Brief Report

Towards Rigorous Diagnostics for Vaccine Injury

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Towards Rigorous Diagnostics for Vaccine Injury

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Abstract: Delineating the epidemic of vaccine injury from the coterminous condition long covid is a challenging prospect, but one with many implications not just for treatment, but also has important legal considerations for settlements of vaccine injury. The shared etiological factor of the spike protein in both vaccine injury and long covid make differentiation difficult, and while treatment is largely similar between vaccine injury and long covid, there are important distinctions. Furthermore, diagnostics are important for monitoring treatment progress and assessing the extent of subclinical vaccine injury in population having received a covid-19 vaccine. The development of rigorous diagnostics is an important step towards the recognition of both long covid and vaccine injury, as those suffering these conditions have faced immense challenges in having their conditions recognized, treated, and compensated by insurance companies or national health services.

Introduction

Adverse events after vaccination have been far more common with Covid-19 vaccines than any other licensed vaccine[1]. Not only are the rates of clinical conditions associated with Covid-19 vaccines high, but there is also extensive subclinical damage. Due to the pathological mechanisms of the vaccine encoded spike protein, the potential for damage can exist at low levels for long periods of time, and those having received a vaccine can be in a 'sword of Damocles' situation for years or even decades. Frankly, there is a lot left unknown about the long-term effects of Covid-19 vaccines[2].

The extent of subclinical danger, as well as the increase in sudden and unexplained deaths motivates the diagnosis of vaccine injury through biomarkers. One immediate biomarker that comes to the fore is testing for the presence of the spike protein or its subunits in plasma[3], as it is a major pathological agent driving vaccine injury, long covid, as well as acute covid-19 infection[4].

This conflation has important legal implications for those seeking compensation after injury following Covid-19 vaccination. Pathologists can assign causality to covid-19 vaccination (as opposed to infection) by immunostaining for the nucleocapsid (N) protein in addition to the spike (S) protein[5]. Since Covid-19 infection will express both N and S proteins, whereas vaccination only expresses the S protein, the simultaneous presence of the S protein and absence of the N protein is strong evidence for vaccine induced causation[5].

Furthermore, there are two important differentiations between the spike protein induced by vaccination and the spike protein from infection. First, the viral spike protein will change as the virus mutates, whereas the vaccine spike protein only changes when the sequence is updated (as with the bivalent booster). Secondly, the vaccine spike protein is locked into a prefusion conformation through two proline mutations, and will adopt a more rigid conformation than the viral spike protein[6].

There are three important ways to differentiate spike protein from viral infection from that of the vaccine. The following factors can be used to differentiate vaccine damage from viral damage (Table 1).

Table 1. Basis of diagnostic difference between vaccine damage and damage from SARS-CoV-2.

Vaccine Spike	Viral Spike
No N protein present	N protein present
Sequence identical to vaccine sequence	Sequence much les constrained, reflects currently circulating variants
Locked into prefusion conformation	Conformationally flexible

Diagnostics

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General

In guiding treatment, there are multiple biomarkers that one can test with to gain insight into the progression of the injury sustained from the vaccine. These are non-specific to vaccine injury and are general biomarkers of cardiovascular risk. These include troponin, D-dimer and C-reactive protein[3].

[4]. These biomarkers are specific to cardiac injury, and will not be able to determine disease aetiology.

Troponin is a general biomarker associated with diagnosis of acute coronary syndromes[7,8], as troponins are released into the blood following damage to cardiac muscle[9]. D-dimer is a biomarker associated with the breakdown of fibrin clots by the fibrinolytic system [10]. As the test measures breakdown of clots, a high measure can indicate a high level of clot burden, as well as a high degree of breakdown[11], and this must be taken into consideration by the clinician.

C-reactive protein is an inflammatory biomarker, and higher values are associated with increased cardiovascular risk[12].

Biomarker	Upper limit of normal
Peak cardiac troponin (T)	14 ng/L [3]
Brain natriuretic Peptide (BNP)	100pg/mL [3]
N-terminal prohormone of brain natriuretic peptide (NT-proBNP)	450pg/mL [3]
C-reactive protein (CRP)	8mg/L [3]
D-dimer	(patient's age in years x 10mcg/L) [13]

Specific

A recent paper by Yonker surveyed the biomarkers of vaccinated individuals, both with and without post-vaccination myocarditis. The main differentiator between the group with myocarditis and those without was the persistence of full length spike protein, unbound by antibodies[3]. Given that this is the sole gene encoded by most of the vaccines and has multiple documented pathological mechanisms[4], it is a likely aetiological factor in post-vaccination syndrome.

Cases of blood thrombosis after vaccination typically occur within one month of receiving the injection[14,15]. A test for spike protein contains two important quantities, the concentration of spike protein, as well as the time since vaccination. While most often after injection spike protein concentration drops off quickly after one week [16], persistence of high levels of spike protein for months after injection has been documented in a subset of vaccinated individuals [17]. It is unclear what the individual factors are affecting long-term spike protein levels; we propose a model for the long term persistence of spike protein.

The first factors are the variations in the initial dose of spike protein encoding mRNA, which can vary due to storage, dilution and administration. Once the mRNA is in the body, the level of spike is in competition between mRNA degradation and protein expression from the mRNA. We also propose a third alternative between degradation and expression, that of conversion to a reservoir. Reverse transcription into the genome is possible [18]. Additionally, a discovery of DNA contamination in a broad swathe of mRNA vaccine vials[19], potentially opening the possibility of but microbiota transfection through the mechanism of horizontal gene transfer [20].

While the half-life of RNA is well known, and endogenous mRNA has a half life of approximately 10 hours [21], it is known that pseudouridinylated RNA is far more persistent[22,23], and less is known about the degradation of the N1-methyl-psuedouridnylated RNA used in the mRNA vaccines[2] and persistent spike protein appears to be the factor which differentiates those with post-vaccination myocarditis vs vaccinated people without myocarditis[3].

Considerations

Causation

There is an unprecedented wave of vaccine injury, in addition to any disease burden from long Covid. The origins of Covid notwithstanding, establishing causation for those experiencing vaccine injury is an important step both in allowing them to receive just compensation, as well as to establish the true role of vaccination in the mortality and morbidity burdens. The latter is useful both for informing regulatory policy going forward, as it is necessary for regulators and the public to know the true risk profile of this class of intervention. Additionally, establishing causation is useful for legal settings, including compensation for injured recipients as well as prosecution of any wrongdoing. The extent of liability is important, as those seeking treatment often have few options, and little resources, owing to the often debilitating nature of their illness, and its lack of acknowledgement and subsequent compensation by health systems[24]. Vaccine injury compensation schemes are uncommon [25,26].

The experience of the vaccine injured has largely been one of gaslighting and being ignored, and only now are their concerns being heard[24]. Still, treatment is limited, and limited resources exit for injury compensation [27]. Treatment of long Covid is receiving some attention and research funds [28], while treatment of vaccine injury is limited. For example, in the US clinical trials database (clinicaltrials.gov, accessed July 11, 2023) there is currently one study to test treatment of Covid-19 vaccine injury; the study is not yet recruiting and was last updated May 24, 2022. Multiple studies exist to treat long Covid, reviewed in [29]. No large university hospital or academic medical center has published a treatment protocol for vaccine injury, and the current literature is scant [29].

Conclusion

While the situation of the vaccine injured presents a pessimistic view, the situation is improving. Vaccine injury is increasingly being recognized, as a recent acknowledgement by German health minister Karl Lauterbach exemplifies [30]. Still, for those affected, it is a long road to recovery. Diagnostics are a necessary part of the path towards health in those experiencing post-vaccination syndrome and long covid. In some cases, the diagnostics are similar, but the potential also exists to discriminate the two conditions with diagnostics, as well as by patient history.

Developing rigorous diagnostics is an important step towards gauging treatment progress and informing the science of treating vaccine injury, as well as long covid. Diagnostic development ensures that those affected receive the recognition and treatment they deserve, and ensures the integrity of compensation claims, a and can inform legal action against regulators, pharmaceutical manufacturers and public health officials.

References

1. Kim, M.S.; Jung, S.Y.; Ahn, J.G.; Park, S.J.; Shoenfeld, Y.; Kronbichler, A.; Koyanagi, A.; Dragioti, E.; Tizaoui, K.; Hong, S.H.; et al. Comparative Safety of MRNA COVID-19 Vaccines to Influenza Vaccines: A Pharmacovigilance Analysis Using WHO International Database. *Journal of Medical Virology* **2022**, *94*, 1085–1095, doi:10.1002/jmv.27424.
2. Halma, M.T.J.; Rose, J.; Lawrie, T. The Novelty of MRNA Viral Vaccines and Potential Harms: A Scoping Review. *J* **2023**, *6*, 220–235, doi:10.3390/j6020017.
3. Yonker, L.M.; Swank, Z.; Bartsch, Y.C.; Burns, M.D.; Kane, A.; Boribong, B.P.; Davis, J.P.; Loiselle, M.; Novak, T.; Senussi, Y.; et al. Circulating Spike Protein Detected in Post–COVID-19 MRNA Vaccine Myocarditis. *Circulation* **2023**, *147*, 867–876, doi:10.1161/CIRCULATIONAHA.122.061025.
4. Theoharides, T.C.; Conti, P. Be Aware of SARS-CoV-2 Spike Protein: There Is More than Meets the Eye. *J Biol Regul Homeost Agents* **2021**, *35*, 833–838, doi:10.23812/THEO_EDIT_3_21.
5. Mörz, M. A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 MRNA Vaccination against COVID-19. *Vaccines* **2022**, *10*, 1651, doi:10.3390/vaccines10101651.
6. Riley, T.P.; Chou, H.-T.; Hu, R.; Bzymek, K.P.; Correia, A.R.; Partin, A.C.; Li, D.; Gong, D.; Wang, Z.; Yu, X.; et al. Enhancing the Prefusion Conformational Stability of SARS-CoV-2 Spike Protein Through Structure-Guided Design. *Frontiers in Immunology* **2021**, *12*.
7. Mahajan, V.S.; Jarolim, P. How to Interpret Elevated Cardiac Troponin Levels. *Circulation* **2011**, *124*, 2350–2354, doi:10.1161/CIRCULATIONAHA.111.023697.
8. Melanson, S.E.F.; Morrow, D.A.; Jarolim, P. Earlier Detection of Myocardial Injury in a Preliminary Evaluation Using a New Troponin I Assay with Improved Sensitivity. *Am J Clin Pathol* **2007**, *128*, 282–286, doi:10.1309/Q9W5HJTT24GQCXXX.

9. Apple, F.S.; Sandoval, Y.; Jaffe, A.S.; Ordonez-Llanos, J.; IFCC Task Force on Clinical Applications of Cardiac Bio-Markers Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. *Clin Chem* **2017**, *63*, 73–81, doi:10.1373/clinchem.2016.255109.
10. Thachil, J.; Lippi, G.; Favaloro, E.J. D-Dimer Testing: Laboratory Aspects and Current Issues. In *Hemostasis and Thrombosis: Methods and Protocols*; Favaloro, E.J., Lippi, G., Eds.; Methods in Molecular Biology; Springer: New York, NY, 2017; pp. 91–104 ISBN 978-1-4939-7196-1.
11. Linkins, L.-A.; Takach Lapner, S. Review of D-Dimer Testing: Good, Bad, and Ugly. *International Journal of Laboratory Hematology* **2017**, *39*, 98–103, doi:10.1111/ijlh.12665.
12. Ridker, P.M. A Test in Context. *Journal of the American College of Cardiology* **2016**, *67*, 712–723, doi:10.1016/j.jacc.2015.11.037.
13. Urban, K.; Kirley, K.; Stevermer, J.J. PURLs: It's Time to Use an Age-Based Approach to D-Dimer. *J Fam Pract* **2014**, *63*, 155–158.
14. Bilotta, C.; Perrone, G.; Adelfio, V.; Spatola, G.F.; Uzzo, M.L.; Argo, A.; Zerbo, S. COVID-19 Vaccine-Related Thrombosis: A Systematic Review and Exploratory Analysis. *Front Immunol* **2021**, *12*, 729251, doi:10.3389/fimmu.2021.729251.
15. Mani, A.; Ojha, V. Thromboembolism after COVID-19 Vaccination: A Systematic Review of Such Events in 286 Patients. *Annals of Vascular Surgery* **2022**, *84*, 12-20.e1, doi:10.1016/j.avsg.2022.05.001.
16. Ogata, A.F.; Cheng, C.-A.; Desjardins, M.; Senussi, Y.; Sherman, A.C.; Powell, M.; Novack, L.; Von, S.; Li, X.; Baden, L.R.; et al. Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of MRNA-1273 Vaccine Recipients. *Clinical Infectious Diseases* **2022**, *74*, 715–718, doi:10.1093/cid/ciab465.
17. Bansal, S.; Perincheri, S.; Fleming, T.; Poulson, C.; Tiffany, B.; Bremner, R.M.; Mohanakumar, T. Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer–BioNTech) Vaccination Prior to Development of Antibodies: A Novel Mechanism for Immune Activation by MRNA Vaccines. *The Journal of Immunology* **2021**, *207*, 2405–2410, doi:10.4049/jimmunol.2100637.
18. Aldén, M.; Olofsson Falla, F.; Yang, D.; Barghouth, M.; Luan, C.; Rasmussen, M.; De Marinis, Y. 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, doi:10.3390/cimb44030073.
19. McKernan, K.; Helbert, Y.; Kane, L.T.; McLaughlin, S. Sequencing of Bivalent Moderna and Pfizer MRNA Vaccines Reveals Nanogram to Microgram Quantities of Expression Vector DsDNA per Dose. *OSF Preprints. April* **2023**, *10*.
20. Brito, I.L. Examining Horizontal Gene Transfer in Microbial Communities. *Nat Rev Microbiol* **2021**, *19*, 442–453, doi:10.1038/s41579-021-00534-7.
21. Lugowski, A.; Nicholson, B.; Rissland, O.S. Determining MRNA Half-Lives on a Transcriptome-Wide Scale. *Methods* **2018**, *137*, 90–98, doi:10.1016/j.ymeth.2017.12.006.
22. Karikó, K.; Muramatsu, H.; Welsh, F.A.; Ludwig, J.; Kato, H.; Akira, S.; Weissman, D. Incorporation of Pseudouridine Into MRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability. *Molecular Therapy* **2008**, *16*, 1833–1840, doi:10.1038/mt.2008.200.
23. Leppek, K.; Byeon, G.W.; Kladwang, W.; Wayment-Steele, H.K.; Kerr, C.H.; Xu, A.F.; Kim, D.S.; Topkar, V.V.; Choe, C.; Rothschild, D.; et al. Combinatorial Optimization of MRNA Structure, Stability, and Translation for RNA-Based Therapeutics. *Nat Commun* **2022**, *13*, 1536, doi:10.1038/s41467-022-28776-w.
24. Fairgrieve, D.; Rizzi, M.; Kirchhelle, C.; Halabi, S.; Howells, G.; Witzleb, N. No-Fault Compensation Schemes for COVID-19 Vaccines: Best Practice Hallmarks. *Public Health Rev* **2023**, *44*, 1605973, doi:10.3389/phrs.2023.1605973.
25. Fairgrieve, D.; Borghetti, J.-S.; Dahan, S.; Goldberg, R.; Halabi, S.; Holm, S.; Howells, G.; Kirchelle, C.; Pillay, A.; Rajneri, E. Comparing No-Fault Compensation Systems for Vaccine Injury. *Tul. J. Int'l & Comp. L.* **2023**, *31*, 75.
26. Crum, T.; Mooney, K.; Tiwari, B.R. Current Situation of Vaccine Injury Compensation Program and a Future Perspective in Light of COVID-19 and Emerging Viral Diseases. *F1000Res* **2021**, *10*, 652, doi:10.12688/f1000research.51160.2.
27. Demasi, M. Covid-19: Is the US Compensation Scheme for Vaccine Injuries Fit for Purpose? *BMJ* **2022**, *377*, o919, doi:10.1136/bmj.o919.
28. Long Covid Is a 'National Crisis.' So Why Are Grants Taking so Long to Get? Available online: https://www.science.org/content/article/long-covid-national-crisis-so-why-are-grants-taking-so-long-get (accessed on 11 July 2023).
29. Halma, M.T.J.; Plothe, C.; Marik, P.; Lawrie, T.A. Strategies for the Management of Spike Protein-Related Pathology. *Microorganisms* **2023**, *11*, 1308, doi:10.3390/microorganisms11051308.
30. Post-Vac Syndrome — the Forgotten COVID Victims – DW – 03/21/2023 Available online: https://www.dw.com/en/post-vac-syndrome-the-forgotten-covid-victims/a-65051748 (accessed on 11 July 2023).

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