INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 202
2022 March; 28(2): 202-208
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Immunogenicity Assessment on Clinical Trials of SARS-CoV-2 Vaccines

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ABSTRACT

Various strategies for dealing with COVID-19 have been carried out since the WHO declared COVID-19 as an international health emergency. One of the preventive strategies is the development of vaccines. Various vaccines have been developed worldwide. As of April 13, 2021, there were 184 vaccine candidates in the pre-clinical phase and 16 vaccine candidates currently in phase III clinical trials using several platforms, such as inactivated viruses, vector viruses, and protein subunits, and mRNA. Clinical trials of the SARS-CoV-2 vaccine include a screening test consisting of thorough physical examination and laboratory tests. The safety of clinical trials is evaluated based on laboratory test results referring to the standard toxicity grading scale. Immunogenicity assessment at the stage of clinical trials of vaccines includes assessment of humoral and cellular immunogenicity. The humoral immunogenicity test measures the ability of antibodies to neutralize the virus with the live virus neutralization test, Pseudo Virus Neutralization Test (pVNT), and Surrogate Virus Neutralization Test (sVNT) method. The cellular immunogenicity response aims to assess the immune response that leads to the Th1-cell phenotype. The COVID-19 vaccine under development is expected to trigger a helper 1 (Th1) cell response. Th1-producing Interferon-γ (IFNγ) is formed during acute viral infection, and Th1-type immune response correlates with milder disease. This is one of the considerations in vaccination. Th1-cell phenotype as part of cellular immunogenicity can be evaluated with ELISPOT, interferon-gamma release assay, and flow cytometry using blood samples that have been cultured with the administration of specific SARS-CoV-2 peptides. This literature review aims to study various immunogenicity assessments in the laboratory for clinical trials of COVID-19 vaccines.

Keywords: COVID-19, vaccine, immunogenicity

INTRODUCTION

The rapid increase in COVID-19 cases on March 11, 2020, made the World Health Organization (WHO) declare Coronavirus Diseases 2019 (COVID-19) a pandemic.[1] Several therapeutic modalities to overcome this pandemic have been developed by researchers worldwide, such as intravenous immunoglobulin (IVIG), monoclonal antibody (mAb), convalescent plasma, antiviral agent, immunosuppressant, immunomodulator, and vaccine.[2] It is agreed that vaccines are a therapy that needs to be widely developed to overcome the spread of this pandemic.[3] Vaccine manufacturing is a long and complicated process that requires evaluation in several long-term clinical trials.[4]

The development of various COVID-19 vaccines is a serious attempt to prevent a pandemic. Most of the vaccine candidates use the S-protein from SARS-CoV-2. One hundred and sixty-six SARS-CoV-2 vaccine candidates are in the pre-clinical or clinical phase worldwide. Vaccines with inactivated viral platforms or live attenuated viruses, protein sub-units, Virus-Like Particles (VLP), viral vectors (replicating and non-replicating), DNA vaccines, RNA vaccines, and nanoparticles vaccines have their typical characteristics. The percentage of the COVID-19 vaccine platform, which has been developed from various types of vaccines worldwide, is illustrated in Figure 1.[3]

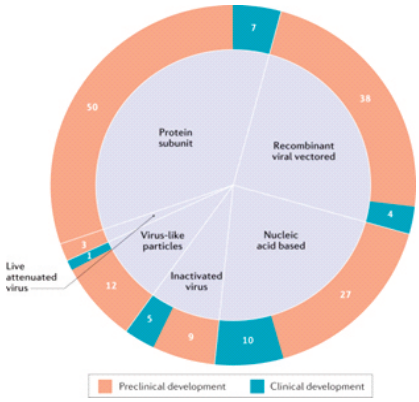

Figure 1. Pie chart showing various COVID-19 vaccine platforms in development[3]

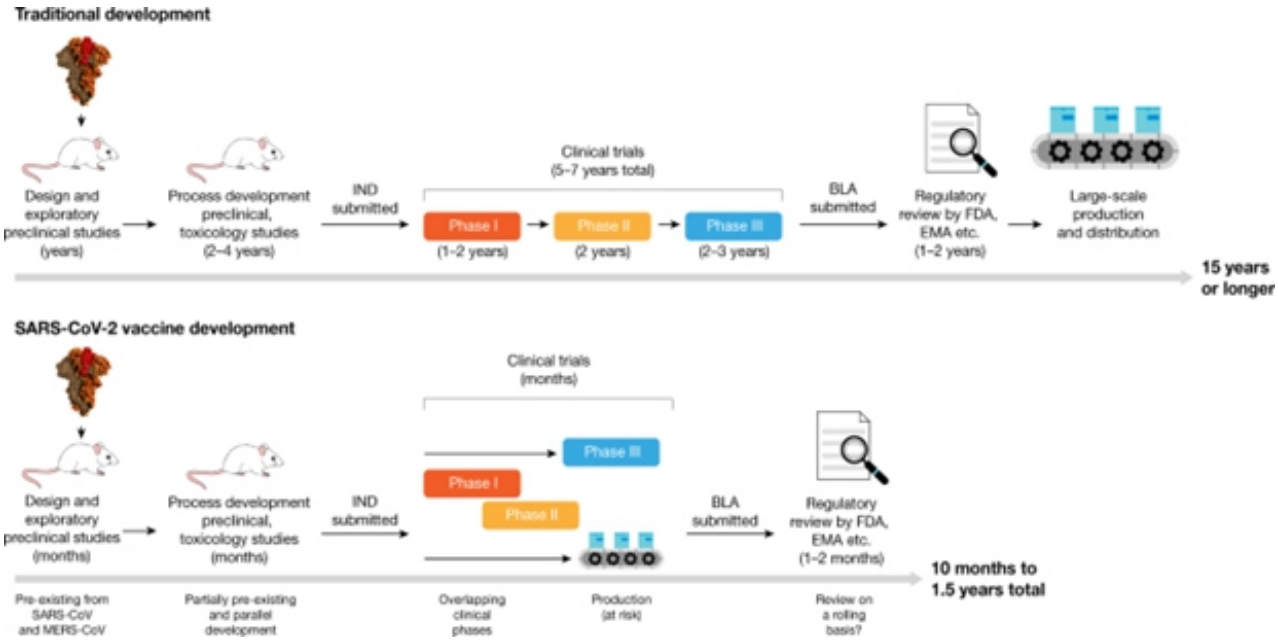

Figure 2. Comparison between the development of conventional and SARS-CoV-2 vaccines[5]

The development of the SARS-Cov-2 vaccine has accelerated due to the urgency of the need. This development is carried out based on knowledge from previous vaccines for SARS-CoV and MERS-CoV to finish the drug discovery phase quickly. Subsequently, phase I and II clinical trials are carried out, and phase III is started after the interim analysis of phases I/and II, thereby enabling the parallel performance of various phases (Figure 2).[5]

Pre-clinical phase is carried out before trials in humans. The first stage is to identify the vaccine candidate by selecting the proper antigen. This phase requires a comprehensive test both in-vitro and in-vivo (in experimental animals), including the determination of vaccine immunogenicity, the ability of the vaccine to induce a specific immune response (antibody levels and T cell response).[6,7]

Phase I clinical trials involve relatively few participants, about less than 100 volunteers. The focus of phase I clinical trials is safety, tolerability, and initial immunogenicity assessment. Vaccine safety in phase I is evaluated based on the incidence of vaccine-related adverse events (local reactions at the injection site, body temperature, systemic side effects, and laboratory results).[5-7]

Stratification can be used in phase I to assess several factors that influence vaccination response. For example, if it is suspected that the vaccine antibody response is influenced by age, stratification is carried out on the younger and older ages (>45 years). If the result in phase 1 is proved to be safe, it proceeds to phase II trials.[6,7]

Phase III clinical trials refer to the success of vaccine candidates in phase II; phase III clinical trials involve approximately 3000 to 10,000 participants whose aim is to evaluate vaccine safety and efficacy on a large scale of people who are targeted for vaccination. In this phase, vaccine safety is also evaluated at administration close to other vaccines. Evaluation of immunogenicity and vaccine safety of one or both doses are carried out continuously after phase I.[6,7]

Two concepts of testing that are frequently carried out respectively are phase II (small scale) as a proof of efficacy to obtain initial evidence of vaccine effectiveness before phase III (large scale) as a confirmatory test; a combination of direct phase II/III on a large scale. Immunological biomarkers (T cell and antibody response) used to assess immunogenicity can also be used as markers to assess vaccine efficacy. Vaccine efficacy can be assessed as a clinical protective outcome and/or immunological endpoint based on the immunologic response. If the result of phase III trials meets the pre-defined endpoints, a biologics license application is processed.[5-7]

Phase IV studies, also known as ongoing studies, are post-licensure safety trials to gather additional information on immunogenic safety and efficacy to meet the intended goals. Studies on immunological persistence also need to be conducted to determine how long vaccine-induced immunity can last and whether a booster is needed after several years.[6]

In various clinical trials of vaccines, screening tests are carried out, consisting of a thorough physical examination including heart rate, blood pressure, body temperature, height and weight, medical history (history of therapy or other vaccinations in the previous 30 days), and laboratory tests. Therefore, it is expected to find subjects who meet the criteria for clinical trials and baseline data. The laboratory parameters tested are as follows: Complete blood count, serum creatinine, Aspartate Aminotrasterase (AST), Alanine (ALT), total bilirubin, lipase Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), HBsAg, anti HCV, anti HIV; Additional tests such as pregnancy test needs to be carried out on female subjects of childbearing age, generally carried out before randomization and every time before vaccination.[8,9]

Safety assessment

Safety outcomes could be determined as primary or secondary endpoints. A thorough medical history at the time of subject screening. It is necessary to ascertain the history of allergies, cancer, immunodeficiency, psychiatric disorders, drug abuse, and autoimmune diseases. At each subject's visit, anamnesis and physical examination are carried out to find any changes in recent medical conditions which indicate the possibility of Medically-Attended Adverse Events (MAAEs) and New-Onset Chronic Medical Conditions (NOCMCs).[8,9]

An adverse event is observed after each dose immediately (commonly 20-60 minutes) for any severe immediate reactions. Assessment of all Adverse Event (AE), including solicited, also monitored for any AE that occurs at each vaccination injection up to the 7th post-injection day. This assessment includes post-injection erythema, edema/induration, pain, and systemic symptoms such as fever, fatigue, chills, myalgia, arthralgia, headache, and nausea. Laboratory tests on the screening panel are carried out at baseline (before vaccination) and post-vaccination according to the schedule set out in the clinical trial protocol. According to the standard toxicity grading scale, safety assessments of clinical trials are evaluated based on laboratory results to determine possible side effects.[8-10]

Immunogenicity assessment

Immunogenicity assessments are conducted at all stages of pre-licensure vaccine development and additional in post-licensure stages. Immunogenicity data can be used to describe the magnitude of immune response for the prediction of vaccine efficacy and Immune Correlate of Protection (ICP). Immune responses to vaccination are routinely measured in serum (humoral immune responses) and blood (cellular immune responses). Pre-vaccination and post-vaccination sampling is needed to evaluate the magnitude of the immune response. Immunogenicity testing at the vaccine clinical trial includes assessment of the humoral immune response such as antibody concentrations or antibody titers that are commonly measured as the Geometric Mean Titer (GMT) of SARS-CoV-2 neutralization antibody and the Geometric Mean Concentration (GMC) of IgG-RBD SARS-CoV-2 and the cell-mediated immune response (such as percentages of sensitized T cells) after the first and second doses vaccine.[8,10,11]

Humoral Immunity

Neutralizing Antibodies (NAbs) are a type of antibody produced by B lymphocytes against invading microorganisms. Neutralizing antibodies can inhibit viral infection during the viral replication cycle, primarily through the inhibition process of virus attachment and entry into host cells. Neutralizing antibodies can facilitate the aggregation of viral particles, reduce internalization through endocytosis, and inhibit metabolic processes (replication or transcription). Live virus neutralization assays are still the gold standard for measuring Nabs, while pseudovirus neutralization assays, Surrogate Virus Neutralization Test (sVNT), and high-throughput versions of neutralization assays or binding antibody assays are the alternatives assay each own advantages and disadvantages.[12]

The live virus neutralization test measures antibodies in the serum with the ability to neutralize the virus. A mixture of antibodies and viruses is incubated under certain conditions and then inoculated (in cells, chicken, and animal embryos). Test using cell culture will reveal how the antibody inhibits the cytopathic effect or viral plaque formation. Several tests included in neutralization using live viruses are Focus Reduction Neutralization Test (FRNT), Plaque Reduction Neutralization Test (PRNT), and live virus microneutralization assay (MN assay).[12] PRNT is considered the gold standard for measuring neutralizing antibody levels in various viral infections. Neutralizing antibodies is one of the tests needed to determine the optimal vaccine candidate. The principle of this PRNT assay is that live virus is added to serial dilutions of the patient's serum and then cultured on plates. For several days, antibodies with neutralizing ability will prevent the virus from making "plaques" or small areas without cell growth on the plate. Antibody titer is defined as

the highest serum dilution resulting in a 90% (PRNT90) or >50% (PRNT50) reduction in viral plaque count.[12,13]

PRNT using a measurement of reciprocal antibody titer with the ability to neutralize the SARS-CoV-2 virus and reduce plaque by up to 50% compared to this negative control is reported as PRNT 50. PRNT needs about five days for the time to result. The principle of FRNT is similar to PRNT, but the detection of the virus is using specific antibodies that are conjugated to Horseradish Peroxidase (HRP), and the results are presented by observing the foci of SARS-CoV-2-infected cells with microanalyzer.[12-14] An illustration of neutralizing antibody testing with the PRNT method can be seen in Figure 3A.

The requirement of the BSL-3 facility, difficulty to perform on a large scale, the long processing time to obtain plaque visualization, and no automation procedure remain the limitations of PRNT, thereby making it difficult to be used for routine tests and many purposes such as phase III vaccine clinical trials.[13]

This Pseudovirus Neutralization Test (pVNT) is based on the engineering of non-infectious viral particles, making the virus unable to express its surface proteins. Pseudotype virus particles are created by integrating the surface proteins of highly infectious viruses without retaining their pathogenic properties. Pseudoviruses are designed (are usually engineered) to carry a reporter gene encoding NanoLuc luciferase or Green Fluorescent Protein (GFP) to facilitate the reading of the results. This method does not require a BSL-3 laboratory, but it still requires a specialized laboratory setup, and this procedure is complicated and time-consuming, but it can be applied for large-scale testing.[12,14]

Human Immunodeficiency Virus (HIV)-1 and Vesicular Stomatitis Virus (VSV) are the most commonly used pseudovirus vectors. pVNT is similar to a PRNT principle, but instead of a live virus, a virus-like HIV or VSV is transfected with the S protein of SARS-CoV-2 and a luminescent reporter. The luminescence units are sites of infection, and a reduction in luminescence units means that neutralizing antibodies are present (Figure 3B).[14]

The SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) is developed as an alternative to the gold standard live virus neutralization test or pseudovirus neutralization test. SVNT is based on the blocking Enzyme-Linked Immunosorbent Assay (ELISA) that resembles virus-cell interaction to detect the presence of NAbs in a sample (Fig 3C). Specific antigens (protein S or RBD that can be attached to biotin) are attached to the plate and incubated with serum, then soluble human ACE2 conjugated with HRP is added, and the substrate 3,3′, 5,5′-Tetramethylbenzidine is added. Another principle is to attach ACE-2 to the plate and add soluble RBD that competes with antibodies in serum or plasma. Antibodies that inhibit RBD-ACE2 interaction can be detected by decreasing the HRP luminescence signal. The initiation test can quickly measure the level of neutralizing antibodies in a

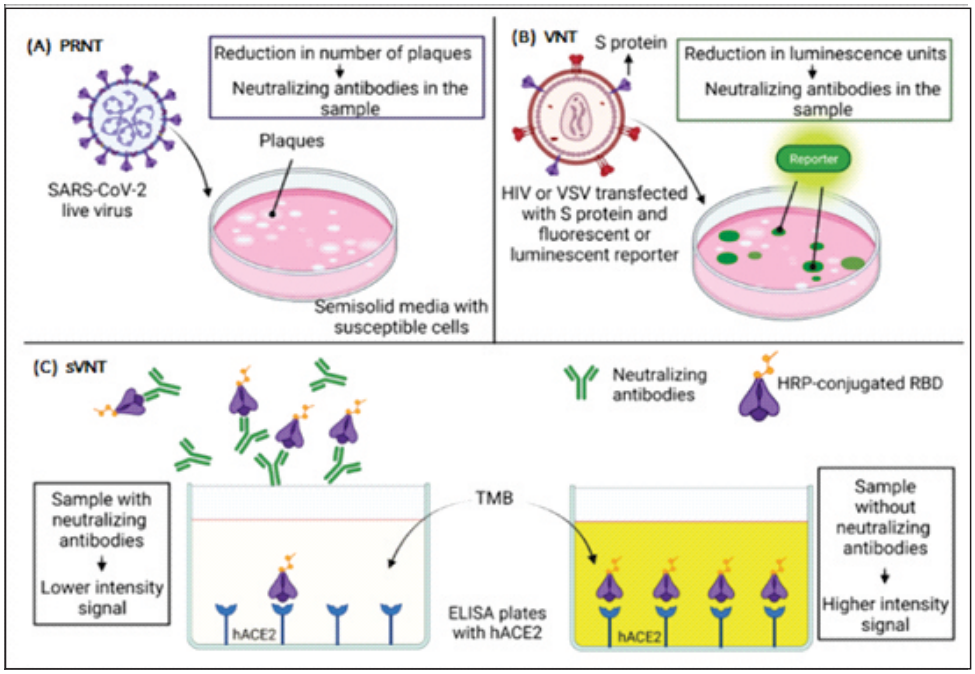

Figure 3 A-C. Comparison of neutralization assay method in COVID-19[14]

sample and is an alternative to cell-based neutralization tests.[12,14]

The advantages of sVNT are that it can be rapidly conducted in most laboratories without the need to use live biological materials, cells, and biosafety containment; and enable high-throughput testing and/or fully automated testing after minimal adaptation. The performance of SVNT is similar to pVNT and PRNT.[12,15]

Cellular Immunity

The cell-mediated immune response is commonly assessed by detecting and quantifying sensitized T cells in the blood. These investigations would enable characterization of the predominant cytokines released and detection of differences in sensitization between T cell subpopulations. Several methods for examining cellular immunity are typically based on measuring the production of a range of cytokines following in vitro stimulation of T cells with individual or pooled antigens.[8]

A successful COVID-19 vaccine requires both neutralizing antibodies and Th1-driven cellular component responses. Vaccines that generate high neutralizing antibodies, Th1 responses, and balanced CD4/CD8 and polyfunctional T cell responses are less likely to cause immunopathology.[16] In reply to viral infection, T cells are essential mediators that kill infected cells and support B cell function and antibody responses. Helper T cells that produce Interferon-γ (IFN-γ) or also known as Th1 cells, are formed during the process of acute viral infection associated with milder disease severity. Helper 1 (Th1) cells that produce IFN-γ are formed during acute viral infection, and the correlation between Th1-type immune response and milder disease becomes a consideration in vaccination. The currently developed COVID-19 vaccine is also expected to trigger a Th1 response. Individuals with higher levels of IFNγ-secreting T cells against SARS-CoV-2 S protein, a nuclear protein, and membrane protein have better protection against disease.[17]

An essential consideration in COVID-19 is that the developed vaccine induces an immune response that leads to a Th1 cell phenotype that can be tested by several methods as follows:

IFN-γ Release Assay

Ex-vivo IFN-γ ELISpot assay is reported to be used to measure cellular immunity in clinical trials of the ChAdOx1 nCoV19 vaccine. ELISPOT test is carried out on fresh Peripheral Blood Mononuclear cells (PBMC) samples before and after vaccination. This test uses Multiscreen IP ELISpot plates (Millipore) to which 10 g/mL human anti-IFN-γ coating antibody (clone 1-D1K, Mabtech) is attached overnight at 4°C in carbonate buffer followed by washing three times with PBS and blocking using R10 medium for 2-8 hours. The prepared PBMC specimens with a cell density of 2.5×10^6 are inserted into each well along with 13 pools of SARS-CoV-2 peptide with a certain concentration and incubated for 16-18 hours at 37°C with 5% CO_2. ELISPOT calculations are performed using the automated ELISpot counter. The examination is carried out in triplicate, and the IFN-γ response is calculated based on the average of the three wells minus the average negative control response (unstimulated well).[18]

The results are SFCs/106 PBMCs. ELISPOT responses to specific peptides are positive if >40 SFUs/106 PBMCs. Negative control (PBMC without antigen) and positive control were also used for the analysis.[18]

T cell responses can also be evaluated with the Quantiferon (QFN) SARS-CoV-2, which measures Interferon-γ Release Assay (IGRA). This test uses three kinds of tubes containing antigens, such as SARS-CoV-2 Ag1, Ag2, and Ag3, which use a combination of SARS-CoV-2-specific antigen peptides to stimulate lymphocytes in the blood, which plays an essential role in cellular immunity. The QFN tube also consists of Nil and Mitogen BCTs, which act as negative and positive controls. The SARS CoV-2 Ag1 QFN tube contains the Cd4 epitope of the spike protein S1 subunit (receptor binding domain), the Ag2 tube contains the CD4 and CD8 epitopes of the S1 and S2 spike protein subunits, and the Ag3 tube contains the CD4 and CD8 epitopes of the S1 subunit, S2 and the immunodominant CD8 epitope from the whole genome. Plasma from stimulated samples can be used to measure IFN-γ using the ELISA method. Plasma is then harvested after antigen stimulation for 6-24 hours at 37°C (5% CO_2) and then stored at -80°C for measurement of IFN-γ levels with ELISA. The results are reported as quantitative levels (IFN-γ levels in IU/mL). Improved response > 0.20 IU/mL are compared to Nil QFN-SARS-CoV-2 tube levels.[19]

Flow Cytometry

Assessment of T cell responses to vaccine candidates can be carried out by intracellular cytokine assays performed at the time specified in the clinical trial protocol. The samples used are PBMC prepared with a specific cell density (generally 10^6 cells/mL), cultured under stimulation with specific SARS-CoV-2 peptide at 37°C with 5% CO_2 incubated

for ± 4-6 hours. The step is followed by readings using a flow cytometer. T cell responses are evaluated based on the expression of helper T cell cytokines (CD4). The th1-type immune response shows high expression of TNF α, IL-2, or IFN-γ cytokines by CD4 T cells, while Th2 expression is evaluated based on the expression of IL-4 and or IL-13 from CD4 T cells.[9]

Most COVID-19 vaccines are intended to induce immunological responses, typically neutralizing antibodies (NAbs) against the SARS-CoV-2 spike protein. An mRNA vaccine BNT162b2 developed by Pfizer/BioNTech elicits an immunological response including IgG, IgA, CD8 cells, or CD4 cells.[16] Humoral immune response in this vaccine showed as presentation of S1-binding antibody after the first dose and increased responses following the second dose. Also, a significant NAb was present after the second dose, while the cellular immune response to this vaccine was reported as increased in antigen-specific IFN-γ+ CD4 and CD8 T cells after the second dose with the predominance of IFN-γ and IL-2 secretion, compared with IL-4, suggesting TH1 cell polarization.[17] Another mRNA-1273 vaccine that Moderna developed induces an S-binding antibody, which was detected 14 days after the first dose, and the levels increased slightly by 28 days, with a marked increase after the second dose, minimal NAb present after the first dose then reached the peak at 14 days after the second dose and also elicited CD4 T cell responses that on stimulation by S-specific peptide pools were strongly biased toward the expression of Th1 cytokines (tumor necrosis factor α > IL-2 >IFN-γ), with minimal type 2 helper T cell (Th2) cytokine expression (IL-4 and IL-13).[9,17] Adenoviral-vectored vaccines such as the ChAdOx1 nCoV-19 vaccine developed by Oxford/AstraZeneca also induce a variety of immunological responses. It produced anti-IgA and IgG antibodies, T cell, Th1-biased T cell, IFN-γ and IL-2, and Cd4 T cell response.[16]

REFERENCES

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of Coronavirus Disease (COVID-19) outbreak. J Autoimmun, 2020; (PG-102433): 102433.
2. Iqbal Yatoo M, Hamid Z, Rather I, Nazir QUA, Bhat RA, *et al.* Immunotherapies and immunomodulatory approaches in clinical trials-a mini-review. Hum Vaccin Immunother, 2021; 17(7): 1897-1909.
3. Jeyanathan M, Afkhami S, Small F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. Nat Rev Immunol, 2020; 20(10): 615-632.
4. Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. The complexity and cost of vaccine manufacturing-An overview. Vaccine, 2017; 35(33): 4064-4071.
5. Krammer F. SARS-CoV-2 vaccines in development. Nature, 2020; 586: 516-527.
6. The path Malaria Vaccine Initiative (VMI). Clinical trials steps in malaria vaccine development. Available from: https://vaccine-safety-training.org/tl_files/vs/pdf/MVI.pdf. (accessed March 12, 2022).
7. IFPMA. The complex journey of a vaccine. International Federation of Pharmaceutical Manufacturers & Association. Available from: https://www.ifpma.org/wp-content/uploads/2019/07/IFPMA-ComplexJourney-2019_FINAL.pdf (accessed October 23, 2021).
8. WHO. Guidelines on clinical evaluation of vaccines: Regulatory expectations. WHO, 2016; 1-92.
9. Jackson L, Anderson E, Rouphael N, Roberts P, Makhene M, Coler R. An mRNA vaccine against SARS-CoV-2-preliminary report (supplementary protocol). The New England Journal of Medicine 2020; 383: 1920-1931. Available from: https://www.nejm.org/doi/suppl/10.1056/NEJMoa2022483/suppl_file/nejmoa2022483_protocol.pdf (accessed October 23, 2021).
10. European medicines agency. Guideline on clinical evaluation of vaccines. 2018. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-clinical-evaluation-vaccines-revision-1_en.pdf (accessed October 16, 2021).
11. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, *et al.* Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature, 2020; 586: 589-593. Available from: https://doi.org/10.1038/s41586-020-2639-4 (accessed November 18, 2021).
12. Lu Y, Wang J, Li Q, Hu H, Lu J, Chen Z. Advances in neutralization assays for sARS-CoV-2. Scand J Immunol, 2021; 94: e13088.
13. Bewley KR, Coombes NS, Gagnon L, McInroy L, Baker N, Shaik I, *et al.* Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization, and pseudotyped virus neutralization assays. Nat Protoc, 2021; 16: 3114-3140.
14. Morales-Núñez JJ, Muñoz-Valle JF, Torres-Hernández PC, Hernández-Bello J. Overview of neutralizing antibodies and their potential in COVID-19. Vaccines, 2021; 9(12): 1376.
15. Tan CW, Chia WN, Qin X, Liu P, Chen MIC, Tiu C *et al.* A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol, 2020; 38: 1073-1078.
16. Goyal K, Goel H, Baranwal P, Tewary A, Dixit A, *et al.* Immunological mechanisms of vaccine-induced protection against SARS-CoV-2 in Humans. Immuno, 2021; 1(4): 442-456.
17. Manish Sadarangani, Arnaud Marchant, R. Kollmann.

Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Immunology, 2021; 21: 475-484.
18. Katie JE?, Jordan RB?, Sandra BR, Hannah ST. Cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. Nature Medicine, 2021; 27: 270-278.
19. Martínez-Gallo M, Esperalba-Esquerra J, Pujol-Borrell R, Sanda V. T-cell responses as a correlate of COVID-19 vaccination. A pilot study in health care workers. MedRxiv, 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.03.31.21254472v2.full-text (accessed November 18, 2021).