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No changes of parameters nor coagulation activation in healthy subjects vaccinated for SARS-Cov-2

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ABSTRACT

Background: Recent reports of thrombotic events after SARS-Cov-2 vaccination raised concern. However, modifications of the most common coagulation parameters after vaccination are unknown.
Aims: We measured parameters of coagulation including (i) basic coagulation tests, (ii) procedures aimed to assess the ex-vivo potential capacity to generate thrombin and (iii) in vivo thrombin activity. We also assessed anti-platelet factor 4 (aPF4) with two methods.
Design: Laboratory measurements were performed for a cohort of subjects (n = 30) before (baseline) and after (7 and 21 days after first dose, and 14 days after second dose) SARS-Cov-2 vaccination.
Results: All subjects received the Pfizer-BioNTech vaccine, and none developed symptomatic thrombotic events during the study period. None of the parameters showed clinically relevant variations at different time-points before and after vaccination. Only platelet count showed a slight increase, and F1.2 and the thrombin generation parameters ETP and ETP-TM ratio, showed a small decline, at the last time-point after vaccination when compared to baseline. aPF4 was negative in all the subjects, except two, who were positive (one with the chemiluminescent and the other with the ELISA assay).
Conclusions: The study shows no modifications of the coagulation parameters nor the presence of biochemical signs of coagulation activation following the administration of the Pfizer-BioNTech vaccine.

1. Introduction

Recent news on cerebral vein thrombosis (CVT) and splanchnic vein thrombosis associated with thrombocytopenia in subjects immunized with nonreplicating adenovirus vector–based DNA vaccines (ChAdOx1 nCoV-19 [Oxford–AstraZeneca] and Ad26.COV2.S [Johnson & Johnson/Janssen]) sparked concerns among the population, healthy authorities and care givers about the safety of vaccination. Unfortunately, these concerns slowed down the vaccination campaign currently ongoing in many countries across the World. Although information is still scanty and mainly reported on newspapers, on April 7th the European Medicines Agency (EMA) evaluated so far 169 cases of CVT and 53 cases of splanchnic vein thrombosis among close to 34 million subjects who received the AstraZeneca vaccine [1] and 8 reports of serious cases of unusual blood clots associated with low levels of blood platelets, over

7 million people who received Janssen's vaccine in USA as of 13 April 2021 [2]. In Italy, 11 cases have been so far reported, which are under investigation by the Italian regulatory drug agency (AIFA) [3]. EMA's safety committee (PRAC) concluded that unusual blood clots with low blood platelets should be listed as an exceedingly rare side effect with COVID-19 Vaccine AstraZeneca and Janssen. Since hemostasis parameters have been shown to be altered during SARS-Cov-2 infection [4], our study aimed to investigate changes of coagulation parameters and activation of coagulation in a cohort of health professionals working at the hospital, who underwent SARS-Cov-2 vaccination using the mRNA Pfizer-BioNTech vaccine.

2. Materials and methods

Thirty subjects who received the Pfizer-BioNTech vaccine were

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Table 1

Median (IQR) values of parameters of coagulation for subjects at different time points after vaccination. PT, prothrombin time. APTT, activated partial thromboplastin time. VWF, von Willebrand factor. FVIII, factor VIII. F1.2, prothrombin fragment 1 + 2.

	Baseline	7days after first dose	21days after first dose	14 days after second dose	P value[a]
PT (sec)	13 (13–14)	13 (13–14)	14 (13–14)	13 (13–14)	0.354
APTT (sec)	30 (29–32)	31 (30–31)	30 (29–31)	30 (29–32)	0.793
D Dimer (ng/ml)	273 (175–360)	214 (144–431)	314 (167–513)	286 (160–514)	0.633
VWF:Ag (U/dL)	113 (92–136)	118 (93–132)	118 (89–140)	104 (97–130)	0.943
Adamts13 (U/dL)	103 (92–117)	107 (97–116)	107 (97–116)	109 (96–119)	0.964
FVIII (U/dL)	104 (89–116)	100 (90–116)	101 (90–127)	103 (89–120)	0.348
Protein C (UdL)	105 (95–130)	105 (89–130)	105 (91–124)	109 (94–124)	0.381
F1.2 (pmol/L)	155 (117–212)	149 (97–193)	170 (122–229)	152 (120–236)	0.029
P-selectin (ng/mL) n.v. (30–101)	60.2 (45.9–74.7)	61.0 (48.2–68.7)	58.4 (51.8–67.3)	58.8 (48.3–69.1)	0.784
Platelet count X 10^9/L	252 (220–306)	258 (220–297)	252 (222–282)	270 (236–307)	0.041

[a] Comparison (baseline vs 14 days after second dose).

enrolled in this study after informed consent. Blood samples were collected at the specified time points (before the vaccination, 7 days after the first dose of the vaccine, 21 days after the first dose and 14 days after the second dose) into vacuum tubes containing 1/10 vol of 0.109 M trisodium citrate or plain tubes and centrifuged at 3000 g for 15 min at (controlled) room temperature. Supernatant plasma and serum were aliquoted in plastic capped tubes and stored frozen at −70 °C until testing. The prothrombin and activated partial thromboplastin time (PT and aPTT) were measured by Recombiplastin 2G or Synthasil aPTT (Werfen, Orangeburg, NY). Factor (F)VIII was measured as activity by one-stage clotting assay (Werfen). Von Willebrand factor (VWF) antigen was measured by an immunoturbidimetric assays (Werfen) and ADAMTS-13 was measured by the FRETS assay. Thrombin generation (TG) procedure was carried out by a home-made method [5,6] which triggers test plasma by small amounts (1pM) of tissue factor (Werfen) and 1 μM of blended synthetic phospholipids (Avanti Polar, Alabaster, AL), in presence or absence of 2 nM rabbit thrombomodulin (TM) (Hematologic Technologies, Essex, VT). Thrombin was continuously monitored with a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Bubendorf, Switzerland) (617 μM) by a fluorometer (Fluoroskan Ascent®, ThermoLabsystem, Helsinki, Finland). The dedicated software (ThrombinoscopeTM, Thrombinoscope BV, Maastricht, Netherlands) was used to obtain the curve of thrombin concentrations as a function of time and to calculate the following parameters. The lag-time, defined as the time (minutes) between the addition of the triggers and the initiation of thrombin generation. The thrombin peak (nM). The time needed to reach the peak. The area under the curve, defined as endogenous thrombin potential (ETP) (nM x min). Fragment 1.2 (F1.2), a breakdown product, which derives from the prothrombin-to-thrombin conversion, was measured by a commercially available ELISA system (F1.2 Enzygnost, Siemens, Marburg, Germany). D Dimer (DD), which derives from the plasmin mediated breakdown of stabilized fibrin, was measured by a commercially available turbidimetric assay (HS D Dimer, Werfen). Both F1.2 and DD possess limited half-life in vivo and are considered as biochemical markers of coagulation activation at the time of blood

drawing. They are considered as biochemical indexes of hypercoagulability and have also been associated with the risk of thrombosis (reviewed in Refs. [7,8]). Antibodies against platelet factor 4 (aPF4) were measured by chemiluminescent assays (Werfen) and by the ELISA method (Immucore, GTI Diagnostics, Waukesha, WI). P-selectin was measured with a commercially available ELISA system (Life Technologies Thermo Fisher Scientific, UK) and represents an index of platelet and endothelial activation.

Continuous variables were expressed as medians and interquartile ranges (IQR). Differences in medians were evaluated with Wilcoxon tests for paired-data. Statistical analyses were performed using SPSS v27.

3. Results and discussion

The most prevalent symptoms reported by the investigated subjects were mild headache, fever, muscle or joint pain during the next few days after vaccination, mainly after the second dose. The hemostasis parameters obtained for the subjects available for analysis are in Tables 1, 2. None of the parameters did show significant variations at different time-points before and after vaccination. Exceptions were platelet count that showed a slight increase at the last time point (Table 1), and F1.2 (Table 1) and the TG parameters ETP and ETP-TM ratio (Table 2) that showed a small (but significant (p < 0.05)] decline at the last time point after vaccination. Furthermore, one subject was positive to aPF4 before vaccination and remained positive after vaccination using the chemiluminescent assay but negative with the ELISA assay. Conversely, another subject who was negative with the chemiluminescent assay was slightly positive with the ELISA assay. Both subjects were negative when tested with a home-made functional confirmatory assay based on platelet aggregation (not shown); platelet counts were normal in both subjects and both denied recent exposure to heparin.

Although normal parameters of coagulation, absence of biochemical signs of coagulation activation and negative confirmatory assays for the HIT-like disease do not represent per se low or no risk of clinical

Table 2

Parameters of thrombin generation for subjects at different time points after vaccination. LT, lag time. ttpeak, time to peak. ETP, endogenous thrombin potential. ETP-TM ratio (ETP with/without TM). TM, thrombomodulin.

	Baseline	7days after first dose	21days after first dose	14 days after second dose	P value[a]
LT (min)	7 (6–8)	7 (7–8)	7 (7–8)	8 (7–9)	0.167
LT + TM (min)	9 (8–10)	9 (8–10)	8 (8–10)	9 (7–10)	0.909
ttPEAK (min)	11 (10–12)	11 (10–12)	11 (10–12)	11 (10–12)	0.657
ttPEAK + TM (min)	11 (10–12)	12 (11–13)	11 (11–13)	11 (10–13)	0.982
ETP (nM^{a}min)	1929 (1798–2190)	1950 (1773–2101)	1891 (1768–2208)	1823 (1694–2092)	0.001
ETP + TM (nM^{a}min)	1328 (1129–1717)	1413 (1090–1816)	1442 (1182–1808)	1214 (831–1421)	0.008
ETP-TM Ratio	0.72 (0.58–0.85)	0.77 (0.59–0.87)	0.78 (0.62–0.84)	0.64 (0.47–0.78)	0.036
PEAK (nM)	270 (232–325)	285 (250–323)	291 (254–337)	262 (217–329)	0.374
PEAK + TM (nM)	259 (211–318)	273 (193–354)	275 (202–369)	220 (127–305)	0.088

[a] Comparison (baseline vs 14days after second dose).

thrombosis, the above results cast doubts on the causal relationship between vaccination with Pfizer vaccine and thrombotic events. There are limitations of the study. First, while the investigated population was treated with the Pfizer-BioNTech vaccine, most thrombotic events were reported for the AstraZeneca and Janssen, although few events have been more recently reported even with the Pfizer vaccine. Second, the sample size and the follow up after vaccination was relatively limited to assess the real prevalence of rare side effects. However, it should be recognized that most of reported events do occur within the period of 7–14 days after vaccination. It is therefore unlikely that we missed clinically significant events. Third, the investigated population was free from known risk factors of thrombosis and was not on anticoagulant treatment; it is therefore unknown whether the same conclusions could apply to subjects with known or unknown abnormalities of coagulation and/or during anticoagulant treatment. In conclusion, the study shows no modifications of the coagulation parameters nor the presence of biochemical signs of coagulation activation following the administration of Pfizer-BioNTech vaccine. Until new information is available the above data support the reassuring statement on the safety of vaccination for SARS-Cov-2, recently issued by the International Society on Thrombosis and Haemostasis [9].

Authors' contribution

F. Peyvandi designed the study and wrote the manuscript. E. Scalambrino performed the laboratory assays and statistical analysis. M. Clerici, A. Lecchi, and N. Ravelli performed the laboratory assays. R. Palla interpreted the results and critically reviewed the manuscript. R. Gualtierotti and D. Prati collected clinical data and critically reviewed the manuscript. A. Tripodi interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

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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.

References

[1] Last access, April 26th, 2021, https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood.

[2] Last access, April 26th, 2021, https://www.ema.europa.eu/en/news/covid-19-vaccine-janssen-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood.

[3] Last access, April 26th, 2021, https://www.aifa.gov.it/documents/20142/1315190/Rapporto_sorveglianza_vaccini_COVID-19_3.pdf.

[4] F. Peyvandi, A. Artoni, C. Novembrino, S. Aliberti, M. Panigada, M. Boscarino, R. Gualtierotti, F. Rossi, R. Palla, I. Martinelli, G. Grasselli, F. Blasi, A. Tripodi, Hemostatic alterations in COVID-19, Haematologica (2020), https://doi.org/10.3324/haematol.2020.262634.

[5] V. Chantarangkul, M. Clerici, C. Bressi, P.L. Giesen, A. Tripodi, Thrombin generation assessed as endogenous thrombin potential in patients with hyper- or hypo-coagulability. Effects of phospholipids, tissue factor and residual platelets on the measurement performed in platelet-poor and platelet-rich plasma, Haematologica 88 (2003) 547–554.

[6] A. Tripodi, C. Legnani, V. Chantarangkul, B. Cosmi, G. Palareti, P.M. Mannucci, High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of recurrent venous thromboembolism, J. Thromb. Haemostasis 6 (2008) 1327–1333.

[7] A. Tripodi, P.M. Mannucci, Markers of activated coagulation and their usefulness in the clinical laboratory, Clin. Chem. 42 (1996) 664–669.

[8] A. Tripodi, D-dimer testing in laboratory practice, Clin. Chem. 57 (2011) 1256–1262.

[9] Last access, March 13, 2021, https://www.isth.org/news/556057/ISTH-Statement-on-AstraZeneca-COVID-19-Vaccine-and-Thrombosis.htm.