

Presence of viral spike protein and vaccinal spike protein in the blood serum of patients with long-COVID syndrome

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Abstract. – OBJECTIVE: COVID-19 patients experience, in 10-20% of the cases, a prolonged long-COVID syndrome, defined as the persistence of symptoms for at least two months after the infection. The underlying biological mechanisms of this syndrome remain poorly understood. Several hypotheses have been proposed, among which are the potential autoimmunity resulting from molecular mimicry between viral spike protein and human proteins, the reservoir and viral reproduction hypothesis, and the viral integration hypothesis. Although official data state that vaccinal spike protein is harmless and remains at the site of infection, several studies proposed spike protein toxicity and found it in blood circulation several months after the vaccination.

To search for the presence of viral and vaccine spike protein in a cohort of long-COVID patients.

PATIENTS AND METHODS: In this study, we employed a proteomic-based approach utilizing mass spectrometry to analyze the serum of 81 patients with long-COVID syndrome. Moreover, viral integration in patients' leukocytes was assessed with a preliminary study, without further investigation.

RESULTS: We identified the presence of the viral spike protein in one patient after infection clearance and negativity of COVID-19 test and the vaccine spike protein in two patients two months after the vaccination.

CONCLUSIONS: This study, in agreement with other published investigations, demonstrates that both natural and vaccine spike protein may still be present in long-COVID patients, thus supporting the existence of a possible mechanism that causes the persistence of spike

protein in the human body for much longer than predicted by early studies. According to these results, all patients with long-COVID syndrome should be analyzed for the presence of vaccinal and viral spike protein.

Key Words:

Viral Spike Protein, Vaccinal Spike Protein, SARS-CoV-2, COVID-19, Long-COVID syndrome, Mass spectrometry, Viral reservoir, Viral integration.

Introduction

CoronaVirusDisease-2019 indicates the disease caused in humans by the SARS-CoV-2 virus, characterized by fever, cough, breathing difficulties, severe acute respiratory syndrome, and even death¹⁻⁴. 10-20% of COVID-19 patients manifest long-COVID syndrome, defined as the persistence of symptoms two months after the infection⁵⁻⁸. The most common symptoms associated with long-COVID include fatigue, breathlessness and cognitive dysfunction^{9,10}. Notably, even seven months after the initial infection, patients with long-COVID continue to experience cardiovascular and neural problems, indicating a prolonged and complex disease course, and highlighting the significant impact of long-COVID on individuals' health and quality of life¹¹. Extensive research¹²⁻²² has been conducted to elucidate the underlying mechanisms and pathophysiology of long-COVID. However,

the exact cause of long-COVID and the factors contributing to its diverse symptomatology are still not fully understood. Several hypotheses have been proposed, among which potential autoimmunity resulting from molecular mimicry between viral spike protein and human proteins^{12,13}, the reservoir and viral reproduction hypothesis¹⁴⁻¹⁷, and the viral integration hypothesis¹⁸⁻²². Finally, it has also been proposed that the spike protein, the primary antigen targeted by COVID-19 vaccines, could have a potential toxicity that is linked to the development of long-COVID symptoms²³⁻²⁸.

The spike protein used in vaccines differs from the viral spike protein found in SARS-CoV-2 because it has been modified to enhance its stability and immunogenicity through prefusion stabilization with a double proline substitution²⁹. Both the viral and the vaccine spike protein are considered harmless and are not expected to circulate freely in the bloodstream, this being one essential aspect of vaccine safety as official data report³⁰⁻³². Indeed, the vaccine spike protein is synthesized by cells, it remains bound to the cellular membrane, and it is presented on the cell surface to the immune cells^{33,34}. Moreover, as from official data, the spike protein should remain in the vicinity of the injection site and local lymph nodes, where the immune response is initiated³⁵, and it may persist up to a few weeks after vaccination^{36,37}. The official data about spike protein have been challenged by recent studies^{38,39} that proposed that the spike protein has inherent toxicity, acting as an inflammagen and thus stimulating inflammation and blood hypercoagulation. Moreover, viral and vaccine spike proteins have been found in the bloodstream of individuals even months after infection clearance and vaccination⁴⁰⁻⁴⁵.

Considering the proposed persistence of spike protein in long-COVID syndrome, this study aimed to specifically investigate the presence of viral and vaccine spike proteins in the blood serum of long-COVID syndrome patients using mass spectrometry analysis^{46,47}. Secondly, polymerase chain reaction (PCR) was used for a preliminary study to check for SARS-CoV-2 integration in the long-COVID patients' leukocytes¹⁸, without further investigation^{48,49}.

Patients and Methods

Patient Recruitment

Patient recruitment was conducted based on clinical history and symptoms. We aimed to include a diverse cohort of 81 long-COVID syndrome patients,

ensuring representation across different age groups, genders, and disease severity. Informed consent was obtained from each participant, and ethical guidelines were strictly followed throughout the study. The study was approved by the Ethics Committee of Brescia (Italy) Prot. No. NP4588. All research process was conducted according to the ethical guidelines of the Declaration of Helsinki. A written informed consensus was obtained from all patients at the time of enrollment, and each of them was anonymized.

Mass Spectrometry

Mass spectrometry analysis was performed on the serum samples obtained from the recruited long-COVID syndrome patients, with the aim of detecting and quantifying spike protein fragments present in the samples. To achieve this, trypsin digestion was employed, generating specific tryptic fragments for each spike protein variant. The distinct tryptic fragments identified in the samples allowed for discrimination between the vaccine spike protein and the viral spike protein. The analysis was conducted using an HPLC Surveyor system (ThermoFisher, Waltham, MA, USA) equipped with a Halo Peptide ES-C18 column (2.1 x 50 mm, 2.7 μ m). A two-phase gradient was utilized, with Phase A consisting of H₂O with 0.2% Formic Acid (HCOOH) and Phase C consisting of acetonitrile (CH₃CN). A volume of 5 μ L of the sample was injected for analysis. Data acquisition was performed using a "SANIST" mass spectrometer, utilizing electrospray ionization (ESI) as the ionization source.

Data Analysis

Statistical analysis was not performed due to the descriptive design of our study. Data analysis was carried out to analyze the mass spectrometry data and draw meaningful conclusions. The analysis was processed by SANIST Hb software using a database containing the glycoprotein spike and other proteins randomly selected to increase accuracy. For the detection of the LDPPE-AEVQIDR fragment, ion extractions of the child ion fragments at m/z 577 of the ions at m/z 979.4 and m/z 830.3 (MS3 technique) were performed.

Results

Patient Recruitment and Clinical Data Analysis

The study included a total of 81 patients with long-COVID syndrome. Clinical data were available for 70 patients (Table I).

Mass Spectrometry Analysis

Out of the 81 long-COVID patients analyzed, fragments of the vaccine spike protein were found in 2 patients, while fragments of the viral spike protein were found in 1 patient (Table II). Control samples from unvaccinated individuals were negative for spike protein. The areas of the identified fragments were quantified

to assess the presence and abundance of the spike protein. Table III provides the areas of the standard and of the samples in which the vaccine protein was identified, as well as the corresponding percentages.

The samples in which vaccine spike protein was identified were collected at least two months after the administration of the second dose (Table IV).

Table I. Summary of clinical data for 70 patients.

Characteristics		Case subjects (n=70)
Sex	Male	35 (50%)
	Female	46 (65.71%)
Age (year)		52
BMI		26.1
Vaccine (YES)		51 (72.86%)
Severity score	Asymptomatic	0 (0%)
	Mild symptoms	34 (48.57%)
	Severe symptoms	35 (50%)
	Intensive care	1 (1.43%)
	Asthenia (during COVID)	7.8%
	Asthenia (long-COVID)	5.1%
	Headache (during COVID)	4.4%
Reinfection	Headache (long-COVID)	2.1%
	Yes	27 (38.57%)
Clinical data	No	43 (61.43%)
	Pneumonia (NO)	31 (44.29%)
	Pneumonia (YES)	39 (55.71%)
	Fever (NO)	16 (22.86%)
	Fever (YES)	54 (77.14%)
Serology	Not done	35 (50%)
	Negative	13 (18.57%)
	Doubtful	0 (0%)
	Positive	22 (31.43%)
Therapy	Paracetamol	50 (71.43%)
	Hydroxychloroquine	13 (18.57%)
	Antibiotics	42 (60%)
	Antivirals	18 (25.71%)
	Corticosteroids	23 (32.86%)
	Eparine	18 (25.71%)
	Ventilation	44 (62.86%)

BMI: body mass index.

Table II. Spike analysis processing results.

ID	Viral Spike Protein	Vaccinal Spike Protein (PP)
1	N.D.	Low signal
8	Signal	N.D.
37	N.D.	Low signal

N.D. = Not Detected.

Table III. Areas of the samples in which vaccine protein was identified and area of the standard.

ID	Area m/z 830.3	Area m/z 979.4	830.3%	979.4%
1	12.42	35.27	26.04	73.96
37	15.94	13.05	45.02	54.98
Std	6554	3385	65.94	34.06

Table IV. Vaccine and sample data for patients

ID	Type of vaccine	Date of 2 nd vaccine dose	Date of sample collection	Vaccine Spike protein	Viral Spike protein
1	Pfizer	02/2021	26/04/2021	Yes	No
37	Pfizer	02/2021	30/04/2021	Yes	No

Discussions

This study employed mass spectrometry analysis to investigate the presence of viral and vaccine spike proteins in the blood serum of patients with long-COVID syndrome. As reported in Table II, the mass spectrometry analysis revealed the presence of both viral and vaccine spike protein fragments in a subset of patients with long-COVID syndrome even two months after vaccination or after infection clearance and negativity of the COVID-19 test (Table IV). Official data sustain that the vaccine spike protein remains in the vicinity of the injection site and local lymph nodes and that it may persist in the body up to a few weeks after vaccination²⁰⁻²⁴. Our findings, in alignment with other studies and in contradiction with official data, show the presence of both the vaccine and the viral spike protein in the bloodstream even after infection clearance and several months after vaccination^{40-42,45-49}. Furthermore, viral integration in patients' leukocytes was assessed with a preliminary study following the protocol of Merchant¹⁸, without further investigation (**Supplementary Data**). Having detected the vaccinal protein in two subjects and the viral protein in one subject in a cohort of 95 patients, this study has mainly a descriptive function. Nevertheless, these results are aligned with many other already published literature performed on other independent cohorts. We conclude that considering the proposed toxicity of the spike protein and that official data sustain that it should not persist in blood circulation a few weeks after vaccination, blood samples of long-COVID patients should be routinely tested for the presence of vaccine and viral spike protein. Future research should focus on investigating the specific pathways and interactions through which viral and vaccine spike protein can circulate and persist in blood circulation several months after viral clearance or vaccination and on its possible negative effects.

Conclusions

This study, in accordance with other published investigations, shows the persistence in blood

circulation of viral spike protein in one patient after infection clearance and the negativity of the COVID-19 test, of vaccine spike protein in two patients two months after vaccination. This study underscores the importance of mass spectrometry analysis of long-COVID patients to detect spike protein persistence. Further research is needed to understand the underlying mechanisms of spike protein persistence.

Availability of Data and Materials

The data are within the test or in the supplementary materials document.

Funding

This research was funded by the Provincia Autonoma di Bolzano, in the framework of LP 14/2006.

Authors' Contributions

Conceptualization, M.B.; Methodology, S.C.; Investigation, F.F., A.C., A.P., M.G.D.A., G.A., and S.N.; Writing- original draft preparation, K.D., and M.C.M.; Writing, review and editing, C.M., K.D., A.M., F.F., A.C., A.P., M.G.D.A., G.A., S.C., S.N.; Project administration, M.B.; Funding acquisition, M.B. All authors have read and agreed to the published version of the manuscript.

Ethics Approval

The study was approved on the 12th of January 2021 by the Ethics Committee of the University of Brescia (Italy), Prot. No. NP4588.

Informed Consent

A written informed consensus was obtained from all patients at the time of enrollment, and each of them was anonymized.

Conflicts of Interest

The authors declare no conflict of interest.

References

- 1) Dennis A, Wamil M, Alberts J, Oben J, Cuthbertson DJ, Wootton D, Crooks M, Gabbay M, Brady M, Hishmeh L, Attree E, Heightman M, Banerjee

- R, Banerjee A; COVERSCAN study investigators. Multiorgan impairment in low-risk individuals with post-COVID-19 syndrome: a prospective, community-based study. *BMJ Open* 2021; 11: e048391.
- 2) Alkodaymi MS, Omrani OA, Fawzy NA, Shaar BA, Almamlouk R, Riaz M, Obeidat M, Obeidat Y, Gerberi D, Taha RM, Kashour Z, Kashour T, Berbari EF, Alkattan K, Tleyjeh IM. Prevalence of post-acute COVID-19 syndrome symptoms at different follow-up periods: a systematic review and meta-analysis. *Clin Microbiol Infect* 2022; 28: 657-666.
 - 3) Al-Aly Z, Bowe B, Xie Y. Long COVID after breakthrough SARS-CoV-2 infection. *Nat Med* 2022; 28: 1461-1467.
 - 4) Rando HM, Bennett TD, Byrd JB, Bramante C, Callahan TJ, Chute CG, Davis HE, Deer R, Gagnier J, Koraischy FM, Liu F, McMurry JA, Moffitt RA, Pfaff ER, Reese JT, Relevo R, Robinson PN, Saltz JH, Solomonides A, Sule A, Topaloglu U, Haendel MA. Challenges in defining Long COVID: Striking differences across literature, Electronic Health Records, and patient-reported information. *medRxiv [Preprint]* 2021 Mar 26:2021.03.20.21253896.
 - 5) Kumar S, Veldhuis A, Malhotra T. Neuropsychiatric and Cognitive Sequelae of COVID-19. *Front Psychol* 2021; 12: 577529.
 - 6) Huang L, Yao Q, Gu X, Wang Q, Ren L, Wang Y, Hu P, Guo L, Liu M, Xu J, Zhang X, Qu Y, Fan Y, Li X, Li C, Yu T, Xia J, Wei M, Chen L, Li Y, Xiao F, Liu D, Wang J, Wang X, Cao B. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet* 2021; 398: 747-758.
 - 7) Korompoki E, Gavriatopoulou M, Hicklen RS, Ntanas-Stathopoulos I, Kastritis E, Fotiou D, Stamatelopoulos K, Terpos E, Kotanidou A, Hagberg CA, Dimopoulos MA, Kontoyiannis DP. Epidemiology and organ specific sequelae of post-acute COVID-19: A narrative review. *J Infect* 2021; 83: 1-16.
 - 8) Mehandru S, Merad M. Pathological sequelae of long-haul COVID. *Nat Immunol* 2022; 23: 194-202.
 - 9) WHO. Coronavirus disease (COVID-19): Post COVID-19 condition. (Accessed on 07/09/2023) Available at: [https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-\(covid-19\)-post-covid-19-condition#:~:text=The%20most%20common%20symptoms%20associated,as%20work%20or%20household%20chores](https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-(covid-19)-post-covid-19-condition#:~:text=The%20most%20common%20symptoms%20associated,as%20work%20or%20household%20chores).
 - 10) Moldofsky H, Patcai J. Chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep in chronic post-SARS syndrome; a case-controlled study. *BMC Neurol* 2011; 11: 37.
 - 11) Pinzon RT, Wijaya VO, Buana RB, Al Jody A, Nunsio PN. Neurologic Characteristics in Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis. *Front Neurol* 2020; 11: 565.
 - 12) Churilov LP, Normatov MG, Utekhin VJ. Molecular Mimicry between SARS-CoV-2 and Human Endocrine Cells: A Prerequisite of Post-COVID-19 Endocrine Autoimmunity? *Pathophysiology* 2022; 29: 486-494.
 - 13) Son K, Jamil R, Chowdhury A, Mukherjee M, Venegas C, Miyasaki K, Zhang K, Patel Z, Salter B, Yuen ACY, Lau KS, Cowbrough B, Radford K, Huang C, Kjarsgaard M, Dvorkin-Gheva A, Smith J, Li QZ, Wasserman S, Ryerson CJ, Nair P, Ho T, Balakrishnan N, Nazy I, Bowdish DME, Svenningsen S, Carlsten C, Mukherjee M. Circulating anti-nuclear autoantibodies in COVID-19 survivors predict long COVID symptoms. *Eur Respir J* 2023; 61: 2200970.
 - 14) Petrillo M, Brogna C, Cristoni S, Querci M, Piazza O, Van den Eede G. Increase of SARS-CoV-2 RNA load in faecal samples prompts for rethinking of SARS-CoV-2 biology and COVID-19 epidemiology. *F1000 Res* 2021; 10: 370.
 - 15) Brogna C, Brogna B, Bisaccia DR, Lauritano F, Marino G, Montano L, Cristoni S, Prisco M, Piscopo M. Could SARS-CoV-2 Have Bacteriophage Behavior or Induce the Activity of Other Bacteriophages? *Vaccines (Basel)* 2022; 10: 708.
 - 16) Brogna C, Brogna B, Bisaccia DR, Giuliano M, Montano L, Cristoni S, Petrillo M, Piscopo M. SARS-CoV-2: Reinfection after 18 Months of a Previous Case with Multiple Negative Nasopharyngeal Swab Tests and Positive Fecal Molecular Test. *Medicina (Kaunas)* 2022; 58: 642.
 - 17) Proal AD, VanElzakker MB, Aleman S, Bach K, Boribong BP, Buggert M, Cherry S, Chertow DS, Davies HE, Dupont CL, Deeks SG, Eimer W, Ely EW, Fasano A, Freire M, Geng LN, Griffin DE, Henrich TJ, Iwasaki A, Izquierdo-Garcia D, Locci M, Mehandru S, Painter MM, Peluso MJ, Pretorius E, Price DA, Putrino D, Scheuermann RH, Tan GS, Tanzi RE, VanBrocklin HF, Yonker LM, Wherry EJ. SARS-CoV-2 reservoir in post-acute sequelae of COVID-19 (PASC). *Nat Immunol* 2023; 24: 1616-1627.
 - 18) Merchant HA. Comment on Aldén. Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr Issues Mol Biol* 2022; 44, 1115-1126. *Curr Issues Mol Biol* 2022; 44: 1661-1663.
 - 19) Smits N, Rasmussen J, Bodea GO, Amarilla AA, Gerdes P, Sanchez-Luque FJ, Ajjikuttira P, Modhiran N, Liang B, Faivre J, Deveson IW, Khromykh AA, Watterson D, Ewing AD, Faulkner GJ. No evidence of human genome integration of SARS-CoV-2 found by long-read DNA sequencing. *Cell Rep* 2021; 36: 109530.
 - 20) Zhang L, Richards A, Barrasa MI, Hughes SH, Young RA, Jaenisch R. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proc Natl Acad Sci U S A* 2021; 118: e2105968118.
 - 21) Whitehead Institute. Genomic Integration of the SARS-CoV-2 Virus and the Potential Relevance for the Course of COVID-19. Available at: <https://wi.mit.edu/events/video-genomic-integration-sars-cov-2-virus-and-potential-relevance-course-covid-19>.
 - 22) Kyriakopoulos AM, McCullough PA, Nigh G, Seneff S. Potential Mechanisms for Human Genome Integration of Genetic Code from SARS-CoV-2 mRNA

- NA Vaccination. Available at: <https://www.authorea.com/users/455597/articles/584039-potential-mechanisms-for-human-genome-integration-of-genetic-code-from-sars-cov-2-mrna-vaccination>.
- 23) Halma MTJ, Plothe C, Marik P, Lawrie TA. Strategies for the Management of Spike Protein-Related Pathology. *Microorganisms* 2023; 11: 1308.
 - 24) Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, Bullock TA, McGary HM, Khan JA, Razmpour R, Hale JF, Galie PA, Potula R, Andrews AM, Ramirez SH. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood-brain barrier. *Neurobiol Dis* 2020; 146: 105131.
 - 25) Lei Y, Zhang J, Schiavon CR, He M, Chen L, Shen H, Zhang Y, Yin Q, Cho Y, Andrade L, Shadel GS, Hepokoski M, Lei T, Wang H, Zhang J, Yuan JX, Malhotra A, Manor U, Wang S, Yuan ZY, Shyy JY. SARS-CoV-2 Spike Protein Impairs Endothelial Function via Downregulation of ACE2. *bioRxiv [Preprint]* 2020; 2020.12.04.409144.
 - 26) Murphy WJ, Longo DL. A Possible Role for Anti-idiotypic Antibodies in SARS-CoV-2 Infection and Vaccination. *N Engl J Med* 2022; 386: 394-396.
 - 27) Idrees D, Kumar V. SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration. *Biochem Biophys Res Commun* 2021; 554: 94-98.
 - 28) Trougakos IP, Terpos E, Alexopoulos H, Politou M, Paraskevis D, Scorilas A, Kastritis E, Andreaskos E, Dimopoulos MA. Adverse effects of COVID-19 mRNA vaccines: the spike hypothesis. *Trends Mol Med* 2022; 28: 542-554.
 - 29) Carnell GW, Ciazynska KA, Wells DA, Xiong X, Aguinam ET, McLaughlin SH, Mallery D, Ebrahimi S, Ceron-Gutierrez L, Asbach B, Einhauser S, Wagner R, James LC, Doffinger R, Heeney JL, Briggs JAG. SARS-CoV-2 Spike Protein Stabilized in the Closed State Induces Potent Neutralizing Responses. *J Virol* 2021; 95: e0020321.
 - 30) Mascellino MT, Di Timoteo F, De Angelis M, Oliva A. Overview of the Main Anti-SARS-CoV-2 Vaccines: Mechanism of Action, Efficacy and Safety. *Infect Drug Resist* 2021; 14: 3459-3476.
 - 31) Oliver SE, Gargano JW, Marin M, Wallace M, Curran KG, Chamberland M, McClung N, Campos-Outcalt D, Morgan RL, Mbaeyi S, Romero JR, Talbot HK, Lee GM, Bell BP, Dooling K. The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Moderna COVID-19 Vaccine - United States, December 2020. *MMWR Morb Mortal Wkly Rep* 2021; 69: 1653-1656.
 - 32) Cai C, Peng Y, Shen E, Huang Q, Chen Y, Liu P, Guo C, Feng Z, Gao L, Zhang X, Gao Y, Liu Y, Han Y, Zeng S, Shen H. A comprehensive analysis of the efficacy and safety of COVID-19 vaccines. *Mol Ther* 2021; 29: 2794-2805.
 - 33) Corbett KS, Edwards D, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, Himansu S, Schäfer A, Ziwawo CT, DiPiazza AT, Dinnon KH, Elbashir SM, Shaw CA, Woods A, Fritch EJ, Martinez DR, Bock KW, Minai M, Nagata BM, Hutchinson GB, Bahl K, Garcia-Dominguez D, Ma L, Renzi I, Kong WP, Schmidt SD, Wang L, Zhang Y, Stevens LJ, Phung E, Chang LA, Loomis RJ, Altaras NE, Narayanan E, Metkar M, Presnyak V, Liu C, Louder MK, Shi W, Leung K, Yang ES, West A, Gully KL, Wang N, Wrapp D, Doria-Rose NA, Stewart-Jones G, Bennett H, Nason MC, Ruckwardt TJ, McLellan JS, Denison MR, Chappell JD, Moore IN, Morabito KM, Mascola JR, Baric RS, Carfi A, Graham BS. SARS-CoV-2 mRNA Vaccine Development Enabled by Prototype Pathogen Preparedness. *bioRxiv [Preprint]* 2020; 2020.06.11.145920.
 - 34) Cosentino M, Marino F. Understanding the Pharmacology of COVID-19 mRNA Vaccines: Playing Dice with the Spike? *Int J Mol Sci* 2022; 23: 10881.
 - 35) Ols S, Yang L, Thompson EA, Pushparaj P, Tran K, Liang F, Lin A, Eriksson B, Karlsson Hedestam GB, Wyatt RT, Loré K. Route of Vaccine Administration Alters Antigen Trafficking but Not Innate or Adaptive Immunity. *Cell Rep* 2020; 30: 3964-3971.e7.
 - 36) Horwitz JR, Wiley LF. Not Ready for the End Game - Why Ending Federal Covid-19 Emergency Declarations Will Harm Access to Care. *N Engl J Med* 2022; 386: e40.
 - 37) Stein SR, Ramelli SC, Grazioli A, Chung JY, Singh M, Yinda CK, Winkler CW, Sun J, Dickey JM, Ylaja K, Ko SH, Platt AP, Burbelo PD, Quezado M, Pittaluga S, Purcell M, Munster VJ, Belinky F, Ramos-Benitez MJ, Boritz EA, Lach IA, Herr DL, Rabin J, Saharia KK, Madathil RJ, Tabatabai A, Soherwardi S, McCurdy MT; NIH COVID-19 Autopsy Consortium; Peterson KE, Cohen JI, de Wit E, Vannella KM, Hewitt SM, Kleiner DE, Chertow DS. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. *Nature* 2022; 612: 758-763.
 - 38) Grobbelaar LM, Venter C, Vlok M, Ngoepe M, Laubscher GJ, Lourens PJ, Steenkamp J, Kell DB, Pretorius E. SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. *Biosci Rep* 2021; 41: BSR20210611.
 - 39) Pistollato F, Petrillo M, Clerbaux LA, Leoni G, Ponti J, Bogni A, Brogna C, Cristoni S, Sanges R, Mendoza-de Gyves E, Fabri M, Querci M, Soares H, Munoz A, Whelan M, Van de Eede G. Effects of spike protein and toxin-like peptides found in COVID-19 patients on human 3D neuronal/glia model undergoing differentiation: Possible implications for SARS-CoV-2 impact on brain development. *Reprod Toxicol* 2022; 111: 34-48.
 - 40) Ogata AF, Cheng CA, Desjardins M, Senussi Y, Sherman AC, Powell M, Novack L, Von S, Li X, Baden LR, Walt DR. Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients. *Clin Infect Dis* 2022; 74: 715-718.
 - 41) Swank Z, Senussi Y, Manickas-Hill Z, Yu XG, Li JZ, Alter G, Walt DR. Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike

- Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. *Clin Infect Dis* 2023; 76: e487-e490.
- 42) Angeli F, Spanevello A, Reboldi G, Visca D, Verdecchia P. SARS-CoV-2 vaccines: Lights and shadows. *Eur J Intern Med* 2021; 88: 1-8.
- 43) Yonker LM, Swank Z, Bartsch YC, Burns MD, Kane A, Boribong BP, Davis JP, Loisel M, Novak T, Senussi Y, Cheng CA, Burgess E, Edlow AG, Chou J, Dionne A, Balaguru D, Lahoud-Rahme M, Arditi M, Julg B, Randolph AG, Alter G, Fasano A, Walt DR. Circulating Spike Protein Detected in Post-COVID-19 mRNA Vaccine Myocarditis. *Circulation* 2023; 147: 867-876.
- 44) Castruita JAS, Schneider UV, Mollerup S, Leinewer TD, Weis N, Bukh J, Pedersen MS, Westh H. SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination. *APMIS* 2023; 131: 128-132.
- 45) Brogna C, Cristoni S, Marino G, Montano L, Viduto V, Fabrowski M, Lettieri G, Piscopo M. Detection of recombinant Spike protein in the blood of individuals vaccinated against SARS-CoV-2: Possible molecular mechanisms. *Proteomics Clin Appl* 2023; e2300048.
- 46) Sun Z, Ren K, Zhang X, Chen J, Jiang Z, Jiang J, Ji F, Ouyang X, Li L. Mass Spectrometry Analysis of Newly Emerging Coronavirus HCoV-19 Spike Protein and Human ACE2 Reveals Camouflaging Glycans and Unique Post-Translational Modifications. *Engineering (Beijing)* 2021; 7: 1441-1451.
- 47) Cardozo KHM, Lebkuchen A, Okai GG, Schuch RA, Viana LG, Olive AN, Lazari CDS, Fraga AM, Granato CFH, Pintão MCT, Carvalho VM. Establishing a mass spectrometry-based system for rapid detection of SARS-CoV-2 in large clinical sample cohorts. *Nat Commun* 2020; 11: 6201.
- 48) Smits N, Rasmussen J, Bodea GO, Amarilla AA, Gerdes P, Sanchez-Luque FJ, Ajjikuttira P, Modhiran N, Liang B, Faivre J, Deveson IW, Khromykh AA, Watterson D, Ewing AD, Faulkner GJ. No evidence of human genome integration of SARS-CoV-2 found by long-read DNA sequencing. *Cell Rep* 2021; 36: 109530.
- 49) Brogna C, Cristoni S, Petrillo M, Querci M, Piazza O, Van den Eede G. Toxin-like peptides in plasma, urine and faecal samples from COVID-19 patients. *F1000 Res* 2021; 10: 550.