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RESEARCH ARTICLE

PERSISTENT COAGULATION ABNORMALITIES IN RECOVERED COVID-19 PATIENTS FOLLOWING VACCINATION: A RETROSPECTIVE ANALYSIS

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Abstract

This retrospective observational study assessed persistent coagulation abnormalities in individuals who recovered from COVID-19 and subsequently received two doses of COVID-19 vaccination. The study enrolled 250 individuals, aged 20–50 years, and evaluated four primary coagulation markers: prothrombin time, activated partial thromboplastin time, D-dimer, and fibrinogen. Blood samples were analyzed using the STA-R coagulation analyzer. Results indicated that 10% of participants exhibited elevated D-dimer levels, 34% had elevated prothrombin times, and 85% displayed prolonged activated partial thromboplastin times, while 7.2% showed increased fibrinogen levels. Statistical analysis revealed significant deviations in D-dimer, activated partial thromboplastin time, and fibrinogen from standard reference ranges. These abnormalities suggest a possible hypercoagulable state post-vaccination, potentially due to vaccine-induced mechanisms that activate coagulation pathways. Findings align with reports of vaccine-induced thrombotic complications, especially in adenovirus-vectored vaccines. This study underscores the importance of monitoring coagulation parameters in vaccinated individuals with prior COVID-19 infection to ensure vaccine safety and efficacy. Future studies should investigate the underlying mechanisms of these coagulation changes and their clinical implications.

Introduction:-

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was recognized as a global pandemic that originated in Wuhan, China in late 2019. Severe cases are prone to serious complications such as pneumonia, acute respiratory distress syndrome (ARDS), and even death (Hu et al., 2021). To reduce the severity, risk and spread of the primary infection, several vaccines such as mRNA, DNA, viral vector vaccines, have been developed by many countries. In preventing severe cases and deaths from COVID-19, vaccines such as Oxford-Astrazeneca (ChAdOx1) have been shown to be very effective (Bernal et al., 2021). Although vaccines have a striking effect against disease, thrombosis also increases following vaccination (EMA, 2021). Alongside thrombosis, a rare incidence of cerebral venous sinus thrombosis (CVST) has been reported in recent studies (Medicherla et al., 2021). Another study found a direct association between developed blood clots and thrombocytopenia after immunization with Astrazeneca, and reported cases of arterial thrombosis and splanchnic vein thrombosis (EMA, 2021). The Medicine and Healthcare Products Regulatory Agency of the UK received 79 cases of thrombosis associated with thrombocytopenia, of which

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44 cases were CVST, and all occurred after the first dose (Public Health England, 2021). Pottegard et al. (2021) reported 122 cardiovascular and haematological events in 158 individuals who received the Pfizer vaccine. There are several studies which indicated post-vaccination thrombotic events including venous and arterial thrombosis with thrombocytopenia, pulmonary embolism, deep vein thrombosis etc (Tobaiqy et al. 2021) (Schultz et al. 2021) (Blauenfeldt et al. 2021). Furthermore, Greinacher et al. (2021) reported positive anti-platelet factor 4 antibodies and evidence of DVT, such as elevated levels of D-dimer, prothrombin time (PT), INR, and fibrinogen in patients who had thrombotic complications after Astrazeneca vaccination (Shima et al. 2021). Some studies suggest that certain individuals may have persistent coagulation issues following immunization, although the majority of these changes are temporary (Yamada et al. 2022).

It is essential to understand the long-term impact of COVID-19 vaccination on coagulation markers for multiple reasons. First, it can be beneficial for recognizing those who could be more susceptible to problems resulting from coagulation abnormalities. Second, it can help in the formulation of management and prevention plans for these issues. Finally, this study advance our knowledge of the pathways through which the coagulation system may be affected by the COVID-19 vaccine.

In this study, we conducted a retrospective analysis to investigate persistent coagulation abnormalities in individuals who recovered from COVID-19 and received the COVID-19 vaccination.

Methodology:-

This was a retrospective observational cohort study conducted between February 2024 and June 2024. This study aimed to investigate the coagulation parameters in recovered COVID-19 patients who received two doses of vaccination.

A total of 250 volunteers aged 20-50 years who had recovered from COVID-19 and received two doses of the vaccine were enrolled. The inclusion criteria for the study comprised of a verified history of SARS-CoV-2 infection and full recovery from the acute stage of the illness. Any underlying conditions that could independently influence coagulation markers, such as continuous anticoagulant medication, aggressive cancer, or chronic liver disease were excluded.

Blood samples from every volunteer were collected into blue-top vacutainer tubes with 3.2% sodium citrate added as an anticoagulant. Blood was kept from clotting until the analysis using sodium citrate to chelate calcium. Collected blood samples were immediately transported to the laboratory for processing. The samples were centrifuged at 2,500-3,000 RPM for 15 min to separate the plasma from the cellular components. Plasma was carefully extracted and stored at -80˚C until further analysis.

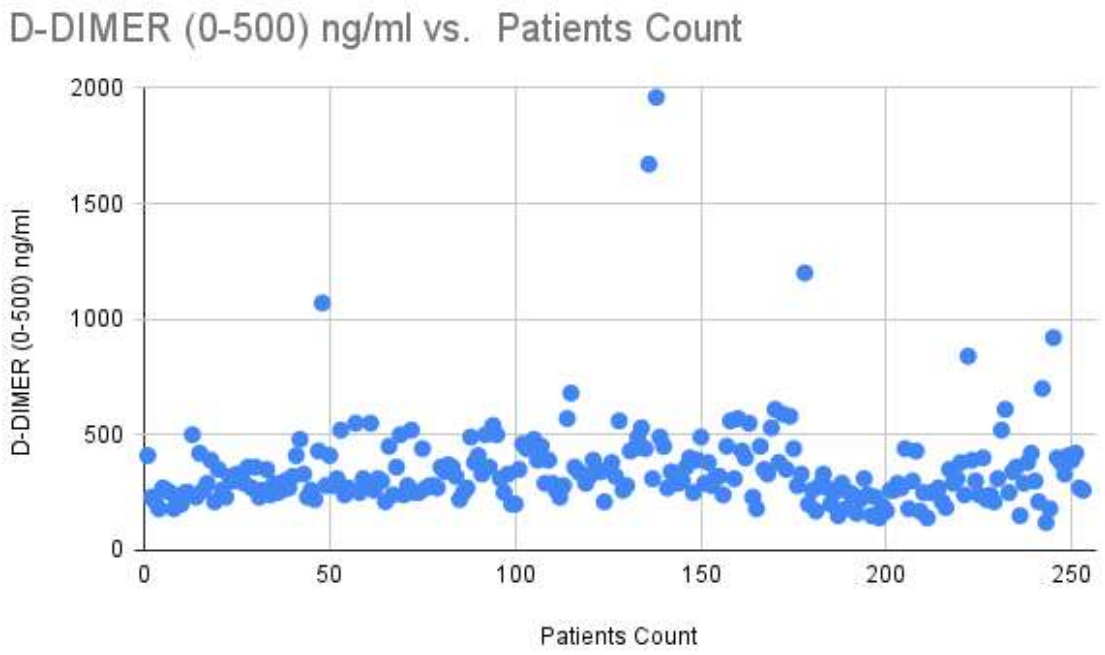
The coagulation markers prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, and fibrinogen were measured using specialized coagulation assay kits. Stago kits were used and measurements were conducted using the STA-R coagulation analyzer, an automated system designed for high-throughput and precise determination of coagulation parameters. The analyzer was calibrated according to the manufacturer's instructions and quality control was ensured using standard reference materials.

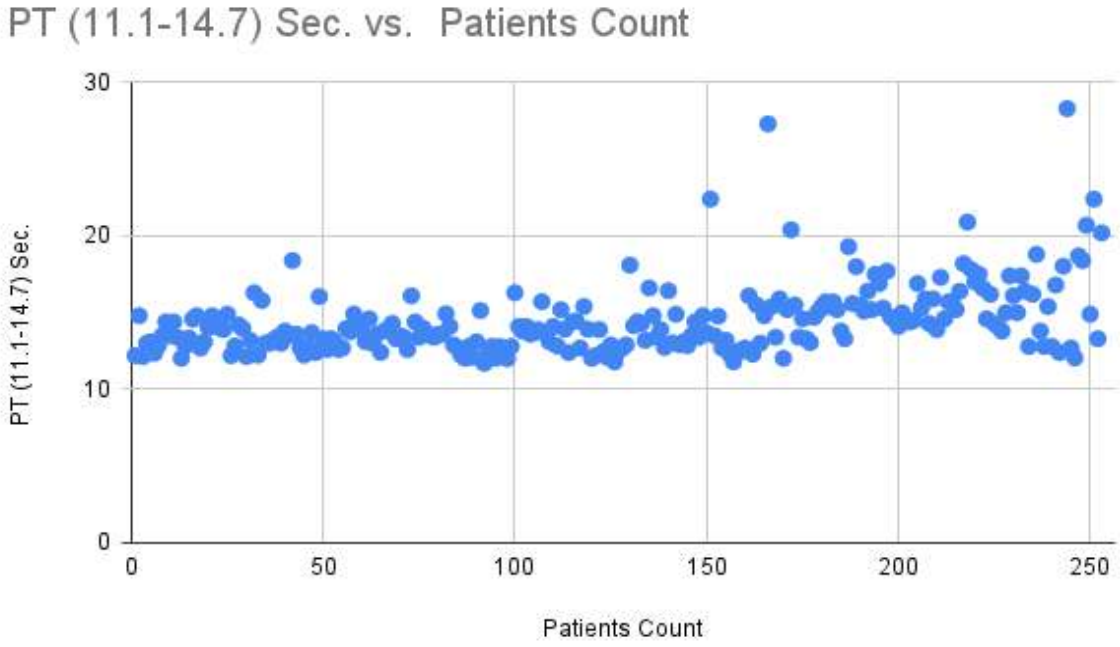
The data obtained from the STA-R analyzer were recorded and statistically analyzed. Descriptive statistics, including mean, median, and standard deviation, were calculated, and a one-sample t-test was performed to compare the mean or median of each coagulation parameter to its respective reference range. Statistical significance was set at p-value <0.05.

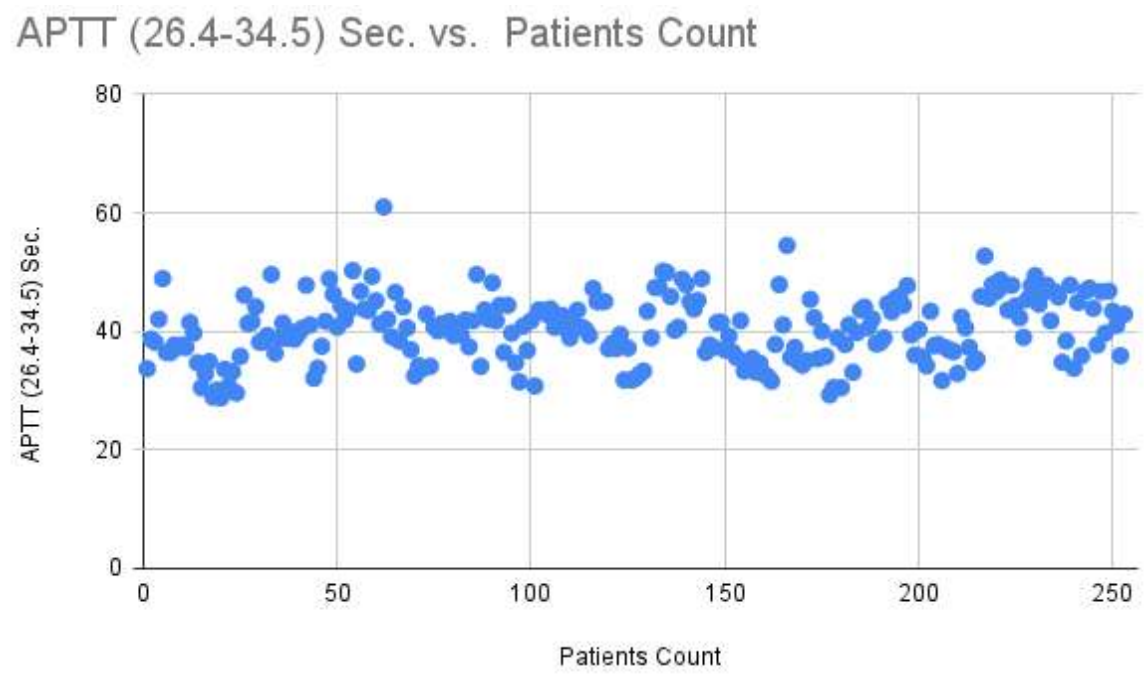
This study was conducted in accordance with the ethical standards of the institutional research committee. Informed consent was obtained from all volunteers prior to participation in the study. All data were anonymized to ensure participant confidentiality.

Results:-

In this retrospective analysis, we evaluated the coagulation profiles of vaccinated COVID-19 patients after a long recovery period. The study cohort had a mean age of 37.71 years. Our analysis focused on four key coagulation parameters: D-dimer level, PT, APTT, and fibrinogen level.


D-DIMER (0-500) ng/ml vs. Patients Count
D-DIMER (0-500) ng/ml
2000
1500
1000
500
0
0
50
100
150
200
250
Patients Count


PT (11.1-14.7) Sec. vs. Patients Count
PT (11.1-14.7) Sec.
30
20
10
0
0
50
100
150
200
250
Patients Count



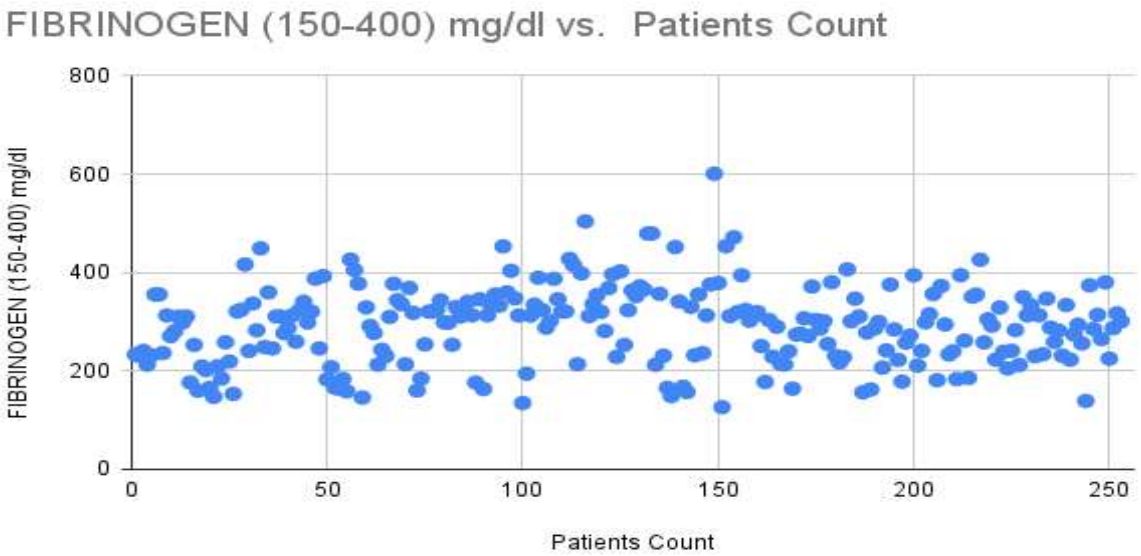


Table 1:- Descriptive statistics.

Parameters	Mean (SD)	Median	Minimum	Maximum
D-dimer (ng/mL)	349.34 (191.44)	310	120	1.960
PT (sec)	14.43 (2.252)	13.9	11.7	28.3
APTT (sec)	40.31 (5.38)	40.4	28.8	61
Fibrinogen (mg/dL)	291.45 (78.60)	298.9	126.4	600.9

Table 2:- No. of patients with elevated values from reference range.

Parameters	Patients with elevated values	Percentage of patients with elevated value
D-dimer	25	10%
PT	85	34%
APTT	212	84.80%
Fibrinogen	18	7.20%

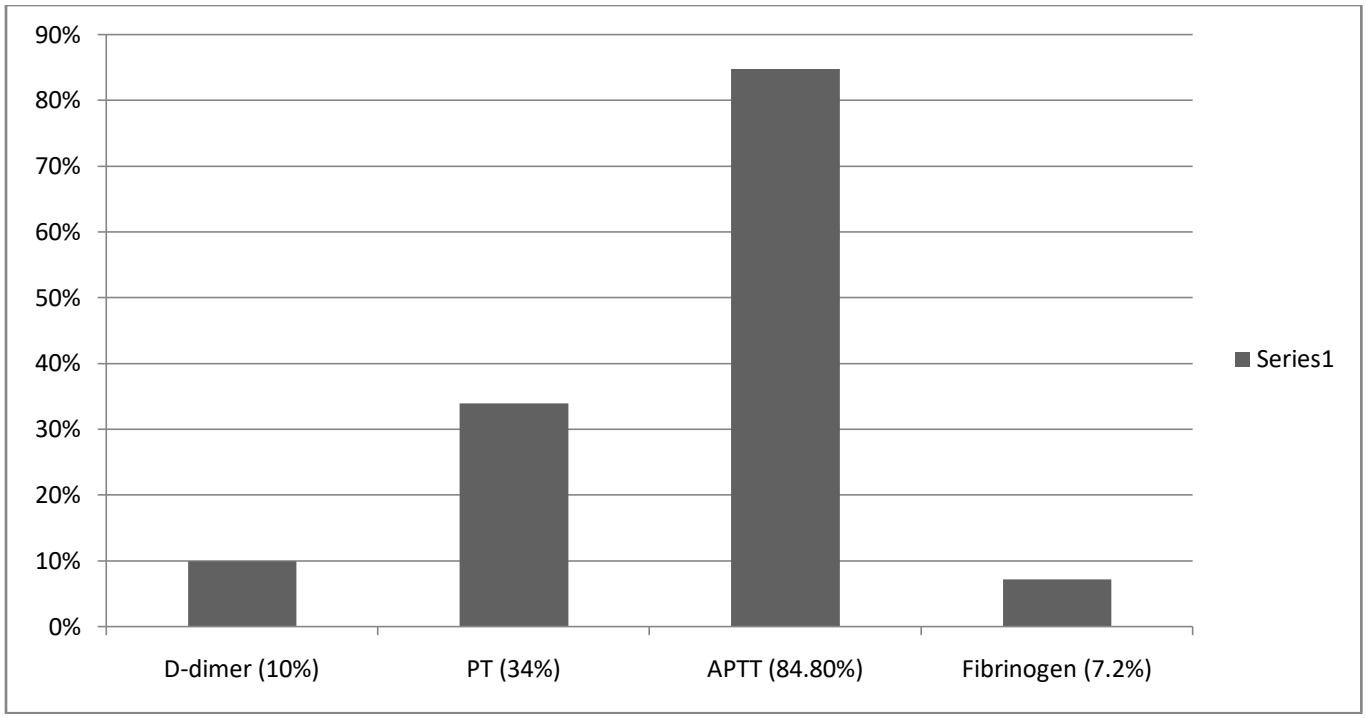


Table 3:- One-sample t-tests were used to compare the mean of each coagulation parameter with its respective reference range.

Parameters	t-statistics	p-value
D-dimer	-12.542	0
PT	-1.843	0.06649
APTT	17.187	2.42E-44
Fibrinogen	-21.966	1.78E-60

The mean D-dimer level was 349.34 ng/ml (SD: 191.44 ng/ml), with a median of 310 ng/ml, ranging from 120 to 1960 ng/ml. elevation from the reference range was observed in 29 patients (10%). The t-statistic for D-dimer was -12.542, with a p-value of 0.00, indicating a statistically significant deviation from the expected reference range. The mean PT was 14.43 seconds (SD: 2.252 seconds), with a median of 13.9 seconds. The minimum recorded value was 11.7 seconds and the 28.3 seconds. Notably, 85 patients (34%) exhibited elevated PT levels. The t-statistics was -1.843, with a p-value of 0.06649, suggesting a significant trend. The mean value of APTT was 40.31 seconds (SD: 5.38 seconds), with a median of 40.4 seconds, ranging from 28.8 to 61 sec. A significant no. of patients, 212 (84.8%) had elevated APTT levels. The t-statistic was 17.184, with an extremely significant p-value of 2.41968e-44, which indicates a marked prolongation of the APTT. The mean level of fibrinogen was 291.45 mg/dL (SD: 78.60 mg/dL) with a median of 208.9 mg/dL, ranging from 126.4 to 600.9 mg/dL. Elevated fibrinogen levels were observed in 18 patients (7.2%). The t-statistic was -21.966, with a p-value of 1.782707e-60, indicating a significant increase in fibrinogen levels, which may reflect an acute-phase response post-vaccination.

All coagulation parameters showed a significant elevation in the notable no. of patients, suggesting a potentially hypercoagulable state following vaccination.

Discussion

The findings of our study revealed a significant elevation in coagulation parameters, specifically, elevated D-dimer, PT, APTT, and fibrinogen levels in recovered COVID-19 patients who received two doses of vaccination. In our study, we found that there is a highly elevated D-dimer level up to 1960 ng/mL in some patients, which is four-fold higher than the higher reference value. PT was also elevated in a large proportion of patients, while APTT was elevated in 85% of vaccinated patients which shows that there is a persistent abnormality in the intrinsic and extrinsic pathways of coagulation and increased levels of fibrinogen were also noticed in some patients. There was

no significant association between the parameters; therefore, the elevation or reduction in one biomarker did not predict elevation or reduction in the other within this cohort. These findings align with those of a previous study that reported prolonged PT and APTT and increased D-dimer and fibrinogen levels in COVID-19 patients who are facing the problem of thrombosis following vaccination (Greinacher et al. 2021).

Many studies has reported low platelet counts in many patients after vaccination with adenovirus-vectored vaccine (Tiede et al. 2021) (See et al. 2021) (Muir et al. 2021) and termed the condition as vaccine-induced thrombotic thrombocytopenia (VITT) (Sharifian-Dorche et al. 2021). VITT is characterized by a lower platelet count, abnormalities in coagulation parameters such as D-dimer, PT, and APTT, and positive enzyme-linked immunosorbent assay (ELISA) results for anti-platelet factor-4 (PF4) antibodies (Greinacher et al. 2021). Adenoviral vector vaccines such as Astrazeneca, introduce viral proteins into the body, which interact with PF4 released from activated platelets. The adenoviral vector components bind with PF4 and form complexes, which are recognized as foreign bodies by the immune system and activated B-cells, which then produce anti-PF4 antibodies. The binding of anti-PF4 antibodies to PF4 on the surface of platelets leads to increased platelet activation, resulting in the release of prothrombotic microparticles from activated platelets and the promotion of thrombin production (Scully et al. 2021) (Greinacher et al., 2021). PF4 also enhances prothrombotic state by binding to heparin sulfate and chondroitin sulfate on vascular endothelial cells, and binding of antibodies at these sites leads to endothelial activation. Activated endothelial cells express tissue factors and activate the coagulation cascade. Kowarz et al. (2021) used a term "vaccine-induced COVID-19 mimicry" syndrome (VIC19M syndrome), in which they explained that soluble spike protein variants of vaccine mimic the mechanism of thromboembolic events caused by spike protein of SARS-CoV-2 virus. Soluble spike protein variants bind to ACE 2 receptors on endothelial cells in blood vessels and induce endothelial cell activation, ultimately causing thrombotic problems.

Although a direct link between vaccination and coagulation problems has not been established, these studies suggest an indirect relationship. The observed elevations may be caused by vaccine-induced mechanisms that mimic the viral effects on endothelial function, platelet activation, and inflammation. Future research is necessary to clarify the specific cause-and-effect relationship and identify potential risk factors.

Conclusion:-

Our research highlights a significant correlation between the elevation of coagulation parameters and the post-vaccination effects observed in the study population. These findings suggest that the immune response elicited by vaccination may influence coagulation pathways, leading to measurable changes in the coagulation parameters. This relationship underscores the importance of monitoring coagulation status in individuals following vaccination, as it may provide valuable insights into the safety and efficacy of vaccine interventions. Future studies should aim to further elucidate the mechanisms which underlying these correlations and explore their clinical implications, ultimately contributing to a more comprehensive understanding of vaccine responses and their impact on hemostatic balance.

References:-

1. Bernal JL, Andrews N, Gower C, et al. 2021. Early effectiveness of COVID-19 vaccination with BNT162b2 mRNA vaccine and ChAdOx1 adenovirus vector vaccine on symptomatic disease, hospitalisations and mortality in older adults in England.MedRxiv 2021.
2. Blauenfeldt RA, Kristensen SR, Ernstsen SL, Kristensen CCH, Simonsen CZ, Hvas A. Thrombocytopenia with acute ischemic stroke and bleeding in a patient newly vaccinated with an adenoviral vector-based COVID-19 vaccine. J Thromb Haemost. 2021;19(7):1771-1775.
3. EMA. Covid-19 vaccine AstraZeneca: PRAC preliminary view suggests no specific issue with batch used in Austria, 10 March 2021
4. EMA. AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets. 2021.
5. Favaloro, E.J. Laboratory testing for suspected COVID-19 vaccine–induced (immune) thrombotic thrombocytopenia. Int. J. Lab. Hematol. 2021, 43, 559–570.
6. Greinacher, A.; Thiele, T.;Warkentin, T.E.;Weisser, K.; Kyrle, P.A.; Eichinger, S. Thrombotic Thrombocytopenia after ChAdOx1nCov-19 Vaccination. N. Engl. J. Med. 2021, 384, 2092–2101.
7. Hu, B., Guo, H., Zhou, P. et al. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 19, 141–154 (2021).

8. Kowarz E, Krutzke L, Reis J, Bracharz S, Kochanek S, Marschalek R (2021). Vaccine-Induced Covid-19 Mimicry" Syndrome: Splice reactions within the SARS-CoV-2 Spike open reading frame result in Spike protein variants that may cause thromboembolic events in patients immunized with vector-based vaccines.
9. Medicherla CB, Pauley RA, de Havenon A, Yaghi S, Ishida K, Torres JL. Cerebral venous sinus thrombosis in the covid-19 pandemic. J Neuroophthalmol2020;40:457-62.
10. Muir, K.-L.; Kallam, A.; Koepsell, S.A.; Gundabolu, K. Thrombotic Thrombocytopenia after Ad26.COV2.S Vaccination. N. Engl. J. Med. 2021, 384, 1964–1965.
11. Public Health England. Guidance: covid-19 vaccination and blood clotting. 2021.
12. Pottegård A, Lund LC, Karlstad Ø, et al. Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study. BMJ 2021
13. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130
14. **Shima, H., Kawashima, Y., Hirano, T., et al.** (2021). Persistent hypercoagulable state after COVID-19 infection. **Blood Advances** 5(20), 3606-3610.
15. See, I.; Su, J.R.; Lale, A.;Woo, E.J.; Guh, A.Y.; Shimabukuro, T.T.; Streiff, M.B.; Rao, A.K.; Wheeler, A.P.; Beavers, S.F.; et al. US Case Reports of Cerebral Venous Sinus Thrombosis with Thrombocytopenia After Ad26.COV2.S Vaccination, March 2 to April 21, 2021. JAMA 2021, 325, 2448–2456.
16. Sharifian-Dorche, M.; Bahmanyar, M.; Sharifian-Dorche, A.; Mohammadi, P.; Nomovi, M.; Mowla, A. Vaccine-induced immune thrombotic thrombocytopenia and cerebral venous sinus thrombosis post COVID-19 vaccination; a systematic review. J. Neurol. Sci. 2021, 428, 117607.
17. Scully, M., Singh, D., Lown, R., et al. (2021). "Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination." New England Journal of Medicine, 384(23), 2202-2211.
18. Tobaiqy M, Elkout H, MacLure K. Analysis of thrombotic adverse reactions of COVID-19 AstraZeneca vaccine reported to EudraVigilance database. medRxiv. 2021
19. Tiede, A.; Sachs, U.J.; Czwalinna, A.;Werwitzke, S.; Bikker, R.; Krauss, J.K.; Donnerstag, F.G.;Weißenborn, K.; Höglinger, G.U.; Maasoumy, B.; et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccination. Blood 2021, 138, 350–353.
20. Yamada, S.; Asakura, H. Coagulopathy and Fibrinolytic Pathophysiology in COVID-19 and SARS-CoV-2 Vaccination. Int. J. Mol. Sci. 2022, 23, 3338.