)	0.567
	0.362	0.021	
D-dimers (mg/L FEU)			
Before vaccination	0.27 (0.20–0.40)	0.33 (0.20–0.50)	0.434
7 days after 1st dose	0.31 (0.20–0.50)	0.31 (0.20–0.50)	0.885
14 days after 1st dose	0.31 (0.20–0.40)	0.30 (0.20–0.50)	0.834
P#	0.054	0.042	
VWF activity (VWF:Ac) (%)			
Before vaccination	131 (95–148)	121 (102–144)	0.914
7 days after 1st dose	134 (105–149)	126 (107–153)	0.885
14 days after 1st dose	127 (88–145)	107 (98–141)	0.778
P#	0.001	0.023	
Platelets (10^9/L)			
Before vaccination	260 (214–293)	278 (229–297)	0.370
7 days after 1st dose	257 (211–290)	252 (205–300)	0.834
14 days after 1st dose	258 (227–311)	255 (205–306)	0.680
	0.010	0.258	

P* - differences between patients without AH and with HA; P# - differences between different time points after vaccination. AH** arterial hypertension. The results are expressed as medians and interquartile ranges.

and ultrasonic changes of the arterial stiffness in 32 subjects after BNT162b2 vaccination is evidence of a transient subclinical deterioration of endothelial function [33]. The downside of this study is the number of subjects and the lack of more specific laboratory parameters to quantify the possible endothelial dysfunction.

Lim et al. found no changes in the markers of endothelial dysfunction (ICAM-1, VCAM-1, P-selectin) in subjects after BNT162b2 vaccination [34]. Even though there were three time points of laboratory investigation the study conclusion can be challenged by a small number of participants ($N = 18$).

Robles et al. demonstrated a higher in vivo expression of leukocyte adhesion molecules (ICAM-1, VCAM-1) and ACE2 on endothelial cells after incubation with Spike protein which supports the hypothesis of an endothelial injury after the mRNA vaccination [35].

Whether the rise of big ET-1 in the mRNA group is a part of a mild inflammation and in concordance with the coagulation changes, or a sign of transient endothelial dysfunction is to be investigated more in the future. However, the changes in big ET-1 in our study were subtle and without any clinical repercussion.

In the vector group, besides a rise in D-dimer levels, several parameters have shown a statistically significant changes by time points; CRP ($P = 0.006$), VWF:Ac ($P < 0.001$) and platelets ($P = 0.021$).

A subtle rise of CRP and VWF:Ac can be contributed to the mild local or systemic reaction post vaccination.

The platelet levels slightly decreased with the lowest point noted 7 days after the vaccination. In the light of the mild proinflammatory reaction to the vaccination hypothesis, a rise would be much more expected. However, the platelets are known to be elevated in the circumstances of a more prominent inflammation so the lack of elevation could be explained by an insufficient stimulus.

Due to the reported changes in the blood pressure (BP) after COVID-19 vaccination we included a history of arterial hypertension (AH) as a possible adverse factor for the changes in coagulation and proinflammatory parameters [36,37].

In our study, subjects in the mRNA group with AH had statistically higher D-dimers at baseline ($P = 0.048$) and after the 1st vaccination ($P = 0.005$). The levels of F1 + 2 were higher in the AH group at almost all time points, including baseline ($P < 0.001$).

When observing the changes in the parameters within each group, IL-6 and VWF activity showed a statistically higher levels after vaccination in the AH group, both peaking 7 days after the 2nd dose ($P = 0.008$ and $P = 0.028$, respectively).

Having in mind a small number of subjects with AH, we cannot postulate a potential aggravation of the pre-existing endothelial injury with the mRNA vaccination. Future studies with a larger pool of subjects with AH could shed more light on this issue.

When observing the vector group, besides baseline levels of big ET-1, there were no statistically significant differences between the subgroups (with or without AH).

Subjects with AH experienced a rise in CRP, peaking 14 days after the vaccination ($P = 0.019$). Fibrinogen ($P = 0.021$) and VWF:Ac ($P = 0.023$) peaked 7 days after receiving the vaccine. D-dimers were statistically higher before vaccination ($P = 0.042$).

Subjects without AH had a slight increase of VWF:Ac after vaccination ($P = 0.001$) and a slight decrease of the platelets ($P = 0.010$).

In our study laboratory parameters have been determined 7 days post vaccination so more prominent changes could have been potentially missed. This was shown by Hong et al. who reported a more prominent rise of the coagulation parameters (TAT, PAP and PAI-1) in the first few days (day 1 and 3) post-vaccination [38]. Clearly more evident changes were present in the AH vector subgroup but a future investigation with more AH subjects and several time points closer to the vaccine inoculation could perhaps provide a better information on the effect of vaccination in the settings of pre-existing AH, as we stated earlier.

Both mRNA and vector group did not experience changes in their blood pressure after vaccination.

All the subjects tested negative for the presence of the antibodies to PF4. None of the subjects experienced more than usual side effects of the vaccination stated by the manufacturers. No thromboembolic events were noted in the early postvaccination period nor within 1 year after vaccination.

Based on our observations, there is not enough evidence to support

hypothesis of significant coagulation activation post SARS-CoV-2 vaccination. However, one should be aware of the statistically significant rise of D-dimers post both mRNA and vector vaccination at several time points.

This is the first study in real life that compared head-to-head two different SARS-CoV-2 vaccines; mRNA BNT162b2 vaccine and recombinant vector Ad26.CoV2.S. However, our study has some limitations. First, the study included relatively small number of subjects which we tried to encompass by sequential venepunctures in the precisely defined time points. Second, we have not tested the differences between subjects who had COVID-19 before vaccination and those who were COVID-19 naïve because majority of our subjects had COVID-19 after the vaccination.

6. Conclusion

Our study implicates that there is no plausible scientific evidence of significant disruption in the coagulation and inflammatory processes after vaccination with BNT162b2 mRNA and Ad26.CoV2.S vector SARS-CoV-2 vaccines.

However, although clinically irrelevant, in both vaccine groups changes in D-dimers after vaccination were stated at several time points. None of the subjects in our study experienced more than usual side effects of the vaccination stated by the manufacturer.

Further studies are needed to elucidate the possible effect of the SARS-CoV-2 vaccines on the hemostatic and proinflammatory markers.

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.

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