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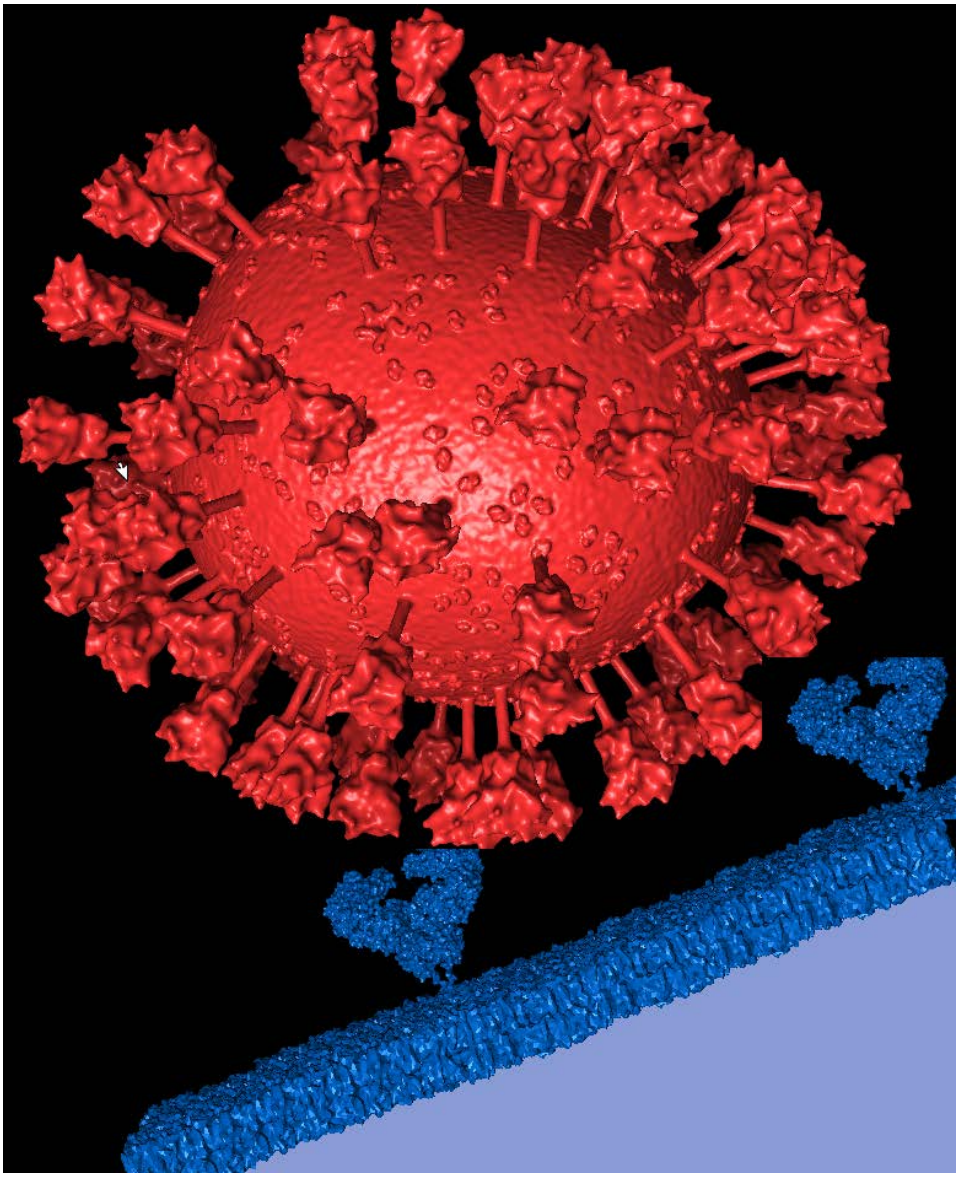
SARS-CoV-2 Special Edition



Eureka!

The great mathematician Archimedes reportedly exclaimed “EUREKA! EUREKA!” (which roughly translates to “I HAVE FOUND IT, I HAVE FOUND IT”) when the solution to a complex problem was revealed in his mind. As all scientists know, the *moment of discovery* is a cherished event; the prospect of discovery is the reason to get up in the morning, and it is what carries scientists through long nights of struggle and frustration. It is this Eureka! moment we wish to share with our students. “Eureka!” is therefore the perfect distillation of the spirit of science and an appropriate title for a journal whose goal is to provide a forum in which students can share their Eureka! moments.

To those who reviewed manuscripts for this publication;

Thank you for your time and effort on the behalf of our students. We know the density of your schedule and understand the sacrifice you have made to review our work. This sacrifice is greatly appreciated. The students participating in this research program are enthusiastic, and their work strives to reveal interesting and pertinent things about the world around us. Each manuscript published herein is the result of input from at least three faculty reviewers and the interpretation of this input by the student researchers. We have done our best to address the concerns expressed in each review, and your comments and suggestions have greatly improved the quality of our manuscripts. Please understand that final manuscripts are the result of the efforts of reviewers, students and mentors, and that not all suggestions may be incorporated, but were certainly considered. We will continue to recruit new students, and therefore hope that this is not the last time we call upon you to review such work.

We sincerely thank you for your help,

Cover Art: SARS-CoV-2 viral particle (in red) binding to Angiotensin Converting Enzyme 2 (ACE2) on a human host cell (in blue). Credit to Dr. Andrew C. Vinal, M.S., Ph.D.

A Note from the Eureka! Editorial Board

The coronavirus family contains hundreds of different viruses that can infect a wide variety of animals, but prior to 2003 only four coronaviruses were known to infect humans. These four viruses typically induce very common diseases resulting in cold-like signs and symptoms. However, in February 2003, there was an outbreak of a new coronavirus, eventually named Sudden Acute Respiratory Syndrome Coronavirus (SARS-CoV), that infected 8098 people (killing 774) before being controlled (Centers for Disease Control and Prevention, 2013). Then in 2012 another novel coronavirus outbreak started in the Middle East, eventually infecting 2494 people (killing 858) (Munyua et al., 2021). This virus was named Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Luckily, although both SARS-CoV and MERS-CoV have frighteningly high mortality rates, neither appear to be easily transmitted.

In late 2019, another novel coronavirus began infecting people in Wuhan, China. Unfortunately, Sudden Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is more easily transmitted than its cousins SARS-CoV and MERS-CoV (Petrosillo et al. 2020). Like most diseases, the disease caused by SARS-CoV-2 (Coronavirus Disease-19; COVID-19) will vary in severity from person-to-person. Most who are infected with SARS-CoV-2 will suffer only cold- or flu-like symptoms, if they suffer at all. Although many people show no signs or symptoms when infected (i.e. they are asymptomatic), and are still able to transmit the virus, in approximately 20% of those infected, COVID-19 is a life threatening disease (World Health Organization, 2020). While recent advances in the treatment of the disease has reduced the COVID-19 mortality rate, it is still a deadly disease killing more than 1% of those infected in most countries (Hasan et al., 2021). The ease and stealth with which SARS-CoV-2 is transmitted combined with the likelihood of severe disease has forced humans to alter their behaviors in order to decrease the spread of the disease.

It is certainly an understatement to say our world has been completely changed by SARS-CoV-2, and of course the Wake Technical Community College community has been drastically affected as well. With few exceptions, Wake Tech courses had to rapidly adapt to fully online instruction in March 2019 and remained so through Spring 2021. These changes also affected the Wake Tech research community as well, with lab and field work temporarily halted. However, our research students were still eager to contribute to the scientific and Wake Tech communities. One suggestion was to create a series of review articles about the basic biology and epidemiology SARS-CoV-2, as well as review some aspects of COVID-19 disease and treatments. To say the least, this proposed venture posed an extremely daunting task. The students pursuing these reviews were adjusting to the personal and academic changes forced on

them by the pandemic. Information about SARS-CoV-2 and COVID-19 quickly became voluminous, with each day bringing new revelations. However, the authors of the following papers found the time to research their chosen topics, and have done their best to summarize what we know about SARS-CoV-2 and COVID-19 in terms that all can understand. While the authors have worked hard, two factors beyond their control will limit the completeness of their work. First, some papers were submitted, reviewed, and accepted for publication in 2020. This is to say that the Eureka! peer review process is lengthy enough to preclude the inclusion of some more recent studies in some reviews. Secondly, the pace of scientific discovery also limits the inclusion of even more recent discoveries. Regardless, the authors offer the following review articles as a service to the Wake Tech community in hopes that we may better understand why the world around us has so radically changed, and what the path forward may look like.

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COVID-19 and You

Sam Teague
Wake Technical Community College Nursing Program
Raleigh, North Carolina, 27616

Introduction

COVID-19 is the name of the disease caused by the novel coronavirus SARS-CoV-2, and has been reported worldwide since late December of 2019. The symptoms and severity of the disease varies considerably from person to person. As with most diseases, COVID-19 progresses through four stages; *incubation*, which lasts from the time that the virus enters the host until symptoms begin to present, which on average lasts between 4.1 to 5.8 days (Lauer et al., 2020). In the incubation period a person might be unaware they are infected and may also be able to infect other people. Incubation is followed by *prodrome*, in which an individual first begins to present symptoms, which in the case of COVID-19 will most commonly be dry cough, shortness of breath, and/or fever. However, other more generalized symptoms such as fatigue, muscle and body aches, nausea, vomiting, sore throat, diarrhea, congestion, and loss of taste and smell may also be present (CDC, 2020). Following the prodromal period, the virus will replicate within a host to its full potential, causing the full expression of the disease within the host. This is called the stage of *invasion*. With COVID-19, the stage of invasion may be characterized by similar symptoms as experienced during the prodromal stage, but can progress into a more serious infection, causing pneumonia or tissue damage to several systems across the body, such as the lungs and heart (Carlos et. Al, 2020). *Convalescence* follows the stage of invasion, and is when the individual recovers from the disease, unless the disease results in mortality, when it is termed a *terminal infection* (Timberlake, 2002). However, SARS-CoV-2 does not affect everyone in the same way, and there appear to be several risk factors involved with the development of severe COVID-19 symptoms.

Risk Factors for COVID-19

While many young and healthy people tend to recover from COVID-19 reasonably quickly with few complications, there are some factors that can worsen the syndrome in some individuals. First and foremost, trends indicate that the older an individual is, the higher the risk of death from COVID-19 (CDC, 2020). A 13.4% fatality rate has been suggested with individuals over 80 years of age, as compared to an average fatality rate of 1.38% for those younger than 80 (Ruan, 2020). Regardless of age, people already affected by other diseases (called comorbidities) such as cardiovascular diseases (e.g. hypertension, congestive heart failure, coronary artery disease), lung diseases (e.g. asthma, and chronic obstructive pulmonary disease), diabetes mellitus, and obesity are at a greater risk for morbidity and mortality. While the mortality rate for patients with comorbidities is not uniform, a study of 5700 COVID-19 patients in the New York City metropolitan area showed a 21% mortality rate for patients with the aforementioned comorbidities (Richardson, et. al, 2020). While older people and those with comorbidities often make up the majority of hospital admissions for COVID-19, it is important to note that patients aged 20-44 make up 20% of COVID hospital admissions, and up to 12% of intensive care unit (ICU) admissions (Auwaerter, 2020). Statistics have also shown that African Americans are hospitalized more for COVID-19 infections than any other racial group in the United States (Auwaerter, 2020). While no physiological link is known between COVID-19 and African Americans, there are some factors that place them at a higher risk, such as high occurrence of cardiovascular disease (Mozaffarian et. Al, 2015), high rates of obesity and diabetes (Marshall, 2005), as well as a large presence of African Americans in industries that are considered essential (USBLS, 2019).

How SARS-CoV-2 Infects Host Cells and The Body's Immune Response

While news stories about COVID-19 are readily available, information regarding the pathophysiology of the disease is not often on the forefront. In order to understand how SARS-CoV-2

can infect and cause COVID-19 in a human host, one must first understand how this virus infects human cells. On its outer covering, a virus has protein receptors called spikes that allow the virus to bind to a host cell. SARS-CoV-2 spikes bind to a specific protein called angiotensin converting enzyme 2 (ACE 2) that is found on some human cells. If a cell does not make ACE 2, then it is unable to be infected by the SARS-CoV-2 virus. That being said, there are many cells throughout the human body that produce substantial amounts of ACE 2 and are therefore able to be infected (Bourgonje et al., 2020).

Once a human cell is infected, the virus takes over, subverting the needs of the host cell and prioritizing the production of new viral particles. Some viruses, like SARS-CoV-2, do not kill cells outright, but keep the host cell alive indefinitely to serve as a virus factory. In this state, the host cell may not be able to perform its normal functions. This loss-of-function can lead to deleterious effects within the body, but is sometimes not the direct cause of severe signs and symptoms.

Human cells are capable of detecting when they are infected by viruses and will then signal surrounding cells by releasing chemical messengers called cytokines. The human immune system is a complex mix of cells, chemicals and structures that together protect us from harmful agents. Because of this complexity, it is essential that immune cells communicate with each other, and with the cells they are tasked with protecting. This is accomplished through the cytokine system. Cytokines released by infected cells attract immune cells to the area of infection. Once there, these immune cells destroy infected cells in an effort to prevent the virus from spreading to uninfected cells. To promote this activity, other cytokines promote other immune processes, including the stimulation of more immune cells, and the activation of inflammatory response (Tisoncik et al., 2012).

When released at the proper time and concentration, cytokines are a critical part of our immunity. But in response to some infections, as it is in some COVID-19 patients, cytokines can spill over from the site of infection (such as the lungs) into other parts of the body, causing the release of

even more cytokines, which then activates even more immune cells, that release even more cytokines. This spiraling activation of the immune communication system can lead to a syndrome called a “cytokine storm”, characterized by systemic inflammation and in some cases shock (for cytokine storm reviews, please see Tisoncik et al., 2012; Jose and Manuel, 2020; Song et al., 2020).

Inflammation is a critical response of our body to infections. In response to cytokines and other chemical messengers, blood vessels dilate and become leaky, allowing fluid and our immune cells to more easily enter infected tissue. However, systemic and simultaneous activation of inflammation can flood even uninfected tissues, reducing their ability to perform their tasks. For example, inflammation in the lungs can lead to a buildup of fluid in the normally air-filled spaces that are necessary for gas exchange. Adding to the damage, immune cells called into uninfected tissues can release substances that can inadvertently kill cells. Most serious of all, a cytokine storm can lead to a sudden and significant drop in blood pressure, such that all tissues rapidly become starved of nutrients and oxygen, a serious life-threatening condition.

While cytokine storms are occurring, another chemical phenomenon is taking place alongside of them, bradykinin storms. In patients with severe cases of COVID-19, ACE (a protein that is similar to ACE 2, but has a different function) was found to be significantly downregulated while ACE 2 was found to be significantly upregulated. This downregulation is particularly detrimental given that ACE degrades peptides known as bradykinins, potent vasodepressors that can cause hypotension and vasodilation. In a healthy individual, bradykinins would function as part of the normal inflammatory process by inducing pain, recruiting neutrophils, and increasing vascular permeability. However, that functionality becomes hyperactive in cases of COVID-19 leading to significant tissue damage. While ACE degrades bradykinin, ACE 2 produces peptides known as angiotensin 1-9 which potentiate bradykinin, and upregulate its receptors. Upregulation of bradykinin receptors not only leads to higher levels of bradykinins, but also

higher levels of interleukin 2 (IL-2), which also potentiates vascular leakage leading to inflammation of the surrounding tissues, and impaired gas exchange from impaired perfusion. Bradykinin storms not only promote states of inflammation and decreased perfusion, but also promote the production and action of a substance known as hyaluronic acid. Hyaluronic acid can absorb 1000 times its weight in fluid to form a hydrogel that when secreted into the lungs gets trapped in bronchoalveolar membranes and physically blocks gas exchange, resulting in respiratory distress. Greater amounts of hyaluronic acid can enter the bronchoalveolar spaces during a bradykinin storm due to the increased vascular permeability caused by the effects of bradykinin and IL-2. To make matters worse, he enzyme that degrades hyaluronic acid (hyaluronidase) is downregulated during a bradykinin storm. Due to the downregulation of many chemicals that suppress bradykinin, and the upregulation of many chemicals that potentiate bradykinin, the body remains in a suspended state of vascular leakage, inflammation, impaired gas exchange, and impaired perfusion until said chemicals reach an equilibrium again (Garvin et al., 2020).

Mild to Moderate Cases of COVID-19

Fortunately, it is estimated that 80% of COVID-19 cases are classified as “mild”. A mild to moderate case of COVID-19 is defined by the CDC as a case without the presentation of pneumonia or hypoxia (CDC, 2020), and at worst would present with dry cough, shortness of breath, fever, nausea and vomiting, fatigue, muscle and body aches, headache, congestion, diarrhea, and/or loss of taste and smell. These cases can be treated at home, depending on clinical presentation, age, comorbidities and condition. Generally, mild to moderate cases of COVID-19 only require basic supportive care, such as fluids, and antipyretics/analgesics like acetaminophen (Tylenol), similar to treating other viral infections (Harvard Health Publishing, 2021). Oropharyngeal and/or nasopharyngeal suctioning may be beneficial for clearing secretions in patients with moderate cases of COVID-19 if they are having trouble being able

to clear their own secretions. Patients with more severe shortness of breath or trouble breathing may benefit from BiPAP (BiLevel positive airway pressure) therapy, a form of one-way noninvasive, mechanical ventilation that helps push air into the lungs. A BiPAP pushes air at a greater pressure on inspiration, and lessens on expiration, helping the patient keep an adequate rate, depth, and rhythm of breathing (Singh & Sardana, 2020). It is also important to note that an individual with a mild to moderate case of COVID-19 will need to isolate themselves in their home for at least 10 days after the onset of symptoms and until they test negative for the SARS-CoV-2 virus (CDC, 2021).

Severe Cases of COVID-19 and Respiratory Complications

As previously mentioned, cytokine and/or bradykinin storms damage and weaken tissues targeted by SARS-CoV-2, and are generally the cause of the majority of most complications leading to severe cases of COVID-19. One of the largest concerns for complications caused by COVID-19 is Acute Respiratory Distress Syndrome (ARDS), which is triggered by the buildup of gel-like fluid in the air sacs of the lungs (the alveoli) where air and carbon dioxide are exchanged (Li and Ma, 2020). As the respiratory tissue has already been damaged by the unsuppressed immune response, fluid can leak from surrounding tissues into the alveoli, impairing gas exchange, which leads to lower levels of oxygen in the blood (hypoxemia) and by default, lower oxygen levels in the body as a whole (hypoxia). ARDS will generally manifest with symptoms such as severe shortness of breath, heavily labored breathing, confusion, extreme fatigue, hypotension and cyanosis (Matthay et. al, 2012). Gattinoni et. al (2020) showed that 20-30% of COVID-19 ICU admissions presented with ARDS. With ARDS, if a patient is unable to adequately breathe on their own, they may need endotracheal intubation, in which a patient is sedated and a tube (called and endotracheal tube, or ET tube) is inserted into a patient's trachea through their mouth, in order to provide positive pressure airflow into the lungs, which helps keep the patient's tissues and blood oxygenated, protecting the patient's organs, especially their brain.

Severe Cases of COVID-19 and Cardiac Complications

Due to the large amount of ACE 2 in the heart, it is a prime location for the SARS-CoV-2 virus to infect, making the heart a target for damage over the course of a severe COVID-19 infection. Cardiac complications vary significantly, from abnormal heart rhythm (arrhythmias), to heart attack (myocardial infarction), inflammation of the heart muscle (myocarditis), and congestive heart failure. Arentz et. al (2020) showed damage to heart muscle in up to 33% of COVID-19 ICU admissions.

Arrhythmias are thought to be caused by the body's inability to meet oxygen demands, therefore increasing heart rate (tachycardia), an impairment to the perfusion of the coronary arteries, the inflammatory response, hypokalemia (low blood potassium) from increased potassium excretion from increased availability of angiotensin II. It is also believed that arrhythmias in COVID-19 patients can be caused by medications, such as hydroxychloroquine (Ranard et. al, 2020). China reported that 16.7% of patients with severe COVID-19 developed arrhythmias, although this study did not specify the cause or type of arrhythmia (Wang et. al, 2020).

Myocardial infarction is thought to also be caused by a mismatch in the supply and demand of blood and oxygen (i.e. the heart is damaged because it is not getting enough blood). Myocardial infarction is also thought to be caused by an increased demand on the heart to pump more blood to the rest of the body (cardiometabolic demand). Myocardial infarctions are time critical events requiring rapid treatment; the collection of data is therefore not a priority and quantitative data on the subject is lacking (Ranard et. al, 2020).

Myocarditis is thought to be caused directly by the inflammation cascade and cytokine storm, as well as viral entry into the myocardium through ACE 2 receptors. Quantitative data is also difficult to collect on myocarditis, as diagnoses have been made through clinical data from judgment,

electrocardiogram, cardiac markers in the blood, echocardiogram, and ejection fraction (the percentage of blood volume leaving the left ventricle and going into systemic circulation) (Ranard et. al, 2020).

The cause of congestive heart failure in COVID-19 is unknown, however it is thought to be related to tissue damage from COVID-19 related factors such as ARDS, and the aforementioned cardiac events (Ranard et. al, 2020). A retrospective study of patients in Wuhan, China showed that congestive heart failure was present as a complication in 23% of patients overall (Zhou et. al, 2020).

In extremely catastrophic situations, the heart's muscle becomes so damaged that the heart is not able to pump out enough blood to meet the body's demands (cardiogenic shock), usually a fatal experience if not treated rapidly and aggressively. Patients with cardiogenic shock present with rapid breathing, severe shortness of breath, tachycardia, loss of consciousness, a weak or absent pulse, low blood pressure, sweating (diaphoresis), pale skin (pallor), and cold extremities (Cardiogenic, 2017). If a patient were to go into cardiogenic shock that could not be corrected, or their heart was damaged to the point of not being able to meet the perfusion demands of the body, then the patient could be placed on a heart-lung bypass, known extracorporeal membranous oxygenation (ECMO). ECMO can take over the role of both the heart and lungs, by creating pressure to circulate blood through the patient's circulatory system while also swapping oxygen for carbon dioxide. while passing through specialized exchange surfaces within the device. The type of ECMO given for severe cardiac injuries with impairments in oxygenation is called Veno-Arterial, with a tube taking blood from a patient's vein, delivering it into the machine for gas exchange, and then reintroducing the blood back into a patient's body through an artery, forming a closed circuit. However, ECMO will only be used in extreme cases, as the risks of infection sometimes outweigh the benefits (Bartlett et al., 2020).

Severe Cases of COVID-19 and Inappropriate Clotting

SARS-CoV-2 has been known to cause an increased tendency for blood to clot, a syndrome called hypercoagulopathy. In one study, around 10% of 400 hospitalized COVID-19 patients developed an irregular clot, and about 5% developed thromboembolisms (Al-Samkari et. al, 2020). It is thought that this hypercoagulative state is caused by the activation of the clotting and complement cascade due to the hyperactivation of the immune system. It should also be noted that patient immobility from fatigue or sedation also increases the risk of clot formation. The clotting cascade has been known to form large clots in the veins, known as venous thromboembolisms, which have the ability to block larger vessels. The clotting cascade in COVID-19 patients can also form very small blood clots in small vessels known as microvascular thrombosis, which have the potential to block blood flow going to important organs, such as the kidneys, or even more importantly the brain, starving the target tissue of nutrients and oxygen. Unfortunately there is not very much evidence to support the use of clot busting medications (anticoagulant drugs) for the prevention or treatment of microvascular thromboses. On the other hand, prophylaxis and treatment of venous thromboembolisms with clinical anticoagulants such as Enoxaparin, Heparin, or Warfarin is effective (Connors and Levy, 2020)

Severe Cases of COVID-19 and Renal Damage

Much like the heart and lungs, the kidneys also express a lot of ACE 2, making them a target for infection by SARS-CoV-2, and a subsequent inflammation cascade from cytokines chasing the virus. Along with damage to lung and heart tissues, damage to the kidneys is also present in a significant number of patients presenting with a severe COVID-19 infection. Guangchang et. al (2020) showed that 75.4% percent of patients with COVID-19 related pneumonia also presented with renal complications ranging from proteins in the urine (proteinuria), blood in the urine (hematuria), and acute kidney injury. Like many other COVID-19 related pathologies, the direct cause of COVID-19 related renal damage is not

clear. However, as with heart and lung tissue, it is thought that this damage is related to destruction of tissue from the direct infection of renal tissue from SARS-CoV-2, the cytokine storm, hypoxia related renal dysfunction, and/or clogged filtration tissue (nephrons) related to microvascular thrombosis (Kant, 2020). Significant renal damage might signify the need for temporary or permanent hemodialysis, which is a process where blood is removed from the body from an artery through a surgically created connection, filtered of waste by an external machine called a dialyzer, and pumped back into the body through a vein, forming a closed circuit.

Severe Pediatric Cases of COVID-19 and MIS-C

Pediatric cases of COVID-19 are generally less severe than their adult counterparts, however infection with the SARS-Cov-2 virus in children has been linked to the development of a phenomenon known as multisystem inflammation syndrome in children (MIS-C) (Schvartz et al., 2020). This presents as: severe inflammation, body rash, high fever, nausea and vomiting, and changes in cardiac function. This syndrome manifests itself in a way very similar to other serious diseases, like toxic shock syndrome (TSS), Kawasaki disease, and macrophage activation syndrome. A study conducted in Spain showed that 12% of pediatric patients admitted for COVID-19 related symptoms were diagnosed with MIS-C by a physician (Moraleda et. al, 2020).

Final Stage: Convalescence

For the majority of people who have had a mild or moderate COVID-19 infection, recovery will be uneventful. One may feel weak for a few days, and for the majority of mild to moderate level cases, no complications arise. In this stage of convalescence, an individual will have built some degree of immunity to the specific virus. During convalescence, the immune system becomes less active, inflammation reduces, and immune cells recede from the sites of former infection. Patients with more severe cases of COVID-19 who suffered extensive tissue damage, may have life-altering complications

(e.g. congestive heart failure, irreparably damaged lung tissue or a permanent reduction in kidney function) (Renu et al., 2020), and may require a transplant or permanent supportive therapy. At this point, it is much too early to determine the proportion of COVID-19 patients that will have such permanent physical or functional damage.

Conclusions

COVID-19 is a widespread disease with wildly varying effects on individuals, from making someone slightly ill, to requiring mechanical ventilation, or even death. The SARS-CoV-2 virus is highly virulent and as of March 5, 2021 it has claimed around 520,000 lives in the United States (CDC, 2021) and 2.56 million worldwide (WHO, 2021). It is important to realize that it is unknown to what extent COVID-19 will present itself in an individual until they actually contract the disease. Therefore, the best way to avoid a severe case of COVID-19 is to not become infected. Since the first case of COVID-19 presented itself in 2019, many advances have been made towards its treatment and eradication. For example, three vaccines have now been approved for use in the United States and recommended precautions such as: wearing a mask, social distancing, following proper hand hygiene protocols have been shown to be effective in decreasing SARS-CoV-2 transmission (CDC, 2021). In order to stop the spread of SARS-CoV-2, one must take necessary precautions, follow guidelines published by government and medical organizations, and if eligible, get vaccinated.

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A Short History of Vaccines and Their Role in the SARS-CoV-2 Pandemic

Younes Labsh Abazid
North Carolina State University
Raleigh, North Carolina, 27695.

Introduction

Pandemic pathogens that affect large numbers of the world population have been sporadic health issues since recorded history. In more modern times, scientists have brought forth means to fight against pathogenic intrusions by a variety of methods, one that captures the most attention are vaccines. Most recently, the SARS-CoV-2 is the name of the new virus that is affecting the health of people around the globe. SARS-CoV-2 is transferred through droplets of saliva and nasal discharge (World Health Organization [WHO], 2021a). Since the emergence of SARS-CoV-2, in 2019 from Wuhan, China, the virus has caused more than 242 million cases and approximately 5 million deaths worldwide (Johns Hopkins Coronavirus Resource Center [JHCRC], 2021). The rapid spread of SARS-CoV-2 has led many research companies around the world to join in the development of vaccines against SARS-CoV-2. While many are still in the developmental stage, some vaccines have been approved and are currently in use.

History of Vaccines

A vaccine can be described as a biological preparation that provides active acquired immunity to a particular infectious disease, be it bacterial, viral or other microorganism. Vaccines are typically have been produced by taking microorganisms and weakening them so that they cannot reproduce (or replicate) themselves very well or so that they can't replicate at all.

The first attempt to produce immunity was thousands of years ago, people were inoculating healthy people by taking live smallpox from sick people and smearing it into deliberate cuts. This was because they knew that most of the time the intentionally inoculated people would get a much less severe form of the disease that would not leave scars all over the body. Inoculation was practiced in Asia and Africa, but in the 1700s inoculation reached Europe and America, and they start calling it variolation, after the Latin name for smallpox (variola) (CDC, 2021a). As was practiced in many parts of the world, Dr. Edward Jenner (1749-1823) inoculated patients with smallpox by rubbing pox material from smallpox patients into cuts in their arms (Reidel, 2005). These individuals usually developed a less serious form of smallpox, and were protected from further infections. Surprisingly, Jenner noticed that the milkmaids in the countryside were mostly immune to smallpox. Milkmaids were in contact with cowpox, a much milder disease that resembles smallpox. Many people understood that if they inoculated themselves with cowpox material, they would be immune to the more deadly smallpox (CDC, 2021a). In 1796, Dr. Jenner tested this hypothesis by taking some cowpox pus from a milkmaid and injecting the pus into an arm of a child; the child developed some symptoms such as developing a scab, soreness, and a mild fever for a day. Six weeks later, Dr. Jenner inoculated the child with smallpox pus and the kid did not show any signs of the disease. Dr. Jenner called the process vaccination after the Latin word for cow (vacca). Although Dr. Jenner was successful with his vaccine, no one understood why it worked, as there was no knowledge of viruses and very little about the human immune system (Reidel, 2005).

In 1885, Louis Pasteur, was the first to develop a modern vaccine, while simultaneously providing evidence for the theory that infectious diseases are caused by biological pathogens. His experiments spearheaded the development of live attenuated cholera vaccine and inactivated anthrax vaccine in humans (1897 and 1904, respectively). Pasteur believed bacteria were responsible for causing anthrax, as he was able to identify the microorganism in infected blood. He then made a

solution that has a weakened form of the anthrax bacteria (i.e., one that would not cause disease) to develop a vaccine for anthrax (Smith, 2012). Pasteur defined the word *vaccine* as a "suspension of live (usually weakened) or inactivated microorganisms (bacteria or viruses) or fractions thereof administered to induce immunity and prevent infectious disease or its sequelae (such as an infection from a disease, injury, or trauma)" (Stern & Markel, 2005). Childhood deaths that are caused by measles, throat disease (diphtheria), smallpox and many infectious diseases were decreased due to the effectiveness of vaccines that were subsequently developed.

How Vaccines Work

The administration of vaccines is called vaccination. Vaccination is the most effective method of preventing infectious diseases (United States Centers for Disease Control and Prevention, 2011). To understand how vaccines have saved millions of lives, it is important to understand how vaccines work to stimulate the human immune system. If a pathogen enters a person, and survives, they can multiply and cause harm to that person (i.e., they can cause an infection). However, the human body has a plethora of defenses to fight the pathogen, including a variety of white cells. The part of the immune system that recognizes and responds to specific pathogens is called the adaptive immune system, and is based on the activity of cells called lymphocytes. These cells can mount a very powerful response against a specific disease, but they must first be exposed to antigen (i.e., very small, discrete parts of a pathogen). Each lymphocyte will recognize only a very specific antigen, which is important to limiting the immune response to the immediate pathogenic threat. In other words, we do not want to activate the lymphocytes that recognize anthrax if infected by the flu virus, or vice versa. However, once activated, lymphocytes will make clones of themselves. Some of these clones will continue to fight the current infection, while other clones (called memory cells) will take up station throughout the body so they can respond to the next infection by that particular pathogen (CDC, 2018). In summary the first

encounter with a pathogen elicits a slow response from the adaptive system, but the second encounter with the same pathogen elicits a much faster and more robust response. A vaccine mimics the first encounter with antigen without the threat of the disease, so when a person then encounters the pathogen for real, they are protected by a rapid, robust response (Pulendran and Ahmed, 2011).

Types of Vaccines

Currently, when a new pathogen begins to affect large populations of people, scientists immediately collect data to learn about the pathogen and begin developing control measures, including vaccines. Each pathogen will present unique and different antigens to which the body will develop the best immune response (Seib, 2012). The different types of vaccines currently in use are live-attenuated vaccines, inactivated vaccines, toxoid vaccines, subunit vaccines, conjugate vaccines, nucleic acid vaccines, and viral vector vaccines.

Live-Attenuated Vaccines

The live-attenuated vaccines are weakened strains of a pathogen; therefore, the side effects of the live-attenuated vaccines are closest to the symptoms of the real infection. Live-attenuated vaccines (e.g., the chickenpox vaccine) are not suitable for those who have weak immune systems such as children who are undergoing chemotherapy (U.S. Department of Health and Human Services [HHS], 2021). Currently, there are no live attenuated vaccines for SARS-CoV-2. However, there are two candidates that are being developed that include Codagenix/Serum Institute of India and Meissa Vaccine (WHO, 2021b). Neither vaccine is currently available in the United States.

Inactivated Vaccines

Inactivated vaccines (e.g., the polio vaccine) are vaccines that are developed from killed strains of a pathogen. This type of vaccine needs to be given multiple times in order to build up immunity (HHS,

2021). Currently there are two inactivated vaccines against SARS-CoV-2. One was developed by China (Sinovac and Sinophram) and one developed by India (Bharat) (WHO, 2021b). Neither vaccine is currently available in the United States.

Toxoid Vaccines

Toxoid vaccines (e.g., the tetanus vaccine) are vaccines that are made from purified inactivated toxic compounds (toxins), weakened so that they do not cause harm, but can still initiate an immune response. When the immune system recognizes the weakened toxin, it learns how to fight the natural toxin (HHS, 2021). Currently, there are no toxoid vaccines against SARS-CoV-2.

Subunit Vaccines

Subunit vaccines (e.g., Bordetella and Hepatitis B vaccines) are vaccines made of specific antigens from a pathogen, instead of the entire pathogen (HHS, 2021 and The Children's Hospital of Philadelphia, 2014). There are 29 subunit vaccine candidates against SARS-CoV-2 in development, but none are in use at this time (WHO, 2021b).

Conjugate Vaccines

Conjugate vaccines (e.g., *Haemophilus influenza* type B (Hib) Vaccine) are vaccines that attack the outer layer of some bacteria, known as the capsule. The layer consists of repeated units of simple sugars to protect the bacterium, and this layer can disguise antigens, making it difficult for the body to develop an immune response. These simple sugars do not usually evoke a strong immune response either, so a conjugated vaccines works by attaching (i.e. conjugating) the polysaccharide layer of the bacteria to a known strong antigen (CDC, 2018). Because these types of vaccines are specific to bacteria, and SARS-CoV-2 is a virus, there are currently no SARS-CoV-2 conjugate vaccines.

Nucleic Acid Vaccines

The nucleic acid vaccine is a new vaccination technique whereby a person's immunity is stimulated by injecting viral or bacterial DNA in human or animal cells that express one or more of a pathogen's genes (CDC, 2018). Currently there are two nucleic acid vaccines to SARS-CoV-2 being used (Moderna and Pfizer) (WHO, 2021b). Since most of the vaccine choices for SARS-CoV-2 in the United States are nucleic acid vaccines it is important to understand how they work. Nucleic acid vaccines take advantage of the fact that an infected host cell can warn the immune system that they are infected, and activate the adaptive system in response to a specific pathogen. This is done by displaying antigens specific to the pathogen on the infected cell's outer surface, so that lymphocytes can detect these antigens. A nucleic acid vaccine takes advantage of the fact that human cells can take up nucleic acids from the fluid surrounding them. Nucleic acid vaccines use nucleic acids that encode antigens from pathogens; once injected, a person's cells can take up the nucleic acid. Once the nucleic acid is inside the cell, it can be used to make the pathogen's antigen, which can be displayed on the surface of the cell, and is seen by immune cells as a signal that the cell is infected. The immune cells responding to the "infected" signal usually will kill the "infected" cell, but will also begin producing increasing numbers of adaptive immune cells that respond to that specific pathogen, protecting the person from a real exposure to the pathogen (CDC, 2021b).

Viral Vector Vaccines

A viral vector vaccine uses a modified version of a different virus (known as the "vector") that is responsible to deliver instructions to our cells. For example, in a COVID-19 viral vector vaccine, a harmless piece of the SARS-CoV-2 genome is inserted into a different and harmless virus (e.g., cold virus). People are then purposefully infected with the engineered viruses and may develop minimal and mild symptoms while developing immunity to the SARS-CoV-2 protein. The Johnson and Johnson (which

is used in the United States) and University of Oxford & AstraZeneca vaccine are of this type (CDC, 2021c).

Process of Developing Vaccines

The process of developing a vaccine traditionally has been complex and slow. Vaccines have to be approved by the Food and Drug Administration (FDA) in the United States (and similar agencies in other countries). The FDA approval process takes years and large amounts of documentation. Vaccine development starts in a research laboratory, then progresses to clinical testing, and only after being proven safe then can pharmaceutical companies start producing and distributing the vaccine. There are different phases of vaccine development and there are many problems that researchers face during these phases (Institute of Medicine (US) Committee on the Children's Vaccine Initiative: Planning Alternative Strategies [IMCCVI], 1993).

The decision to make a vaccine is often established after an assessment of the public health needs. There are many reasons why we do not have a vaccine for every pathogen including lack of understanding of pathogens, the impact of the pathogen on the population, the developmental time for vaccines and the expense of vaccine development.

Before a vaccine can be developed, basic research must be done on the pathogen to identify and isolate antigens, sequencing the pathogen's genome, and develop reagents targeting the pathogen. This stage is often done by academic and government scientists who are funded by the federal government and usually takes 2-4 years.

Once more is known about the pathogen, the process of developing an actual vaccine can begin. This is done often by private companies using the basic information about the pathogen. This preclinical research is often done on animal models. During this stage, scientists use tissue-culture and animal

testing to test the safety of the candidate vaccine, and *immunogenicity* (the ability to provoke an immune response). This usually gives scientists an idea on the response they might except in humans. This stage usually takes 1-2 years (IMCCVI, 1993).

A vaccine has to be approved by the FDA and requires well-documented clinical evaluations before distribution. A good vaccine should meet basic criteria of safety, purity, potency, and efficacy (IMCCVI, 1993). When a manufacturer believes it has met these four criteria they submit an Investigational New Drug Application to the FDA. Once approved by the FDA they can start testing the vaccine on humans. There are four phases of human testing (see table 1 (IMCCVI, 1993)). Phase I is often a short study involving a small number of people. During this phase the manufacturer tests the safety of the candidate vaccine, how well the body develops immunity, the optimal dose, and how it should be administered. Phase I most often involves healthy individuals and typically takes one to two years.

After successful completion of phase I, companies can move to phase II. Phase II trials involve more individuals, usually in the hundreds between 25 to 1000 individuals divided into two groups. One group gets the vaccine and the other group receives a placebo. To avoid bias, individuals are randomly assigned treatment, and neither the researchers nor the patients know who receives the vaccine or the placebo treatment (i.e., a “double blind” experiment). During phase II, the focus is on the safety of the vaccine, the strength of the immune response, and the consistency of the results. This phase usually takes two years.

After completing phase II, vaccine developers can move to phase III, the final phase. During phase III research is conducted on thousands of people in an attempt to replicate the target population. Research focuses on how long the vaccine stays in the body and side effects associated with the process. This phase usually takes from 1-4 years.

Table 1: Summary of the Clinical Phases of Vaccine Research

Phase	Number of Individuals	Length of the Phase	Purpose	Characteristics of Study Population
I	5 to 50	1-2 years	To assess safety, and optimal dosage.	Conducted on individuals who are at low risk of infection. May require placebo controlled, double blinded.
II	25 to 1000	1-4 years	To expand safety and optimal dosage	May include at risk individuals. Usually, double blinded and placebo controlled.
III	100 to 10,000	3 years	To assess safety	Includes at risk individuals. Usually, placebo controlled and randomized design
IV	100,000 to millions	15-20 years	To assess safety and effectiveness and detect rare adverse events.	Post licensure vaccinated individuals, observational study design.

After completing phase III, the manufacturer submits to the FDA an application known as Product License Application, which requires the company to provide details about the vaccine development process and all the data gathered during the first three testing phases. The manufacturer also submits a second application known as the Establishment License Application, where they describe the facilities, the equipment, and the personnel involved in the manufacturing process. Then the FDA has to further analyze the data, which can take up to three years. If approved, the manufacturer can get a license to start manufacturing and distributing the vaccine.

Phase IV starts when these applications are approved. This involves research on the effectiveness of the vaccine in the general population and side effects that might have been missed during the clinical trials. Phase IV takes an average of 15 to 20 years to complete and develop a vaccine (IMCCVI, 1993).

Current Efforts to Develop COVID-19 Vaccine

When SARS-CoV-2 pandemic widened, governmental agencies like the CDC in the US, and the World Health Organization began recommending people to adapt to new norms of living such as wearing masks, social distancing, limiting the number of people in stores, and the use of curbside pickups. The safest path to normalcy is to get a vaccine for SARS-CoV-2 .The normal process takes 15 years to develop a vaccine, but the Trump Administration launched Operation Warp Speed (OWS), where the Federal government would partner with the scientific community and private sectors to develop a vaccine by 2021 (The White House, 2020). OWS partnered with the Department of Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC), the National Institutes of Health, the Biomedical Advanced Research and Development Authority, and the Department of Defense. The main goals of OWS were to accelerate the development of an efficient and safe vaccine and to produce 300 million doses. OWS established sets of safety protocols to be followed, which

allowed trials to proceed more quickly. Then the trial's protocols were supervised by the federal government, which is not the case in a normal vaccine development where public-private partnerships allow pharmaceutical companies to decide on their own protocols. In March 2020, HHS approved funding for Johnson & Johnson's vaccine and Phase 1 development started in July 2020; Phase 3 started in September 2020 when 60,000 volunteers participated. In April 2020, HHS funded Moderna and it began Phase 1 on March 2020 and Phase 3 in July 2020. In May 2020, the HHS funded AstraZeneca working with the University of Oxford. The agreement states that at least 300 million doses were to be made available for the United States. In August 2020 AstraZeneca began Phase 3. In July 2020, the HHS funded Novavax and Pfizer to manufacture 100 million vaccine doses each (HHS, 2020)

The FDA has approved three vaccines for use in the United States: Pfizer- BioNtech COVID-19 vaccine, Moderna's COVID-19 vaccine and Johnson and Johnson's COVID-19 vaccine. There still two candidates that have not been approved: AstraZeneca COVID-19 vaccine and Novavax COVID-19 vaccine (HHS, 2020). Merck & Co in September of 2020 started testing a COVID-19 vaccine based on a weakened measles virus that delivers genes from the new coronavirus into the body to stimulate an immune response to the coronavirus. Other developmental vaccines are underway that use protein-based vaccines that use purified pieces of the virus to spur an immune response. Vaccines against whooping cough and shingles employ this approach. Novavax and French drugmaker Sanofi are developing protein-based COVID-19 vaccines (HHS,2020).

How Are the Coronavirus Vaccines Different?

Pfizer-BioNTech

Pfizer-BioNTech developed a nucleic acid (mRNA) vaccine. It is given in the muscle of the upper arm in 2 shots, 21 days apart. Before taking the Pfizer-BioNTech COVID-19 vaccine it is important to inform the provider of the vaccine about any allergies, fever, bleeding disorder or if taking a blood

thinner, immunocompromised or medications that affect the immune system, pregnant or planning to become pregnant, breastfeeding, or have received another COVID-19 vaccine. It is authorized to be given for individuals who are 12 years and older. Some symptoms or risks that are associated when taking Pfizer-BioNTech COVID-19 vaccine include: injection site pain, tiredness, headache, muscle pain, chills, joint pain, fever, injection site swelling, injection site redness, nauseas, feeling unwell, and swollen lymph nodes. Pfizer-BioNtech COVID-19 vaccine could cause severe allergic reaction which occurs after few minutes to an hour after taking the vaccine, vaccination providers may ask you to stay for at least 15 minutes to monitor you looking for signs of severe allergic reaction which may include: difficulty breathing, swelling of the face and the throat, fast heartbeat, bad rash all over the body, dizziness and weakness. Pfizer- BioNTech COVID-19 vaccine cannot give the recipient COVID-19 because it does not contain active SARS-CoV-2 virus. (CDC, 2021c).

Moderna COVID-19 Vaccine

The Moderna COVID- 19 vaccine is also an mRNA vaccine. It is given in the muscle of the upper arm in two doses about one month apart. The vaccine may be given for individuals who are 18 years of age or older. As mentioned before, a person should tell their provider of their medical history before getting the vaccine, and it has similar side effects and chances of severe allergic reaction as the Pfizer-BioNTech COVID-19 Vaccine (CDC, 2021c).

Johnson and Johnson/ Janssen COVID-19 Vaccine

The Janssen COVID-19 Vaccine is a viral vector vaccine. The virus vector in this vaccine is an adenovirus (a common cold virus) that is modified to include the SARS-CoV-2 spike gene (Johnson and Johnson, 2021). It is given in the muscle of the upper arm in a single dose. The vaccine is may be given for individuals who are 18 years of age or older. Individuals who have history of anaphylaxis should not take this vaccine, women who are younger than 50 years of should be aware that in rare occasions blot

clots may occur after being vaccinated. It has similar side effects as Moderna and Pfizer-BioNTech COVID-19 vaccine (CDC, 2021c).

Who Should Get Vaccinated?

When first released for distribution, the supply in the United States was limited. Therefore, the CDC structured some recommendations to the federal, state and local governments about who should get the vaccine first. These recommendations were made to decrease death and serious complications of COVID-19 in the most vulnerable populations, and to protect first responders and health care workers who were most likely to be exposed to the virus. Healthcare personnel and residents of long-term care facilities were offered the first doses of COVID-19 vaccines, then the frontline essential workers such as fire fighters, police officers, correction officers and people who are 75 years of age and older because of their higher risk of hospitalizations and serious complication from COVID-19. The next group to be prioritized are people who are between 65-74 years of age, then people age 16-64, and lastly other essential workers such as people who work in transportation, food service, housing and other professions that put people at risk of exposure (CDC, 2021c). As of June 24, 2021, everyone 12 years or older is eligible to get the Pfizer-BioNTech COVID-19 vaccine (CDC, 2021d).

Should you get the vaccine?

The virus is still very active in all populations. Vaccinations are currently the only way to generate an immune response to SARS-CoV-2 without risking the signs and symptoms of COVID-19. All current studies indicate that for the majority of people, the vaccinations produce mild, if any, side effects. Very rarely, more serious problems can develop. Anaphylaxis, a life-threatening allergic reaction, occurred in only 11 people out of 18 million people receiving the Pfizer-BioNTech vaccination (Katella, 2021). Much was made of the blood clotting events in people who had received the Johnson & Johnson or AstraZeneca vaccines, but this serious side-effect occurred in only 1 in 2 million and 1 in 1

million recipients, respectively (Wallis, 2021). By contrast, two people out of every one hundred who contract SARS-CoV-2 may die (JHCRC, 2021).

While the vaccines have proven both safe and effective, there are guidelines one should follow before deciding to take any vaccination. A person known to be allergic to any ingredient in the vaccine should not receive the vaccine, and if a person has an allergic reaction after the first dose, they should not take the second dose. Before taking the vaccine, an individual should ask their primary care health provider, as they know the medical history of the individual (CDC, 2021c). In the end, it is a personal decision, and one should thoughtfully weigh the benefits and risks before any medical procedure.

Debunking myths about COVID-19 Vaccines

Because the entire SARS-CoV-2 genome is not present in the vaccine, the mRNA vaccines cannot give COVID-19 to those who receive it. These vaccines do not affect or interact with our DNA. As mentioned before, mRNA vaccines are a new type of vaccine to protect against infectious diseases. They teach our cells how to make a protein that may trigger an immune response, which produces antibodies, and ultimately protect the body from getting infected if exposed to the actual virus. COVID-19 mRNA vaccines give instructions (in the form of mRNA) for our cells to make a "spike" protein. The spike is what a whole virus uses to attach to a host cell, but alone it is a harmless protein. Because the spike is found on the outside of a virus, it is exposed to the immune system, which can recognize it and develop an immune response to it. When the COVID-19 mRNA vaccine is administered, the spike mRNA goes into the human cells, which then use the mRNA to make the spike. Once the spike is made, the cells break down the spike mRNA. Next, the cells display the protein spike on its surface and the immune system recognizes it as foreign and begins an immune response. This mimics what happens during a natural COVID-19 infection. In the end, the body has learned how to fight and to protect itself against

SARS-CoV-2 infections. The main benefit of a COVID-19 vaccine is that our bodies gain protection without ever getting seriously ill with COVID-19 (CDC, 2021c).

Conclusions

The current available vaccines are safe for most people and very effective in reducing the risk of SARS-CoV-2 infection and severe COVID-19. Several different companies are manufacturing vaccines, but as of now only Pfizer-BioNTechis fully approved by the FDA, while Moderna and Johnson & Johnson are authorized by the FDA for emergency use. Getting vaccinated is important as it is the only way to develop immunity without suffering the disease; it is the best way to avoid sustained person-to-person transmission. Also, the more people that infected, even if they develop only mild symptoms, the more likely there will be a mutation event that produces a more deadly variant; mutations can also lead to variants that are not affected by the current vaccinations.

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Transmission of SARS-CoV-2

Marissa Dellinger
North Carolina State University, Raleigh, NC, 27695.

Introduction

SARS-CoV-2 is a highly transmissible respiratory virus that causes the disease COVID-19. As of October 7, 2021 there have been over 44 million SARS-CoV-2 infections so far in the United States (Johns Hopkins University of Medicine [JHUM], 2021). SARS-CoV-2 can affect the lungs, heart, and other physiological systems (Cascella et al., 2021) as SARS-CoV-2 can bind to receptors throughout the human body (Hamming et al., 2004). While many who get infected by SARS-CoV-2 experience little to no symptoms, the case-fatality rate for COVID-19 in the United States is 1.8 % (JHUM, 2021), or about 18 times more lethal than the seasonal flu (World Health Organization [WHO], 2020).

SARS-CoV-2 is in the coronavirus family; most of these viruses cause benign cold-like illnesses (V'kovski et al., 2020). Colds are spread through respiratory droplets and most adults will contract a cold virus 2-3 times per year (Centers for Disease Control and Prevention [CDC], 2020). Infectious diseases are transmitted either directly (i.e. the disease agent is transferred through direct contact with a sick individual or infected animal) or indirectly (i.e. transferred through interactions with something that merely carries the disease agent, such as an inanimate object or an organism that isn't affected by the virus). The efficiency and mode of transmission plays a critical role in how a viral disease affects populations, and how the disease is prevented and treated. The transmission efficiency, sometimes called R_0, is the average number of people that can be infected by one infected individual (Randolf and Barreiro, 2020). For example, seasonal influenza has a R_0 of 1.5-3, meaning one contagious individual could infect one to three other individuals (Toner, 2006). The R_0 is contextual, meaning the transmission of the pathogen will depend on the biological properties of the contagion, the characteristics of the host

population, and the efforts to reduce transmission of the pathogen. Biological properties of the pathogen that affect transmission include the ability of the virus to survive environmental conditions, the ability to bind its receptors in the body of the host, and the number of pathogens produced and released from the host (University of Michigan School of Public Health [UMSPH], 2020). The overall health of a host population, density of a host population, and resources available to a host population impinge on the R_0. For the original SARS-CoV-2 variant, the R_0 appears to be in the range of 2.43 to 3.1 (D'Arienzo and Coniglio, 2020), although other studies indicate the R_0 might be as low as 1.4 or as high as 3.58 (Rabi et al., 2020). This means that a single person infected with SARS-CoV-2 can infect approximately 3 individuals (UMSPH, 2020). However, the recently emerged Delta variant is much more contagious, with an R_0 of 3.5-4 (Yale Medicine, 2021). Because of the exponential nature of transmission, the Delta variant will spread much more quickly, especially in unprotected populations.

Direct Transmission of SARS-CoV-2

Transmission through respiratory droplets

Direct transmission means the virus is transferred through intimate and immediate contact with contaminated bodily fluids (Asadi et al., 2020). SARS-CoV-2 is primarily transferred through small droplets of mucus and saliva, called respiratory droplets which can leave the mouth of an infected person through talking, laughing, sneezing, and coughing (WHO, 2020b). These droplets can then be breathed in by other individuals or land on inanimate surfaces, contaminating them. Respiratory droplets are defined as droplets larger than 5 µM in size, but they don't necessarily have to be visible to the human eye (Asadi et al., 2020). Therefore, it is recommended that people remain six feet apart, not only because respiratory droplets can travel that far (Sun and Zhai, 2020), but also because the droplets can be invisible to the naked eye. Respiratory droplets appear to be the primary means of SARS-CoV-2

transmission, but the virus has been detected in other bodily fluids, including blood, feces and urine (San Diego State University School of Public Health [SDSUSPH], 2020).

Transmission through blood contact

SARS-CoV-2 can infect and replicate in the cells that line blood vessels, and subsequently be shed into the blood to be carried to other parts of the body (SDSUSPH, 2020). In fact, SARS-CoV-2 can be found in the blood of infected individuals for over 16 days after the initiation of symptoms (Cevik et al., 2021). Because of this, donated blood products are routinely screened for the presence of SARS-CoV-2, therefore the risk of contracting SARS-CoV-2 through blood is minimal (Gaussen et al., 2021; SDSUSPH, 2020).

Transmission through feces and urine

SARS-CoV-2 can also replicate in the digestive tract, and can be found in anal swab samples weeks after the onset of symptoms (SDSUSPH, 2020). Anal swabs from patients can test positive for SARS-CoV-2 long after their nasal swabs test negative (Xu et al., 2020). Although there is some evidence of transmission through fecal aerosols generated when flushing and draining toilets (Kang et al., 2020), transmission of SARS-CoV-2 through direct contact with feces appears to be minimal (SDSUSPH, 2020).

Receptors for SARS-CoV-2 are also widely distributed in the human urinary tract, and SARS-CoV-2 can be found in human urine (SDSUSPH, 2020). Because of the presence of SARS-CoV-2 in both feces and urine, it is important to thoroughly disinfect toilets and other surfaces in bathrooms to further reduce the probability of transmission (Ong et al., 2020).

Transmission through animal vectors

Zoonotic transmission (i.e. disease transfer from an animal host to a human) is another form of direct transmission**.** However, in the case of viruses (such as SARS-CoV-2), there must be similarities between receptors found in infected animals and humans. The current SARS-CoV-2 outbreak is thought

to have begun in a wet market in Wuhan, China, possibly through exposure to an infected animal (Ashour et al., 2020; Wu 2020). There is evidence of some zoonotic spillover, meaning an infected animal can pass the agent to a human, though it is still unknown exactly which animal may have caused the outbreak in Wuhan (Dhama et al., 2020). It is known that common household pets, such as cats and dogs, may also contract SARS-CoV-2 but it doesn't seem to be a significant form of transmission (SDSUSPH, 2021).

Indirect Transmission of SARS-CoV-2

Indirect transmission involves the disease agent being transferred through an intermediate, for example through contact with inanimate objects (called fomites) or with carrier animals (called vectors) that are not infected by the virus (Asadi et al., 2020). Fomites can play a key role in transmission because some viruses remain viable on surfaces for long periods. The material that the object is made from will determine how long the virus can last on that surface without a host.

Fomites represent an important means of indirect transmission of SARS-CoV-2 (WHO, 2020b). For example, if someone contagious with SARS-CoV-2 were to cough into their hands and then use a doorknob, people who subsequently touched that doorknob may pick up SARS-CoV-2 on their hands. If these previously uninfected people at any point touch their mouth, nose or eyes before washing their hands, they could be infected with SARS-CoV-2. It is therefore important to disinfect possible fomites (Ong et al., 2020), but with the correct disinfectants. Many common chemicals, including ethanol (62-71 %), isopropyl alcohol (greater than 70%), hydrogen peroxide (0.5 %) and bleach (0.1 %) inactivate SARS virus within 1 minute (Kampf et al., 2020). Soap and warm water can also be effective SARS-CoV-2 as the soap can lift the virus off fomites and hands, allowing the water to carry away any viral particles. It is important to read the labels on store-bought disinfectants to see what it's composed of and whether it would be useful against coronaviruses (CDC, 2021a).

There are many surfaces where SARS-CoV-2 can successfully survive and be transferred to a host, but on other surfaces, SARS-CoV-2 only lasts a short while, so transfer is less likely. A SARS-CoV-2 virus in a respiratory droplet will survive a bit longer on non-porous surfaces since the respiratory droplet protects the virus from dehydration. For example, on steel, plastic, and glass SARS-CoV-2 can survive up to 48 hours, 72 hours, and 4 days, respectively (van Doremalen et al., 2020). These non-porous surfaces may therefore represent effective fomites for SARS-CoV-2 transmission. On-the-other hand, on porous surfaces, such as cotton (van Doremalen et al., 2020), the respiratory droplet will be absorbed, making it harder for SARS-CoV-2 to be transferred to a new host. Surfaces such as cardboard, although SARS-CoV-2 can survive up to 24 hours, are unlikely to transmit SARS-CoV-2 (van Doremalen et al., 2020). It's important to note that survival of the virus is affected by many other factors, such as temperature, humidity, and surface porosity. While viral particles can survive on these surfaces, they won't necessarily do so if the other conditions are not optimal for viral survival.

Another indirect pathway of transmission is through respiratory nuclei. In contrast to respiratory droplets, respiratory nuclei are <5 µm (i.e. 0.0002 inches) (Asadi et al., 2020; WHO, 2020b). Because of their extremely small size, respiratory nuclei can be suspended in the air for extended periods, unlike larger respiratory droplets (Asadi et al., 2020). Although SARS-CoV-2 was not thought to be transmitted through respiratory nuclei, a recent study tested SARS-CoV-2 for nuclei transmission and found the virus could remain suspended in air for three hours (van Doremalen et al., 2020). However, the equipment used in this study to generate the nuclei may not have accurately mimicked a human cough or sneeze. While transmission of SARS-CoV-2 through respiratory nuclei is a possibility, there currently isn't strong evidence to indicate widespread transmission through this pathway (Asadi et al., 2020).

Contagious Period in Infected Humans

The shedding of virus particles in different types of samples (e.g. upper respiratory, lower respiratory, stool) persisted for around 17 days in people with COVID-19, but live virus (i.e. infectious virus) is generally not detected after 9 days, and people with COVID-19 are generally most infectious within the first five days of infection (Cevik et al., 2020).

However, SARS-CoV-2 is especially difficult to control despite being only a moderately contagious disease because individuals don't need to have symptoms to spread the virus. The CDC (2021c) estimates that 30% of SARS-CoV-2 infections are transmitted by infected people who never display symptoms (i.e. asymptomatic carriers), and 50% of SARS-CoV-2 infections are transmitted by pre-symptomatic people who eventually do show signs and symptoms. This is why the disease is so hard to control because so many perceived "healthy" people are going about their daily routines and transmitting SARS-CoV-2 unknowingly. Precautions to limit transmission are extremely important, because it is difficult to discern when infected individuals are actively transmitting SARS-CoV-2.

Limiting the Transmission of the Disease

The most important means of protecting against SARS-CoV-2, including the Delta variant, is vaccination (Yale Medicine, 2021). However, while vaccination lowers the risks of infection, hospitalization with COVID, and death from COVID, some vaccinated individuals may still become infected, and may be able to transmit the virus to others (CDC, 2021b). The virus itself is not resilient without a host but still can spread quickly so the best course of action to curb the spread would be to adhere to quarantine restrictions and avoid contact. However, if you must interact with others, social distancing and wearing a mask have been proven to help decrease the transmission of SARS-CoV-2 (Brooks and Butler, 2021). Masks constructed from porous materials absorb respiratory droplets, thus

keeping the virus from being transmitted to the mask-wearer. The viral particles, however, still accumulate on the mask and the mask should therefore be frequently washed or exchanged for unused masks daily.

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Potential Courses of Treatment for SARS-CoV-2

Cameron Ulmer
Hillman Scholars Program in Nursing Innovation
University of North Carolina
Raleigh, North Carolina, 27599

Introduction

SARS-CoV-2, the virus that causes COVID-19, forced the world to reassess work and social interactions. Initially, there was little information about the virus itself and even less information available to direct experts on how to treat the sometimes severe illness that can result from the virus. As we pass through the second year of this pandemic, research and medical professionals have been able to shed light on SARS-CoV-2, COVID-19, and its potential treatments, bringing hope of new ways to avoid transmission, and of new treatments for those who are infected.

A recent study estimates that about one third of people infected with SARS do not display signs or symptoms of the disease (i.e. are asymptomatic) (Oran and Topol, 2021). Furthermore, approximately 80% of those that display symptoms will have mild to moderate disease (World Health Organization, 2020). A minority of people will therefore develop severe infection. For those people who develop moderate to severe infections, the concern progresses beyond simply the replication of the virus, but also includes the steps that our immune system takes to get the virus under control. Because of this shift in concern associated with COVID-19, treatment plans that focus on disrupting viral replication in the early stages of the illness will shift to mediating immune responses in the moderately to severely ill. This article seeks to review the current information about treatments for SARS-CoV-2

that have been explored as possibly effective at various stages of the infection, and some that have even been approved for emergency use by the Food and Drug Administration (FDA).

Basic SARS-CoV-2 Biology

A virus is, in biological terms, a very simple entity composed of very few parts (Figure 1). All viruses must have a set of instructions (i.e. a genome) with which to make new viruses in the host cell; it can be made of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). In a mature virus, this genome is packaged up in a protein shell called a capsid. In some viruses (i.e. enveloped viruses), the capsid is enclosed in a lipid membrane called the envelope. While other proteins are made by viruses, one that is absolutely essential is called the spike. This is the protein that allows a virus to recognize and stick to its specific host cell, thereby allowing infection to occur. Once inside, a virus will take over its host cell, using the viral genomic information to synthesize, assemble and release new viral particles. This process is called viral replication.

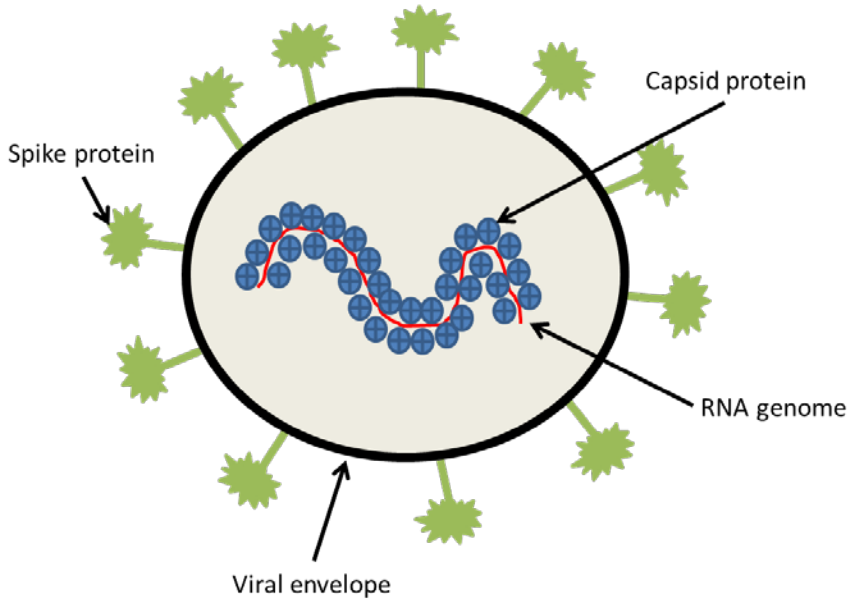

Figure 1: Cartoon representation of the SARS-CoV-2 virus. The red line represents the RNA genome which stores the genetic information with which new viral particles can be made. The RNA genome is surrounded by a helical shell formed from the interaction of multiple capsid proteins. This is turn is surrounded by a lipid viral envelope that is derived from the host's cell membrane. Spike proteins in the envelope are necessary to attach to and infect new host cells.

The SARS-CoV-2 virus is a member of the coronavirus family of enveloped RNA viruses. The SARS-CoV-2 spike is found in the viral envelope and binds to a protein found on the host cell surface called angiotensin converting enzyme type 2 (ACE2). ACE2 is expressed in cells found in the respiratory, cardiovascular, urinary and digestive tracts, among other tissues, at least partially explaining the effects SARS-CoV-2 can have on those infected (V'kovski et al., 2021). Understanding the basic biology of viruses like SARS-CoV-2 has greatly assisted the search for effective chemotherapies and immunotherapies that interfere with the basic viral replication processes, or reduce the signs and symptoms produced by viral infection.

Antiviral Treatments

Lopinavir-Ritonavir

Lopinavir and Ritonavir are antivirals that have been used in the treatment of the human immunodeficiency virus (HIV) (Trivedi, 2020). They inhibit enzymes called proteases that are critical to replication in many viruses. These antivirals were first used in coronavirus treatment in Hong Kong during the 2003 SARS outbreak and led to a milder disease course and lower mortality rate (Trivedi, 2020). There are two completed studies evaluating its use with the current SARS-CoV-2 outbreak. In one study, patients treated with Lopinavir-Ritonavir showed mild improvement compared to "standard care" (Cao et al., 2020). However, the standard care given was not well defined, and varied between patients, making it hard to evaluate the comparison (Trivedi, 2020). In a more rigorous study, the WHO Trial Consortium Study found no effect of Lopinavir on the outcome(s) of hospitalized COVID-19 patients (WHO, 2020). Another concern is that these drugs can also have serious side effects, including renal failure and liver toxicity (Trivedi, 2020). It has not been shown whether sufficiently high concentrations of Lopinavir-Ritonavir can be reached in patients to inhibit SARS-CoV-2 (Gandhi, 2020), but there are more studies underway that will shed more light on its use as against COVID-19 (Trivedi, 2020).

Remdesivir

Remdesivir is a broad-spectrum antiviral that was originally developed for the treatment of Ebola. It works by blocking the action of RNA-dependent RNA polymerase, causing the premature termination of RNA strands and preventing the virus from successfully replicating (Amirian, 2020; Trivedi, 2020). It is hypothesized that there is yet another unidentified mechanism of action which may make Remdesivir especially effective against coronavirus (Amirian, 2020).

In vitro, Remdesivir was shown to be efficacious against SARS-CoV-2 with a low level of cytotoxicity, and *in vivo* studies with SARS-CoV-2 have revealed that Remdesivir reduces the length of illness, but it does not lessen the likelihood that an individual will die (Trivedi, 2020). The WHO Solidarity Trial Study has confirmed the unpromising results of earlier studies (WHO, 2020). More studies on the effectiveness of Remdesivir in treating COVID-19 are currently underway.

It should be noted that people with renal and/or hepatic insufficiencies should avoid Remdesivir. Remdesivir is processed (activated) by the liver and can create a strain on this organ. The preparation of Remdesivir includes the use of a solubilization agent which restricts its use in those with renal impairment (Trivedi, 2020). Other drawbacks of Remdesivir include the fact that it must be administered intravenously, and that it is under patent until 2038 (leading to high cost), both of which could limit access to the drug for COVID-19 patients (Trivedi, 2020).

Interestingly, a drug closely related to Remdesivir called GS-441524 has shown to be efficacious *in vitro* against two coronaviruses (SARS-CoV-2 in humans and Feline Infectious Peritonitis in cats). GS-441524 is activated by several organs (including the lungs), which both reduces the likelihood of adverse effects on the liver but also increases the delivery of the activated medication to the virus in the lungs (Yan, 2020a). Once activated and in the blood stream, Remdesivir and GS-441524 behave similarly (Yan, 2020a). Other potential advantages of this newer drug include (1) GS-441524 is a smaller molecule, and

therefore has the potential to be aerosolized for direct delivery to lung tissue (Yan, 2020b), improving ease of administration and potential for out-patient delivery; (2) the synthesis of Remdesivir is more expensive and more complicated than GS-441524, so GS-441524 could potentially be cheaper, allowing more universal access (Yan, 2020a).

Favipiravir

Favipiravir is another anti-viral that has been evaluated for use against SARS-CoV-2 and its mechanism of action is like Remdesivir's: it inhibits RNA-dependent RNA polymerase, the enzyme needed by the virus to replicate its genome (Trivedi, 2020). Favipiravir has not yet been used to treat coronaviruses, but it was previously used in Japan to treat influenza and in New Guinea to treat Ebola (Trivedi, 2020). There are a limited number of completed studies of Favipiravir, but one Chinese study evaluating its use against SARS-CoV-2 revealed the virus was cleared faster and there appeared to be less lung damage resulting from the infection. Favipiravir also has fewer side-effects in comparison to other drugs, but it should be noted that Favipiravir can cause birth defects, so it should be avoided in women that are, or may become, pregnant (Trivedi, 2020). It is administered orally (Trivedi, 2020), making it a potential option for outpatient treatment (as opposed to drugs like Remdesivir, which must be administered intravenously). Studies are still underway in China (Trivedi, 2020), so we will have to wait for more information on the potential use of Favipiravir for the treatment of SARS-CoV-2.

Immunomodulatory Treatments

Corticosteroids

As opposed to drugs like Remdesivir, corticosteroids (e.g. dexamethasone, methylprednisolone) are not used to attack the virus itself, but to help manage the response of the patient's immune system to the infection (Zhao, 2020). Corticosteroids are immunomodulators, meaning that they help to

dampen the immune response and its associated inflammation. A patient's immune system can respond aggressively to a SARS-CoV-2 infection, and trigger a massive inflammatory response, consequently causing damage to tissue, with lung tissue being the largest concern. Corticosteroids can help to quell the immune system, reducing inflammation in the lungs of patients with severe COVID-19 and thereby reducing the chance of Acute Respiratory Distress Syndrome (Zhao, 2020).

Dexamethasone is the first drug shown to reduce mortality in COVID-19 patients (Trivedi, 2020). Dexamethasone reduces mortality in COVID-19 patients who require some sort of pulmonary support (The RECOVERY Collaborative Group, 2021). The effectiveness of dexamethasone treatment was greater in patients receiving mechanical ventilation (mortality reduced by one third), than in patients receiving oxygen support (mortality reduced by one fifth). Additionally, evidence suggests that dexamethasone can help keep some patients with moderate COVID-19 from progressing to severe disease, as patients who were not on mechanical ventilation but received dexamethasone were less likely to progress to mechanical ventilation (The RECOVERY Collaborative Group, 2021). Patients with less severe symptoms that did not require pulmonary support did not recover from COVID-19 any more quickly when given dexamethasone, suggesting that dexamethasone was most beneficial for patients further in the progression of COVID-19, when increased inflammation is more likely (The RECOVERY Collaborative Group, 2021).

Care should be taken when administering corticosteroids. They have shown to be helpful in low to moderate doses, but in higher doses, they can reduce the speed of viral clearance (Trivedi, 2020), meaning that recovery from the virus will be slowed. This may be the reason complications are more likely when it is given to people with mild to moderate COVID-19, who become more likely to develop secondary bacterial infections (Trivedi, 2020). It is also important to note that dexamethasone should be

given with consideration to pregnant mothers as it can cross the placental barrier to the unborn child (Trivedi, 2020).

Convalescent Plasma and Monoclonal Antibodies

Most people will begin to mount what is called an active response to a specific infectious agent shortly after infection. An important part of this active immune response is the production of proteins called antibodies. These antibodies will bind to the infectious agent, neutralizing it and/or marking it for destruction by other elements of the immune system. Unfortunately, the active immune response takes time to develop fully, and some people succumb to the disease before antibodies are produced in sufficient quantities to help. However, others will survive and go through a period of recovery (i.e. convalescence). Such survivors usually produce sufficient antibodies to ward off subsequent infections, or at least reduce the chances of a serious infection, by the same agent. This is the underlying concept behind the effectiveness of vaccinations, a process by which a clinical intervention stimulates a person's immune system against a specific infectious agent without giving that person the disease caused by the infectious agent (for a review of the current SARS-CoV-2 vaccination strategy, please see Abazid, 2021, this issue). However, antibodies produced by one person who has convalesced from an infectious disease (i.e. convalescent plasma) can be used to treat another who is in the initial stages of the same disease. In contrast to active immunity, passive immunity is when antibodies made by one individual's immune system are administered to another person, immediately giving the recipient immune tools to help protect them against the virus. While this therapy does not last long in the recipient, it can sometimes greatly reduce the risk of serious disease. Luckily, many antibodies shown to be effective against COVID-19 neutralize the portion of the virus that allows them to bind to host cells, making them useful in treating patients in the early stages of COVID-19 (Marovich, 2020).

Convalescent plasma that has been harvested from people that have recovered from COVID-19 can be administered via a single intravenous infusion to patients, either prophylactically or as a viral treatment during hospitalization (Chen, 2020; Marovich, 2020). Donor antibodies could help the recipient temporarily gain passive immunity against the virus (Marovich, 2020), but convalescent plasma comes with complications: potential infectious pathogen contamination, fluid overload, harvesting difficulties from the donor, and deleterious infusion reactions in the patient (Marovich, 2020).

Sometimes, antibodies to specific infectious agents (usually referred to as monoclonal antibodies) can be produced in the lab. Monoclonal antibodies may be preferred as they contain purified neutralizing antibodies, produced by cells taken from human donors or infected mice (Marovich, 2020). Monoclonal antibodies can therefore be thought of as purified versions of convalescent plasma and because of this, monoclonal antibodies do not carry risk of infectious contamination (Marovich, 2020). They also do not run the risk of fluid overload in the patient, with one study of the monoclonal antibody LY-CoV555 showing adverse effects to be equal to that of the placebo hospitalization (Chen, 2020).

The effectiveness of monoclonal antibodies and convalescent plasma is dose-dependent, with relatively high-titer doses being the most effective (Chen, 2020; Marovich, 2020). Effectiveness may also be dependent on the timing of administration within the course of the disease. Convalescent plasma has been shown to be most effective when administered closer to initial diagnosis, with no reduction in mortality rate when it was administered to those that are already in the throes of severe illness (Marovich, 2020). Another study showed that convalescent plasma given to individuals with less severe illness did help with symptom severity, which could possibly be due to viral replication being the main concern at that point in the disease course (Marovich, 2020). A study of neutralizing antibody LY-CoV555 use in outpatients revealed that patients had reduced symptoms after receiving the antibodies

and the rate of eventual hospitalization due to the infection was 1.6% in low-risk patients. This is significantly lower than the placebo group, which carried a 6.3% rate of eventual hospitalization (Chen, 2020).

Antiviral and Immunomodulatory Treatment

Chloroquines and Hydroxychloroquines (with and without Azithromycin)

Chloroquine and its derivative hydroxychloroquine are approved by the FDA for the treatment of lupus and rheumatoid arthritis, along with other inflammatory disorders, and for the prevention of protozoal infections (National Center for Biotechnology Information, 2020). When considered for use in treating COVID-19, the main hindrance is insufficient evidence of its effectiveness. *In vitro* studies have shown that both chloroquine/hydroxychloroquine change the pH at the host-cell binding site for the virus, thereby disrupting the viral particle's ability to enter the host cell (Reilly, 2020; Boulware, 2020). They have also shown some ability to inhibit RNA synthesis, meaning that these drugs were able to stop the virus from replicating its genome if they did manage to enter the host cell (National Center for Biotechnology Information, 2020). In addition, chloroquine and hydroxychloroquine have anti-inflammatory properties which may be useful in preventing and treating excessive lung inflammation caused by the immune system's response to SARS-CoV-2 (Reilly, 2020). This lung inflammation can contribute to the myriad of respiratory symptoms displayed in the moderately to severely ill.

While this sounds promising, there have been few studies that have adequately shown these drugs' *in vivo* effectiveness. Qaseem et al. (2020) recommended neither chloroquine or hydroxychloroquine be used to treat COVID-19 due to known harms and lack of evidence of its benefits. A 2020 study by the WHO Solidarity Trial Consortium revealed hydroxychloroquine had little to no effect on patients hospitalized with COVID-19. In addition, the potential side-effects associated with chloroquine and hydroxychloroquine (potentially fatal heart arrhythmias) (National Center for

Biotechnology Information , 2020) are, to some experts, considered too severe to risk on a drug with questionable effectiveness, causing the FDA to pull the emergency-use authorization for these drugs (Food and Drug Administration, 2020).

There have been reports of some individuals using these drugs prophylactically, but studies have shown there is no evidence to support the use of these drugs in this manner (Boulware, 2020). The bottom line is that high quality research is still required to determine whether these medications can be used safely and effectively for the prevention or treatment of COVID-19 (Hernandez, 2020). The use of azithromycin in combination with hydroxychloroquine to treat SARS-CoV-2 has also been proposed but is largely not prescribed by physicians due to a lack of evidence suggesting any benefit. Additionally, the side effects of hydroxychloroquine are potentially compounded when used in conjunction with azithromycin (Reilly, 2020). Medical investigators cite the unnecessary use of antibiotics in the development of antibiotic resistant bacteria as another reason to be cautious with the use of azithromycin to treat COVID-19 without solid evidence as to its effectiveness (Qaseem, 2020).

Conclusions

Although the world is still struggling to gain control over SARS-CoV-2, it is safe to say that there are more tools at our disposal to improve patient outcomes than in March 2020. Not all potential treatments have survived rigorous testing and study, but those that have survived increase survival rates, while reducing the overall burden on global healthcare systems. There is no magic bullet that will save every patient, but a hospitalization with COVID-19 is different than it was a year ago: providers have more tools in their arsenal to keep patients alive. Studies have shown which treatments are beneficial and which treatments are a waste of time, which is critical when time is limited. Studies are still in progress regarding the COVID-19 to further hone our treatments and an effective vaccine is now in use. Let's all hope that the year 2022 looks drastically different than 2020.

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The Importance of SARS-CoV-2 Testing

The Antibiotic Resistance Research Group
Wake Technical Community College START Program
Raleigh, North Carolina, 27616

Introduction

SARS-CoV-2, the virus that causes the respiratory disease COVID-19, is easily transmitted between humans through respiratory droplets. Even worse, COVID-19 has a substantially higher mortality rate than the seasonal flu (to which it is often mistakenly compared), especially among the most vulnerable humans. To this end, the spread of this virus from country to country has wreaked havoc on the health and financial stability of millions of humans across the globe. Because of its ease of transmission and potential to do harm, the world has reacted quickly and robustly to meet its challenge. One key aspect of studying and combating pathogenic threats such as SARS-CoV-2 is the ability to quickly identify those that are infected through the application of direct tests, so called because such tests look *directly* for the pathogen. But *indirect testing,* the ability to identify those who have survived and developed natural immunity to the pathogen, is also important to understanding the disease. We therefore also look for markers of previous exposure and/or infection (such as antibodies to a specific pathogen) in people. Both direct and indirect testing is important to our understanding of SARS-CoV-2 and COVID-19.

Importance of testing to individuals

The first application of testing that probably comes to mind is direct diagnostic testing (i.e. testing a patient to determine the cause of their illness). This is, of course, critical to the proper treatment of all sick individuals. We can treat sick individuals more effectively and more efficiently if we

know with what they are infected. Identifying the pathogen responsible for an infectious disease also allows us to predict with more accuracy how the disease will progress, to prepare for the most likely clinical events and to educate the patient about their condition. Correctly identifying the cause of an illness and communicating the risks of transmission to the patient is also critical to breaking the chain of transmission and protecting others. Even if an infected person is not sick enough to require hospitalization, diagnostic testing allows infected individuals to protect friends, family and coworkers. In addition to limiting exposure to other people, a person who has tested positive for SARS-CoV-2 may prompt others who may have been exposed to seek testing.

On the other hand, an individual may believe they have been exposed in the past, and may seek indirect testing to determine their immune status and future risk of infection. In most cases, indirect testing examines a person's blood for the presence of antibodies to a specific pathogen. Antibodies are proteins produced by immune cells called B-lymphocytes (B-cells). Antibodies are important to our immune response because specific antibodies are produced in response to specific pathogens, and when made, will bind only to that specific pathogen. That is to say, our immune system can tailor its response to a specific threat, providing a much more efficient and potent response. Although the process of producing antibodies for the first time can take weeks, it involves the production of thousands of B-cells, all making these specific antibodies. Once produced, these B-cells can remain abundant in our bodies, and can be rapidly mobilized when we are exposed to the same pathogen in the future. In fact this mobilization happens so quickly that we normally never realize we have again been exposed.

Once made, antibodies decrease the ability of a pathogen to infect host cells in several ways. When bound to a pathogen, antibodies serve as a signal to immune cells that a foreign particle needs to be removed. Some antibodies can bind to a lot of particles at once and clump them together (agglutination), making it easier for immune cells to remove them. And importantly in the case of

viruses like SARS-CoV-2, antibodies can bind to viral particles and keep them from entering host cells, basically rendering them inert.

Because there is a lag of at least a few days following exposure to an infectious agent and the production of antibodies, the initial antibody response does not keep people from getting sick. Also, this lag time does not allow for rapid detection of active infections. However, indirect testing does allow some predictive ability about the risk of future infection by a pathogen. If you are producing a significant amount of antibody to a pathogen, you most likely have immunity to that pathogen and will not suffer from the disease again.

Importance of Testing to Institutions

Diagnostic testing is obviously important to individuals, but testing is also critical to the local, regional, national and worldwide responses to outbreaks of infectious diseases. At the local level, identifying patients infected with easily transmitted pathogens such as SARS-CoV-2 allows these patients to be isolated in special wards or rooms in order to provide more efficient care and to protect other patients. To help control the outbreak in Wuhan, China, officials established “fever clinics”, where patients having COVID symptoms could go to be assessed by medical professionals and then be tested for SARS-CoV-2. If they tested positive, they were transferred to a hospital designated as a COVID unit (Zhang et al. 2020). Identifying and isolating COVID patients helps to stop the spread of the disease, but also allows medical professionals with infectious disease expertise, and medical equipment and supplies, to be concentrated at specialized facilities.

Dr. Larry Brilliant, who was instrumental in the successful global effort to eradicate smallpox, believes that early detection of novel infectious diseases, and an early, rapid response to such diseases are key to avoiding pandemics (Brilliant, 2006). Cataloging the signs and symptoms of a specific infectious disease and educating health care workers about them is always an important aspect to

tracking and combating a disease. However, there are drawbacks to relying solely on clinical information for diagnosis. Many diseases share signs and symptoms, making it difficult to discern the actual causative infectious agent. Also, as is true with SARS-CoV-2 infections, the signs and symptoms of a single disease may vary dramatically between infected individuals. Therefore, the ability to accurately and precisely determine the causative agent of a disease within infected individuals is critical to detecting, combating and defeating outbreaks.

When rapidly and accurately reported, direct diagnostic testing data is useful in many ways. When used in conjunction with other patient data, it can be used to discern other aspects of an infectious disease, as how patients with other, unassociated, underlying clinical conditions fare when infected. It can help determine whether certain parts of the human population are more susceptible or more resistant to the disease. It can also indicate whether there are environments in which the agent thrives or does poorly. Such tests can also sometimes be used on non-human species to determine the types of animals that can serve as vectors and/or reservoirs of infectious diseases. Of course once direct diagnostic tests have been created and optimized, they help limit the scope of future outbreaks by identifying infected individuals and allowing proper procedures to be followed to reduce exposure to others.

Indirect testing is also important to the institutional study and management of disease. "Herd immunity" is a term that defines when a certain percentage of a population within a geographical area has obtained immunity to a particular infectious disease, either through surviving an infection, or by vaccination, limiting the spread of this disease. The percentage of the population that needs to be immune to significantly decrease disease transmission varies between infectious diseases, and depends on the ease with which the disease is transmitted (sometimes referred to as R_0 or R-naught). Basically, R_0 is a number that indicates how many people an infected person is likely to infect over the period of

their illness. For example, the R_0 for measles is 12-18, although it depends on several different factors (Guerra, 2017). Practically, this means that in a population that has not been exposed to the virus and in the absence of public safety interventions, an infectious person could transmit the measles virus to 18 other people during the course of their personal infection.

Once the R_0 is known for a disease, the herd immunity threshold (HIT) can be calculated with the equation: $HIT = 1 – (1/R_0)$. Because of the novelty of the virus, the R_0 for SARS-CoV-2 is not well defined, and estimates of the R_0 vary from 1 to 6, but if an R_0 of 3 is assumed, then HIT = 0.67 for SARS-CoV-2 (Randolf and Barriro, 2020). In other words, two thirds of a population must be immune in order to significantly decrease SARS-CoV-2 transmission. However, as the R_0 approaches 1 (as happens when public safety initiatives are introduced), then HIT moves closer to zero (i.e. transmission ceases). Indirect testing is therefore essential to determining if a population has reached HIT.

What the Numbers Mean

The topic of testing has received a lot of attention during the course of the SARS-CoV-2 pandemic. The absence of an accurate, rapid SARS-CoV-2 test hampered the tracking of and response to the outbreak in the United States (Pulia et al., 2020). Testing scarcity can lead to false perceptions of the seriousness of outbreaks. For example, because there were so few tests, early testing was limited to those displaying severe signs and symptoms, first responders, and other prioritized individuals. This most likely provided a perception that the extent of the outbreak was limited, while simultaneously inflating the COVID-19 mortality rate. While reporting the absolute number of tests performed is useful in gauging efforts to monitor an outbreak, and reporting the absolute number of positives provides an indication of the extent of the problem, these can be misleading and confusing statistics. Instead, when testing becomes easier and more widespread, reporting the percentage of positive SARS-CoV-2 tests relative to the overall number of tests (i.e. the % positive statistic) provides a more normalized picture

of the infection rate at a given time. Likewise, the number of hospitalized, COVID-19 cases and overall mortality of testing-confirmed COVID-19 cases are perhaps more telling statistics regarding the current pandemic. Most of the data released to the public currently is also averaged over a seven-day window (i.e. the seven-day average) in order to reduce the influence of short term variability and highlight long term trends.

Sample Collection

Both direct and indirect testing requires an uncontaminated patient sample. The type of sample that is taken from a person depends on what question is being asked of the specific patient. For example, for direct diagnostic testing for SARS-CoV-2 (i.e. does a patient have the virus that causes COVID-19) a sample of mucous is taken from an area in the back of the nasal cavity, referred to as the nasopharyngeal area. In order to take this sample, a health care professional must insert a clean, sterile swab into the nasal canal to a depth that approximates the opening of the nasal cavity to the opening of the outer ear (i.e. a depth of several inches). It is recommended that the swab be left in place for several seconds in order to obtain sufficient material (Marty et al. 2020). The sampling should not be painful, but in most cases is very uncomfortable. However, a recent study indicates that a simple saliva sample may not only be easier to obtain, but more sensitive than nasopharyngeal samples (Wyllie et al. 2020).

Some studies have indicated that cells within the digestive tract are also susceptible to infection by SARS-CoV-2 (San Diego State University School of Public Health, 2020). In fact, SARS-CoV-2 particles can sometimes be detected in stool samples, when samples from the respiratory tract are negative (Xu et al., 2020). This implies that the infection may linger in other parts of the body even after the respiratory tract has recovered. While stool samples will probably not be routinely used for diagnosis, it has been suggested that direct testing of stool samples might be necessary to determine if a patient is

completely clear of SARS-CoV-2 infection before they are released from clinical care, to avoid SARS-CoV-2 transmission through the fecal-oral route (Amirian, 2020).

In addition to nasopharyngeal, oral and fecal samples, other types of human samples may be tested for the presence of SARS-CoV-2. For example, post-mortem testing of different organs may reveal specifics regarding the cause of death in individual cases, and provide a better overall picture of how the virus affects the human body. Because SARS-CoV-2 has the ability to enter and alter the function of cells within a range of human tissues, including those of the respiratory, cardiovascular, urinary, and digestive tracts, taking samples from such organs to determine the presence and number of viral particles can be revealing.

Samples Taken for Indirect Testing

Because antibodies are found at high concentrations in our blood, indirect testing is usually performed on plasma or serum. Many tests require that blood be drawn from a patient's vein into a tube or syringe. The blood is centrifuged to separate the blood cells from the serum or plasma, which is then tested for the presence of antibodies. However, other tests are based on small amounts of whole blood (i.e. plasma plus blood cells) obtained from a simple finger stick (Kobokovich et al. 2020).

Direct Testing Methods

Reverse transcription-polymerase chain reaction testing

Living organisms store the information that allows the maintenance of existing living cells and production of new, living cells in molecules of deoxyribonucleic acid (DNA). All the DNA within a cell is called the genome, and in humans is mostly found in long linear chromosomes. Chromosomes are actually two long, complementary strands of DNA loosely held together by weak chemical bonds. In order to make new cells, an existing cell must pry the two complementary strands apart and then copy

each strand, a process called replication. At the end of replication, a cell will have two full copies of its genome, one it can keep for itself, and another to pass onto a new cell. In living cells, replication is performed by a complex system of proteins.

The polymerase chain reaction (PCR) was conceived by Dr. Kary Mullis in the 1970's to mimic the natural replication process, but in a laboratory test tube (Garibyan and Avashia, 2013). However, PCR does not seek to copy the entire genome, but only a very specific segment of it (i.e. a target sequence). To do this, there must be some prior knowledge of the target sequence. This is because the proteins that replicate DNA (proteins called DNA polymerases) must know where to start the replication process, and is told so by other proteins which create short segments of nucleic acid (called primers) at specific points along the separated strands of DNA in a living cell. Dr. Mullis knew that if you knew the target DNA sequence, one could make these primers in the lab, and include them in the test tube so the DNA polymerase would know where to start. He also figured out that using a DNA polymerase isolated from bacteria growing in hot springs at boiling hot temperatures would allow scientists to use heat to separate the replicated strands of DNA. He then devised a procedure where a mix of the basic building blocks of DNA, genomic DNA isolated from specific cells, and primers designed to initiate the replication of a specific gene would allow the production of millions of copies of that gene within an hour or two (See Mullis, 1990 for a review of PCR and its discovery). This procedure completely revolutionized the study of all aspects of biology, and Dr. Mullis deservedly won the 1993 Nobel Prize in Chemistry for this work.

One of the most practical uses of PCR is in the detection of infectious diseases. Because of its ability to greatly amplify a target sequence (theoretically from a single copy of a DNA template), PCR has incredible sensitivity. And because amplification is driven by the specific primers included in the reaction, which are in turn designed based on a known target sequence, PCR can also offer incredible

specificity. Sensitivity and specificity are the two most important qualities of a diagnostic test. So in theory, one can develop a PCR-based direct test that should be able to detect even a few pathogens, and provide an accurate diagnosis.

However, some of the most virulent viruses do not have DNA genomes. Influenza, polio, Ebola, human immunodeficiency virus (HIV), SARS-CoV-2 and many other viruses use ribonucleic acid, or RNA, to store their biological information. This creates a problem for direct testing using PCR, as DNA polymerase will not recognize RNA, and therefore will not copy it. Fortunately, we can use other proteins to solve this problem. Retroviruses (RNA viruses such as HIV) have a protein called reverse transcriptase that can create DNA copies of RNA genomes (Mullard, 2008). This process is called reverse transcription, and because SARS-CoV-2 is a RNA virus, PCR-based direct testing methods start with this process, and these types of tests are referred to as reverse transcription-polymerase chain reaction (RT-PCR) tests.

RT-PCR tests entail first obtaining a sample suspected of harboring SARS-CoV-2 (e.g. nasopharyngeal, saliva, stool, etc.). Reagents are then used to extract the RNA from the sample, and the purified RNA is then used as template in reverse transcription. The DNA created in the RT reaction can then be used in PCR. However, for the test to be useful there must be a way to ascertain whether amplification has occurred. There are basically two ways to visualize amplified PCR samples. The sample can be analyzed by gel electrophoresis, a procedure where the DNA is pulled through a semisolid gel by an electrical current. The gel acts as a molecular sieve, allowing smaller fragments of DNA to move faster through the gel than larger fragments, thus separating the DNA by size. We can then treat the gel with a DNA stain that glows when exposed to ultraviolet light. Because we know the size of the target we are amplifying, and we can compare our PCR product to a set of DNA standards of known sizes (sometimes called a DNA ladder) we can see if a sample has amplified, and if so, if it is the correct size

(figure 1). This is a qualitative analysis that tells us whether a target sequence is there or not, but nothing more. Another type of assay allows us to tell more, however.

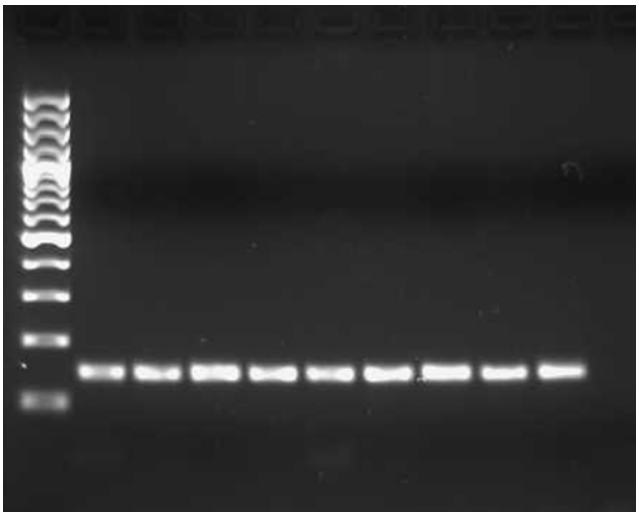

Figure 1: Example of DNA fragments analyzed by gel electrophoresis. The first lane contains a DNA size standard (the DNA ladder), while the other lanes represent PCR amplification of a target sequence. In this case, all samples are positive. This image was released into the public domain by Zaimon and obtained from Wikimedia Commons.

Quantitative reverse transcription-polymerase chain reaction testing

Another way to detect the amplification of target DNA is through the use of a PCR instrument that can monitor changes in the intensity of the light that the DNA stain emits. This instrument will take a reading of light intensity at different times during the PCR cycle over the entire course of the reaction. At the start, the light emitted should be minimal, as very little DNA is present, and if the target sequence is not present, there should be no increase in light emitted over time. However, if the target sequence is present in the sample, the light emitted should predictably increase over the course of the reaction. In this regard, this method is similar to gel electrophoresis in that it can tell us whether or not a target sequence is present or not. However, this method can also sometimes tell us how much of the target sequence was there to start with, as the rapidity with which the light increases can be correlated to the

initial starting target concentration (figure 2). This method is referred to as quantitative RT-PCR (qRT-PCR), and SARS-CoV-2 qRT-PCR tests can tell us not only whether a patient is harboring the virus, but provide some indication of how many viral particles are present.

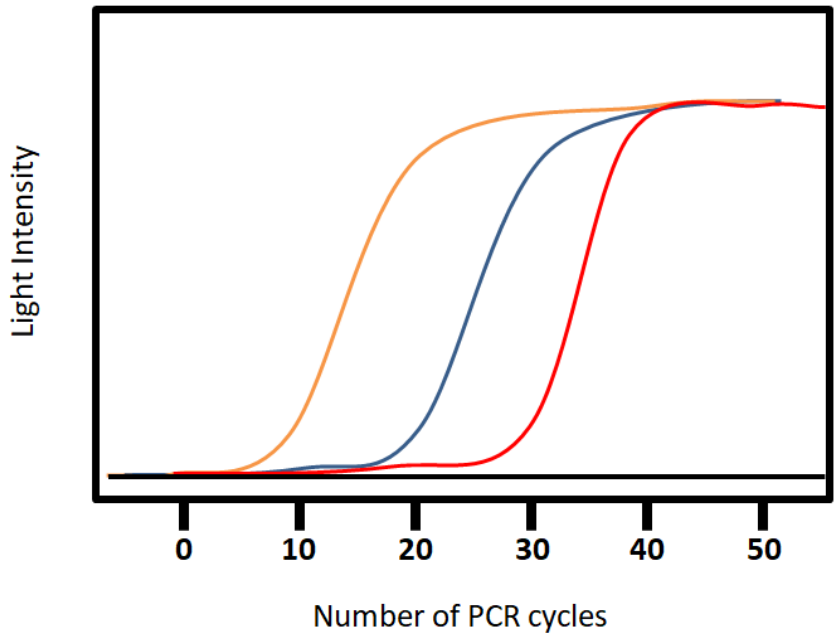

Figure 2: A graphical representation of the results of qRT-PCR. In three samples, light intensity rapidly increases at some point during the PCR cycle. However, in the sample represented by the yellow line, the intensity increases sooner in the cycle than in the other samples, indicating more target sequence was present at the start in this sample (i.e. there was more virus present to start). Conversely, the sample represented by the red line had much less target sequence, and therefore light intensity increased slower (i.e. there was less virus present to start). The black line represents a sample that has no target sequence (i.e. a negative sample).

Indirect Tests for SARS-CoV-2

Indirect tests are not based on DNA or RNA, but rather on the detection of antibodies specific to SARS-CoV-2. Many of these tests use a method called enzyme linked immunosorbent assay (usually referred to as an ELISA or EIA). In general, these tests rely on attaching a part of the pathogen that is exposed to the immune system when the pathogen enters a person. Using SARS-CoV-2 as an example,

this might be the “spike” protein that the virus uses to attach to host cells. The spike is present on the outside of the virus, and can therefore be detected by B-cells, which when activated can begin producing antibodies. To create an ELISA, we can purify SARS-CoV-2 spike proteins and physically attach them to the wells of our test plate so that they cannot be removed. We then take a sample of serum or plasma from a patient and add it to the test well and incubate it for several minutes. This allows any antibodies that recognize the SARS-CoV-2 spike to find and attach to the immobilized spike protein in the well. Once attached, we can wash all other proteins out of the well, leaving only the antibodies that recognize the SARS-CoV-2 spike protein. As with PCR, we are then faced with the problem of visualization. To solve this problem, we add another protein to the test well. This new detection protein has two functions. It is able to bind to human antibodies, so once added to the test well the detection protein will bind the antibodies that in turn are bound to the spike. The other function of the detection protein is to produce color or light, thus providing a means for detection. For example, some kits have a detection protein that is combined with a fluorescent molecule that glows when excited by ultraviolet light. In brief, the total procedure would then be to add the patient serum or plasma to the test well containing the immobilized spike protein and incubate for several minutes. After washing the well to remove any antibodies that do not bind the spike, the detection protein is added and incubated for several minutes. A second wash will remove any unbound detection protein, leaving only the detection protein that is binding antibody, which in turn is binding the immobilized spike (figure 3). The test well is then placed into an instrument that will expose the well to ultraviolet light, and be able to detect the resulting glow from the detection protein. If the samples glow, then those samples contain antibodies to SARS-CoV-2. Like qRT-PCR, this can be a quantitative method, as an increase in the light emitted is correlated to an increase in the amount of SARS-CoV-2 specific antibodies in the sample.

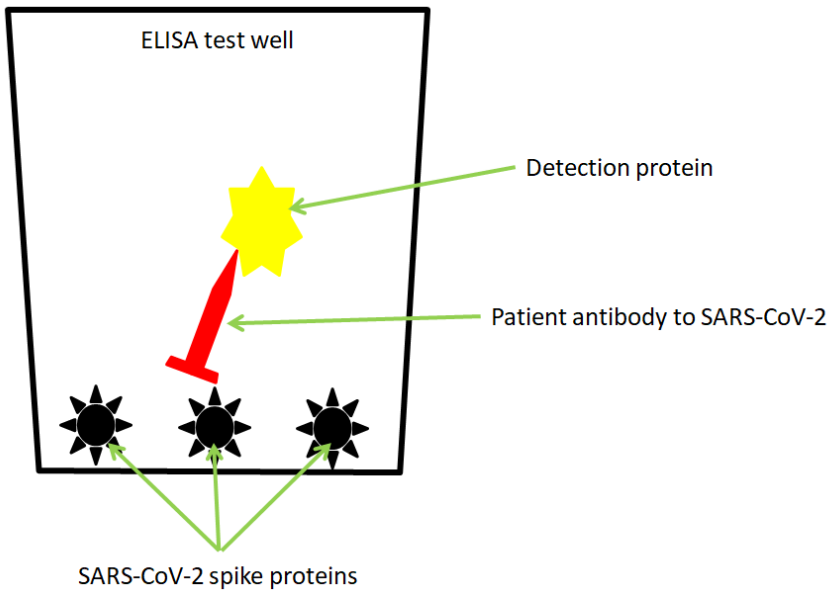

Figure 3: An ELISA designed to detect patient antibodies (the red figure) that recognize the SARS-CoV-2 spike protein (the black stars), which have been immobilized to the well of the test plate. A detection protein (the yellow star) that binds specifically to human antibodies and glow when excited by UV light is used to detect the presence of SARS-CoV-2 antibodies.

Rapid Diagnostic Tests

Modifications of this approach are being developed for use in the rapid direct detection of the SARS-CoV-2 virus and of antibodies developed to the virus. You or someone you know may have used a pregnancy test, or had a rapid strep test performed. These tests are rapid tests that use antibodies developed in and purified from laboratory animals for detection and visualization. These types of assays do not usually require special equipment with which to read them, but usually rely on a simple, visible color change to signal a positive result.

Another test uses purified antibodies to SARS-CoV-2 attached directly to sample collection swabs. These swabs are used to collect nasopharyngeal samples, and the swab is then washed to remove excess material. The swab is then bathed in a solution containing a second antibody that also

recognizes SARS-CoV-2. This second antibody is attached to an enzyme that produces a blue color when provided the proper reagent. Therefore, if a patient sample contains SARS-CoV-2, it will bind to the antibody on the swab. The second antibody is then able to bind the SARS-CoV-2 virus particles trapped on the swab, and when provided the colorless molecule, turns the swab blue (Kammila et al. 2020). While extremely simple and very rapid, a drawback of this particular assay is the lack of assay controls, making it hard to validate results.

Problems associated with direct and indirect tests

Several things can go wrong when using diagnostic tests (Ferran, 2020). The worst outcome for a direct diagnostic test is a false negative result (i.e. when a test fails to detect a pathogen in someone who is actually infected). This potentially deprives someone of treatment, and may lead to a false sense of security that in turn leads to an increase in transmission. False positives can result in several ways. The test may not be sensitive enough to detect low levels of pathogen in the patient sample, or there may be some fault in the test that does not allow consistent detection. The sample may not have been collected correctly, or there may be chemicals in the sample that inhibit the test.

False positives (i.e. a test indicates a person harbors the pathogen, but is not actually infected) are also serious. False positives can lead to unnecessary (and sometimes dangerous) treatments. Especially when threatened with a potentially life threatening disease, a diagnosis based on a false positive result can also lead to psychological distress and isolation. The isolation can also lead financial stress if the person cannot continue employment because of the diagnosis.

Because so much hinges on the accuracy and precision of these tests, diagnostic assays are usually rigorously tested and certified by federal governmental agencies (in the United States it is the Food and Drug Administration). However, due to the seriousness of the SARS-CoV-2 outbreak and the need for testing, many kits have provisional permission to produce and sell SARS-CoV-2 diagnostic tests

through Emergency Use Authorization (EUA) (United States Department of Human and Health Services, 2020). EUA does not release the manufacturer of such kits from validating their kits (i.e. they still must show key test characteristics such as specificity, sensitivity and reproducibility), but the rigor with which such validation is conducted may be lacking due to the speed in which these tests are designed, produced and distributed.

There are normally built in controls that help reduce false test outcomes. For example, most kits come with a sample of SARS-CoV-2 RNA that can be used as a positive control. Dilutions of positive controls can also be used to test the sensitivity of the diagnostic test. Negative controls, either with no RNA or DNA, or perhaps genomes from viruses unrelated to SARS-CoV-2 are also used to make sure there are no contaminants in the kit reagents that could yield false positive. Indeed, the initial diagnostic kits produced by the Centers for Disease Control and Prevention had to be recalled because of contamination in the negative controls (Sharfstein, 2020). Although most test manufactures are reporting rates of sensitivity and specificity reaching or exceeding 90%, the real picture of the effectiveness of such kits will only be revealed after more rigorous and comparative studies (Ferran, 2020).

Diagnostic testing facilities run thousands of tests for different clinical issues each day. Many of these diagnostic tests require special instrumentation and in many cases must be performed by certified, skilled technicians. During a pandemic in which thousands of people may be infected in a limited geographical region, testing facilities may become overwhelmed by samples. Therefore, two other issues regarding diagnostic testing are important during a pandemic. Because such a high volume of samples must be tested quickly, the speed at which each test can be completed correctly is an important characteristic. For example, once received by a testing facility, a single sample can be processed and tested for SARS-CoV-2 by qRT-PCR within a few hours. However, other rapid tests can be

completed within 15 minutes. If both tests perform equally well, it might seem obvious that the rapid test would be preferable. However, many rapid tests must be processed one sample at a time (i.e. four tests and hour), while most qRT-PCR instrument can handle 96 or more samples concurrently. Therefore, throughput (the number of samples processed and tested within a given time) is extremely important when dealing with an abundance of samples over a short time (Durner, 2020).

Cost and Insurance Coverage

Despite the importance of diagnostic testing to our understanding and response to the SARS-CoV-2 pandemic, not all individuals with the signs and symptoms of COVID-19, and/or those who have had direct contact with SARS-CoV-2 initially had the opportunity to be tested. Due to a lack of Federal coordination, the criteria for testing varied at the local and state level. The Families First Coronavirus Response Act (FFCRA; United States Congress, 2020a) and the Coronavirus Aid, Relief, and Economic Security Act (CARES; United States Congress, 2020b) established that most providers of insurance (both private and governmental) cover COVID-19 testing, while also eliminating all cost sharing associated with testing services associated with the disease. Although this may seem straightforward, differences in the evaluation of COVID-19 cases lead to patients paying for significant percentages of the testing costs (Chua and Conti, 2020). Although the FFCRA has provisions for increasing Medicaid coverage for COVID-19 testing, this is left up to individual state governments, and aid to the uninsured therefore varies between states (Tolbert, 2020). Many state and local governments, and some private institutions have begun to offer free, criteria-based SARS-CoV-2 testing (i.e. you must meet at least one criteria in order to be tested). However, although FFCRA and CARES also appropriated monies to reimburse health care providers for treatment of COVID-19, many who are diagnosed with and treated for COVID-19 may accrue thousands of dollars in medically related debt (Merelli, 2020). Additionally, many individuals may

fear that a positive test may jeopardize their employment. The financial uncertainty associated with COVID-19 testing, treatment and other issues may dissuade many from seeking diagnostic testing.

SARS-CoV-2/COVID Testing in North Carolina

Initially, testing in North Carolina was initially restricted to those that met a very stringent set of criteria, and many who displayed signs and symptoms very suggestive of COVID-19 were not able to be tested (Martin et al., 2020). As of October, 2021, both rapid and PCR-based tests are readily available. In most cases, testing for North Carolina residents who have insurance will be free. The uninsured can make an appointment at a federally qualified health center, and some commercial sites offer free testing for anyone meeting the testing criteria (North Carolina Department of Health and Human Services, 2020b). However, because of the complexities of our health care and insurance systems, individuals should be very careful to obtain precise information about what financial liabilities actually accompany testing. This is especially true if procedures and processes besides testing are involved (e.g. telephone/video consultation, testing for other illnesses) or if "out-of-network" professionals are used.

Conclusions

As with any contagious disease that is easily spread, diagnostic testing is essential to reducing the transmission of SARS-CoV-2, efficiently treating those with COVID-19, and to our understanding of the virus and the disease it causes. Direct diagnostic testing allows for the identification and more precise treatment of infected individuals, and a better understanding of the ease and modes of transmission, thereby helping protect the uninfected. Indirect testing can inform individuals of their immune status toward SARS-CoV-2, and help governments and other institutions determine how well populations as a whole are protected from the virus. While initial efforts were hampered by a lack of SARS-CoV-2 tests and poor quality assurance of those that were produced, several different types are now available from several different manufactures, allowing much greater access to SARS-CoV-2 testing.

While the response to the SARS-CoV-2 outbreak in United States could charitably be described as halting, the lessons learned about testing and its importance to limiting the severity of an infectious disease outbreak should serve to educate those in government and health care and increase the rapidity and efficiency with which respond to such challenges in the future.

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