**LESSONS FOR SARS-COV-2 VIRUS AND VACCINATION FROM OTHER RESPIRATORY VIRUSES**

**Running title: NOTES ON THE SARS-COV-2 VIRUS AND VACCINATION**

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**SUMMARY**

# At the beginning of the Coronavirus disease (COVID-19) pandemic due to the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), scientists primarily have focused on diagnostic tests. At the 9th month of the pandemic, discussions are being continued on preventive and therapeutic drugs. Meanwhile, vaccine development strategies like "operation warp speed" gave their early results in developed countries. Nowadays, these vaccines are at the forefront of the world health agenda. Numerous social media posts, conspiracy theories, and some health professional's statements that raise vaccination hesitancy are the equipment of anti-vaxxers propaganda. On the other hand, declarations like ballyhoo of nationalism with the claims of scientific quacksalvers have intertwined, and that has been led to the loss of trust in the scientific community to the vaccine. We have to admit that this propaganda has affected millions of people in an unwanted direction.

# Scientific declarations have to rely 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 reproducibility to provide replicability.

# Here, we scrutinize the biological features of the respiratory transmitted and successfully prevented measles virus (MV) and variola virus (smallpox 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.

# Biological concept of respiratory virus infection and vaccines

Vaccination is a passive immunization method that largely or permanently removes the transmission of the epidemic pathogen within the species and prevents epidemics. Classically, this immunization is obtained by inoculating the attenuated or inactivated form of the pathogen or the proteins that determine the virulence of the pathogen (capsule, toxin, etc.) 1. Modern vaccines are based genomic (mRNA, DNA vb ) and proteomics.

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 and implementation of widespread vaccination programme resulted in diseases disappearance to a large extent and eradication at the world, respectively.2 However, every virus and developed vaccine should be evaluated in its subjectivity. For instance, the mismatch of influenza vaccine strains with circulating viruses have limits the effectiveness of the vaccine3. For this reason, new chimeric influenza vaccine studies continue even 80 years after the first influenza vaccine was developed.

The similarities and main differences regarding measles, smallpox, and SARS-Cov-2 virus, disease and vaccines are summarized in **Table 1**.

İThe coexistence of humans with microflora members has been possible as a result of the co-evolution developed after repeated encounters with these agents for billions of years.4 Epidemics are incurred under the combination of appropriate biological factors by the some pathogens that are not non-members of microflora. İntra-species antigenic shifts or drifts and inter-species spillover are the main feature of the epidemics. It is thought that the archaic ancestors of both viruses evolved by transitioning from possible zoonotic sources (bats) to human and close species (distemper virus infecting cats and dogs, cowpox virus infecting cattle) 5–7. To date, human measles and human smallpox viruses have not been shown to cause non-human disease. The animal reservoir of the SARS-CoV-2 virus before human transmission is not yet known. Its current form presence has also been shown in some animal species8. Measles and smallpox vaccines are **live vaccines** developed against limited genotypes. Both measle virus and smallpox virus have only one serotype that responsible for the epidemics. Meanwhile, in the SARS-COV-2 virus, many strains have been identified and new mutated strains (501.V2 Variant, Cluster 5, VOC-202012/01) continue to be defined.9

Vaccine-elicited antibodies prevent disease development by controlling viremia, not by controlling local replication of the virus and tissue inflammation10. Vaccines induced immunity must primarily block the mucosal entry of relevant pathogens especially in respiratory viruses. Transcutaneous immunization to these viruses provides relatively partial mucosal immunity11. Researchers have observed influenza virus-specific sIgA responses are weak with inactivated influenza vaccines (IIV) compared with live attenuated influenza vaccines (LAIV) that based on mucosal challenge12. In a Respiratory syncytial virus (RSV) vaccine research, while inactivated virus or subunit F glycoprotein based vaccine induced Th2-like lymphocyte response, the live RSV vaccine induced a Th1-like pattern of cytokine mRNA expression, especially with mucosal route13.

Additionally, passive immunization strategies in the treatment of COVID-19 have not shown the expected effect.14 Neutralizing antibody titers to the coronaviruses rapidly wane while specific T-cells activity more durable 15. This suggests to us that the long-term protection should be provided through cellular immunity. Additionally, cross-reactive T cell memory implications to the COVID-19 disease severity and herd immunity may be one of underlying reason for the epidemiological differences among countries16. But, a live attenuated vaccine does not seem in the foreground of SARS-COV-2 vaccine pipeline17

It will be controversial whether attenuated live vaccination would be more effective in totally vulnerable world's population who have not cross-reactive immunity to SARS-COV-2 without examining the pathophysiology of the diseases. In an in vitro study, 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)18. Researchers contend that adaptation of SARS-CoV-2 to human airway is strongest and distinctive from other coronaviruses in this study. In an in vivo study, researchers have been detected the virus only in the upper and lower respiratory tract but not the spleen, liver, kidneys, or small intestine after intranasal administration of SARS-COV virus to the mice19. Additionally, in autopsy studies, involvement of regional lymph nodes is not emphasized and the COVID-19 remains mostly limited in the respiratory system20. On contrary, both T-cells and B cells are permissive for measle virus infection that showed in lymphocytes subset derived from tonsils and bloods21. Immune cells permission to the measle virus and and smallpox virus led to viral dissemination (trojan horse) to the other organs22. However, researchs regard to in vitro infectivity assays of blood components failed to detect virus in blood that may be related that immune cells are not permissive for SARS-COV-2 virus23.

We wanted to emphasize the importance of cellular immunity, especially immune response at the mucosal level for eliminating the SARS-COV-2 virus infection.

## Statistical concept

The aim of the Vaccine Phase-III studies is 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 multicenter. Volunteer individuals participate in the study. Vaccine and Placebo applications are made according to double-blind and the principles of randomness. In order to avoid confounded with the effects of baseline features measured, unmeasured and/or unobservable in the study, the treatment is allocated randomly29. There have been two ways to estimate the effects of a vaccine in Phase-III studies; this could by obtaining the estimates of the efficacy from randomized control trials (RCTs) and also estimates of effectiveness from observational studies 24

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, requiring a rigid design reduces external validity and results are generalized to a limited group. In some cases, it may not be possible to establish standardized conditions, randomization or blinding, for various ethical and technical reasons. In these conditions, 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 occur25.

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 26. 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 3 or 4 secondary endpoints can be used in order to evaluate the vaccine effectiveness has been examined. If co-primary endpoints are to be used, require some adjustment for multiple testing such as false discovery rate.

To achieve reliable results in the real world vaccine effectiveness study, the following considerations should be considered. The sample size to be included in the study should be calculated according to interaction terms and primary endpoints in the model by prior power analysis. The sampling schema should be planned as a multistage which has to combine a stratified random sample and cluster random sample. The participants should be taken from all over the country to cover regional differences. The subjects should be randomly assigned to the vaccine and placebo groups. Hence 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 correlation not implying causation27.

Both clinical/biological and statistical significance should be taken into consideration when deciding whether to include a variable in the model. It can be used Purposeful Variables Selection algorithm at each step of the modeling process. This algorithm will provide the retention of significant covariates as well as confounding ones 28.

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 doesn't 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 nuisance25, 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 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 used 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. We may calculate the more accurate predictions with regard to vaccine effectiveness along with the risk factors for the different time points such as 1 month, 3 months, 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 confounding, including demographic details of the participant's profiles, clinical features, and other factors related to the infection should 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 event29

In conclusion, 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 of great importance to obtain high-quality and valid results. Although problems may arise in 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 30. We believe that humanity will pass this critical threshold in the light of true science.

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