EXPERT REVIEW OF VACCINES
https://doi.org/10.1080/14760584.2021.1965884

Taylor & Francis
Taylor & Francis Group

PERSPECTIVE

Sars-Cov-2 virus and vaccination; biological and statistical framework

Ferhat Arslan[a] and Handan Ankaralı [b]

[a]Department of Infectious Diseases and Clinical Microbiology, Istanbul Medeniyet University, Istanbul, Turkey; [b]Department of Biostatistics, Istanbul Medeniyet University, Istanbul, Turkey

ABSTRACT

Introduction: The Development of the SARS-CoV-2 virus vaccine and its update on an ongoing pandemic is the first subject of the world health agenda.

Areas covered: First, we will scrutinize the biological features of the measles virus (MV), variola virus (smallpox virus), influenza virus, and their vaccines to compare them with the SARS-CoV-2 virus and vaccine. Next, we will discuss the statistical details of measuring the effectiveness of an improved vaccine.

Expert opinion: Amidst the pandemic, we ought to acknowledge our prior experiences with respiratory viruses and vaccines. In the planning stage of observational Phase-III vaccine effectiveness studies, the sample size, sampling method, statistical model, and selection of variables are crucial in obtaining high-quality and valid results.

ARTICLE HISTORY
Received 30 April 2021
Accepted 5 August 2021

KEYWORDS
SARS-CoV-2; COVID-19; coronavirus; severe acute respiratory syndrome; vaccination

1. Introduction

Vaccination is the process of introducing live (often weakened) or inactivated forms or some fractions of microbes to the host immune system [1]. An effective vaccine can be developed by understanding the germ, its reservoir, the transmission route, and factors of disease pathogenesis. In this article, we will try to present a biological perspective on SARS-CoV-2 vaccines and a statistical concept for vaccine studies in light of our knowledge and experience in other respiratory viral infections.

1.1. Biological concept

Vaccination studies against RNA viruses, such as human immunodeficiency virus (HIV), hepatitis C (HCV), and respiratory stress virus (RSV), which cause widespread disease worldwide, have so far failed. After the discovery of an effective and immunogenic vaccine for measles and smallpox viruses, the implementation of a widespread vaccination program lead to an eradication globally [2]. However, every virus and developed vaccine should be evaluated within context. For instance, the divergence of influenza vaccine strains with circulating viruses has limited the effectiveness of the vaccine [3]. For this reason, new chimeric influenza vaccine studies continue despite 80 years having passed since the development of the first influenza vaccine. Measles and smallpox vaccines are live vaccines developed for a limited number of genotypes, and both have only one serotype that is responsible for the epidemics. In case of the SARS-CoV-2 virus, many strains have already been identified and new mutated strains continue to be reported (B.1.1.7 lineage, B.1.351 lineage, B.1.1.28 lineage, B.1.232/B.1.427/B.1.429) [4]. The animal reservoir of the SARS-CoV-2 virus before human transmission is not yet known. The presence of its form has also been seen in some species [5]. It is thought that primitive ancestors of some viruses evolved by transitioning from possible zoonotic sources (bats) to human and close species (distemper viruses infecting cats and dogs, cowpox virus infecting cattle) [6–8]. To date, human measles and smallpox viruses have not been shown to cause non-human diseases. This discrepancy supports the non-biological root theories of SARS-CoV-2 virus. No definite data have yet been revealed about the zoonotic source of SARS-CoV-2. As mentioned above, this brings the question of an evolutionary transition process to an end at the starting point. On the contrary, it has been shown that this virus is transmitted from human source to ferrets, minks, hamsters, and felines [9]. S-protein and DNA-based vaccines for SARS-CoV and DNA-based vaccines for the MERS (Middle East Respiratory Syndrome) virus were discontinued at the Phase 1 study level [10]. In animal model studies, antibody-mediated injuries developed with these vaccines have led to an inquisition of the safety of the vaccine [11]. The mRNA vaccines that are based on in situ transcriptomic technologies are developed under operation warp speed and may be the trigger of non-physiological processes due to mRNA localization challenges [12].

The dissimilarities and similarities between measles, smallpox, influenza and SARS-Cov-2 viruses, diseases, and vaccines are summarized in Table 1.

The cytopathic effect associated with SARS-CoV-2 was confirmed by demonstrating the destruction of basal epithelial integrity and cilia shrinkage in cultured organotypical human airway epithelial cells (HAE) [13]. Researchers dispute that the

CONTACT Ferhat Arslan ferhatarslandr@hotmail.com Infectious Diseases and Clinical Microbiology, Istanbul Medeniyet University, Eğitim Mah. Dr. Erkin Cad., Kadıköy/İstanbul 34722, Turkey

Article highlights

- Although SARS-CoV-2 vaccines were put into practice with the emergency use code, basic biological and statistical knowledge should be prioritized. Experiences of viral infections, which have the potential to cause epidemics and are transmitted by respiratory tract, should not be ignored.
- Neutralizing antibody titers of the coronaviruses rapidly waned, while specific T-cell activity was more durable. In both clinical efficacy studies and in vitro conditions, neutralizing antibodies have shown to be less effective in invariant laboratory viruses (B.1.1.7 variant) than in non-variant viruses. This suggests that long-term protection should be provided through cellular immunity.
- The vaccination efficacy measured in RCTs (Random Controlled Test) under ideal conditions may differ from the vaccination effectiveness is expected to be an observational study normally in non-ideal conditions and in different populations.

adaptation of SARS-CoV-2 for human airway is the strongest and most distinctive of the other coronaviruses in this study. In vitro infectivity was assessed for blood components, failing to detect a virus in blood that may have been related to immune cells is not permissive for SARS-COV-2 virus [14]. Additionally, the involvement of regional lymph nodes is not emphasized and COVID-19 remains mostly limited in the respiratory system in an autopsy study [15]. On the contrary, both T-cells and B cells are permissive for measles virus infection that showed in lymphocytes subset derived from tonsils and bloods [16]. Immune cells permission to the measles virus and smallpox virus led to viral dissemination (Trojan horse) to the other organs [17]. Complications of viremia are relatively rare in both influenza and SARS-CoV-2 virus [18,19]. Vaccination strategy should not be determined according to this rare patient group. Vaccines induced immunity must primarily block the mucosal entry of these viruses. Transcutaneous immunization to these viruses provides relatively partial mucosal immunity [20]. Based on mucosal challenge testing, influenza virus-specific sIgA responses are weak with inactivated influenza vaccines (IIV) compared to live attenuated influenza vaccines (LAIV) [21]. Neutralizing antibody titers to the coronaviruses rapidly wane, while specific T-cells activity is more durable [22]. This suggests that the long-term protection should be provided through cellular immunity. In both clinical efficacy studies and in vitro conditions, neutralizing antibodies have been shown to be less effective in variant laboratory viruses (B.1.1.7 variant) than in non-variant viruses [23]. Additionally, cross-reactive T cell memory implications to the COVID-19 disease severity and herd immunity can partially explain the epidemiological differences among countries [24]. However, a live attenuated vaccine does not seem to be in the foreground of SARS-COV-2 vaccine pipeline [25].

1.2. Statistical concept

The vaccine Phase-III studies aim to evaluate the clinical efficacy and effectiveness and also the safety of the vaccine in a large population. Phase-IV studies are planned in order to predict the long-term effects of vaccines and to examine the rare side effects. These studies are conducted as multinational and multicentric. Volunteers who participated in the study were given the vaccine and placebo applications; this was to reinforce the double-blind and randomness principles. In order to avoid confounded results with the effects of baseline features measured, unmeasured and/or unobservable in the study, the treatment is allocated randomly [26]. There have been two ways to predict the effects of a vaccine in Phase-III studies; this could be by obtaining the estimates of the efficacy from randomized control trials (RCTs) and also estimates of effectiveness from observational studies [27]. The vaccination efficacy measured in RCTs under ideal conditions may differ from the vaccination effectiveness estimated in the observational study normally in non-ideal conditions and in different populations. The greatest strength of RCTs is that they produce results with high internal validity. However, a rigid design reduces the external validity and limits the generalizability of the results. Ethical and technical reasons delay the establishment of standardized conditions, randomization, or blinding, etc. For these reasons, well-designed observational studies are recommended. Especially in prospective cohort studies, when the appropriate sample size and follow-up period are determined, they give reliable results like RCTs and their external validity is better than RCTs. It is also generally less expensive than RCTs and is more advantageous for investigating rare results. Because observational studies are always non-randomized and volunteer individuals participate in the study, undesirable situations, such as selection bias and confounders occur [28]. Statistical methods, such as propensity scores, regression adjustment, or marginal structural models, are used in data analysis to eliminate bias and confounder effects. The propensity score method targets causal inference in observational studies in a manner similar to randomized experiments by facilitating the measurement of differences in outcomes between the vaccinated and placebo participants [29]. Although these two groups are distributed with similar characteristics, whether by randomization or other matching methods, the interaction effect should be evaluated in the model to be established. In general, a single primary endpoint and three or four secondary endpoints can be used in order to evaluate the vaccine effectiveness has been examined. If co-primary endpoints are to be used, they require some adjustment for multiple testings such as false discovery rate.

To achieve reliable results in the real-world vaccine effectiveness study, the following considerations should be taken into account. The sample size should be calculated according to interaction terms and primary endpoints in the model by a priori power analysis. The sampling schema should be planned as a multistage that has to combine a stratified random sample and cluster random sample. The participants should be taken from all over the country to account for regional differences. The subjects should be randomly assigned to the vaccine and placebo groups. Hence, they should be roughly equal in terms of behaviors, opinions about the pandemic, and how seriously they take precautions. Also, randomization helps avoid the problems associated with correlations not implying causation [30]. Both clinical/biological and statistical significance should be taken into

Table 1. Virological, pathophysiological, and vaccine features of smallpox, measles, influenza, and Sars-Cov-2 viruses.

Virus	Genotypes	Cell entry receptor/cells	Pathophysiology	Vaccine type
Smallpox (**DNA virus**)	**Variola majör,minor**	Entry-fusion complex[a]	• Respiratory epithelium • primary viremia (?) • Macrophages • **Regional lymph node amplification** • **Secondary viremia** • Bacterial superinfections	***Live virus***
Measle (**single-stranded, negative-sense, enveloped RNA**)	8 clades of measles (A–H) **There is only one measles serotype**	CD46[b] SLAMF1[c] Nectin-4[d]	• Respiratory epithelium • primary viremia • MHC class II + CD11 c + dendritic cells • **Regional lymph node amplification** • **Secondary viremia** • Bacterial superinfections	***Live virus***
Influenza A	Types, A, B and C 16 HA subtypes and 9 NA subtypes	α2,6- or α2,3-linked sialic acid	• Respiratory epithelium • Diffuse alveolar damage • **Viremia ?** • Bacterial superinfections	IIV3 IIV4 LAIV4 RIV4
SARS-COV-2 (**single-stranded, Positive-sense RNA**)	Seven main strains: O, L, S, V, G, GR, and GH 23 subtypes New mutant variants	ACE-2 receptor	• Respiratory epithelium • Pulmonary II pneumocytes • Diffuse alveolar damage • **Viremia ?** • Bacterial superinfections	İnactive virus, mRNA, DNA, Vector intermediate

[a]Four proteins are involved in attachment to glycosaminoglycans and laminin, and a complex of 11 proteins that are conserved in all poxviruses mediates the hemifusion and entry steps.
[b]CD46 is a regulator that normally prevents cells from complement-mediated self-destruction, and is found on the surface of all human cells, with the exception of erythrocytes
IIV3 = inactivated influenza vaccine, trivalent; IIV4 = inactivated influenza vaccine, quadrivalent; LAIV4 = live attenuated influenza vaccine, quadrivalent; RIV4 = recombinant influenza vaccine, quadrivalent

consideration when deciding whether to include a variable in the model. The Purposeful Variables Selection algorithm can be used at each step of the modeling process. This algorithm will provide the retention of significant covariates, as well as confounding ones [26]. Before the modeling process, it should be discussed which variables are related to primary and secondary objectives, and the relation between these variables and outcome, and also their relationships with each other should be defined. The variables in the model are named as confounder, effect modifiers (or interaction), according to their current relationships. A confounder is a variable that influences both the exposure and outcome, causing a relationship that does not actually exist, and confounding factors are a nuisance. Confounding factors need to be eliminated to avoid misinterpretation of the results. Effect Modification is not a nuisance [28]. It provides important information. If there is an effect modifier in the model, the analysis performed by ignoring the values of this variable is misleading. Stratified analysis is required.

Vaccine effectiveness studies contain a data structure similar to survival analysis that is commonly used in cancer research. Time-to-event data (survival data) analysis techniques can be used to estimate the effectiveness of a vaccine using data from an observational study. Therefore, it is recommended to use multiple and/or multivariate regression models with survival times for evaluating vaccine effectiveness and safety, one of which is a Cox proportional hazard regression model. Calculating the more accurate predictions with regard to vaccine effectiveness, along with the risk factors for varied time points, such as 1 month, 3 months, and 6 months with this model. In addition, by estimating the mean or median immunization period, risk factors affecting this period, and their interactions with each other can be revealed. For minimizing or eliminating bias and incoherency, including demographic details of the participant's profiles, clinical features, and all other factors related to the infection ought to be considered together during the data analysis. In a Cox proportional hazards regression model, the measure of effect is the hazard ratio. This ratio is frequently interpreted as RR, but they are not technically the same. RR does not care about the timing of the event but only about the occurrence of the event by the end of the study. Alternatively, hazard ratio takes account not only of the total number of events but also of the timing of each event [31].

1.3. Conclusion

Here, we aim to emphasize the importance of cellular immunity, especially immune response at the mucosal level for eliminating the SARS-CoV-2 virus infection. Additionally, in the planning stage of observational Phase-III vaccine effectiveness studies, the suitable sample size, suitable sampling method, appropriate statistical model, and selection of variables are crucial in obtaining high-quality and valid results.

2. Expert opinion

In developed and developing countries, the vaccination process continues rapidly, especially for the risk group. Finally, the child

age groups are included in the population that should be vaccinated. The efficacy and safety reports of vaccine phase III studies conducted in these populations are under close monitoring within the global community. The lifting of COVID-19 restrictions in countries like Israel that has vaccinated the majority of its population has been promising for the end of the pandemic. At the same time, it is another topic for discussion, on whether vaccines are effective on variant viruses. However, the question arises whether widespread vaccination allows more variant viruses to occur. Moreover, the medium and long-term risks of genetic technology-based vaccines, which the global population had to test in large numbers, for the first time during this pandemic, are a matter of scientific debate.

Numerous social media posts, conspiracy theories, and some health professional's statements have elevated vaccination hesitancy. This has been the main tool for anti-vaxxers propaganda. On the other hand, declarations such as the overt promotion of nationalism with the claims of scientific impersonators have intertwined and have been led to the loss of trust in the scientific community to the vaccine. Realty that needs addressing is that this propaganda has affected millions of people in an unwanted direction.

It is obvious that vaccines developed with new technologies have promising results. However, the most important feature of every medical application is that proof of its safety. Although SARS-CoV-2 vaccines are put into practice with the emergency use code, basic biological and statistical knowledge should not be ignored.

Scientific declarations have relied on researches that have accurate and reliable statistical methods supported with basic biological knowledge. Scientific research results should be disclosed transparently, and its methods must have reproducible to provide replicability. Although problems are to be expected, the large-scale vaccination campaigns, international collaborations that provide vaccines for the world's poor countries against the SARS-COV-2 virus are promising steps for the future of humanity [32]. New ethical and methodological standards should be encouraged to move from individual countries to collective global responsibility, and promote diversity. Studies should enable wider inclusion to address global disparities in the access to health care. The much-needed vaccines ought to be planetary public goods and seen as a public utility goods, rather than a commodity. This is needed so that vaccines are available and accessible for all, and no region is left behind which can serve as a reservoir for the coming waves of the pandemics.

Funding

This paper was not funded

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Authors contribution

FA: conception and design of the review article and interpreting the relevant literature. FA and HA have been involved in writing the review article or revised it for intellectual content.

ORCID

Handan Ankaralı http://orcid.org/0000-0002-3613-0523

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) or (•=) of importance to readers.

1. Epidemiology and Prevention of Vaccine-preventable Diseases. Department of Health & Human Services, Centers for Disease Control and Prevention; 2006.
2. Moss WJ, Strebel P. Biological feasibility of measles eradication. J Infect Dis. 2011;204(Suppl 1):S47–53.
3. Chan MCW, Wang MH, Chen Z, et al. Frequent genetic mismatch between vaccine strains and circulating seasonal influenza viruses, Hong Kong, China, 1996–2012. Emerg Infect Dis. 2018;24:1825–1834.
4. Yuan M, Huang D, Lee -C-CD, et al. Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. Science. [Internet]. 2021 [cited 2021 Jun 18];eabh1139. Available from: https://science.sciencemag.org/content/early/2021/05/19/science.abh1139
5. Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARS-CoV-2. Nature. 2020;586:776–778.
6. Haller SL, Peng C, McFadden G, et al. Poxviruses and the evolution of host range and virulence. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2014;21:15–40.
7. Norrby E, Sheshberadaran H, McCullough KC, et al. Is rinderpest virus the archevirus of the morbillivirus genus? Intervirology. 1985;23:228–232.
8. Drexler JF, Corman VM, Müller MA, et al. Bats host major mammalian paramyxoviruses. Nat Commun. 2012;3:796.
9. Maurin M, Fenollar F, Mediannikov O, et al. Current status of putative animal sources of SARS-CoV-2 infection in humans: wildlife, domestic animals and pets. Microorganisms. 2021;9:868.
10. Lin J-T, Zhang J-S, Su N, et al. Safety and immunogenicity from a phase I trial of inactivated severe acute respiratory syndrome coronavirus vaccine. Antivir Ther. 2007;12:1107–1113.
•• **The article summarized the former coronavirus vaccine trial's safety.**
11. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in humans elicited both enhancing and neutralizing effects on infection in non-human primates. ACS Infect Dis. 2016;2:361–376.
12. Das S, Vera M, Gandin V, et al. Intracellular mRNA transport and localized translation. Nat Rev Mol Cell Biol. 2021.
13. Zhu N, Wang W, Liu Z, et al. Morphogenesis and cytopathic effect of SARS-CoV-2 infection in human airway epithelial cells. Nat Commun. 2020;11:3910.
14. Leblanc J-F, Germain M, Delage G, et al. Risk of transmission of severe acute respiratory syndrome coronavirus 2 by transfusion: a literature review. Transfusion (Paris). 2020;60:3046–3054.
15. Vasquez-Bonilla WO, Orozco R, Argueta V, et al. A review of the main histopathological findings in coronavirus disease 2019. Hum Pathol. 2020;105:74–83.
16. Laksono BM, Grosserichter-Wagener C, De Vries RD, et al. In vitro measles virus infection of human lymphocyte subsets

demonstrates high susceptibility and permissiveness of both naive and memory B cells. J Virol. 2018;8:92.

• **The article scrutinizes the biological characteristics of measle virus that deemed for disease effective control**

17. De Vries RD, Mesman AW, Geijtenbeek TBH, et al. The pathogenesis of measles. Curr Opin Virol. 2012;2:248–255.
18. Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine. 2008;26:D59–D66.
19. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun. 2020;11:5493.
20. Belyakov IM, Hammond SA, Ahlers JD, et al. Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. J Clin Invest. 2004;113:998–1007.
21. Hoft DF, Lottenbach KR, Blazevic A, et al. Comparisons of the humoral and cellular immune responses induced by live attenuated influenza vaccine and inactivated influenza vaccine in adults. Clin Vaccine Immunol. 2017;24.

•• **The article point out the live attenuated influenza vaccines additive immune enhancement in in adults with extensive and partially cross-reactive preexisting influenza immunity**

22. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature. 2020;584:457–462.
23. Emary KRW, Golubchik T, Aley PK, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet Lond Engl. 2021;397:10282:1351–1362.

•• **of considerable interest: The article emphasize that the new mutant viruses may be threaten the protective efficacy of current vaccines**

24. Lipsitch M, Grad YH, Sette A, et al. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. Nat Rev Immunol. 2020;20:709–713.
25. Parker EPK, Shrotri M, Kampmann B. Keeping track of the SARS-CoV-2 vaccine pipeline. Nat Rev Immunol. 2020;20:650.
26. Bursac Z, Gauss CH, Williams DK, et al. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008;3:17.

•• **The purposeful variable selection method discussed in this article should be a preferred variable selection method, especially in biological research such as vaccine studies. Other statistical methods used for variable selection may choose the wrong variables in selecting variables.**

27. Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet Lond Engl. 2021;397:1819–1829.
28. McNamee R. Confounding and confounders. Occup Environ Med. 2003;60:227–234;quiz 164, 234.
29. Rubin DB. Causal inference using potential outcomes: design, modeling, decisions. J Am Stat Assoc. 2005;100:322–331.

•• **In this article, statistical methods of removing bias and confounder effects have been explained.**

30. Hodgson SH, Mansatta K, Mallett G, et al. What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. Lancet Infect Dis. 2021;21:e26–e35.
31. Stare J, Maucort-Boulch D. Odds ratio, hazard ratio and relative risk.: 9.
32. Mackenzie D. US smallpox vaccination plan grinds to a halt [Internet]. New Sci. [cited 2020 Dec 27]. Available from: https://www.newscientist.com/article/dn4074-us-smallpox-vaccination-plan-grinds-to-a-halt/