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EVALUATION OF THE ABILITY OF CYTOKINE LEVELS TO INFLUENCE IMMUNITY IN GROUP VACCINATED WITH COVID-19 PFIZER VACCINE

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EVALUATION OF THE ABILITY OF CYTOKINE LEVELS TO INFLUENCE IMMUNITY IN GROUP VACCINATED WITH COVID-19 PFIZER VACCINE

By Maha Kadhim Ali ABOKSHOT

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EVALUATION OF THE ABILITY OF CYTOKINE LEVELS TO INFLUENCE IMMUNITY IN GROUP VACCINATED WITH COVID-19 PFIZER VACCINE

Maha Kadhim Ali ABOKSHOT

Master of Science in Biology

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In addition to all the information obtained in studies on the SARS agent SARS-CoV and the MERS agent MERS-CoV, during the COVID-19 pandemic caused by SARS-CoV-2, the immunogenic capacity of the S and N structural proteins of coronaviruses and also vaccine candidates using the S protein as the antigen have been shown to induce a protective immune response mediated by neutralizing antibodies. The primary cause of death in COVID-19 patients was initially defined as acute respiratory infection with severe pneumonia. Cardiovascular problems and coagulation problems due to severe systemic inflammatory state have attracted attention as aggravating factors in the clinical picture of people infected with SARS-CoV-2. Biomarkers that can guide prognosis have also been investigated. It has been reported that serum levels of MCP-3 (monocyte chemotactic protein-3) and IP-10 (interferon gamma-induced protein-10) chemokines are elevated in studies conducted in COVID-19 patients hospitalized in the intensive care unit and progressing to a fatal process. During the pandemic process, it has emerged that protective vaccines against the COVID-19 epidemic must be rapidly produced on a large scale and distributed all over the world.

2023, 83 pages

Keywords: Cytokine levels, Immunity, COVID-19 vaccine

ÖZET

COVID-19 PFIZER AŞISI İLE AŞILANMIŞ OLAN BİR GRUPTAKİ SİTOKİN DÜZEYLERİNİN BAĞIŞIKLIK YANITINI ETKİLEME KABİLİYETİNİN DEĞERLENDİRİLMESİ

Maha Kadhim Ali ABOKSHOT

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SARS etkeni SARS-CoV ve MERS etkeni MERS-CoV üzerine yapılan çalışmalarda elde edilmiş olan tüm bilgilere ek olarak, SARS-CoV-2'nin neden olduğu COVID-19 pandemisi döneminde koronavirüslerin yapısal proteinlerinden S ve N'nin immünojenik kapasitesi ve antijen olarak S proteinini kullanan aşı adaylarının, nötralize edici antikorların aracılık ettiği koruyucu bir bağışıklık tepkisini indüklediği ortaya konulmuştur. COVID-19 hastalarında birincil ölüm nedeni başlangıçta şiddetli pnömoni ile akut solunum yolu enfeksiyonu olarak tanımlanmıştır. SARS-CoV-2 ile enfekte kişilerin klinik tablosunda şiddetli sistemik inflamatuar duruma bağlı kardiyovasküler sorunlar ve pıhtılaşma sorunları, ağırlaştırıcı faktörler olarak dikkat çekmiştir. Prognoza rehberlik edebilecek biyobelirteçler de araştırılmıştır. Yoğun bakım ünitesine yatırılan ve ölümcül sürece ilerleyen COVID-19 hastalarında yapılan araştırmalarda, kemokinlerden MCP-3 (monosit kemotaktik protein-3) ve IP-10'un (interferon gama ile indüklenen protein-10) serum düzeylerinin yükseldiği bildirilmiştir. Pandemi sürecinde COVID-19 salgınına karşı koruyucu aşıların büyük ölçekte hızla üretilerek tüm dünyaya dağıtılması gerekliliği ortaya çıkmıştır.

2023, 83 sayfa

Anahtar Kelimeler: Sitokin seviyeleri, Bağışıklık, COVID-19 aşısı

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Maha Kadhim Ali ABOKSHOT

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CONTENTS

LIST OF SYMBOLS

%	Percentage
<	Less than
>	Greater than
℃	Degree celsius
mL	Milliliter
pg	Picogram
µg	Microgram
µL	Microliter

LIST OF ABBREVIATIONS

ACE2	Angiotensin-2 converting enzyme
ADE	Antibody dependent enhancement
ARDS	Acute respiratory distress syndrome
CQ	Chloroquine
DNA	Deoxyribonucleic acid
FDA	Food and drug administration
HCQ	Hydroxychloroquine
HLH	Hemophagocytic lymphohistiocytosis
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LNPs	Lipid nanoparticles
MAS	Macrophage activation syndrome
MERS	Middle east respiratory syndrome
NAs	Nucleoside analogues
NIAID	National institute of allergy and infectious diseases
PBS	Phosphate buffered saline
RBD	Receptor-binding domain
RNA	Ribonucleic acid
SAH	Systemic arterial hypertension
SARS	Severe acute respiratory syndrome
WHO	World health organization
XLA	X-linked agammaglobulinemia

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1. INTRODUCTION

1.1 Introduction to COVID-19

The *Coronaviridae* family known as coronaviruses is commonly responsible for respiratory illnesses. The SARS-CoV-2 coronavirus was first detected in humans in Wuhan, China, in December 2019, following an infectious outbreak of an acute respiratory syndrome of zoonotic origin called COVID-19 (Coronavirus disease-2019). COVID-19 emerged and spread far more rapidly than previous coronavirus diseases; three months later, the World Health Organization declared COVID-19 as a pandemic in March 2020 (Pal *et al.* 2020). Infected people were defined to be the most common carriers of the disease, which spreads through the air and person-to-person contact (Jayaweera *et al.* 2020).

Initially, the source of contamination was suspected to be an animal market in Wuhan City, Hubey Province, China. Traditionally, coronaviruses can be found in bats, birds, and other animals, particularly wild animals. Previous epidemics of coronaviruses, which cause the respiratory disorders; SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome), have been shown to be communicable between animals and people. The World Health Organization (WHO) briefly dubbed the virus in question the "2019 new coronavirus" on January 12, 2020 (2019-nCoV) (Machhi *et al.* 2020).

SARS-CoV-2 is the seventh virus in the coronavirus family to infect humans. The coronaviruses HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 cause common cold symptoms in immunocompetent individuals. SARS-CoV, in turn, was the causal agent of the SARS outbreaks in 2002 and 2003 in Guangdong Province, China. In 2012, MERS-CoV was recognized as the etiologic agent of MERS in the Middle East. On March 11, 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO, which recognized its spread on all continents. Unlike SARS and MERS which had their outbreaks controlled, COVID-19 has unfortunately acquired pandemic status 3-5.

Strategies of isolation and social distancing have been adopted around the world in order to control the spread of infection by this new coronavirus (Liu *et al.* 2021).

Clinically, infection with SARS-CoV-2 causes from the common cold to systemic conditions characterized by severe acute respiratory syndrome, with the possibility of concomitant coagulopathies, neurological disorders, in addition to a severe systemic inflammatory state characterized, immunopathologically, by the occurrence of a "storm of cytokines". The severity of clinical manifestations is related to the systemic conditions of infected individuals. Patients with cardiovascular diseases, diabetes, obesity, renal failure and systemic arterial hypertension (SAH) are at high risk of developing severe disease and, consequently, are more likely to die. The elderly, characteristically, also have higher incidences of severe acute respiratory syndrome and mortality (Ye *et al.* 2020).

The involvement of immunosuppressed patients by COVID-19, either for treatment of malignant neoplasms or for control of organ transplant rejection, seems to be proportionately small compared to the total number of cases and, peculiarly, these patients present a favorable clinical evolution compared to others. To date, although there are few published reports, patients with primary immunodeficiencies have not had a worse clinical course with the development of severe disease. Another interesting finding is that patients with respiratory allergies, such as asthma, are probably not more susceptible to SARS-CoV-2 infection and do not belong to the risk group for the development of severe clinical forms of COVID-19 (Gao *et al.* 2022).

A global race for knowledge about COVID-19 had been put underway. Scientists from research laboratories around the world worked tirelessly to obtain, as soon as possible, immunoprophylaxis and therapeutic strategies for SARS-CoV-2 infection. The likely clinical implications arising from knowledge of COVID-19 immunopathology were also started to be investigated (Costela-Ruiz *et al.* 2020).

Although respiratory droplet transmission is the primary mode of SARS-CoV-2 spread, evidence of viral shedding in faeces supports additional modes of transmission, such as fecal-oral. Diarrhea, nausea, vomiting, and abdominal pain have been reported in 11.5%-

32% of patients, and the disease has a wide spectrum of signs and symptoms, with cough and fever being the most common initial manifestations. Some patients with this disease develop acute respiratory distress syndrome (ARDS). This is notable, though, because the condition can be so fatal for certain patients. Hyperinflammation and a picture resembling hemophagocytic lymphohistiocytosis (HLH), a clinical syndrome caused by an inadequate response of the immune system to a trigger, whether infectious, neoplastic, rheumatologic, or metabolic, have been proposed as potential causes of the virus's severe pulmonary involvement. HLH is not a disease in and of itself but a clinical syndrome with many potential causes that all result in the same inflammatory phenotype. The clinical picture is caused by an excessive release of cytokines, often known as a "cytokine storm," as a result of the immune system's hyperactivation (Harapan *et al.* 2020).

Published data (Harapan *et al.* 2020) show that in about 80% of cases, those infected were asymptomatic or with relatively mild symptoms, such as fever, fatigue, and some respiratory signs, including cough, sore throat, and shortness of breath, while the rest (20%) had more severe cases, progressing to severe pneumonia and, in some cases, death. The coronavirus can remain incubated for about 2 to 14 days and still be transmitted through fomites or spraying droplets through coughing or sneezing, which are the most common symptoms in patients affected by the disease (Jones *et al.* 2020).

1.2 How Does SARS-CoV-2 Infect Human Cells

HCoVs (human coronaviruses) are RNA viruses that have as a striking morphological characteristic the presence, on their surface, of the S protein forming projections called spike. Four structural molecules are recognized in the virus: S protein, membrane proteins, nucleocapsid protein and viral envelope protein (Bahadur Khadka *et al.* 2020). Figure 1.1 schematically represents the molecular structure of SARS-CoV-2. The pathogenicity of SARS-CoV-2 correlates with the high affinity of protein S for receptors on target cells. SARS-CoV and SARS-CoV-2 use angiotensin-2 converting enzyme (ACE2) as a receptor to establish infection in host cells. The SARS-CoV-2 S protein has tropism for the ACE2 molecule. Additionally, the enzyme hemagglutinin esterase, present on the viral surface, interacts with sialic acid residues that are part of the human

cell membrane, promoting another type of mechanism for penetration and insertion of SARS-CoV-2 into cells (Luisetto *et al.* 2020).

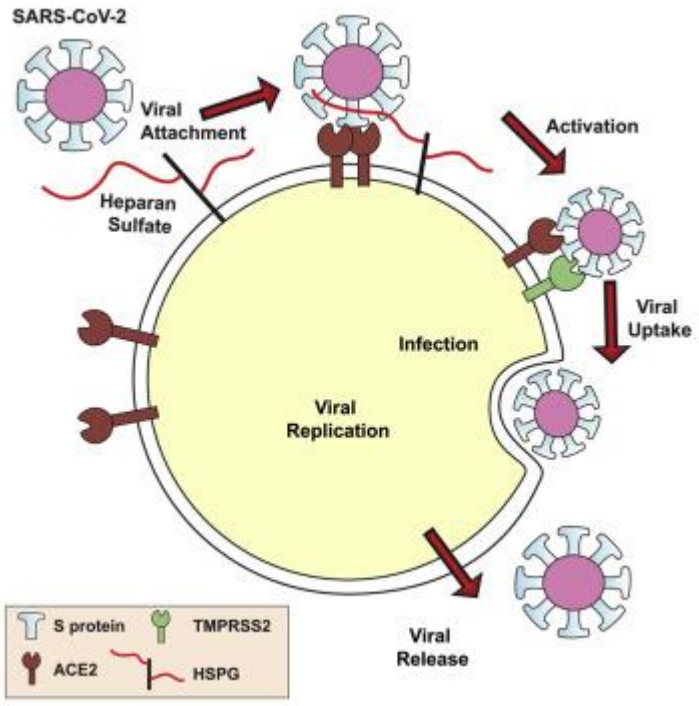

Figure 1.1 Molecular structure of SARS-CoV-2 and interaction with the cell receptor ACE2

The primary cause of death in patients with COVID-19 was initially identified as acute respiratory infection with severe pneumonia. Cardiovascular issues and coagulation issues linked to a severe systemic inflammatory state have gained attention as aggravating factors in the clinical picture of persons infected with SARS-CoV-2 as the pandemic has progressed and investigations have been conducted globally. Pneumocyte II, endothelial cells, small intestine enterocytes, smooth muscle cells, myocardial cells, and proximal renal tubule epithelial cells are only a few of the cells that express the ACE2 protein. As a result, the respiratory, gastrointestinal, renal, and cardiovascular systems all contain the SARS-CoV-2 receptor. A significant discovery is that smoking, diabetes, and systemic arterial hypertension all encourage an increase in ACE2 expression. This information helps to explain why these comorbidities are linked to a higher risk of severe clinical COVID-19 manifestations (Piché-Renaud *et al.* 2022).

1.3 Characteristics of the Immune Response in Patients with Severe Forms of COVID-19

Hypothesized that the natural history of COVID-19 has three phases: the viremia phase that occurs until the 7th day, the acute phase between the 7th and 10th day characterized by the presence of pneumonia, and a third phase that may vary depending on the disease (Chowdhury *et al.* 2020) ability of the immune system to adequately control the viral infection. Most patients, especially children and healthy adults, progress satisfactorily, and between 14th to 21th days they have a recovery phase. However, patients with comorbidities and the elderly may, in this third phase, present severe forms, progressing to death. Patients who progress to severe clinical forms present several laboratory findings, such as: lymphopenia, increased D-dimer expression, increased viral load and increased serum levels of inflammatory cytokines. Chowdhury *et al.* (Chowdhury *et al.* 2020) discussed the indication of using anticoagulant therapy, having as a parameter for its use the increase in serum levels of D-dimer.

In 2020, (George *et al.* 2020) evaluating the production profile of inflammatory mediators of 54 patients with COVID-19, postulated that there are two immunological mechanisms that cause tissue damage in patients who develop severe respiratory injury. The first mechanism corresponds to macrophage activation syndrome (MAS), and the second is represented by an alteration in the antigenic presentation leading to a high production of IL-6 with deregulation of the immune response. Additionally, high serum levels of TNF-α were also detected, amplifying the state of dysregulation of the immune response, which, in addition to not being able to contain the infectious process, ends up promoting the development of a systemic hyperinflammatory response and tissue damage. This study also found a lymphopenia with a reduction in CD4+ T lymphocytes and NK cells, so patients who have an unsatisfactory clinical course have alterations both in the innate immune response and in the acquired immune response.

The presence of lymphopenia and cytokine storm as a maximal expression of immune response dysregulation has been observed in several other studies performed in patients with severe clinical form of COVID-19. In a study with 1.099 patients, lymphopenia was

present in 914 (83.2%) patients at hospital admission. Elevated serum levels of several pro-inflammatory mediators, such as interleukin-1beta (IL-1β), IL-1RA, IL-7, IL-8, IL-9, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IP10 (interferon γ-inducible protein), TNF-α, and vascular endothelial growth factor (VEGF) have been found in patients with COVID-19 (Fathi and Rezaei 2020).

As previously described in SARS, MERS and COVID-19, MAS corresponds to an important immunopathological mechanism that leads to the development of severe respiratory distress, lymphopenia, coagulopathy, transaminase elevation, hyperferritinemia, and multiple organ dysfunction. MAS syndrome is a phenomenon also observed in autoimmune diseases, such as juvenile idiopathic arthritis. The use of systemic corticosteroids, cyclosporine and intravenous immunoglobulin are recommended treatments in these situations. Due to the high production of proinflammatory cytokines induced by SARS-CoV, MERS-CoV and SARS-CoV-2, systemic corticosteroids were initially used in the treatment of patients with severe disease, in order to reduce the inflammatory process in the lungs. Nonetheless, viral clearance. This fact led the WHO not to recommend the routine use of systemic corticosteroids (Idiz *et al.* 2022).

Controlling viral replication concomitantly with the modulation of the inflammatory response is the main challenge in the treatment of COVID-19. The use of systemic corticosteroids and/or immunosuppressive drugs in autoimmune diseases show good results, but in the case of a viral disease characterized by a high replication rate of the etiologic agent, the inhibition of the immune response can favor the evolution of the infectious process. On the other hand, Precision Medicine strategies that selectively inhibit key cytokines in this cytokine storm phenomenon appear to be promising in the treatment of patients with COVID-19. The use of the biological Tocilizumab, which selectively inhibits IL-6, partially rescued the dysregulation of the immune response caused by SARS-CoV infection (Magro 2020). Another promising therapeutic possibility is the combination of antiviral drugs with anti-inflammatory drugs. Quality clinical trials will be able to elucidate in the future the effectiveness of these treatment modalities that,

although promising, still do not have robust scientific evidence to be used as a therapeutic routine.

The search for biomarkers capable of guiding the prognosis has also been researched. Studies performed in patients with COVID-19 admitted to an intensive care unit (ICU) who progressed to fatal outcomes suggest that elevated serum levels of two chemokines, MCP-3 (monocyte chemotactic protein-3) and IP-10 (interferon gamma induced protein 10), may be markers that indicate severity and consequent higher probability of death (Yang *et al.* 2020). The evaluation of the profile of cells involved in the immune response in SARS-CoV-2 infection can help us understand the immunopathology and infer clinical and laboratory findings, aiding in the evaluation of patients.

An interesting laboratory study carried out with patients with COVID-19 revealed important data on the regulation of the immune response, (Kaftan *et al.* 2021) evaluated 65 patients with COVID-19, classified as mild (n=30), severe (n=20) and extremely severe (n=15) forms of the disease. Elevated serum levels of ferritin, lactic dehydrogenase and D-dimer have been detected in critically and extremely critically ill patients. The absolute number of CD4+ T lymphocytes, CD8+ T lymphocytes and B lymphocytes all gradually decreased with increasing disease severity. A reduction in the percentage of natural regulatory T cells (nTreg) was seen in extremely critically ill patients. These findings support the hypothesis that dysregulation of the immune response is a crucial phenomenon in determining an unfavorable clinical course.

The data taken together from the various clinical and laboratory studies carried out in patients infected with SARS-CoV-2 and who progressed to severe acute respiratory syndrome support the theory that there are two immunopathological mechanisms that explain the cases that did not progress satisfactorily (Sharma *et al.* 2020). MAS, characterized by intense macrophage activation and high production of IL-1 in the early stages of the disease, presents a series of events that are observed in the laboratory in the analysis of serum from critically ill patients. Patients with moderate antigen presentation develop lymphopenia associated with a decrease in B lymphocytes and CD4+ T lymphocytes, reduction in serum levels of IgA and IgM, increased production of

cytokines IL-1, IL-6, TNF-α, increased ALT/AST liver enzymes, elevated serum levels of D-dimer, C-reactive protein and ferritin. People who in the initial phase of the disease have a poor presentation of the antigen with lymphopenia (decrease in the peripheral blood of NK cells, B lymphocytes and CD4+ T lymphocytes, and CD8+ T lymphocytes) walk towards the deregulation of the immune response (Skevaki *et al.* 2020).

Figure 1.2 schematically represents the interaction between cells and cytokines that participate in the immunopathological process of COVID-19.

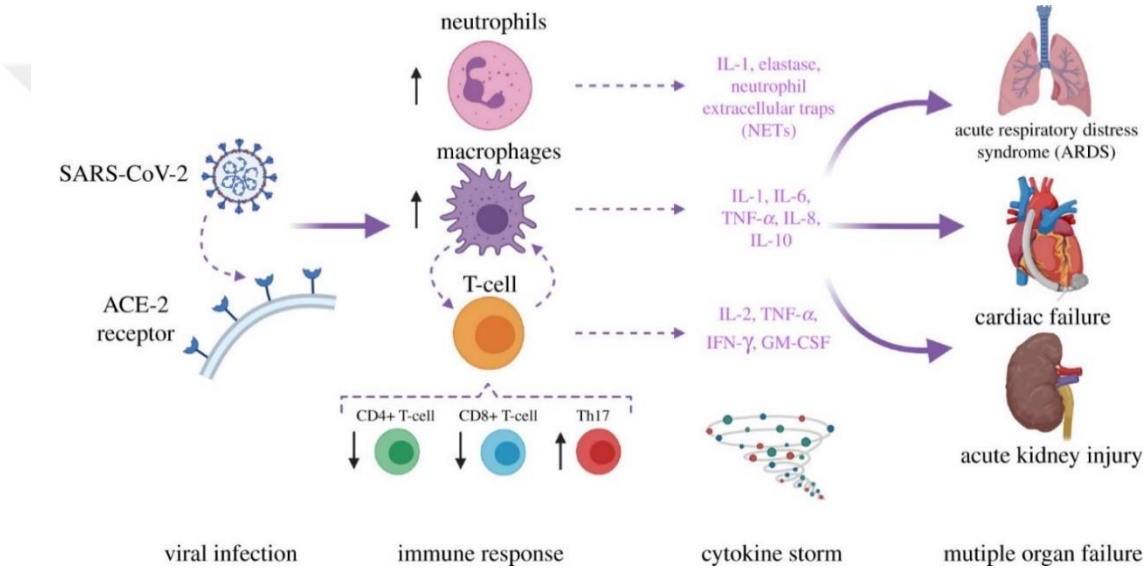

Figure 1.2 Immune mechanisms leading to the cytokine storm associated with severe forms of COVID-19

1.4 What Do We Know About Immunosuppression and SARS-CoV-2 Infection?

Relevant publications on the link between COVID-19 and immunodeficiencies 8 were found in a recent systematic review of the literature (Fung and Babik 2021). Inclusion criteria included reports of SARS-CoV-2 infection in immunocompromised adults and children. The study included data on the clinical progression of 110 patients undergoing immunosuppression for reasons including cancer treatment and organ transplantation. Patients with advanced stages of cancer and a weakened general clinical condition had a more severe course of COVID-19, although this did not necessarily indicate a poor prognosis. The authors draw the conclusion that immunosuppressed patients with

COVID-19 are relatively uncommon and fare well when compared to those with other conditions. Possible protection from a weaker cellular immune response explains this finding.

The intricacy of the immune response in COVID-19 46 was recently highlighted by the report of two clinical cases of patients with X-linked agammaglobulinemia (XLA) who were infected with SARS-CoV-2 and recovered. The raised CRP and ferritin levels and symptoms of fever, cough, and loss of appetite that characterise interstitial pneumonia were present in both patients, but they were able to recover without the use of ventilators and intensive care. The two examples presented here suggest that even in people who lack the ability to produce immunoglobulins, a T-lymphocyte-mediated immune response may be enough to successfully resist the virus. This fascinating and unforeseen development in these patients raises the possibility that the immune system employs numerous pathways to manage SARS-CoV-2 infection (Siddiqi and Mehra 2020).

1.5 Antivirals Against COVID-19

Given that Th17 LTs may play a role in COVID-19, the evaluation of several inhibitors of its transcription factor RORgt (and RORα) is postulated. Alternatively, the use of JAK2 inhibitors (eg fedratinib, used in myelofibrosis) is proposed to restrict the inflammatory function of Th17. Because IL-6 and IL-23 activate STAT3 through JAK2, along with these inhibitors, the Food and Drug Administration (FDA) has approved STAT3 inhibitors, which are useful, but they could affect the activity of IL-21-producing LB. These inhibitors could be used together with type I interferons without affecting them, since these use JAK1 and TYK2 to activate STAT1 and STAT2 (Beigel *et al.* 2020).

Nucleoside analogues (NAs) such favipiravir and ribavirin that are currently in use and those still in the research phase may both be effective against SARS-CoV-2 (remdesivir and galidesivir). Many viruses, including human coronaviruses, are susceptible to NA because adenine and guanine derivatives target RNA-dependent RNA polymerase and inhibit viral RNA synthesis. Among these NAs, the phosphoramidate prodrug of an adenine derivative with possible reverse transcriptase activity, remdesivir (GS5734),

displays broad-spectrum effects against RNA viruses like MERS and SARS, both in culture and in animal models. Recently, it was found to have an EC50 (mean maximal effective concentration) of 0.77 μM against SARS-CoV-2 in infected Vero E6 cells (Biswas *et al.* 2021).

A report (Frediansyah *et al.* 2021) of the first infected 34-year-old American showed clinical improvement after the administration of remdesivir, after the failed attempt of other drugs, so it is concluded that this drug should be clinically and rigorously evaluated. Perhaps it is this case that led the United States National Institute of Health (NIH) to conduct two phase III clinical trials to evaluate this drug in patients with COVID-19. These are randomized, controlled, double-blind studies with 308 and 453 volunteers to evaluate the efficacy and safety in patients with mild or moderate (NCT042552664) and severe (NCT04257656) respiratory disease due to COVID-19. The studies began in February and results should be obtained in April and May 2020. The LOTUS trial was conducted in China to investigate the efficacy and safety of lopinavir/ritonavir (LPV/RTV, protease inhibitors); however, there were no differences in clinical improvement (Patel *et al.* 2021).

Antimalarial therapy began in the mid-17th century by Jesuit missionaries in Peru, which led to the French Pelletier and Caventou obtaining the alkaloid quinine and then synthesizing it in the United States as chloroquine (CQ). The prospects of drugs such as CQ and hydroxychloroquine (HCQ) are promising due to in vitro and animal studies (Sinha *et al.* 2021), but these drugs require solid clinical evidence to be recommended for use against COVID-19, as demanded by several letters to the editor about these studies CQ and HCQ are aminoquinolines, the difference being that HCQ has an N-hydroxyethyl group and CQ has an N-diethyl group.

A French clinical study with many deficiencies, such as sample size, lack of randomization, poor selection of study groups, lack of description of side effects, among others, has been the subject of discussion in the scientific community. The study attributes to HCQ sulfate and also to azithromycin the reduction of viral load from day six in patients with COVID-19; however, the evidence is not strong enough. Although this study

served for the FDA to authorize the prescription of HCQ and CQ to patients with COVID-19, the scientific community calls for clinical trials with better data and that point to a true benefit (Gautret *et al.* 2020).

In vitro studies indicate that CQ blocks SARS-CoV-2 infection at low micromolar concentrations (EC50=1.13 μM) and clinical study records exist conducted in China, demonstrating the safety and efficacy of CQ or HCQ in pneumonia associated with COVID-19. These studies would show that CQ phosphate would be able to inhibit the exacerbation of pneumonia, would improve the findings of pulmonary images, would promote the negative conversion of the virus and would shorten the course of the disease; In addition, no serious adverse effects are reported in recovered patients (Hathout *et al.* 2020).

The proposed mechanisms of action for CQ or HCQ are similar, and many have been evaluated in vitro and some animal models where it has been shown that they increase intracellular pH, which decreases the lysosomal activity of antigen-presenting cells with the consequent decreased activation and costimulation of LT CD4 + and production of IL-1, IL-6 and TNF-α. CQ and HCQ also prevent the binding of TLR receptors to mRNA, which also decreases the production of proinflammatory cytokines (Singh and Vijayan 2020).

Since March 2020, a phase III clinical trial has been carried out at the University of Minnesota endorsed by NIH (NCT04308668) to evaluate post-exposure prophylaxis and preventive therapy of HCQ in SARS-CoV-2. It is a randomized, placebo-controlled study that will enroll 3.000 asymptomatic and symptomatic participants from the United States and Canada (Boulware *et al.* 2020).

Interferons are broad-spectrum antiviral agents and have shown activity against SARS-CoV and MERS-CoV. Ribavirin combined with LPV/RTV has been used against SARS-CoV; however, against SARS-CoV-2 in vitro it requires a high EC50=109.5 μM; therefore, a clinical study is being carried out in China (ChiCTR2000029387) of ribavirin,

LPV/RTV and their combination. Additionally, it has been determined that remdesivir and IFN-β have greater antiviral activity in vitro than LPV/RTV (Dong *et al.* 2020).

1.6 Vaccines Against COVID-19

As a result of the urgent need for vaccinations against COVID-19, proteomics has been used to look for pathogen-specific antigens in the S protein. Bioinformatics has allowed the identification of 933 pentapeptides that are missing from the human proteome; 107 of these peptides are located in the vicinity of protein S, and 66 of these peptides are more immunogenic and hence suitable for use in vaccine development (Felsenstein *et al.* 2020). The World Health Organization (WHO) can choose among as many as 52 potential vaccine candidates developed on protein, RNA, DNA, non-replicating vector, replicating vector, inactivated virus, attenuated virus, and virus-like particle platforms. Only the RNA and non-replicating vector vaccines out of these options have begun human safety trials (Kyriakidis *et al.* 2021).

A vaccination against the current COVID-19 pandemic needs to be manufactured and distributed rapidly on a massive scale. Consideration should be given to nucleic acid-based vaccines like mRNA vaccines due to their suitability for rapid response applications, their ability to elicit systemic protective immune responses, and their rapid and adaptable production timelines. mRNA vaccines induce CD8+ LT and humoral immune responses by expressing vaccine antigens in situ, so simulating a viral infection. Because it is recognised by receptors like TLR, this form of vaccination can also stimulate innate immunity, which in turn promotes the development of antigen-presenting cells that are responsible for boosting adaptive immunity. Unlike traditional immunizations, mRNA vaccines do not produce infectious particles or alter the host cell's genome. After the pathogen's most immunogenic antigen has been isolated, its gene is sequenced, synthesised, and cloned onto a DNA plasmid. Following in vitro transcription of the mRNA, a vaccine is administered to the patient, possibly employing lipid nanoparticles (NPL) as the vehicle (Figure 1.3) (Barzelighi *et al.* 2020).

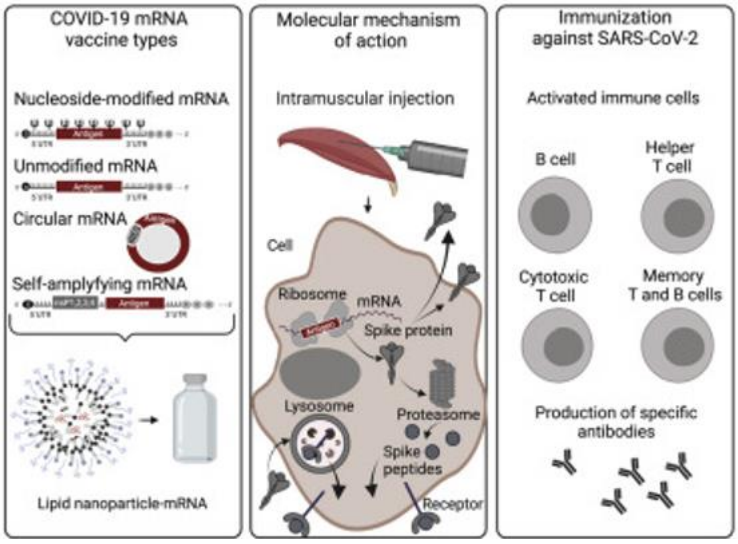

Figure 1.3 mRNA vaccine candidate against SARS-CoV-2. NPLs containing the mRNA with the sequence of a protein S fragment are inoculated IM **(A)**. NPLs are recognized and endorsed by CPAs **(B)**

In March 2020, the NIH, with the support of the National Institute of Allergy and Infectious Diseases (NIAID) of the United States, initiated the clinical trial NCT04283461 on the safety and immunogenicity of the vaccine mRNA-1273 for prophylaxis of SARS CoV-2 infection. This is a phase I, open-label, dose-ranging trial in 45 healthy men and non-pregnant women between the ages of 18 and 55. The mRNA-1273 vaccine has been manufactured by Moderna TX, Inc. (Anderson *et al.* 2020). It is a vaccine consisting of mRNA encapsulated in lipid nanoparticles (NPL) that encodes the fusion protein S of SARS-CoV-2. The vaccine will be administered intramuscularly on days 1 and 29 in three cohorts (25 μg, 100 μg, and 250 μg). Follow-up will be for 12 months.

Protein subunit vaccine: To stimulate the durable protective and/or therapeutic immune response, a subunit vaccination based on synthetic peptides or recombinant antigenic proteins is used (Heidary *et al.* 2022). However, the subunit vaccine is not very immunogenic and needs the help of an adjuvant to produce the desired immune responses.

mRNA vaccine: mRNA is a novel platform that has the advantages of not being infectious and not integrating, therefore there is very little chance of insertional

mutagenesis occurring. Researchers are examining both virally produced self-replicating RNA and non-replicating RNA. To make these vaccines more stable, it is possible to reduce the mRNA's immunogenicity and make other adjustments.

DNA vaccines: Transfected cells produce transgene-specific proteins in a steady stream, mimicking the effects of a living virus. Furthermore, immature dendritic cells endocytose antigenic material, which is then presented to CD4+ and CD8+ T cells in conjunction with MHC 2 and MHC 1 antigens on the cell surface, thereby triggering humoral immune responses. Effective and mediated by cells.

Viral vector vaccines: A vaccine made from a virus holds great potential as a preventative measure against disease. These vaccines successfully stimulate an immune response and are particularly selective in their gene delivery to target cells.

The vaccine developed in Russia, based on adenoviral vectors, was registered by the Ministry of Health of the Russian Federation on August 11, 2020, thus becoming the first vaccine against SARS-CoV-2 to get to market (Callaway 2020).

This vaccine uses a vector that lacks the reproduction gene and uses it to carry genetic material; In the case of SARS-CoV-2, its vector (AD26) contains the S protein gene, enters the cell and in response generates immunity in the first vaccination. After 21 days, the second vaccination is performed with another vector (AD5) that stimulates an immune response and enhances long-term immunity.

Among all this arsenal of advances in the understanding of the disease, the ongoing trials on the imminent vaccine, and the race against time, there is the great fear of reports of reinfection (Callaway 2020).

1.7 Immunological Response

The types of responses generated by T lymphocytes are: T helper lymphocytes (CD4+), which organize the adaptive response by activating B lymphocytes in the production of antibodies and cytotoxic T lymphocytes (CD8+) that are essential to eliminate cells infected by the virus.

Regarding the antibodies produced by B lymphocytes, immunoglobulin M (IgM) is produced when the infection is recent, while immunoglobulin G (IgG) is produced in later stages. SARS-CoV-2 induces IgG production against protein (N), which can be detected in the serum 14 days after the onset of the disease.

The responses of helper T cells (CD4+) were lower in number and function compared to cytotoxic T lymphocytes (CD8+), according to a study with 128 cases. Similar to how clonal expansions of T cells (CD8+) were found in the bronchoalveolar lavage fluid (BALF) of patients with COVID-19, but only in those with mild disease, single cell analysis of BALF from patients with SARS-CoV-2 suggests that the presence of adaptive T cell responses may be protective in SARS-CoV-2 infection.

The initial infection phase of a virus normally lasts between 7 and 10 days, during which time adaptive T cell immune responses prime and expand to control the virus. Patients infected with COVID-19 either make a full recovery or experience a severe decline in health within this time frame.

Based on a meta-analysis of 38 studies, (Deeks *et al.* 2020) found that the sensitivity of IgG, IgM, IgA, total antibody, and IgG/IgM antibody tests was low in the first week after symptom onset (30.1%), rising steadily in the second week, and peaking in the third week. Over 1-7 days, the IgG/IgM combination exhibited a sensitivity of 30.1% (95% CI: 21.4 to 40.7); over 8-14 days, it was 72.2% (95% CI: 63.5 to 79.5); and over 15-21 days, it was 91.4% (95% CI: 87.0 to 94.4). The tests' sensitivity past 35 days after symptoms first appear has not been adequately studied.

Thus, in patients with negative RT-PCR results but high clinical suspicion that more than two weeks have passed since the onset of symptoms, COVID-19 antibody testing may be useful.

1.8 Retention in Immunology

Adaptive immune system cells play a vital role in preserving immunological memory. B and T lymphocytes that recognise viral antigens through their antigen receptors become activated, proliferate, differentiate, and secrete effector molecules in response to the majority of acute viral infections.

Roughly 90% of these virus-specific "effector cells" die off as the infection clears, but 10% survive as long-term "memory" cells (Mukerjee 2020).

It is clear that immune memory cells, such as long-acting antibody-secreting plasma cells, can release a steady stream of effector molecules (LLPCs). Dormant memory lymphocytes, on the other hand, are usually placed in such a way that they can quickly revive in response to reinfection and execute effector programmes imprinted on them during the original response. Pathogen-specific memory B cells (MBCs) that express antigen receptors and the transcription factor T-bet rapidly proliferate and develop into IgG+ antibody-secreting IgG+ plasmablast cells upon reinfection (PBS).

Reactivated T-bet is expressed by memory T cells (CD4+), which then proliferate, "assist" activate MBCs, and release cytokines (such as interferon gamma IFN) to activate innate cells. Memory CD8+ T cells, meantime, can release cytolytic chemicals that directly kill virus-infected cells.

In order to avoid sickness and limit viral transmission, these virus-specific memory populations are coordinated to quickly eliminate the virus.

In order to infect host cells and disseminate, SARS-CoV-2 relies on the contact between the receptor-binding domain (RBD) of its spike (S) protein and angiotensin-converting enzyme 2 (ACE2).

RBD-specific monoclonal antibodies kill the virus in vitro and in vivo, and many studies suggest that the majority of SARS-CoV-2 infected individuals develop these antibodies during the initial immune response. As a result, if LLPC or MBC express RBDs, RBD-specific antibodies will likely aid in protection against reinfection.

That's why it's so important to tell the difference between the initial, waning PB-derived antibodies and the later, permanent CLL-derived antibodies that can neutralise subsequent, potentially lifetime infections (Vabret *et al.* 2020).

1.9 Immune System Dysregulation

In a sample of 127 patients in Wuhan, China, where the presence of COVID-19 was confirmed by laboratory testing (Bai *et al.* 2020). There was no significant change in the number of T cells (CD8+) or B cells among COVID-19 patients, but an increased NLR (neutrophil-lymphocyte-ratio), meaning an increasing neutrophil count and a decreasing lymphocyte count, was commonun among patients and was more evident in severe cases, as was T-lymphopenia, especially decreased T-lymphocytes (CD4+). Patients with COVID-19 may have peripheral lymphopenia because lymphocytes are being recruited to the respiratory tract and adhering to the inflamed respiratory vascular endothelium.

This in vitro investigation found that hyperinflammatory responses may play a role in the aetiology of disease, as greater levels of serum cytokines, chemokines, and increased NLRs in infected patients were associated with disease severity and bad outcomes. COVID-19; this severe inflammation can and often does result in fatalities.

CD4+ and CD8+ T lymphocytes, in particular, have been found to play a significant role in suppressing the overactive immune responses that occur in response to viral infection.

But in some COVID individuals, the ideal state may not be hyperactivation or hypoactivation of T cells or bias toward an inefficient differentiation state (such as TH17 cells, depleted T cells, or terminally developed T cells).

Future research assessing virus replication should give context for understanding the evolution of T cell responses during infection, as viral load can have a major impact on the amplitude and quality of the response by the SARS-CoV-2 virus (Cheemarla *et al.* 2021).

Studies on coronaviruses such as SARS-CoV and MERS-CoV, in addition to all the knowledge generated over a year with SARS-CoV-2, have shown the immunogenic capacity of structural proteins S and N. It is well established that vaccine candidates that use protein S as antigen induce a protective immune response mediated by neutralizing antibodies.

The evidence of vaccine candidates for the aforementioned coronaviruses has allowed rapid development of vaccines against SARS-CoV-2, which mostly use protein S as antigen. However, it is clear that there must be other antigens that also participate in the induction of a protective immune response, such as the N protein, which has been shown to be capable of inducing a protective immune response mediated by T73 cells.

To date, a large number of mutations have been reported in the S protein of SARS-CoV-2, which has generated various variants for this virus. There are reports that show that the antibodies present in vaccinated individuals have a lower neutralizing capacity for the reported variants of SARS-CoV-2. However, the comparative analysis of the sequences of different SARS-CoV-2 isolates do not show variations in the coding sequence for the N protein. This suggests that the use of this protein as a vaccine candidate could induce an immune response against variants, currently circulating in the world. A preclinical study of a vaccine candidate that uses protein N as an antigen report that animals immunized with this protein are capable of establishing an immune response against variants of SARS-CoV-2. This study also indicates that the immune response is cellular in nature and independent of neutralizing antibodies. So far it is unknown whether the N

protein induces neutralizing antibodies, however, it is known to be capable of inducing a memory T cell response. On the other hand, memory T cell response against SARS-CoV N protein has been observed 11 years after a primo-infection. Interestingly, and in contrast, humoral immunity for this same coronavirus has been detected only 3 years after the first infection. These data suggest that cellular immunity persists longer and, therefore, it is important to consider a vaccine candidate that induces both effector arms of the immune system, both cellular and humoral.

Recently, some research groups have proposed the use of other antigens of this virus to generate new vaccine candidates. Among these possible new antigens is the use of the N protein. To date, few works have proposed the combination of more than one antigen for the induction of a more robust immune response.

1.10 Objectives of the Thesis

- In this study, the effectiveness of the COVID-19 Pfizer vaccine was investigated by investigating the cytokine levels in the blood samples taken from 4 groups of Hilla City public, Iraq, who were never vaccinated with the COVID-19 Pfizer (Biontech) mRNA vaccine and were vaccinated with second dose, and who had or did not have COVID-19 infection information is intended.
- To measure the frequency of immediate adverse reactions as a consequence of the application of the **Pfizer/BioNTech (BNT162b2)** vaccine against SARS-CoV-2.
- To establish some factors associated with the appearance of immediate adverse reactions as a consequence of the application of the **Pfizer/BioNTech (BNT162b2)** against SARS-CoV-2.
- To evaluation of post-vaccination antibody titer in male patients infected/non infected/vaccinated/nonvaccinated Covid Patients;
- For the evaluation of cellular immune response by measuring cytokines INF-γ, and TNF-α, IL-17, IL-6 and IL-10 in the culture supernatant of TCD4+ lymphocytes.

2. LITERATURE REVIEW

The immune response observed in COVID-19 is the same as the response observed in previous viral infectious processes, mediated mainly by T lymphocytes (CD4+) and (CD8+) that, by mildly affecting, there is a coordinated response between these groups, which producing the decrease and elimination of the viral load and subsequent formation of antibodies responsible for long-term immunological memory, which are reactivated in the event of a possible reinfection. There is currently a wide genomic variety of SARS-CoV-2, dividing it into strains where type L may be more aggressive and more frequent (Cascella *et al.* 2022).

In the case of primary virus infection, it usually takes 7-10 days for T-lymphocyte adaptive immune responses to be present in the blood, therefore IgG-IgM detection tests are not recommended before of the week after the onset of symptoms, a time that is also expected if a patient with SARS-CoV-2 begins to recover or develops a serious illness (Bar-On *et al.* 2020).

Studies show the development of immunological memory up to 35 days. It was found to be in the research phase after this time in the first data on cases or vaccines, so constant updating of the information is recommended. However, since there are reports of cases in which reinfection is already confirmed, an accurate diagnosis should be made with IgG-IgM and RT-PCR, clinical and imaging (CAT) laboratory tests, as well as; We must thoroughly investigate each case in detail before confirming SARS-CoV-2 reinfection.

2.1 Antigenicity and Immune Response

Protein S (spike) of the viral surface has been identified as an optimal antigen for vaccine development, based on previous studies with SARS-CoV and MERS-CoV. In SARS-CoV and SARS-CoV-2, protein S binds to a receptor in the lung epithelium: angiotensin-converting enzyme (ACE-2), so that antibodies against protein S could neutralize the virus and, consequently, viral entry into the cell, acting as neutralizing antibodies (AcN).

In animal studies of vaccines against SARS-CoV, it was shown that vaccination generated greater survival, lower viral loads in respiratory samples, and less morbidity when compared to those not vaccinated. However, in a small proportion the phenomenon of "antibody dependent enhancement" (ADE) occurred; that is, viral entry facilitated by non-neutralizing antibodies (Li *et al.* 2020).

2.2 Duration and Antibody Titers

In the experience with SARS-CoV in China 2002-2003, it was shown that, although all patients developed a humoral response, IgG titers decreased, on average, 2 years after infection, with significantly lower titers at the third year. Another study, also in China in the same period of time, at the Beijing Police Armed Forces Hospital, showed that the peak of IgG and AcN antibody titers occurred at the fourth month post-infection (Borrion *et al.* 2020). Then, a progressive decrease in IgG began, while AcN titers remained detectable for longer and began to decrease at the 16th month of follow-up. A study in Korea, in 2015, (Kim *et al.* 2017) showed that in most patients with Middle East respiratory syndrome coronavirus (MERS) and severe clinical evolution, they generated high titers of antibodies against MERS-CoV; however, this did not occur with mild cases, despite presenting with pneumonia. In addition, at one year of follow-up, titers decreased and it was found that the duration of viral RNA detection in saliva was inversely related to blood antibody titers.

2.3 Safety Considerations for SARS-CoV-2 Vaccines

Monitoring safety is essential, since most people affected by SARS-CoV-2 have mild or asymptomatic infections. In them, the benefits of the vaccine must outweigh their low risk of serious illness, based on their baseline health status. On the other hand, the aforementioned risk of ADE remains latent, a phenomenon observed in dengue virus infection and not ruled out in this new entity, even though the evidence available to date seems to indicate that this would not occur with vaccination against SARS-CoV-2 (Li *et al.* 2021).

2.4 Pandemic Vaccine Development

The traditional development of a vaccine takes approximately 10 to 15 years to be licensed for use in humans. The experience acquired in 2009 with the influenza A H1N1 and Ebola pandemic in Africa 2013-2016, (Coltart *et al.* 2017) has shown that a reaction capacity, greater manufacturing worldwide, as well as financing, are required to allow research and development of vaccines, not necessarily profitable, or that remain available for possible future threats, even when the contingency has been controlled. In other words, long-term preparation.

To respond quickly enough to a pandemic, the standard development process has had to be sped up, from its usual 6–12-month time frame. Rather than being tested sequentially, as has traditionally been the case, safety, immunogenicity, protection against infection, adverse events, and duration of protection are all studied simultaneously. There should be a window of about 16 months for a pandemic platform from the time viral sequencing is complete to the time when a protective immune response has been demonstrated, safety has been evaluated, and large-scale production has begun.

There are currently four types of vaccines in phase III development (Ehsan *et al.* 2020):

- Inactivated vaccines.
- Vaccines based on purified or recombinant proteins.
- Vaccines based on DNA/RNA nucleic acids.
- Vaccines based on viral vectors.

2.5 Inactivated Vaccines

They are SARS-CoV-2-based vaccines with chemical inactivation, with the aim of producing a non-infectious virus, with or without adjuvant. In technological terms, they are the ones used in more traditional processes, so the know-how is known and the infrastructure to manufacture them is available. They present the host with multiple

antigens and, consequently, can induce broader responses since, unlike other candidates, the immune response is directed at more than one epitope. Both the China Center for Disease Control and Prevention (CDC-China), the Wuhan Institute of Virology, and the Beijing Institute of Biologicals, as well as China's Sinopharm and Sinovac laboratories, began developing inactivated vaccines early of 2020 (Kashte *et al.* 2021).

2.5.1 Preclinical stages of inactivated vaccines

Sinovac Laboratory candidate vaccine

Eleven hospitalised patients (5 from China, 3 from Italy, 1 from Switzerland, 1 from the UK, and 1 from Spain) provided the strains used in its preparation (Schultz *et al.* 2022). The SARS-CoV-2 virus strains found in these 11 samples were indicative of the circulating viruses, showing a broad distribution in the phylogenetic tree. To prove the phylogenetic relatedness of the other 10 strains, CN1, CN3–5, OS1–OS6, CN1 and OS1, 2019–nCoV–BetaCoV/Wuhan/WIV04/2019, and EPI ISL 412973, they used the CN2 strain for purification and inactivation and production of PiCo-Vacc (Gao *et al.* 2020).

Purification, culture and multiplication in Vero cells and inactivation with β-propiolactone were performed and aluminum was used as adjuvant. Subsequently, it was purified with deep filtration and chromatography, confirming the morphological stability of the strains by electron microscopy, and their genetic stability by sequencing and molecular analysis.

Trials in murine models, of inactivated vaccines against SARS CoV2, in lines of BALB/c mice and Wistar rats with different doses of PiCoVacc and adjuvant in the control group (n=10), showed that vaccination with PiCoVacc generates a specific humoral response, IgG type, against the 10 representative strains of SARS-CoV-2, which were initially isolated from clinical samples (Gao *et al.* 2020). Specific antibodies for protein S, RBD and N, were measured between weeks 1 and 6 from the initial immunization, by ELISA technique. IgG against protein S and RBD developed rapidly and peaked at week 6. RBD

is the "dominant" immunogen, with good correlation when compared with plasma from patients recovered from SARS-CoV-2 infection. The presence and function of AcN was measured by microneutralization assay (MC), in the same rodents. Three weeks post vaccination were better: the higher the dose and the longer the time elapsed. Then, this same group tested in non-human primates (Macaca mulatta) 3 IM doses of 3 or 6 μg (medium and high dose) of PiCo-Vacc in a schedule of 0, 7, and 14 days, observing results similar to those obtained in mice. Finally, they were subjected to the challenge ("viral challenge") with intra-tracheal inoculation of SARS-CoV-2, and as expected, those who received placebo developed pneumonia and presented excessive viral excretion, measured by genomic RNA in the pharynx, throat and lung, when compared with the vaccinated, who presented mild symptoms (Thakur *et al.* 2021).

2.5.2 Human clinical trials

CoronaVac (NCT04352608)

Randomized, placebo-controlled, double-blind, phase I and II phase IIA study to evaluate the safety and immunogenicity of the inactivated SARS-CoV-2 vaccine, in healthy adults between 18 and 59 years of age. Trial location: Juining County, Jiangsu Province, China. Enrollment began on April 16, 2020. A total of 600 healthy adults aged 18 to 59 years were randomly assigned to receive two IM injections of study vaccine at doses of 3 μg/0.5 mL, 6 μg/0.5 mL or placebo, in two schemes: 0 and 14 or 0 and 28 days.

Safety: For the safety evaluation, adverse events were collected after each vaccination, requested the first seven days after injection, and unrequested, 28 days after vaccination. Blood samples were obtained to study for antibodies. CoronaVac was well tolerated and without safety alerts.

Immunogenicity: The assay showed good immunity with low doses of 3 μL, generating a seroconversion of 92% with the 0 and 14 day schedule and 97.4% with the 0 and 28 day schedule. Samples were taken on days 0, 28 and 42 for the 0 and 14 day schedule (n=297),

and days 0 and 56 for the 0 and 28 day schedule (n=294). For the study of anti-RBD antibodies by ELISA, a dilution greater than 1:160 was considered positive. AcN measurement was performed with a modified cytopathogenic effect assay. There were no statistically significant differences between the two regimens, and younger subjects tended to have higher AcN15 titers. Given the comparable results of this phase II trial, with both doses (3 μL and 6 μL) and with both schedules, 0-14 and 0-28 days, it was decided in the phase III trial to use the 3 μL dose and the 0-14 days scheme since this would allow a faster response (Jin *et al.* 2022).

Sinopharm and Wuhan Institute of Biological Products Co Ltd and Henan Provincial Center for Disease Control and Prevention (Chinese CDC) (ChiCTR2000031809)

They publish their experience of phases I and II, of a double-blind, randomized, controlled study (Xia *et al.* 2020). This study was carried out between April 12 and May 12, 2020 and included 481 healthy volunteers, 96 in phase I and 224 in phase II. Vaccine strain WIV01 from National Genomic Data Center of the Chinese Academy of Science, accession No. SAMC133237, and GenBank accession No. MN996528 from Jinyintan Hospital, Wuhan was cultured on Vero cell line, inactivated with β-propiolactone (1:4.000 vol/vol) between 2 and 8°C for 48 h. Clarification, ultrafiltration and a second inactivation were carried out. Then, adsorbed on 0.5 mg aluminum, to be distributed in pre-sealed syringes with 0.5 mL of sterile saline solution, without preservatives. The vaccination schedule used was 0-14 or 0-21 days using the average dose of 5 μg, with a 3:1 ratio between vaccine and placebo.

Safety: The safety results and the registry of adverse events showed, in general, mild transient and self-limited reactions, which did not require treatment; there was no major difference between the phase I injection schedule and the phase II schedule. The daily record of local (pain, redness, edema) and systemic (fever, headache, fatigue) adverse reactions was performed for 7 days post injection. Unsolicited symptoms were followed up for 28 days after each vaccination.

Immunogenicity: Seroconversion was shown in 100% of the participants, as well as in those who received injections on day 0 and 21 in phase II. While this was in 85.7% in those who received scheme 0-14 in phase II. There was no seroconversion in the group receiving adjuvant alone. Most of the participants generated antibodies after the second injection.

Phase III studies are scheduled to begin: in Morocco (Rabat and Casablanca) on September 2, with an estimated enrollment of 600 subjects (1:1), in the United Arab Emirates, Abu Dhabi, beginning on July 17, with 1.500 subjects (2:1) and in Argentina on September 16, with 3.000 subjects. In all the volunteers, two IM doses will be applied, separated by 21 days.

2.5.3 Vaccines based on purified proteins

Novavax, a company based in the United States of America (USA) and Sweden

It has developed vaccines based on purified or recombinant proteins (EU2020-004123-16 NCT04583995) rSARS-CoV-2 or NVX-CoV2373, in association with an adjuvant to stimulate the immune response. They are nanoparticle vaccines, developed by genetic engineering, with a complete S protein trimer, associated with an M1 matrix as adjuvant. This molecule was developed using the protein S sequence of SARS-CoV-2, associated with nanoparticle recombination technology to generate a more powerful antigen; in addition, with the M® matrix, as an adjuvant, which is typical of this laboratory (Toback *et al.* 2022).

The molecule is developed in a baculovirus of the insect called “cogollero del maiz” (Spodoptera frugiperda) (Sf9), in which the complete protein is synthesized, including the transmembrane segment, and the matrix M (adjuvant) is purified from the quillay tree (Quillaja saponaria Molina) (saponin from its bark). The PS80 nanoparticle with its “native” conformation of trimers is dispensed into a pre-filled vial and kept at 2-8°C.

In preclinical studies in non-human primates (Macaca fascicularis), vaccination was performed on days 0 and 21, with 5 or 25 μg NVX-CoV2327 associated with 50 μg Matrix-M1 (Novavax AB, Uppsala, Sweden), plus one challenge per day 37, taking serum samples on days 0, 21 (before the second dose) and on day 33. They demonstrated production of AcN (hARA2) in higher titers than in plasma from patients recovered from SARS-CoV-2 infection, ARA2 receptor blockade and viral loads in upper and lower airway samples significantly lower than placebo. After viral challenge, animals were sacrificed on day 42, with signs of infection in those receiving placebo, with moderate to severe inflammation compared to little inflammation in vaccinated animals.

The phase I and II study published on September 2, is a controlled trial versus placebo, blinded to the investigator. It officially began on May 26, 2020. Phase I, conducted in Australia at two sites (one in Herston, Queensland, and one in Melbourne, Victoria), included 131 adults aged 18-59; 83 subjects were randomized to adjuvanted vaccine, 25 to nonadjuvanted vaccine, and 23 to placebo. Preliminary results, delivered in August 2020, show that it was well tolerated, with a robust and numerically superior antibody response to convalescent serum (Formica *et al.* 2021).

Safety: They did not present severe adverse events (SARs), with low local reactivity, self-limited. Only one participant developed fever and there was no dose delay due to adverse events.

Immunogenicity: Subjects had no detectable antibodies prior to immunization, with IgG anti-S (spike) responses (measuring days 0, 7, 21, 28 and 35) and AcN (micro-neutralization) assays at days 0, 21 and 35, with a good correlation between IgG and AcN, higher than the geometric mean of the plasma of patients recovered from COVID-19. Both adjuvant doses generated similar responses. A more potent immune response, predominantly Th1, as measured by intracellular cytokine staining (IFN-γ, IL-2, and TNF-α) was seen in the adjuvant-vaccinated group; in turn, Th2 responses, measured by cytokines (IL-5 and IL-13) were minimal (Heath *et al.* 2021).

The phase III study began on September 23, in the United Kingdom, with two IM doses, separated by 21 days and with an estimated enrollment of 9.000 subjects.

2.5.4 Vaccines based on mRNA nucleic acids

This is a technology whose proof of concept is more than 20 years old; however, there is not yet a vaccine approved and in use with this development platform. In this vaccine, the mRNA of the S protein is transported into the host cells, in a similar way to what happens in a viral infection, where it is transcribed and translated using the human cellular machinery, to finally produce, in this In this case, the S protein of SARS-CoV-2, which, after leaving the cell, stimulates the immune response. Of this type of vaccine there are non-replicating and self-amplified types. The second differs in that it has added an RNA segment that induces replication and, therefore, a lower dose could be used at the beginning, producing a similar immune response. Both express a protein S and induce an adequate immune response (Granados-Riveron and Aquino-Jarquin 2021).

Moderna (NCT04470427) with NIAID (National Institute of Allergy and Infectious Diseases) of the USA

It uses a stabilized protein S optimized mRNA molecule, (SARS-CoV-2 S-2P). The mRNA is purified and encapsulated in lipid nanoparticles (LNPs), through a nanoprecipitation process in ethanol droplets. In pre-clinical studies, rhesus monkeys were vaccinated on day zero and then on day 29 with doses of 10 or 100 μg mRNA-1273 in 1 mL of phosphate buffered saline (PBS) or PBS alone (placebo). They were challenged at week 8, with 7 x 105 SARS-CoV-2 (USA-WA1/2020 strain) plaque-forming units (pfu) 3 mL intra-tracheal and 1 mL intra-nasal (0.5 mL per nostril), with subsequent RNA quantification by bronchoalveolar lavage, histopathology, measurement of antibodies (anti-S and N9), measurement of neutralization and intracellular cytokine staining. Vaccination was shown to induce a humoral response (measured by IgG) greater than convalescent plasma, primarily with a Th1-type cellular immune response and practically undetectable Th2, as well as low or undetectable viral replication in bronchoalveolar lavage on the second day post challenge in vaccinated animals, in which

no viral genome was detected in the nose or lung, no antigens in the lung, and no evidence of inflammation (El Sahly *et al.* 2022).

Human trials. Phase I open-label, non-blinded dose escalation in 45 healthy adults, 18 to 55 years of age, who received two doses of vaccines 28 days apart. Fifteen participants in each group received 25 μg, 100 μg, or 250 μg of mRNA-1273 (Pajon *et al.* 2022).

Immunogenicity: The antibody titers, after the first dose, measured by ELISA anti-S-2P by geometric mean titer (MGT), were 40.227 in the 25 μg group, 109.209 in those that received 100 μg, and 213.526 in the 250 μg group. After the second dose, the mean titers at day 57 were 299.751, 782.719, and 1.192.154, respectively. After the second dose, serum neutralizing activity was detected by two methods in all tested participants, with values similar to those in the upper half of the convalescent serum distribution (El Sahly *et al.* 2022).

Safety: Solicited adverse events, which occurred in more than half of the participants, included: fatigue, chills, headache, myalgia, and injection site pain. Systemic adverse events were more common after the second vaccination dose; In 21.4% of the participants in the 250 μg group, 4 serious adverse events related to the investigational product were reported, 2 systemic and 2 neurological (one syncope and another subject reported feeling "dizzy"). The frequency of CD4+ T lymphocytes producing cytokines characteristic of a Th1 response was seen, both in those vaccinated with 25 μg and 100 μg, also observing practically no cytokines representative of a Th222 response.

In an extension study of the previous one, 40 subjects aged 56 to 70 years or ≥71 years were enrolled to receive two doses of 25 μg or 100 μg, separated by 28 days. Vaccine-related adverse events were mainly mild to moderate. The 100 μg dose induced a greater humoral and AcN response than the 25 μg dose. After the second dose, serum neutralizing activity was detected in all participants by multiple methods, being above the median of convalescent patient serum controls and further demonstrating a predominantly Th1 response by cytokine assay. This supports the use of 100 μg in phase III studies that began

on July 27 of this year, in which two IM doses are administered 29 days apart, hoping to enroll 30.000 subjects (Gilbert *et al.* 2021).

Preliminary phase III results (safety and efficacy) of Moderna's mRNA-1273 candidate, with 30.000 participants in the United States, recorded 95 cases of COVID-19, of which 90 occurred in the placebo group and 5 in the group of vaccinated subjects (Baden *et al.* 2020).

BioNTech-Pfizer of Germany and the U.S.A.

It has also developed modified nucleoside RNA-type vaccines, formulated on the basis of lipid nanoparticles: BNT162b1, which codes for a trimeric RBD of SARS-CoV-2, and the second: BNT162b2, which codes for a complete S protein, with its transmembrane domain stabilized. It began its phase I trial in 2020 (in Germany and the US). They enrolled 332 healthy adults ages 18 to 55 and ages 65 to 85 to receive the vaccine or placebo. 195 participants were randomized into 13 groups of 15 subjects each, 12 received vaccine and 3 placebos; two vaccines (BNT162b1 and BNT162b2), in two age groups (18-55 and 65-85), in three different dosages each, 10 μg, 20 μg, 30 μg, and a placebo group. All participants received two injections of 0.5 mL IM, separated by 21 days. A group aged 18 to 55 years was assigned to receive two 100 μg doses of BNT162b1 or placebo (Patel *et al.* 2022).

Safety: The group aged 18 to 55 years who received BNT162b1 reported mainly local reactions within the first seven days, which were more frequent after the second injection. In the group of older participants, the frequency of local reactions was similar to that of the group of young people, with 92% of reactions reported as local pain with the first dose and 75% after the second dose. No patient in the entire study had a severe local reaction. Regarding systemic events, participants in the group aged 18 to 55 years who received BNT162b1 presented mild to moderate fever with chills. Temperatures greater than 38°C or higher were reported by 75% of participants after the second dose in the group of participants older than 65 years, and systemic events were milder than in younger participants; however, some reported fatigue and headache. A 33% reported temperature

over 38ºC or more after the second dose. As with local reactions, systemic events were dose dependent and occurred more frequently after the second dose. Systemic events were less in response to BNT162b2 versus BNT162b1. There was no serious systemic event. In both age groups and for both vaccine candidates, the use of antipyretics and pain medications increased with increasing dose and with the number of doses administered; furthermore, it was lower in recipients of BNT162b2 compared to BNT162b1 (Gomes *et al.* 2021).

Immunogenicity: The serological response generated by BNT162b1 and BNT162b2 was similar. Synthesis of IgG and AcN in response to vaccination with 10 μg to 30 μg of BNT162b1 or BNT162b2 increased with the second dose in both young and older adults, with lower antibody titers in older adults. The highest AcN titers were those obtained in the sample on day 28 and day 35, observing a similar trend with the geometric means of immunoglobulins in comparable amounts and in a dose-dependent manner, which, in addition, were equal to or greater than the geometric median found in convalescent serum.

In April 2020, the phase III study began, with BNT162b2 (complete spike protein) expecting to enroll 43,998 subjects, with a schedule of two IM doses, separated by 21 days 27. Preliminary results from this study showed 95% efficacy in preventing COVID-19; 162 cases were recorded in the placebo group and 7 in the vaccinated group. In addition, they reported 10 cases of severe COVID-19, 1 in the vaccinated group and 10 in the placebo group (Dooling *et al.* 2021).

2.5.5 Viral vector vaccines

In this type of vaccine, a genetically modified adenovirus (Adv) is used, so that it lacks the necessary genes to replicate. The use of Adv as a vector for vaccines has been used in different studies since the Adv genome has been sequenced and its manipulation is familiar. There are currently four vaccine candidates using this platform:

The vaccine developed in Oxford, UK, uses a genetically modified chimpanzee Adv.

The CanSino (China), Janssen (Belgium) and Gamaleya (Russia) laboratories use Adv vectors of human origin: Ad5, Ad26 and rAd 26 + rAd5, respectively (Granados-Riveron and Aquino-Jarquin 2021).

Adv have a broad tropism for cells of various types. By eliminating the E1 region, its replicative capacity is lost, so it does not generate new viruses and cannot cause infection or disease, which is relevant for the safety of these vaccines. On the other hand, in this case a construct is made with the genetic sequence of the S protein and incorporated into the Adv genome. Then, when the virus enters the cells, they express the S protein in the same way that mRNA-based vaccines do; however, the context in which protein S is presented outside the cell may be more immunogenic. On the other hand, since the Oxford vaccine candidate is a chimpanzee Adv, humans may be less likely to have immunity to the vector used, (in some parts of the world, immunity to human pathogenic Adv may reach to be up to 9%). On the contrary, in the vaccines developed by the Beijing Institute of Biotechnology, in conjunction with CanSino Biological Inc. and Janssen, Adv vectors specific to human pathology are used; Although not very prevalent, they carry the consequent risk of finding an eventual immunity against the vector in vaccinated subjects (Granados-Riveron and Aquino-Jarquin 2021).

ChAdOx from the Oxford Vaccine Institute, UK and AstraZeneca

It is made on a Y25 strain. It is immunogenic in mice and generates a humoral and cellular immune response, mainly mediated by Th1 lymphocytes, demonstrated through the cytokine profiles of IgG subclasses. In studies in non-human primates, using either a single dose or a dose plus booster schedule, a balanced humoral and cellular immune response was developed. In the broncho-alveolar lavage of vaccinated animals, a significant reduction in viral load was observed, in addition to not presenting pneumonia when challenged with SARS-CoV-2. There was no decrease in viral excretion (shedding) by the respiratory route and no evidence of ADE (the aforementioned antibody-dependent increase) was found (Barrett *et al.* 2021).

In the phase I and II study, 1,086 volunteers were enrolled and randomized 1:1 to receive ChAdOx1 nCoV-19 (n: 543) or meningococcal conjugate vaccine (MenACYW) (n: 543).

Safety: Both local and systemic reactions were more frequent in the ChAdOx1 group; the majority decreased with the use of paracetamol, including pain, feverish sensation, chills, myalgia, headache and compromised general condition. There were no serious adverse events.

Immunogenicity: Specific anti-spike T cell responses were strongest on day 14. On the other hand, anti-spike IgGs were elevated on day 28 and increased with the second dose. AcN were detected in 91% of the participants when measured by MNA80 and in 100% by PRNT50. After the booster dose, all participants had neutralizing activity (Singh *et al.* 2021).

Currently under development is phase III of this candidate, ISRCTN89951424, with an IM dose that began on August 31, 2020 with 2,000 participants enrolled, in Oxford, United Kingdom. NCT04516746, which began recruiting on August 2, with a goal of 30.000 participants to be distributed in a 2:1 ratio, vaccine: placebo, who will receive 2 IM doses 29 days apart34. Additionally, CTRI/2020/08/02717035, with the same scheme, began on August 28 at 17 sites in India, aiming to test 1.600 subjects. And finally in Russia, with the difference that it will be open, without a comparator, and the goal of enrolling 100 subjects, has not yet started (NCT04540393) (Shrotri *et al.* 2021).

Beijing Institute of Biotechnology (Beijing, China) and CanSino Biologics (Tianjin, China)

In March, they enrolled 108 participants who were distributed into three groups of vaccine, low, medium and high dose (n: 36 in each one) (Zhu *et al.* 2020).

Safety: Adverse reactions within 7 days of vaccination were reported in 83% of participants in the low-dose group, 83% in the medium-dose group, and 75% in the high-

dose group. The most frequent reaction was pain at the injection site, which was reported in 54% of vaccine recipients. The most frequent systemic adverse events were fever (46%), fatigue (44%), headache (39%), and myalgia (17%). The majority of adverse events reported in all groups were mild to moderate in intensity and there were no serious adverse events within 28 days of vaccination.

Immunogenicity: Antibody and AcN titers increased significantly on day 14 and the maximum peak was at 28 days post-vaccination. The specific T cell response peaked at 14 days post vaccination. Since the vector in this candidate is an Adv5, against which humans could potentially have antibodies, seroconversion was studied in patients who had previous antibodies. Five of 20 (25%) in the low-dose group, 7 of 19 (37%) in the medium-dose group, and 10 of 16 (63%) in the high-dose vaccine group seroconverted. Seroconversion was lower in subjects older than 45 years. There is a booster effect on anti Adv5 antibodies. The authors report that in this phase I study, its limitations are a small sample size, no control group, previous infection against Adv5 that decreases humoral immunity and could have a negative effect on long-term immunity (Lewis 2020).

The results of phase III studies in the United Kingdom have recently been published, comparing ChAdOx nCoV19 versus meningococcal conjugate vaccine, MenACYW. With a total of 560 participants, the most reported local adverse events were: pain at the injection site; feverish feeling; myalgia and headache. 88% in the group from 18 to 55 years old, 73% in the group from 56 to 69 years old and 77% in those over 70 years old. Systemic reactions occurred in 86, 77, and 65%, respectively, in the same age groups. As of October 26, 2020, 13 serious adverse events had been reported, none attributable to the investigational product or the vaccine used as a control. Both the IgG anti-S and the AcN responses were similar in all age groups; At 14 days post-boost, an increase in the response of AcN and a peak response of T37 lymphocytes were observed. Regarding the efficacy data, the results of the interim analysis published in December 2020, showed an efficacy of 70.4% with 62.1% in the group that received standard dose versus 90% in the one that received half dose in the first and standard dose in the second inoculation. The latter results from the analysis of the combination of the subjects enrolled in the United Kingdom and Brazil, with an n of 11.636 enrolled (Muñoz-Valle *et al.* 2022).

Janssen vaccine

Ad26COVS1 is another monovalent candidate, with a non-replicating human pathogenic Ad26 vector encoding protein S. In pre-clinical trials, in non-human primates, 52 rhesus macaques were immunized with vaccine and placebo and challenged with SARS-CoV-2 intra nasal and intra tracheal. The response of Ab and AcN was robust, and protection against pneumonia and decreased viral excretion were demonstrated. Janssen's phase III study (NCT04505722) began recruiting on September 7, with a goal of 60.000 participants on a schedule using one dose IM40 (Roozendaal *et al.* 2021).

Gamaleya vaccine (Russia)

Gamaleya, Russia, has conducted two open-label phase I/II studies at two hospitals in Russia in healthy adults aged 18 to 60 years. They administered a dose of rAd26-S or a dose of rAd5-S on day 1, observing the safety of both components for 28 days. In phase II starting no earlier than 5 days after phase I vaccination, rAd26-S booster was administered IM on day 0 and rAd5-Sen on day 21. Between June 18 and August 3 This year, 76 participants were enrolled in both studies, 38 for each, where 9 volunteers received rAd26-S and 9 received rAd5-S in phase I. Twenty volunteers received both in phase II (Logunov *et al.* 2020).

Safety: Both formulations were safe and well tolerated. The most frequent adverse events were pain at the injection site (58%), fever (50%), headache (42%), asthenia (28%), myalgia and arthralgia (24%). Most were mild, non-serious adverse events. Volunteers who received both vaccines had the highest number of adverse events with the second vaccination.

Immunogenicity: All participants produced anti-RBD antibodies at day 42. The AbNs showed 100% seroconversion, both with the lyophilized and frozen formulation. Cell-mediated response was detected in all participants at day 28, with a better response in the frozen formulation (Dawood 2021).

Two phase III trials are currently underway, one in Russia and one in Pakistan, which began on September 11 and 15, respectively. The schedule is an IM dose and it is expected to recruit 500 participants in Russia and 40.000 in Pakistan. Randomization will be 3:1 and 1:1, respectively (Kytko *et al.* 2022).

The world was in a situation unprecedented in the last century due to the SARS-CoV-2 pandemic. Among the options to deal with this fact, a vaccine or, ideally, several safe, effective and immunogenic vaccines, seemed to be one of the best alternatives to return to lost normalcy within a reasonable time. The vaccine that was licensed first would not be necessarily be the best. It had to be taken into account that the more alternatives approved and in the process of being manufactured, the more opportunities the susceptible population had to access one of them.

Although it is true that the development of a vaccine for SARS-CoV-2 is not starting from scratch, since there are previous platforms being developed, it is relevant to reflect on the opportunities of obtaining vaccines for situations such as the ones we are experiencing. Being prepared has a cost, which today seems to be very justified with respect to the direct and indirect costs produced by the pandemic. It is a historic moment to highlight the importance of good research and science, in order to prepare response structures for future pandemics or future problems that arise on the planet. Along these same lines, it is important to reflect on equity in access to future vaccines, since an imbalance in their availability between developed countries and others with less economic development is expected. Given the rapid spread of the coronavirus, practically throughout the world, it is essential to generate and establish a global distribution strategy and access to these vaccines.

This series of questions, and possibly many more that may exist, must be answered adequately, with serious and well-generated scientific evidence that supports future actions to be followed. For now, pending the stages that must still be completed to have a vaccine against COVID-19 available and approved with all the necessary rigor, it is essential and a priority to insist on self-care measures and appeal to the appropriate

confrontation, on the part of of the authorities of each country and of each inhabitant, so that we have the best possible handling of this pandemic.

3. MATERIALS AND METHODS

3.1 Study Type

Observational, prospective, cross-sectional and exploratory descriptive study.

3.2 Data Collection

A standardized physical examination were performed on all eligible people in this study and data were recorded on the Clinical Assessment Form.

3.3 Location of the Study

Hillah General Teaching Hospital, Hilla City, Iraq. Hillah is an Iraqi city and the center of Babil Governorate, with a population of 970.000 people, according to the 2015 census. It was built by Sadaqah bin Mansour, Prince of the Emirate of Bani Mazyad in 1101 AD. It is about 100 km from Baghdad, and about 60 km from Najaf. It is also located near the ancient city of Babylon, which is one of the most important ancient historical areas in the world.

3.4 Sample Selection and Period of the Study

The study was carried out in the internal medicine workers of Hillah General Teaching Hospital belonging to the health network and people attended to the general medicine and emergency medicine centre in Hillah General Teaching Hospital. In which the vaccination process against the SARS-CoV-2 virus was carried out in the health personnel who work in said hospital and out patients. The study period was March 2022 to April 2022. Sampling was done for convenience, since all Medicine Interns who met the inclusion criteria in the study period were taken.

Process: The survey technique was used. The link of the form, made on the Google forms platform, was sent virtually to all the Medicine Interns who work and outpatients at the Hillah General Teaching Hospital during the study period. Prior to filling out the form, the reason for the investigation was explained, as was the confidentiality of the data collected, and their informed consent was requested in writing. The form consists of three sections, which are: Informed consent, data collection form and survey of adverse reactions to the vaccine against SARS-CoV-2. A pilot test was carried out in 10 medical interns to ensure the correct understanding and completion of the survey. The survey evaluates the appearance of immediate clinical adverse reactions (0-7 days) after the application of each dose of the SARS-CoV-2 vaccine. The appearance of adverse events after the seventh day, treatment to counteract them if received, or changes in the laboratory analysis of the participants were not evaluated.

3.5 Total Sample Selected

Total 80 persons with different age group accepted to participated in the current research study from Hilla City, Iraq. 4 Groups from which blood samples will be taken and cytokine levels will be investigated.

4 groups to work with number of individuals;

- **Group 1:** 20 who have never been vaccinated and have never had a COVID-19 infection
- **Group 2:** 20 never vaccinated and infected with COVID-19
- **Group 3:** 20 who have been vaccinated at second dose and have never had a COVID-19 infection
- **Group 4:** 20 who have been vaccinated at second dose and have had a COVID-19 infection

3.6 Data Analysis

Characteristics of all included samples were described according to second-dose SARS-CoV-2 vaccination status, and the vaccinated group, was compared with the unvaccinated group using the chi-square test. To study the association between SARS-CoV-2 infection and vaccination status (and the rest of the variables: age, sex, reason for study, etc.), the crude Odds ratio (OR) and the adjusted odds ratio (aOR) using logistic regression. In the logistic regression model, those variables that showed statistically significant differences between the vaccinated and unvaccinated PS groups were introduced, as well as those that were significantly associated with the development of SARS-CoV-infection.

Lastly, the vaccine effectiveness (VE) of the second dose of vaccine against SARS-CoV-2 and the adjusted VE and its 95% confidence interval were calculated, with the formula $VE=(1-OR)\times 100$. This calculation was made for the overall positivity risk and for the different groups. The level of statistical significance used was $p<0.05$. The analysis was performed using the statistical program IBM® SPSS® Statistics v.25.0.

3.7 Biochemical Analysis

3.7.1 Collection and preparation of blood samples

The blood samples of the patients were collected by venipuncture and the identifications carried out with the help of a health agent, placing the name and number according to the medical record in five tubes containing Sodium Heparin and one dry tube with gel, each tube holds approximately 10 mL of peripheral venous blood. The tubes were centrifuged at 3000 rpm, for 10 minutes. Serum aliquots were stored at -80°C for further processing for serological screening and confirmatory testing. We obtained peripheral blood mononuclear cells by Ficoll-Hypaque concentration gradient separation (Sigma Chemicals, St Louis, MO USA). Prepared blood samples will be investigated for IL-6, IL17 and IFNγ using enzyme-linked immunosorbent assay (ELISA).

3.7.2 Freezing

After cell counting, the samples were centrifuged, the supernatant was discarded, resuspended in fetal bovine serum (FBS) and 10% DMSO, at a concentration of $20x10^6$ cells in a 500 μL tube of pure fetal serum, then pipetted. the cellular contents in the cryovial and kept in the refrigerator. Pipetting dropwise the DMSO freezing medium. The cryovials were placed in a "freezer-inbox" (kept at 4°C) and after 30 minutes transferred to a freezer at -80°C they were stored for the performance of the study and subsequent experiments. For thawing, the samples were placed in a water bath at 37°C under constant agitation, always observing the flake of ice. The cell suspension was transferred into 10 mls of RPMI medium containing 10% FBS. Soon after, they were centrifuged for five minutes at 1500 rpm, repeating the process twice until all DMSO was removed.

3.7.3 Studies of cytokines

Serum cytokines were measured using the Cytometric Bead Array (CBA) technique (BD Biosciences Pharmingen, San Diego, CA, USA). Cytokines IL-6, IL-10, IL-17, & TNF-α were dosed with the Human Inflammatory Cytokine Kit. Cytokines FN-γ was dosed with the Human Th1/Th2 Cytokine Kit. Flow cytometry (BD FACSArray) and FCAP Array Software (BD Biosciences Pharmingen, San Diego, CA, USA) were used to perform the acquisition and analysis, respectively.

The standard curve for each cytokine was constructed using the reference cytokine concentrations provided by the kits. The maximum detection level considered was the value provided by the manufacturer: 10.000 pg/mL for all cytokines. The minimum detection levels considered were the values provided by the manufacturer. Cytokine measurements were performed twice on the same sample at two different times, by a technician blinded to clinical and etiological information.

Mann-Witnney test (non-normal distribution curve) was used to compare cytokine levels between patients and those in the comparison group and to assess differences in

intragroup cytokine means of dichotomous categorical variables. The Wilcoxon test was used for paired analysis. Spearman's coefficient was used to determine the correlation between cytokines and clinical parameters related to continuous variables.

IgM antibodies against nucleocapsid antigens (N) using the ELISA technique, and IgG antibodies against specular or spike surface antigens (S) using the S-RBD SARS-CoV-2 (CLIA) technique that used reagents against the RBD domain (receptor binding domain) included in the S1 subunit of the S protein (spike) of SARS-CoV-2, as proof of immune response against the vaccine or natural infection. The post-vaccination blood sample was taken a minimum of 15 to 30 days after completing the vaccination. The threshold for positivity for IgG with the CLIA technique was established at titers of ≥1 AU/mL and for IgM with the ELISA technique at titers of ≥0.9 AU/mL (arbitrary units/milliliter from the Eurofins/Megalab analytical laboratory). At the time of screening there were people with second doses of vaccine administered. Information was collected on the variables sex, age, vaccination dates and total number of individuals per center with a history of having been diagnosed with COVID-19 collected by the Social Services Management registration system that counts the number of COVID-19 cases in residences. IgM and IgG antibody titers were tabulated, finding for IgG the mean, range and/or 95% confidence intervals (95% CI) for the series from each location and for the series resulting from the sum of all. In turn, in each center the IgG antibody titers were calculated based on the receipt of two or no doses of vaccine; Within each of these 4 subgroups, a distinction was made between having previously had a COVID-19 infection or not. The values of the percentages and the means were rounded with the rule of 5 for decimals. For the comparison of means, the analysis with the Student's t test was used in pairs, and the Chi Square test for percentages, using the statistical program. All participants signed an informed consent, where they were informed of the treatment of all information confidentially.

3.8 Statistical Analysis

Data collection was performed by the main researcher, as well as data entry and later these data were checked. The results were tabulated in spreadsheets created in Microsoft

Excel (Microsoft Corporation, Seattle, WA, USA) and the collected data were exported to the SPSS statistical program version 19.0 (IBM). The statistical procedure used to describe the epidemiological, clinical and laboratory data of the sample was Descriptive Statistics, with the techniques of frequency calculation, measures of central tendency and dispersion. Tables and frequency graphs were designed for the dependent variables (adverse reactions at the site of inoculation and systemic), as well as cross tables to relate these variables with each potentially associated factor (age, sex, chronic diseases, usual medication, allergies, reactions previous allergies to other vaccines, adverse reaction to the vaccine dose). Chi 2 statistical tests were initially applied, a value of $p < 0.05$ was considered to represent a statistically significant difference. In addition, to identify factors associated with adverse reactions, the odds ratio (OR) and a 95 percent confidence interval (CI) were used.

3.9 Ethical Issues

The study was approved by the Drug Research Ethics Committee of the Hillah General Teaching Hospital, Hilla City, Iraq.

4. RESULTS AND DISCUSSION

4.1 Study Population

A total of 126 Hilla city people were evaluated, among which 80 cases were included in this study. The reasons for excluding cases were: serum sample not available for cytokine measurement; undetected etiology; refusal to sign the free and informed consent form; chronic lung disease except asthma; diagnosis of chickenpox and immunodeficiency. The clinical-epidemiological characteristics of the series are shown in Table 4.1.

Table 4.1 Groups from which blood samples were taken and cytokine levels were investigated.

Group Number*	Total no of Sample	Male	Female	Age (Years)		Average Age (Years)
				(Minimum)	(Maximum)	
1	20	6	14	18	51	34±3.62
2	20	13	7	18	60	31±3.81
3	20	5	15	18	59	40±2.90
4	20	19	1	18	62	52±3.61

- Group-1: Who have never been vaccinated and have never had a COVID-19 infection
- Group-2: Who never vaccinated and infected with COVID-19
- Group-3: Who have been vaccinated at second dose and have never had a COVID-19 infection
- Group-4: Who have been vaccinated at second dose and have had a COVID-19 infection (Table 4.2) (Table 4.3) (Table 4.4) (Table 4.4) (Table 4.5) (Table 4.6) (Table 4.7) (Table 4.8) (Table 4.9) (Table 4.10) (Figure 4.1).

Table 4.2 Distribution of chronic diseases

	Average		Group level			
Chronic diseases	**n**	**Percentage**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Overweight/Obesity	31	38.75	5	7	6	13
Asthma	7	8.75	2	3	2	0
Autoimmune diseases	3	3.75	0	1	1	1
Polycystic ovary syndrome	5	6.25	2	1	1	1
Cancer	2	2.5	0	0	1	1
None	32	40	11	8	9	4
Total	80	100	20	20	20	20

Table 4.3 Vaccination status of the selected people

Vaccination status	**Male**	**Percenta**	**Female**	**Percenta**	**Gap Between First dose and second dose (months.days)**
Total Vaccinated sample with BNT162B2- Bıontech Vaccıne (BioNTech-Pfizer vaccine)	24	60.00	16	40	5.21

Table 4.4 Patients who presented adverse reactions to the vaccine in general and by dose

Presence of adverse reactions	**No**	**%**
1st dose (Group-3& 4)	40	50%
2nd dose (Group-3& 4)	40	50%
Usually	68	85%

Table 4.5 Frequency and distribution of local adverse reactions by dose

	1st dose			**2nd dose**		
Adverse reactions	**n**	**Incidence (%)**	**Proportion of adverse reactions (%)**	**n**	**Incidence (%)**	**Proportion of adverse reactions (%)**
Pain	38	95.00	96.32	39	97.50	98.61
Swelling	11	27.50	27.78	10	25.00	26.00
Erythema	9	22.50	22.73	6	15.00	15.60
Pruritus	8	20.00	20.20	6	15.00	15.60

Table 4.6 Frequency and distribution of systemic adverse reactions by dose

	1st dose			2nd dose		
Adverse reactions	**n**	**Incidence (%)**	**Proportion of adverse reactions (%)**	**n**	**Incidence (%)**	**Proportion of adverse reactions (%)**
Fever	22	55.00	57.20	16	40.00	41.60
Asthenia	19	47.50	49.40	14	35.00	36.40
Hyperoxia	2	5.00	5.20	3	7.50	7.80
Dizziness	9	22.50	23.40	6	15.00	15.60
Nausea	8	20.00	20.80	4	10.00	10.40
Vomiting	2	5.00	5.20	3	7.50	7.80
Constipation	4	10.00	10.40	2	5.00	5.20
Diarrhoea	2	5.00	5.20	4	10.00	10.40
Rash	2	5.00	5.20	2	5.00	5.20
Headache	20	50.00	52.00	11	27.50	28.60
Joint/muscle pain	9	22.50	23.40	13	32.50	33.80
Itching in other regions	3	7.50	7.80	6	15.00	15.60
Anaphylactic shock	1	2.50	2.60	2	5.00	5.20
Angioedema	1	2.50	2.60	1	2.50	2.60

Table 4.7 Association between sociodemographic characteristics and occurrence of adverse reactions

Adverse reactions					
Variables	**Category**	**n**	**Incidence (%)**	χ^2	**P**
Gender	Male (n=24)	21	87.50	0.446	0.368
	Female (n=16)	12	75.00		
Age(years)	≤40Years (n=19)	11	57.89	1.96	0.327
	≥40Years (n=21)	19	90.48		

Table 4.8 Association between pathological background and occurrence of adverse reactions

		Adverse reactions					
Variables	**Category**	**n**	**Incidence (%)**	**χ2**	**P**	**OR**	**CI 95%**
Chronic diseases	Yes	48	60	4.62	0.016	3.59	1.26
	No	32	40				
take medication	Yes	33	68.75	0.64	0.244	1.79	0.298
	No	15	31.25				
Allergic reactions to vaccine	Yes	22	55	0.39	0.254	1.84	0.217
	No	18	45				
Known allergies	Yes	16	20	5.09	0.042	7.76	0.891
	No	64	80				

Table 4.9 Adverse reactions after the second dose in participants with or without adverse reaction after the first dose of vaccination

	2nd dose		
1st Dose	**Yes (%)**	**No (%)**	**Total (%)**
Yes (%)	42.09	20.71	62.8
No (%)	10.36	26.84	37.2
Total (%)	52.45	47.55	100

Table 4.10 CLIA (IgG anti S-RBD)/ELISA (IgM anti-N) serological test results in persons from Hilla city

Group	IgG value Range (AU/mL) found	Mean values of IgG (AU/mL)
Group-1	87.3 to 99.5	81
Group-2	476 to 1180	891
Group-3	58 to 455	255
Group-4	637 to 715	678

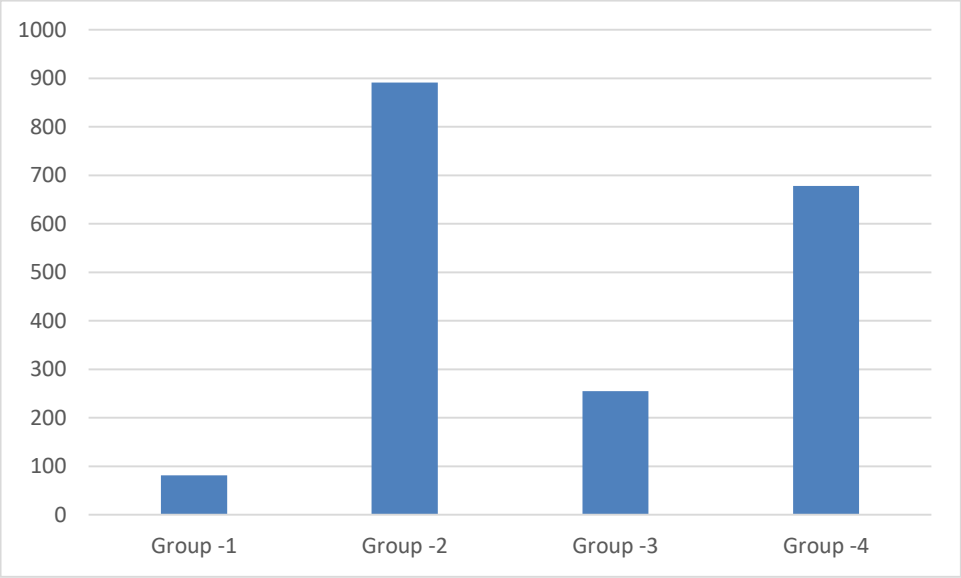

Figure 4.1 Mean values of IgG (AU/mL) in four group of people from Hilla city

Differentiating between “history of COVID-19 infection” and “no history of COVID-19 infection” in each subgroup, vaccinated and non-vaccinated significant differences were observed in the two-dose recipient subgroups; however, these significant differences were not observed in the non-vaccinated group. By gender, there were no statistically significant differences between antibody titers in any of the subgroups (unvaccinated,

vaccinated with two doses), nor in the subgroups of history of infection/no history of infection.

We observed a significant difference between the IgG antibody titers, as an indicator of humoral immune response, between the unvaccinated and the vaccinated, especially after receiving the 1st dose, multiplying the titers practically by ten. After receiving a 2nd dose, a second somewhat milder increase occurs, multiplying the titers with respect to those not vaccinated by approximately 14. The result of 99.8% of people with positive IgG titers, with average values of 678 AU/mL, after vaccination with two doses of the Pfizer/BioNtech BNT162b2 vaccine, indicates a high degree of immunization.

Cytokine study- IL 6

The cytokine IL-6, which belongs to the Th2 profile, had the highest titer among the cytokines described in our study. High production of IL-6 was observed in all groups except group 1, from baseline to the 2nd dose of vaccination. In Figure 4.2, the IL6 levels of the group 2 were much higher compared to other groups of COVID-19 patients, comparing the group 1 with group 3, group 4, it was possible to observe a statistical significance ($p<0.0001$) (Table 4.11).

Table 4.11 Concentration of serum IL-6 in studied groups

Group	**IL-6 (pg/mL)**
Group-1	53.7±8.04
Group-2	317.81±41.26
Group-3	80.75±44.79
Group-4	140.53±43.12

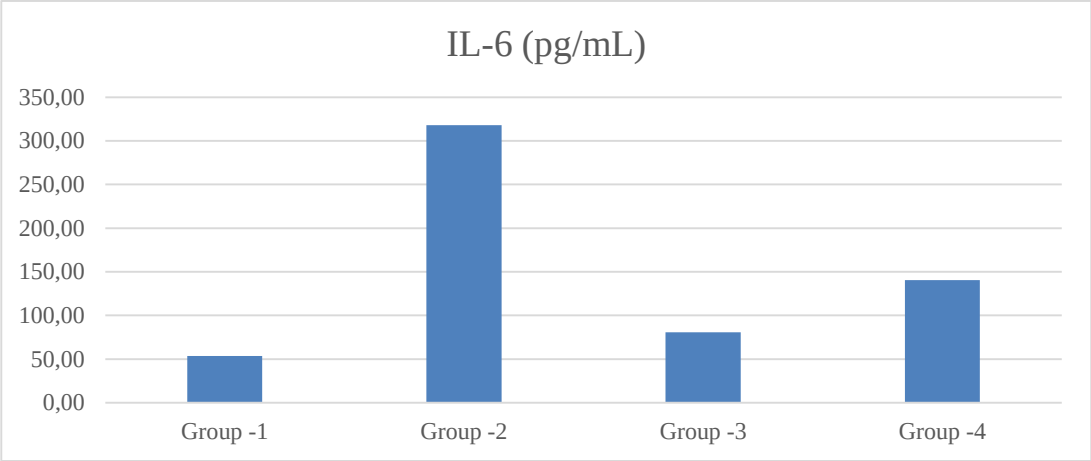

Figure 4.2 Variations in the concentrations of IL-6 (pg/mL) in the selected groups

Serum concentrations of IL-6 were higher than usual in COVID-19 patients. IL-6 concentrations were 53.7±8.04 pg/mL in group 1, 317.81±41.26 pg/mL in group 2, 80.75±44.79 pg/mL in Group 3, and 140.53±43.12 pg/mL in Group 4 (Table 4.11). Compared to Group 1 infected patients, Group 2 COVID-19 infected people who had never had a vaccination had a significantly higher level (Figure 4.2). Although there were no discernible changes between group 1, the vaccinated group 3, and those in group 4 who have had a COVID-19 infection but have only gotten a second dose of vaccination, there was a substantial correlation between the severity of IL-6 and the severity of COVID-19. The increased levels of IL-6, observed in Group 2 patients are consistent with these findings. More than 6 time is more than group 1 persons, 4 times more than group 3, completely double compared to group 4 of infected patients had increased values of these parameters at the time of diagnosis.

Cytokine study- IL-8

Secondary to initial reports of clinical characteristics in which elevated concentrations of cytokines were observed in critically ill patients with COVID-19, the possibility that a self-amplified and unregulated response to infection was the cause of organ dysfunction began to be considered, with a marked interest in focusing medical therapy on immunomodulation. However, there were several problems, one of the most important being the absence of a defined cut-off point or diagnostic criterion for immunomodulatory

intervention. Recently, information was published that measures the concentrations of cytokines in patients suffering from critical COVID-19, comparing them with other critical illnesses or in ARDS of other causes, reporting significantly lower concentrations of IL-8 in patients with COVID-19 compared to the rest of the groups, which for the moment undermines the justification for this management route (Table 4.12) (Figure 4.3).

Table 4.12 Concentration of serum IL-8 in studied groups

Group	IL-8 (pg/mL)
Group-1	279.48±69.36
Group-2	201.96±21.42
Group-3	154.02±21.42
Group-4	253.98±132.6

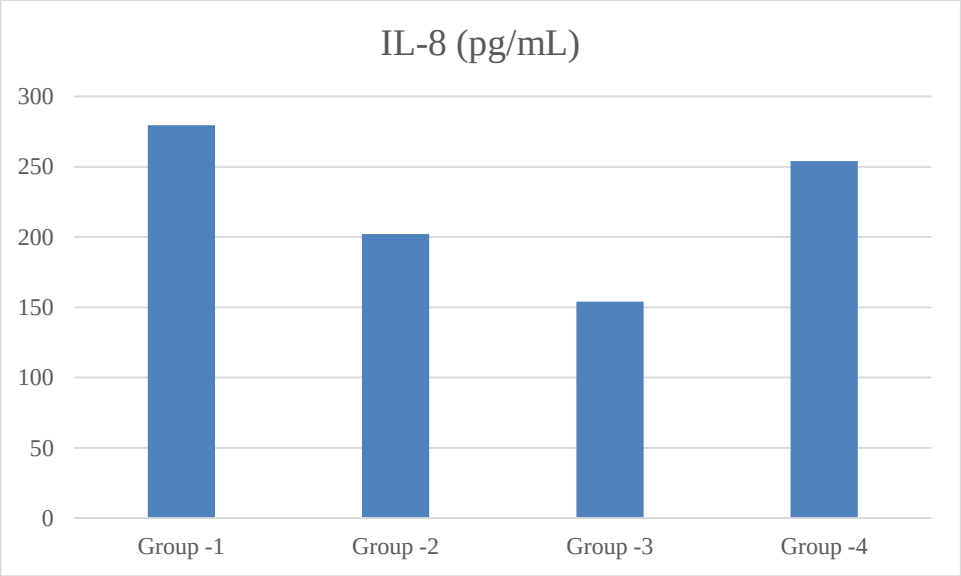

Figure 4.3 Variations in the concentrations of IL-8 (pg/mL) in the selected groups

The findings (Figure 4.3) (Table 4.12) demonstrated that the serum IL-8 levels of COVID-19 patients were low. IL-8 concentrations were 2279.48 pg/mL in Group 1 (non-vaccinated), 201.96 pg/mL in Group 2, 154.02 pg/mL in Group 3, and 253.98 pg/mL in Group 4 (vaccinated and non-infected), respectively. The data showed that IL-8 levels were considerably lower in groups 3 and 4 than in group 1. The lack of differences between groups 1 and 4 indicates that IL-8 responses peak early in a COVID-19 infection, when the viral load is highest, and taper off as patients start to feel better.

Cytokine study: IL-17

Th17 effector cells' primary cytokine, IL-17, appears to be crucial in clearing up microbial and viral illnesses. However, genetic polymorphisms linked to decreased IL-17 secretion or function raise vulnerability to these infections and to clinical states conducive to recurrent mucocutaneous candidiasis. Central functions for Th17 cells have been observed in autoimmune disorders such MS, RA, and psoriasis. In the groups we looked at, IL-17 production was quite high. Patients infected with COVID-19 showed higher levels at baseline than the control group (Group-1) (Table 4.13) (Figure 4.4), but the difference was not significant ($p>0.316$). When comparing the control group to group 2, a statistically significant difference ($p<0.0026$) was detected, showing that group 2 had much higher cytokine production after the first immunisation dose.

There was an increase in IL-17 cytokine levels after the second dose, no change in cytokine levels from the first dose in the control group, a tiny decrease in IL-17 levels in group 4, and a small increase in IL-17 levels in group 4, but neither of these changes was statistically significant. Group 1 had lower IL-17 levels than Group 4, by a significant margin of $p<0.0126$, whereas Group 4 had higher IL-17 levels, $p<0.002$.

Table 4.13 Concentration of serum IL-17 in studied groups

Group	IL17 (pg/mL)
Group-1	64.155±3.15
Group-2	166.95±6.51
Group-3	74.76±2.205
Group-4	78.015±65.1

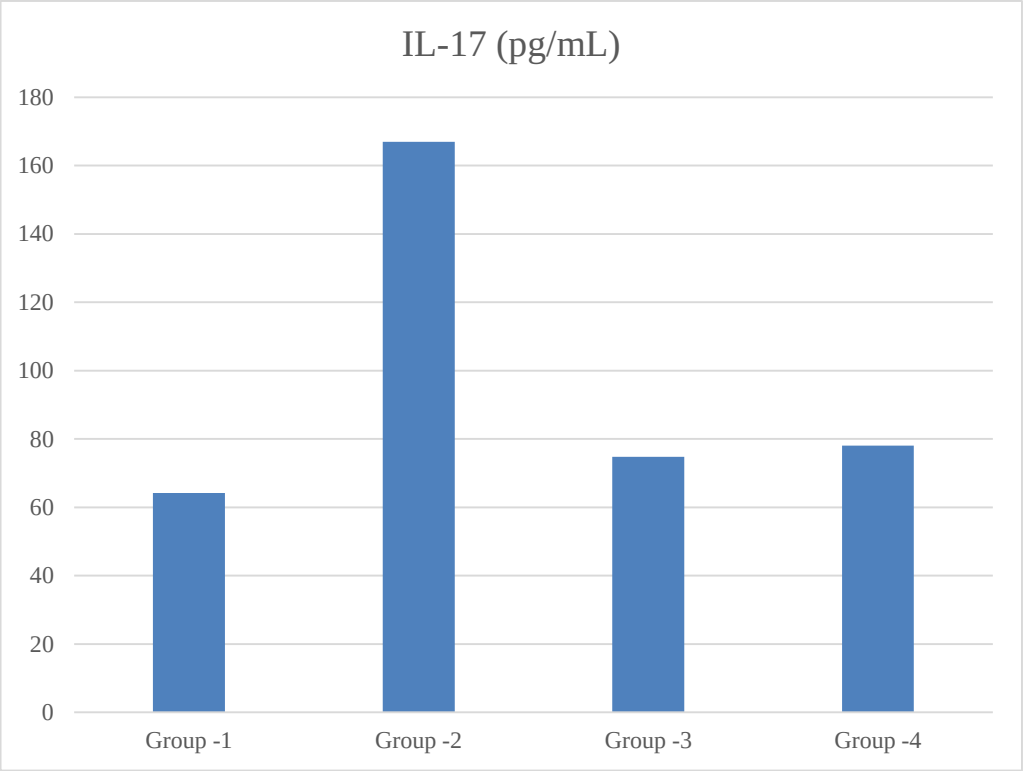

Figure 4.4 Variations in the concentrations of IL-17 (pg/mL) in the selected groups

Cytokine study: Tumor Necrosis Factor (TNF)

It was observed at baseline (Figure 4.5) that TNF-α levels in both groups 2 and 4 (corresponding Covid-19 infected) showed a high production when measured from serum of the selected groups. When comparing the group-1 with group 3, it was possible to observe statistical significance ($p<0.021$), these groups are non-infected with covid-19, one is vaccinated (Group 3) and another is non vaccinated. In group 3 High levels of TNF-α were found corresponding to the 2nd dose of vaccination, it was possible to observe significance between the group -1 and group 4 $p<0.0001$, between COVID-19 groups, $p<0.0001$.

It was possible to observe that after the second dose of vaccination, the TNF levels measured by the lymphocyte culture supernatant, was statistically significant between the group 3 and group 4 with the value of $p <0.003$ (Table 4.14).

Table 4.14 Concentration of serum TNF-α in studied groups

Group	TNF-α (pg/mL)
Group-1	60.79±5.46
Group-2	90.61±5.46
Group-3	71.71±6.51
Group-4	82.63±3.99

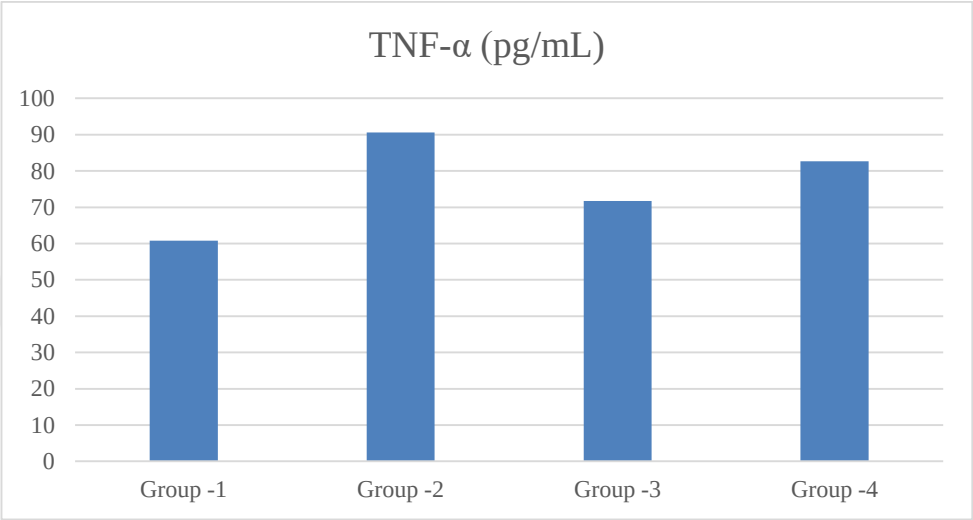

Figure 4.5 Variations in the concentrations of TNF-α (pg/mL)in the selected groups

Interferon (IFN-γ)

IFN-γ levels were measured from CD4+ T lymphocyte culture supernatant. In figure 4.6, we observe that high production of the group-1 compared to group 3 ($p=0.23$) compared group 1 with group 4 ($p< 0.022$). After one month of vaccination, a high production of IFN-γ was observed, group 3 and group 4 compared to group 1 showed a statistically significant difference with $p<0.0002$, compared to groups and 4 ($p<0.000$), group 1 compared to group 2, no statistical significance was demonstrated (Table 4.15).

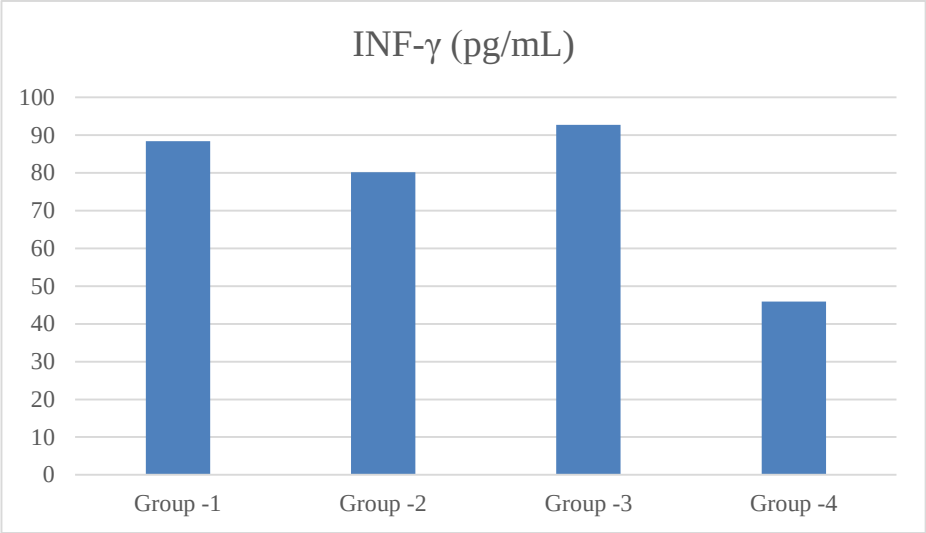

Figure 4.6 Variations in the concentrations of INF-γ in the selected groups

Table 4.15 Concentration of serum INF-γ in studied groups

Group	INF-γ (pg/mL)
Group-1	88.326±47.67
Group-2	80.115±23.20
Group-3	92.6415±23.20
Group-4	45.885±11.76

In Figure 4.6, it was observed that in the second dose of the response to vaccination, IFN-γ levels were high between the group1 and Group 4 with a value of $p>0.561$, Group 3 obtained more discrete levels $p>0.074$; when comparing groups, 3 and 4, statistical significance was observed with $p<0.001$.

4.2 Discussion

4.2.1 Sociodemographic study

Adverse reactions and factors potentially associated with them of the inactivated SARS-CoV-2 vaccine against COVID-19 produced in Vero cells by **Pfizer/BioNTech (BNT162b2) Vaccine** were investigated. This study was carried out in medical interns and people attended at Hilla Teaching Hospital, Hilla city, Iraq after the massive

vaccination campaign at the national level. A total of 80 persons completed the questionnaire. Being 80 those who finally met the inclusion and exclusion criteria.

As seen in Table 4.1, the sample consisted of 43 men (53.75%) and 37 women (46.25%), highlighting that there were higher as many female participants compared to male participants, which coincides with what was found by the (Ahamad *et al.* 2021). In their study of the adverse effects of COVID-19 vaccination (Ahamad *et al.* 2021). The age of the inmates ranged between 18 and 62 years, the average age was 39.25 years, which can be explained by the fact that most students begin their university studies at an average age of 18 years. These data are similar to those found in the study conducted in our environment by Grande Quispe who studied the sociodemographic characteristics of the population of medical interns at HRHDE and Goyeneche Hospital, finding that 98.2% of Arequipa medical interns are between 22-31 years old (Grande Quispe 2020).

A high number of participants, 48 inmates (60%), had a history of having some chronic disease, the most frequent being overweight/obesity 31 (38.75%) and asthma 7 (8.75%); Regarding the frequency of the adverse reactions presented, Table 4.4, we have that a total of 50% participants reported having presented some immediate adverse reaction. 50% recipients of the first dose manifested immediate adverse reactions while a total of 40 (50%) recipients of the second dose presented them, evidencing an apparent lower frequency of adverse reactions in the second dose. These results imply a high frequency of adverse reactions in the population studied, which differs from what was reported in phase 1 of the clinical trial conducted by the Sinopharm laboratory, in which the frequency of adverse reactions was 29% in the first 07 days (Xia *et al.* 2021); which could suggest a greater susceptibility to generate adverse reactions to the vaccine studied in our environment.

Regarding the frequency and distribution of adverse reactions after vaccination, Table 4.5 and 4.6, it is shown that the most common was pain at the inoculation site, with a frequency of 96.32% and 98.61% in the first and second dose; respectively, representing 85% of all adverse reactions in the first dose and 79% of these in the second dose. The most frequent systemic reactions presented were headache, asthenia and joint/muscular

pain, respectively. Headache was reported in 52% persons after receiving the first dose of the vaccine and in 28% persons after the second dose. Asthenia occurred in 49% people after receiving the first dose and in 36% people after the second dose. A total of 23% people presented joint/muscular pain after the first dose inoculation while 33% after the second dose.

These data differ from those found in the phase 1/2 clinical trial conducted by the Sinopharm laboratory, where the most common systemic adverse reaction was fever (Xia *et al.* 2021). However, they agree with the data obtained from the study by (Zhang *et al.* 2021) that evaluated adverse reactions to the CoronaVac vaccine, an inactivated vaccine, where it was found that the most common adverse reaction was localized pain at the injection site, and fatigue, muscle pain and headache were the adverse reactions most frequent systemic (Zhang *et al.* 2021). It should be noted that the adverse reactions found were mild to moderate, none were severe within the first 07 days after inoculation, coinciding with the safety and tolerability studies of the aforementioned vaccine (Zhang *et al.* 2021).

Table 4.7 indicates the incidence and association of sociodemographic characteristics and occurrence of adverse reactions. The analysis indicated that there was no statistically significant difference in the occurrence of adverse reactions by age group or gender.

Table 4.8 shows the incidence and association of pathological antecedents and the occurrence of adverse reactions to the vaccine studied. The analysis suggested that the presence of chronic diseases and having a known allergy influenced the appearance of adverse reactions after the first and/or second inoculation. This is possibly due to the fact that both factors lead to a chronic proinflammatory state that facilitates a faster and more intense response to various stimuli, which would cause a greater predisposition to have adverse reactions to a vaccine (Pahwa *et al.* 2021). In addition, it was found that having a history of a chronic disease (overweight/obesity, Asthma, autoimmune diseases, PCOS, cancer) was 3.6 times more likely to develop an adverse reaction to the vaccine (95% CI 1.21-10.7).

These results are consistent with those found in a study of the safety of the CoronaVac inactivated vaccine (Zhang *et al.* 2021). In Table 4.9, which shows the adverse reactions after the second dose in the participants with or without an adverse reaction after the first dose of vaccination, we observe that 62.8% participants reported having presented an adverse reaction after the first dose; among whom, 52.45 % subjects reported having presented some adverse reaction after the second dose was applied. Participants with and without adverse reactions after the first dose were found to have a significantly different frequency of reactions to the second dose ($P<0.001$). In addition to the fact that adverse reactions to the first dose increased the probability of their occurrence in a second dose (OR=4.71, CI 95% 1.92-11.5). So, we can say that the fact of having presented an adverse reaction after the first dose of the vaccine increases the risk of presenting adverse reactions to the second dose by 4.71 times.

There are two types of adverse reactions: Type A adverse reactions, also called predictable, in which the episode is due to the drug's mechanism of action. For example, drug interactions and adverse events. About 60% of all adverse reactions are due to this type of mechanism. These reactions are more easily recognized. Type B, or unpredictable, adverse reactions are those in which the reaction originates from the drug stimulating the immune system. For example, allergic reactions. Vaccination inactive ingredients including egg protein, gelatin, formaldehyde, thimerosal, and neomycin can cause acute IgE-mediated allergic reactions, so it's not always the vaccine itself that's to blame when someone has an adverse reaction. The European Medicines Agency (EMA) defines an excipient as any non-active ingredient component of a medicinal product. Improve the medications' water solubility with excipients such polyethylene glycol (PEG) and polysorbate, but be aware that these can also trigger allergic reactions (Kounis *et al.* 2021). None of the excipients commonly associated with allergic reactions is included in the composition of the BBIBP-CorV vaccine, which could explain the apparent absence of allergic reactions to this vaccine in this study and in those that precede it.

At the time of this study, COVID-19 candidate vaccines had been developed worldwide, 103 candidate vaccines had been clinically evaluated, and 184 candidate vaccines had been preclinically evaluated according to the WHO Preliminary Outlook of Vaccine

Candidates. COVID-19 (Kounis *et al.* 2021). Currently, there are 8 vaccines that have been approved and received emergency use authorization in various countries, and are being distributed to immunize the world population: Pfizer/BioNTech (USA), Moderna (USA), Sputnik V of the Gameleya Institute (Russia), Novavax (USA), Sinopharm (China), AstraZeneca (UK), Johnson & Johnson (UK), Sinovac (China). In Iraq, agreements have been reached with laboratories for the acquisition of vaccines for COVID-19. The vaccines that have arrived in Iraq and will continue to arrive in the coming months are: Sinopharm, Pfizer and AstraZeneca, through the mechanism of the COVAX Facility (Yesuf *et al.* 2022) So far there are few studies on the safety of the Sinopharm vaccine, on everything in our environment, due to the recent approval for its emergency use. The findings of this study are inclined to consider a favorable safety profile for this vaccine; however, there are various limitations, among which we will mention: The self-administered online questionnaire cannot guarantee the accuracy of the information; the low response rate of the survey, which has repercussions in a small study population; the uncertainty of whether the reported adverse events are attributable to vaccination, so the incidence of adverse reactions may be overestimated; only immediate adverse reactions were taken into account; the sample turned out to be from a single hospital and the surveyed population was younger and healthier than the general population, which could incur a selection bias.

About Pfizer/BioNTech (BNT162b2) Vaccine: It is an mRNA-based vaccine. Its effectiveness against symptomatic covid-19 is 95%. It is administered as two intramuscular doses of 0.3 mL each, 3 weeks apart. Normal storage temperature is between -60 and -80ºC. After being removed from this environment, it can be stored for 2 weeks at a temperature between -15 and -25ºC. It can be stored for one month at a temperature between 2 and 8ºC, which is the storage temperature of normal vaccines. In a study on the efficacy of the Pfizer/BioNTech (BNT162b2) vaccine against variants, the efficacy of two doses with the BNT162b2 vaccine was reported to be 93.7% in individuals with alpha variant and 88.0% in individuals with delta variant (Bernal *et al.* 2021).

The only contraindication to the COVID-19 vaccine is allergic reactions to COVID-19 vaccines or their components. Anaphylactic reactions have been reported after mRNA

vaccines, Pfizer/BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines. 80 percent of anaphylaxis cases occurred in people with a history of allergic reactions and 90 percent occurred within 30 minutes. Other reported allergic reactions include itching, rash, itchy throat and mild respiratory symptoms. Approximately 5 cases of anaphylaxis have been reported per million doses following Pfizer/BioNTech (BNT162b2) vaccination and 2.8 cases per million doses following Moderna (mRNA-1273) vaccines. The mRNA vaccines Pfizer/BioNTech (BNT162b2) and Moderna (mRNA-1273) each contain polyethylene glycol and the vector vaccine Janssen/Johnson & Johnson (Ad26.COV2.S) contains polysorbate. Allergy to these substances in individuals constitutes a contraindication. Depending on the allergic nature of the person, allergic reactions seen in the mRNA vaccine can potentially develop in vector vaccines as well. Similarly, allergic reactions to vector vaccines potentially require caution for mRNA. If there is anaphylaxis after any vaccine or injectable treatment or if there is a history of anaphylaxis for any reason, it should be followed for 30 minutes after vaccination. Vaccines should be administered in settings where immediate allergic reactions can be managed appropriately. Individuals without a history of allergy should be monitored for 15 minutes. The results presented provide evidence that the first dose of the BNT162b2 mRNA vaccine against SARS-CoV-2 is more than 50% effective in preventing symptomatic and asymptomatic COVID-19 cases in a population of Hilla City. The evaluation of LE in different subgroups is also satisfactory, with estimates of LE between 44.3% and 84.1% depending on the subgroup, although imprecise estimates with wide confidence intervals are made. Understanding how and when a vaccine works might be difficult to define. The World Health Organization (WHO) recommended that any COVID-19 vaccine achieve at least a 50% success rate, which might be measured in terms of illness prevention, disease severity, or transmission (Thompson *et al.* 2021). He also noted that a single-dose regimen was preferred and that, in the case of two-dose vaccines, protection after the first dose should be assessed. Likewise, the rapid start of protection stood out, establishing it in less than two weeks. Based on these recommendations, the findings presented in this work are especially relevant since they indicate that a first dose of BNT162b2 vaccine meets the objectives set by the WHO. The effectiveness of the vaccine in this study was evaluated in a context of recommendations for restrictions (social distance, use of a mask, limitation of the permanence of groups of people in public

and private spaces, limitation of mobility, etc. that could decrease exposure to the virus. However, such recommendations should affect case and control groups without distinction. In addition, the study was carried out in a population of active PS in a period of increased incidence of COVID-19 (Cumulative Incidence in the Department of Health on February 8, 2021 of 1.171.9 cases per 100.000 inhabitants - so many in the last fourteen days) (Shimabukuro 2021). This reflects a greater burden on the health system, reaching more than 350 hospitalized patients with COVID-19 and more than sixty of them in Critical Care Units (according to internal data from the COVID-19 Surveillance system). These figures substantiate that the population of PS studied was exposed to SARS-CoV-2 at the time of the study. The coincidence in time of the start of vaccination with the increase in the spread of COVID-19 makes it plausible that some immunized PS develop the infection. Similarly, they reported in a hospital in Israel where, also coinciding with an increase in transmission, of 4.081 vaccinated health professionals, twenty-two (0.54%) developed COVID-19 between one and ten days (median of 3.5 days) after immunization (Pai *et al.* 2021).

This positivity (and their values) may also be influenced by a past natural infection, a frequent circumstance in our series, as derived from the 64% of the unvaccinated group that already had IgG antibodies, although the average of titers of IgG in this group was lower (81 AU/mL). This percentage, moreover, shows a clear under detection and/or underreporting and/or underreporting of cases. One limitation that we encountered was not having collected the particular conditions of pathology or treatment history of each worker, including the condition of immunosuppression, which in the two workers who did not respond serologically to the two doses of vaccine, could be considered as a possible reason for the non-response, that is, an immune escape due to a certain situation of immunosuppression (Case *et al.* 2020).

In other studies, it was seen that the immune response generated by the COVID-19 vaccine in patients who have already overcome the disease generates more antibodies with the first dose than those who received the two doses without having come into contact with the virus (Zhu *et al.* 2020). In our study we were able to corroborate this aspect in the total means (698 AU/mL in two-dose naive recipients versus 891 AU/mL in

one-dose recipients with a history of COVID-19, $p<0.05$), but not separately in each of the centres in a homogeneous way, nor in a statistically significant way, due to the small sample sizes in the subgroups. This "accentuated" humoral response could serve to corroborate any changes that occur in the vaccination policy, offering a single dose to people who have had the disease, instead of two doses, releasing necessary doses for other groups, as some authors point out (Bernal *et al.* 2021).

The high percentage of subjects with elevated IgG levels and the very low casuistry of COVID-19 cases reported among our series of vaccinated subjects, as a reflection of high clinical effectiveness, suggests that the measurement of antibodies Specific IgG with a simple, efficient analytical technique could serve to prove "sufficient" immunity against COVID-19, assuming the correlation between a certain (minimum) level of antibody titers maintained over time and the true neutralizing power, similar to the value with international units of ≥10 IU/mL of anti-HBs antibodies in hepatitis B, still today universally considered protective titers at the individual level in most settings. It would be desirable if this parameter could be standardized and expressed in international units (IU), not only in arbitrary units (AU) of each laboratory. The World Health Organization is trying to precisely establish an international standard for anti-SARS-CoV-2 antibodies, to allow the comparability and harmonization of data obtained with the different techniques by the different laboratories, and to define a reference concentration in international units (IU) for binding and neutralizing antibodies (Wang *et al.* 2021). The WHO has established the first international standard and reference panel for the unit of measurement of the quantity of anti-SARS-CoV-2 antibodies (World Health Organization 2021), although an immunity threshold has not been established in terms of protection against infection and, even less, to transmission.

This parameter should also be systematically measured in all cases of COVID-19 that occurred among vaccinated people to better assess the "immunological escape", especially in symptomatic cases, from mild to severe, and to observe if the thesis of the “validity” of this parameter would falter if the titles remained high. The question remains about the value of the critical antibody threshold that could perhaps be established in this way. In our series, the 7 postvaccinal cases presented positive antibody levels (of the

order of 100, 600, 800, and even 2000 AU/mL on 4 occasions); this casts doubt on the indicative value of immunity of IgG titers, or on the "expressive power" of the result, at least of this diagnostic technique used, although the definition of "case" should also be reconsidered, not allowing it to be limited to an "isolated" positive PCR test, without measuring viral load or considering the clinical situation, perhaps beginning to differentiate between "healthy carrier status" (incidental PCR, with or without transmitting power) and a true "case" (PCR with clinic). In this case, the "post-vaccination cases/failures" would be substantially reduced, which could help reduce the "distrust" regarding vaccine effectiveness, both in the general population and in professionals, as well as adjust incidence rates (worldwide) to this new differentiation. Other analytical techniques should also be studied, such as those that can more accurately measure neutralizing antibodies, or those that measure cellular immunity, especially in people without IgG serological response. The effectiveness of this immunity against new variants should also be observed, which could be demonstrated with the absence of cases in an environment with circulation of these variants and also by measuring techniques for specific neutralizing antibodies, without losing sight of the measurement of cellular immunity, especially in non-responders.

The studies on vaccine effectiveness carried out to date are limited to the decrease in the number of cases, hospitalizations and deaths, and the results coincide with the excellent results of our series (Bernal *et al.* 2021). However, a measure of acquired immunity is not included in the evaluation, a fact that in the case of vaccination was limited in phases one and two of the clinical trials (Logunov *et al.* 2020). Our study is pioneering in measuring postvaccinal seroprevalence in a group of middle-aged workers, comparable to an important sector of the general population. The high humoral response in our series is consistent with the few seroprevalence studies against SARS-CoV-2 carried out after vaccination only in some specific groups and subgroups. The follow-up of the vaccinated people and the evolution of their antibody titers must be carried out, to assess the power to grant the measured antibody titer validity and a validity period, to prove its neutralizing power over time, reflected in the fact of not becoming a case in the face of risk exposures and, where appropriate, in an asymptomatic or very mild clinical course in the event of

contagion, as well as in the effect of mitigating transmission in the community or in specific settings.

4.2.2 Immunity study

In general, the invasion of pathogens, such as viruses, bacteria and fungi, is fought by two major defences of the immune system: an initial defence, commanded by innate immunity; and a later one, mediated by acquired immunity (Abbas *et al.* 2021).

The initial innate defence is premised on preventing invaders from entering the body, using physical barriers (skin and mucous membranes of the respiratory and gastrointestinal tract) and antimicrobial peptides (defensins and cathelicidins) secreted by epithelial cells and some leukocytes. In addition, the innate defense includes cells that detect and phagocytose the invader (tissue macrophages, neutrophils, and monocytes), and sentinel cells that alert the immune system of the invasion (mast cells and dendritic cells) (Li *et al.* 2020).

In most cases, the innate defense destroys the invader by developing an acute inflammatory reaction with the participation of macrophages, which secrete pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12 and chemokines), and of mast cells, which release their inflammatory mediators (histamine, prostaglandin and leukotriene). This joint action promotes the recruitment and migration of neutrophils from the blood to the inflamed tissue and the extravasation of plasma rich in acute phase proteins, such as C-reactive protein and complement protein C3, both produced by the liver in response to endocrine action of the cytokine IL-6, and the cytokines TNF-α and IL-1β may also participate (De Sordi *et al.* 2020, Li *et al.* 2020).

After the phagocytosis of the invaders by the newly arrived neutrophils in the inflamed tissue and the resolution of the inflammation, tissue repair begins, with the phagocytosis of the neutrophils killed in combat and of the invader remains by macrophages. Finally, the return of internal balance occurs, and homeostasis is re-established. In addition to the

acute inflammatory response, there is an innate antiviral defence: a) interferon alpha (IFN-α), a cytokine secreted by infected cells, which plays an important role in inhibiting viral replication in neighboring cells and virus dissemination; and b) Natural Killer (NK) cells, which kill virus-infected cells by degranulating their cytotoxic granules (perforins and granzymes) and secreting interferon gamma (IFN-γ), the main macrophage-activating cytokine (De Sordi *et al.* 2020, Li *et al.* 2020).

The cytokine IL-10 is a polypeptide, considered pro-inflammatory, produced by activated macrophages and monocytes that induce endogenous stimulation of anti-inflammatory cytokines. In our study we can observe a higher production of IL-10 after the second vaccination dose, in the other vaccination doses we observed low levels compared to other cytokines studied in this study.

SARS-CoV-2 replicates rapidly and intensely in some people, particularly when the infecting strain prevents the host cells from producing IFN-. The absence of IFN- promotes viral replication, leading to a greater viral burden. Increased production of proinflammatory cytokines (TNF-α, IL-1-β, IL-6, IL-12, and chemokines) by macrophages and activated dendritic cells is a result of the high viral load, which deregulates the innate immune system (Ragab *et al.* 2020). The pathophysiology of the severe type of SARS-CoV-2 infection is characterised by hyperinflammation, which mostly results in alterations to the lungs. Excessive production of pro-inflammatory cytokines is recognised to be directly related to the patient's clinical status, serving as a predictor of illness severity due to the significant association with multi-organ failure that can ultimately result in death (Fajgenbaum and June 2020). When analysing the serum cytokine profile of patients with COVID-19, (Han *et al.* 2020) found that these patients had increased levels of TNF-α, IFN-γ, IL-2, IL-4, IL-6 and IL-10. Patients with severe COVID-19 had considerably greater IL-6 and IL-10 levels compared to those with mild and moderate COVID-19. As a result, doctors can quickly identify patients at risk for a deterioration of their condition by measuring serum IL-6 and IL-10 levels (Han *et al.* 2020). Serum IL-6 and C-reactive protein levels were found to be positively correlated in another study. However, in the group of non-survivors, IL-6 behaved as a better predictor of disease progression than age and C-reactive protein. For this reason, the serum IL-6

dose was revaluated as a useful prognostic tool, primarily as an outcome predictor (Santa Cruz *et al.* 2021).

In the current study since blood samples were taken from COVID-19 persons who were either vaccinated or unvaccinated, the possible impact of vaccination on serum cytokine levels was examined. Groups 4 (second-dose vaccine recipients) and 2 (non-vaccinated recipients) patients both tested positive for covid. We concluded that there was no evidence of a statistically significant difference in serum cytokines between people with mild COVID-19 who had received the vaccine and those who had not. This finding indicated that immunisation had no impact on the circulating cytokine levels in these COVID-19 patients. Analysis of cytokines in the bronchoalveolar lavage fluid of COVID-19 patients is crucial because they may be involved in the pathophysiology of injured lung tissue.

Our results are in line with those of (Chen *et al.* 2020), who discovered an increase in IL-6 levels but no change in TNF-α concentration in the patients who were the most seriously affected. This, however, conflicts with the findings of studies by (Zhang *et al.* 2021) who discovered increases in IL-6 and IL-8. Diane et al research found that TNF-α and IL-6 may be independent predictors of illness severity. In a recent study, the IL-6 cytokine storm in severe COVID-19 was covered. The observed disparity between IL-8 and TNF- may be influenced by the sample period, the particular stage of the coronavirus lifecycle in the host, and the severity of the illness. But until further research is done, this is debatable. The predominant pathogenic trait of SARS-CoV-2 was immediate lung injury. Coronavirus or other pathogens may start the chain of events that results in lung damage, which then triggers the immune system to produce cytokines and an inflammatory response (Shi *et al.* 2020).

In this study, a high production of cytokines of both the Th1 profile (IFN-γ and TNF-α) and the Th2 profile (IL-10 and IL-6) was analyzed among patients infected with COVID-19, when compared with the group 1. Other studies have shown that CoV-19 infection can cause dysregulation in the production of these cytokines (Gupta *et al.* 2022). The TH1 response is related to cell-mediated immunity and INF-γ (Interferon gamma)

cytokine/interleukin profile. The Th2 response is associated with the humoral profile (IL4, IL6, and IL10), these cytokines have a great role in helping B lymphocytes to secrete antibodies, which are molecules of the humoral system. Th1-type cells secrete interferon, activating cytotoxic effector cells (macrophages, NK cells and CD8+T lymphocytes), stimulating cellular immunity (Kaiko *et al.* 2008).

TNF-α (Tumor Necrosis Factor) is considered one of the main mediators of inflammation in the skin and mucosa, and has an anti-proliferative effect on normal epithelial cells (Jang *et al.* 2021).

The production and amplified release into the circulation of procalcitonin (PCT) comes from extrathyroidal tissues, presumably from cells of the mononuclear phagocytic system as a consequence of the high levels of TNFα and IL-6 that circulate in the body in this situation. However, the synthesis of this biomarker is inhibited by INF-γ concentrations, whose levels are increased in infections of viral etiology (Nielsen *et al.* 2011).

There is already prior evidence that the efficacy of the vaccine begins to become apparent early. Based on phase 2/3 data from the global BNT162b2 vaccine trial, the cumulative incidence of COVID-19 cases over time between placebo and BNT162b2 vaccine recipients begins to diverge by 12 days postpartum. the first dose, being at this point the efficacy of the vaccine of 52% in the prevention of COVID-19(13); and the efficacy data from two weeks after the first dose until before the administration of the second dose reaches 92.6% (Polack *et al.* 2020). Data consistent with that of this trial were obtained in a population of 514 HWs in Israel, where immunogenicity was described twenty-one days after the first dose of BNT162b2 vaccine, and 475 (92%) were found to have antecedents detectable anti-SARS-CoV-2 IgG antibodies (Jabal *et al.* 2021). The protection of the first dose of other vaccines was also analysed: a pooled analysis of four randomized trials showed that the efficacy of the ChAdOx1 vaccine from days twenty-two to ninety after administration of a vaccine dose was 76% (Jabal *et al.* 2021).

Knowing that the effectiveness of the vaccine begins to become apparent after the first dose is useful for making recommendations. Given these data, the WHO suggests that in

situations of limited vaccine supply combined with high morbidity and mortality, postponing the administration of the second dose should be considered in order to maximize the number of people who benefit hundreds of the application of a first dose. In the United Kingdom, on December 30, 2020, the Joint Committee on Vaccination and Immunization endorsed this approach. The CDC also relaxed its recommendations on the timing of the second dose, indicating that a delay of up to six weeks after the first dose would be acceptable (Gurdasani *et al.* 2021).

In Canada, to facilitate decision-making with the vaccination program, a model from the Public Health Agency of Canada examined dose intervals between twelve and twenty-four weeks, and suggested that accelerating vaccination coverage by Extending the dose intervals of mRNA vaccines could have short-term benefits (12 months) in reducing symptomatic disease, hospitalizations, and deaths.

Other authors also position themselves with this strategy, based on the fact that delaying the second dose will make it possible to vaccinate and protect more people in the same period of time, and that the antibodies could last for several months and be reinforced with a second dose administered later. As a general principle of vaccination, the interruption of a vaccine regimen that results in a prolonged interval between doses does not require that the regimen be restarted. Although it remains to be shown that vaccination against SARS-CoV-2 occurs in the same way, in general, a longer interval between the initial dose and the booster dose allows the maturation of memory B cells and gives rise to a greater and more lasting response (Sokal *et al.* 2021).

However, delaying the second dose may also have risks, such as decreased immunity between the first and second doses or that some people may forget to receive their second dose, leading to an incomplete vaccination regimen. Likewise, other authors maintain that suddenly changing the recommendations would put the confidence of patients in vaccination at risk, and that in some populations such as PS it could have an adverse impact on their willingness to work at the same time not being sure of having a high level of protection. Finally, the impact of the extension of the interval between doses on the appearance and circulation of new variants is unknown, although the prevention of

transmission in the community through high vaccination coverage could reduce its appearance and spread. In the first place, the sample size does not allow obtaining precise results, offering results with very wide confidence intervals, fundamentally in the stratified analyses. Another possible limitation could be given because the information on the vaccination status was obtained through the Nominal Vaccination Registry, depending on the collection of information by third parties. However, given the need to monitor the vaccination strategy, the doses administered were recorded with extreme control and in real time by specialized nursing staff. On the other hand, it should be noted that there was no information available on comorbidities and, given that the study population only includes active SPs, there is no representation of the population over 65 years of age, in the same way that the population over 65 years of age is not represented either younger adolescents, children or pregnant women. Lastly, the follow-up time was short and the duration of protection from a single dose of vaccine was not evaluated. As strengths, it should be noted that the test-negative study design minimizes selection bias related to seeking medical attention, by including only people who seek medical attention. Another aspect to note is that SARS-CoV-2 infection can cause mild and non-specific symptoms in many people, which do not lead to contact with a health professional. However, the ease of access to the Preventive Medicine consultation, widely used by PS since the start of the pandemic, means that not only severe cases are diagnosed, but also mild or slightly symptomatic ones.

Evaluating the effects of a new intervention, in this case the administration of the second dose of a vaccine against COVID-19 in a population of Hilla City is essential to know its results and confirm that it is effective. These data could be used to make recommendations on interval extension in a context of limited vaccine supply. At the same time, it would be necessary to know the impact of the extension of the interval between doses on the immune response after the second dose, as well as on the circulation of new worrying variants. It is a priority to continue evaluating the results of vaccination programs against COVID-19 and obtain the best evidence that allows strategies to be adapted, in order to help balance individual protection with the impact at the population level.

5. CONCLUSIONS AND RECOMMENDATION

The history of COVID-19 infection produces a powerful booster effect in the humoral response to the administration of a vaccine dose, which increases slightly more after the administration of a second dose Vaccination with Pfizer's BNT162b2 vaccine/BioNtech grants humoral immunity to a high percentage of those vaccinated, with a significant increase in antibody titers, which goes hand in hand with high clinical effectiveness, expressed in the very few casuistry of COVID-19 cases from 15 days post vaccination. The present study contributes to the possibility of considering the measurement of humoral immunity against SARS-CoV-2 as a parameter of sufficient immunity within the surveillance and control strategy for COVID-19, ideally with a technique standardized at the international level.

The frequency of adverse reactions in general was 52.45%. Medicine Interns presented 63.2% adverse reactions in the second dose 54.7%. The most frequent adverse reaction was pain at the injection site and the most frequent systemic adverse reactions were asthenia and headache. No serious adverse reaction was reported.

The associated factors were having presented a history of chronic diseases and having some known allergy to medications and/or foods, in addition to having presented an adverse reaction to the first dose increased the risk of presenting them in the second dose.

Based on changes in serum cytokines associated with SARS-CoV-2 infection, regardless of vaccination status, it was hypothesised that the host's immune responses to coronavirus inflammation would differ from those reported in other viral infections. Serum IL-6 and IL-17 levels may be an accurate predictor of COVID-19 severity. These findings could also shed light on the pathophysiology of SARS-CoV-2 infection by highlighting a number of immunoregulatory processes that occur both before and after infection. In this study, the production of IL-17 was also analysed, and CoV-19-infected patients had higher levels compared to the group 1. IL-6 showed the highest titers among all the cytokines described in our study. High production of IL-6 was observed in all groups, from the moment of vaccination to the 2nd dose post-vaccination. Elevated IL-10 levels

are associated with cervical tumor biopsies that demonstrated the presence of elevated IL-10 levels in the samples, explaining the immunosuppressive status of patients.

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