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Antiviral Blood Plasma Therapy

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ZADÁNÍ BAKALÁŘSKÉ PRÁCE
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Zadávající katedra:	**Katedra biologických a biochemických věd**

Zásady pro vypracování

V dostupné odborné literatuře mladší 10 let se seznamte se složením krevní plazmy a její rolí v medicíně a imunohematologii. Zpracujte rešerši na téma infekčních onemocnění, ve kterých je možné použít léčbu plazmou obsahující specifické protilátky. Zaměřte se na virová onemocnění. V práci uveďte metody odběru a separace plazmy, její laboratorní testování na obsah účinných látek. Popište rizika spojená s touto antivirovou léčbou.

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V Pardubicích dne 26. února 2021

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In Pardubice on 04.09.2021

Ahmed Salem

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Annotation

This bachelor's thesis aims to discuss an old treatment method, which has recently been introduced to treat and is a feasible method for viral diseases such as Ebola, type A influenza and recently COVID-19 as antiviral plasma therapy or immunotherapy. This thesis discusses its usage in the treatment process for viral infections, how it provides significant immunological information as a way of passive immunization by obtaining specific antibodies against pathogens where have been produced in the host cell, as well as its collection and separation techniques based on the selected criteria in the laboratory and the use purpose, and the consequential risks.

Key words

Plasma therapy, antiviral disease, passive immunization, plasma separation, and neutralization.

Anotace

Cílem této bakalářské práce je diskutovat historicky používanou léčebnou metodu, která byla nedávno terapeuticky zavedena v léčbě virových onemocnění jako např. Ebola, chřipka typu A a nedávno COVID-19 a je označována jako antivirová terapie plazmou nebo také imunoterapie. Tato bakalářská práce je věnována využití plazmy v procesu léčby virových infekcí, tomu, jak poskytuje významné imunologické informace formou pasivní imunizace a získání specifických protilátek proti patogenům, které byly produkovány v hostiteli, a také o jejich technikách sběru a separaci na základě vybraných laboratorních kritérií a účelu použití, a také i následných rizicích jejího použití.

Klíčová slova

Terapie plazmou, antivirová onemocnění, pasivní imunizace, separace plazmy a neutralizace.

Contents

Table of figures and tables

LIST OF ABBREVIATIONS AND SIGNS

Abbreviation	Full name
Ab	Antibody
APC	Antigen Presenting Cell
B-cells	B-Lymphocytes Cells
BSL3	Biosafety Level 3
CDC	Centers Disease Control and Prevention
CP	Convalescent Plasma
CPDA	Citrate Phosphate Dextrose Adenine
DC	Dendritic Cells
DEHP	Di-ethylhexyl Phthalate
EHF	Ebola Hemorrhagic Fever
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
EVD	Ebola Virus Disease
Fc	Fragment Crystallizable
FDA	Food and Drug Administration
GP	General Practitioner
GP	Glycoprotein
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HI	Hemagglutination Inhibition
HIV	Human Immune Deficiency Virus
HLA	Human Leukocytes Antigen
IFA	Immunofluorescence assay
IFN	Interferons
Ig	Immunoglobulin
IL	Interleukin
LDL	Low Density Lipoprotein

Abbreviation	Full name
LFIA	Lateral Flow Immunoassay
Mab	Monoclonal Antibody
MERS	Middle East Respiratory Syndrome
MHC	Major Histocompatibility
N protein	Nucleocapsid protein
NAb	Neutralizing Antibody
NK	Natural Killer
PAb	Polyclonal Antibody
PL	Platelets
PVC	Polyvinyl Chloride
RBC	Red Blood Cell
RBD	Receptor-binding Domain
RVPN	Reporter Viral Particle Neutralization
S protein	Spike protein
SARS	Severe Acute Respiratory Syndrome
sGP	Secreted Glycoprotein
T_c	T-Cytotoxic Cell
T-cells	T-Lymphocytes Cells
T_H	T-Helper Cell
TLR	Toll-like Receptor
WB	Western Blots
WBC	White Blood Cell
WHO	World Health Organization

Introduction

Convalescent plasma (CP) transfusion is a therapeutic approach to treat infectious diseases of its nature that contain immunoglobulins or antibodies, which are produced in order to neutralize pathogens antigens because plasma plays a significant role in inducing the immune response once foreign particles invade the cell. Convalescent plasma is always collected from recovered individuals with specific pathogenic infections and transfused into the treatment of acute or severe infected patients who cannot produce enough of their own antibody titer to fight this infectious molecule including, viruses, bacteria, or fungi. This approach is known as immunotherapy or passive immunization because it has shown good results during outbreaks, reducing the mortality rate and hospitalization period.

This thesis aims to understand how convalescent plasma efficiency and feasibility has in treating viral infections to bust the immunity system to generate a more supportive immune response by understanding its nature, components, mechanism, and risks. It is also discussed the laboratory techniques, protocols, and procedures for collecting, separating, and infusing plasma according to the international plasma transfusion protocol.

1 Blood

Human body contains fluids that circulate around in providing various functions for cells, organs and biological systems. One of the main closed circulating fluids system is blood. Physiologically, blood volume varies in human based on gender and during pregnancy since it is the main nutritional source for the fetus. In vertebrates, blood circulates in closed system known as cardiovascular system including arteries, veins, and capillaries [1]. It is composed of blood cells and blood plasma which is an important component of so many physiological functions and is used in research medicine for treatments and widely in immunology of its immunoglobulin presence.

Depending on age, gender and weight of the body, blood volume has an average of 5 liter circulating blood. Th main function is transporting nutrition to organs and cells and taking waste from them. All blood cells are made in bone marrow as stem cells and mature into different cells within specific organs and specific functions in human's body [2]. All blood cells are suspended in the plasma. They are as following:

1.1 Erythrocytes – Red Blood Cells (RBC)

RBCs are biconcave shape cells without nucleus assisting in carrying more hemoglobin diffusing more oxygen on their surface [3]. They mainly transport oxygen and carbon dioxide between lungs and tissues. They make up 45% estimated average of total blood. They have a lifespan of 120 days [4]. Low hemoglobin volume in blood indicates the diagnosis of anemia.

1.2 Thrombocytes – Platelets (PL)

PL are tiny colorless and small fragments of blood cells that travel to the wound position in order to start clotting blood (blood coagulation) with the help of fibrin which is derived from fibrinogen that is present in blood plasma to stop bleeding in a process called hemostasis [5] and healing wounds.

Pathologically, PL are so important during common medical conditions that indicate the PL counts in the body weather it is caused by the bone morrow or not such as essential thrombocythemia, secondary thrombocytosis (high PL counts), thrombocytopenia (low PL counts), and platelets dysfunction [6]. PL lifespan is from 8-10 days.

1.3 Leukocytes – White Blood Cells (WBC)

WBCs are found in different shapes and sizes. Some of them have nuclei with multiple lobes and some contain packets of granules in cytoplasm known as granulocytes. WBCs play a big role in immune response represented by immunity system. They develop into lymphocytes, monocytes and granulocytes including neutrophils, eosinophils, and basophils [7]. Lymphocytes are important components in inducing specific immune response in immunity by producing B lymphocytes, T lymphocytes and natural killers which all act as defense line in targeting foreigner body invaders such as bacteria, viruses or fungi known as pathogens and they are recognized by their surface antigens in order to develop specific antibodies in a process called neutralization [5]. Monocytes oversee macrophage. Granulocytes are responsible in fighting infections. WBCs have a lifespan of 3 days.

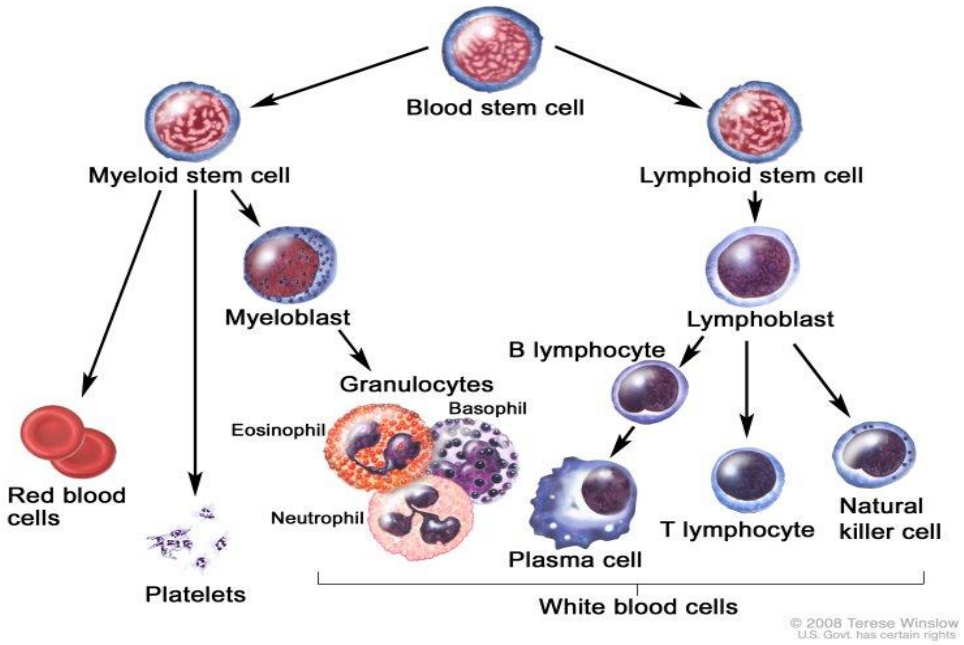

Figure 1: Blood Cell Development [8]

2 Plasma

As the second composition of blood is blood plasma or plasma. Plasma is the medium fluid through which blood cells flow throughout the human body. By the separation methods, the whole blood separates into blood cells that makes up 45% and plasma constitutes 55% of total blood volume [9].

Plasma is described as light-yellowish colored and this color differentiates based on the pathological effects that might look reddish or lipemic. Meanwhile, the main difference between blood plasma and plasma serum is that serum doesn't have the coagulating factor fibrinogen, but plasma has [10]. Plasma has various functions but mainly is to transport blood cells, nutrition, hormones, coagulants, proteins, minerals and water. Also, it facilitates the waste transportation of metabolism to the targeted organs for excretion such as the kidneys, liver and lungs [11].

2.1 Composition

There are many components that are dissolved in plasma in order to perform different functions.

2.1.1 Water

Water makes up around 91-92% of total plasma volume. It helps in providing the fluid medium in the body throughout organs to contribute in generating heat as thermoregulation [12], sustaining the body temperature and helping in the equilibrium of hydration and dehydration process [13].

2.1.2 Mineral salts and ions

Solids are so essential for the biological metabolisms in different ways which are present in form of dissolved molecules in the plasma such as chloride sodium, calcium, potassium, copper, and iron which all involve in maintaining such as pH, osmolality, and pressure. Some of the electrolytes exist in form of soluble gases such as carbon dioxide, nitric oxide and oxygen [1].

2.1.3 Low and high molecular components

This covers so diverse components that are engaged in building base units of many complex biological processes. These components include hormones, vitamins, amino acid, lipids, fatty acids, peptides and polynucleotides that oversee carrying the genetic information (DNA and RNA) or responsible of proteosynthesis which is the process of making proteins [1].

2.1.4 Proteins

They are the most important components in plasma of their significance in coagulation, fibrinolysis, immune response, and carrying cholesterol such as lipoproteins. Same of them are albumin, fibrinogen, immunoglobin and other in small percentage like cytokines where act as cellular communicator or cellular signaling molecules between cells by binding with the available receptors [14].

2.2 Clinical significance

Plasma proteins play a large role in various biological physiological functions throughout the human body. Each protein has a significant purpose and exists in various concentrations. Albumin or serum albumin is a multifunctional protein that changes based on its location [15]. It is a circular protein that contains 610 amino acids and is produced by the liver [16].

It constitutes roughly 50-70% of total plasma proteins because it maintains the oncotic pressure and water cellular concentration as well as it is a transporter for thyroid hormones, fatty acids, unconjugated bilirubin and drugs such as warfarin and penicillin [17]. Clinically, lower albumin concentration is a main indicator of liver diseases, glomerulonephritis, and malnutrition [14].

Secondly, fibrinogen is an essential precursor in the biological coagulating process known as clotting factor I which is converted into fibrin protein [18]. Fibrin coordinates with platelets and other coagulation factors such as prothrombin factor II and Von Willebrand factor VIII to form clotting in order to stop unwanted bleeding at the location of the wound and to prevent any microbe invaders to access the body [19].

Clinically, fibrinogen is considered as biomarker of heart diseases and its lower concentration is an indicator of sophisticated bleeding tendency [14].

Last clinically significant component is globulin. Globulins are insoluble globular group of proteins that are categorized into α-globulins, β-globulins, and γ-globulin fractions and they are respectively in order from small to the largest by separation method known as electrophoresis [20]. Each fraction has its distinct function [14].

Alfa-globulins mostly associate with high-density lipoproteins (HDL) which are responsible for carrying fats to the cells to generate energy metabolism [21]. Beta-globulins are known for the low-density lipoproteins (LDL) that transport fats cellular membrane synthesis and for steroids such as cholesterol.

However, Gamma-globulins are known as antibodies and play a big part in the immune response that are called immunoglobulins. They are discussed further in the immunohematology chapter. Clinically, globulins are biomarker for acute inflammation and infection [22].

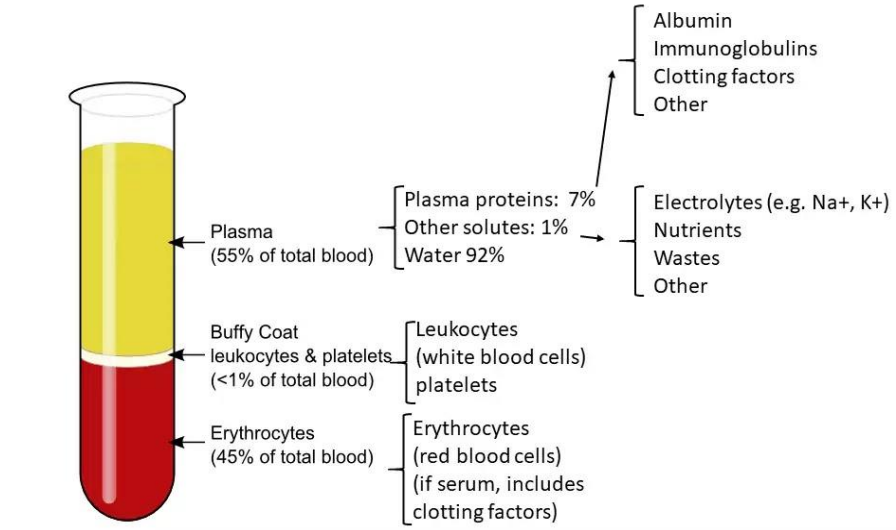

Figure 2: Whole Blood Components *[23]*

3 Immunohematology

In decades, hematology got the attention of clinicians, scientists, researchers and hematologists who are majoring in studying the blood cells and their components in order to find therapeutic approach for hematological diseases or RBCs morphological abnormalities [24]. Whereas, immunology studies the body cells reaction to non-self. This has played a character during transfusion and it introduces the immunohematology as intertwining division of hematology and immunity [25] because it studies the deeper reaction of antigen-antibodies complex reaction in order to avoid the horror autotoxicus (autotoxicity) [26] during commencing the immune response that identifies self from non-self-cells. It is significantly in blood transfusion especially within crossmatching RBCs unites antibodies against the ABO antigen to avoid any post-transfusion acute hemolysis [27].

In addition, immunohematology is an important division that brings the five major disciplines into the medical laboratory including hematology, chemistry, blood banking, immunology, and microbiology [24]. Another critical clinical importance of immunohematology during transfusion and pregnancy is running the test of Rhesus (Rh) factor [28] in relation of ABO group system which plays a greater role in donor-receiver compatibility. This emphasis the immunohematology significance in developing the efficiency and safety of transfusion in order to reduce the risks of immediate or delayed immune reactions.

4 Immunity

Human experiences so many biological processes inside the body that contribute to growth, health, medicinal, metabolism and inner defense. As immunology studies how the body reacts against non-self-cells or in some cases against self-cells that is called autoimmune responses [29]. Basically, immunology studies immunity, immune response between antigen-antibody and its pathological diseases as immunodeficiency, immunosuppression or inflammatory which explains the basic principle of immune response in the immunity system.

The immune system can be identified as a dynamic web of interacting cells and molecules in order to fortress our own bodies from danger against foreigner invaders known as pathogens [26] because the immune system is a collection of mechanical barriers, tissues, cell, organs mainly including spleen, thymus, lymph nodes, and tonsils [30].

Once immune system acts as defense system, it divides into two complete pathways of lines; innate (primarily, nonspecific) immune response and adaptive (secondary, acquired, specific) immune response [31].

4.1 Innate immune response

Innate response is also called non-specific or immediate response because it is the first defense line that is triggered by foreigner invaders (pathogens) such as viruses, bacteria or fungi before stimulating the adaptive immune response [30]. It is composed of principal components that engage in the process of immediate response including mechanical, chemical barrier, cellular and humoral immunity.

Mechanical barrier includes skin of its rough-penetrating epidermis for pathogens unless there is a cut through which becomes an access into the body and the mucous membrane is mostly found lining the respiratory, gastrointestinal, urinary, and reproductive tracts [32]. These membranes are sticky medium for trapping pathogens as mechanical barrier. Whereas, chemical barriers are such as excreting acidic film in form of sweat, tears, gastric acid, and digestive enzymes that digest proteins and eliminate any pathogens that enter the body through the gastrointestinal tract [33]. Humoral innate response starts with immediate recognition of the pathogen by the interaction between complement system and contact cascade [34].

The complement system is a series complex of soluble and cell-linked proteins interacting to enhance the host defense mechanism to kill the pathogens. It contributes to the opsonization and lysis of foreigner cell, thus, it is divided into three categories; classic, alternative and lectin pathway [35].

It plays a role in initiating the inflammatory reactions so the cellular innate response takes place to prevent pathogens from spreading around such as macrophages, neutrophils, basophile, mast cells, dendric cells, and natural killer (NK) cells [36] communicating by intermediate signaling cells such as cytokines and interferons.

Innate immune response can mainly develop inflammatory reactions since it does not have a memory mechanism [37] and it lasts for a short time, so it gives the second defense line the significance to take part as adaptive immune response which is more specific.

4.2 Adaptive immune response

Adaptive immunity is known as specific immunity because it recognizes foreign pathogen as antigen and neutralizes it by producing specific antibody which is remembered by the cells so the immune response can be faster and efficient next time, it invades the host cell again. As innate immune response plays a significant role in recognizing these antigens by the antigen-presenting cell (APC) such as dendric cells, macrophages and Toll-like receptors [38]. They have the functionality of recognizing these antigens and induce the adaptive immune response.

The adaptive immune is mediated by the lymphocytes that are characterized by humoral immunity engaging B-lymphocytes (B-cells) in developing the antibodies and by cellular immunity mediating T-lymphocytes (T-cells) [29]. T-cells are key components in cell-mediated response which are derived from hematopoietic stem cells in the bone marrow and matured in thymus [39].

They are expressed with receptors in their surface known as T-cell receptor which binds with APC in order to form the immune response meanwhile these APCs express series or group of protein on the surface known as the major histocompatibility complex (MHC). MHC are classified into class I, class II, and class III molecule and they are called in human as Human Leukocytes Antigen (HLA) [40].

HLA are important mediated components of adaptive response especially in cancer, transplantation, and autoimmunity. T-cells types are helper, cytotoxic and suppressor T lymphocytes [41]. Helper T lymphocytes (T_H) coordinate in activating cytotoxic cells which kill the infected cells and induce signaling secreting antibodies by B-cells.

Whereas B-cells are derived from bone marrow in adults or liver in fetuses but matured in spleen and lymph nodes [42]. B-cells are the main manufacturer of antibodies known as immunoglobulin (Ig) and producing the memory of these antibodies due to the cloning ability of B-cells recognizing a wider diversity of antigens [43]. B-cells produce 5 types of gamma-immunoglobulins that flow in the plasma including IgA, IgD, IgE, IgG and IgM. Each Ig class has a specific function in the immune system.

In figure 3, there is an illustration on how these antibodies are detecting in relation of the infection and recognizing the immune response phases as IgM represents the innate immune response and IgG reflects the adaptive immune response especially inducing the memory mechanism that gives a faster immediate response in the reinfection period with the same antigen.

4.3 Antibodies (Ab)

Abs are defined as secreted immunoglobulin molecules produced by B-cells in the plasma in order to neutralize antigens and activate complement or bind to Fc receptors [45]. Structurally, they are represented by a Y-shape composition that are made of polypeptides two heavy chains and two light chains [46]. So, each tip of the Y-shape has paratope, which can be referred as a lock, that is specific to bind with the surface of an antigen into epitope representing a key.

Thus, binding antibody-antigen makes an immune complex that describes the idea of a lock and key which has significant usage in life science and medical sciences to understand its functionality better [47].

Antibodies can be divided into specific antibodies, monoclonal antibodies (mAb), and polyclonal antibodies (pAb) [48]. Each classification has important role in immunity, diagnostic methods and treatments.

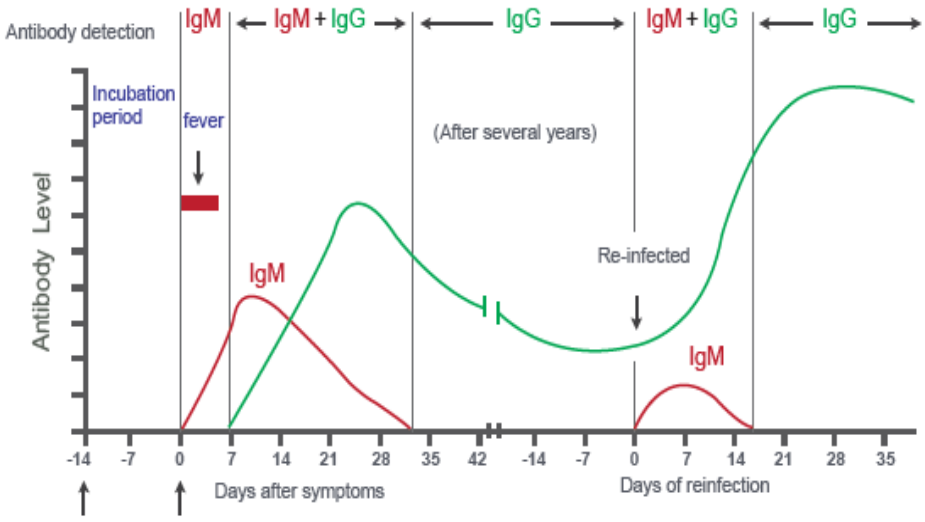

Figure 3: Anti-COVID antibodies Detection Graph *[44]*

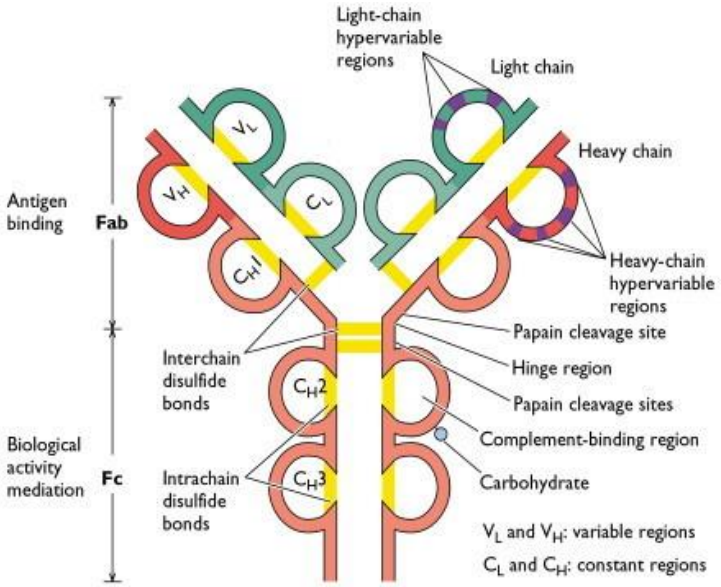

Figure 4: Antibody Structure *[49]*

Specific antibodies are the produced ones by the body itself in defense to the foreigner molecule since their specificity is higher and concise because they bind to one specific antigen. For example, Ebola antibodies cannot neutralize or bind to influenzas A (H1N1) antigens. They exist in form of the main five isotypes IgA, IgD, IgE, IgG, IgM [50].

IgA is higher than IgG especially at mucosal surfaces and in secretions including the saliva and breast milk during breastfeeding. IgD function is not fully known but it is associated with sensitivity of the molecule [45]. IgE exists in low concentration with the shortest half-life and it is associated to hypersensitivity and allergic reactions. IgG is isotype with a long lifespan, and it has subclasses such as IgG1, IgG2, IgG3, and IgG4 [51]. It plays a role in neutralizing toxins and viruses. IgM is a monomeric primarily associated with the first line immune response with functionality of coating the antigen for destruction and fixing the complement and it increases in the early stage of infection [52]. In general, adaptive immune response is important and plays the significant role in keeping our body safe from any foreigner molecules.

Generally, mAb and PoAb differentiate in term of production, structure and usage. MAb are produced by identical B-cells that are clones from a single parent cells for the purpose of passing on the genetic information. They have a monovalent affinity which means their paratope recognizes the same epitope of an antigen and they are produced by hybridoma technique [53]. Hybridoma technique is applied of five steps in order to produce a high number of MoAb by the fusion between antibody producing B cells (injecting an animal with an antigen to produce antibodies spleen cells of that animal) and an immortal myeloma cell (producing a hybridomas) because hybridomas has the ability to continuous growth which get screened for obtaining the desired MoAb [54].

PAbs are mixture of heterogenous antibodies that are derived from distinct B-cells which have the recognition ability of binding to many different epitopes of a single antigen [55]. PAb is known as antiserum of its production nature that is produced by injecting an antigen into a lab animal which is usually rabbit in order to elicit the primary immune response and this process is repeated few times to produce higher titers of antibodies [56].

Once the animal gets the immunization process done, these pAb are obtained straight from the animal serum. PAbs are considered as IgG subclass [57]. As result, antibodies are very significant part of using convalescent plasma as therapeutic approach.

5 Immune response against virus

For a complete comprehension of the immune response, this part is to discuss the viral infection immune response mechanism as antiviral infection from entry the host until initiating the antibodies and eliminating the pathogen.

Viruses are by their chemical and replicating nature classified into DNA such as herpesviruses, smallpox viruses, adenoviruses, and papillomaviruses or RNA such as Influenza, SARS, MERS, Dengue virus, Hepatitis C, Hepatitis E, and Ebola virus disease with single stranded (ss) or double stranded (ds) [58], linear or circular and this leads to differentiate their replication strategies after penetrating the host [59].

Penetrating host can be varied on virus family and damaging cells but mostly on the mechanical barrier. For example, viruses that access by gastrointestinal tract such as Rotavirus, Hepatitis A virus (HAV), by mucus membrane in the respiratory system spreading in the air and transformed through aerosol or person-to-person contact including Influenza viruses, Rhinoviruses, Coronaviruses and Measles virus [60], by skin that can infect oral and genital mucosa such as Herpes Simplex virus (HSV), or by sexual contact including Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) [61].

Virus enters the host cell and immediately innate immune response is initiated as it prepares the body for the specific adaptive immune response and then finishes with killing the foreigner cell (virus) and producing memory immunity.

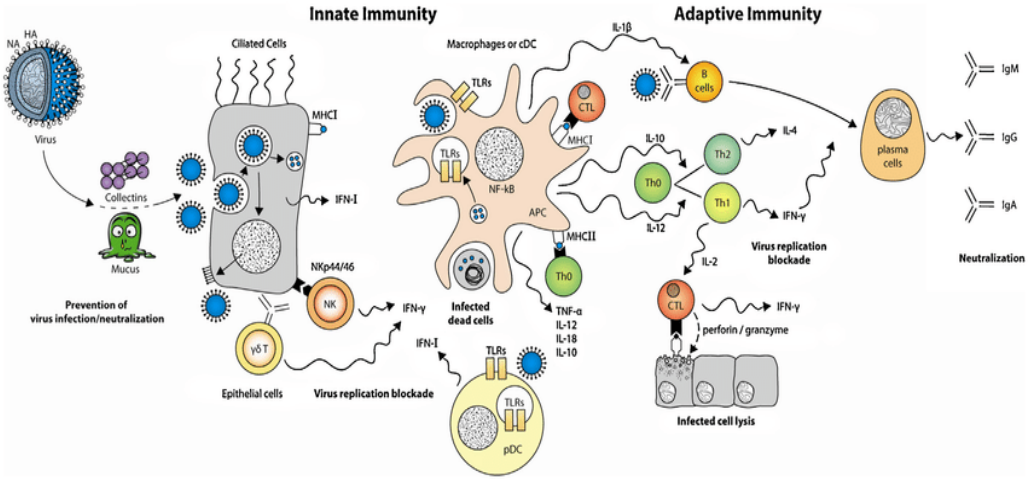

Figure 5: Immune Response Against Virus *[62]*

As shown in figure 5, immune response can be described as below:

- Virus penetrates the host and replicates to start spreading around.
- Innate defense comes into action of blocking or inhibiting the initial infection and it is initiated by pathogen recognition receptors of the Toll-like receptor (TLR) family such as TLR3, TLR7, TLR8 and TLR9 or a family of DExD/H box RNA helicases as first contact with the virus [63].
- TLRs will then promote some sensors that are in charge of expressing interferons (IFN) type I (α/β) along with inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGFβ) and chemokines that are produced by natural killer (NK) cells, dendritic cells (DCs), and macrophages [64]. Inflammatory chemokines can be significant part in innate antiviral defense by managing the process of macrophage, neutrophil, DC, and NK responses at the site of infection.
- Now, the adaptive immune response is generated by T-cells and its different types such as $CD4^+$ T (T_H) cells and $CD8^+$ T (T_c) cells for destructing the foreigner cell directly or extracting tumor necrosis factor (TNF) to destroy the virus-infected cell [65].
- Then, B cells take the role of producing the antibodies into 5 isotypes based on antigen protein which binds with in order to bring the reaction of antibody-antigen into neutralization or opsonization [66].

Overall, once the adaptive immune response ends by neutralizing the virus, antibodies are streamed in the plasma in preparation of another attack by the same virus, so it recognizes the antigen faster with efficiency before causing any viral infection. The most two important antibodies to indicate the process of infection or previous infections are IgM and IgG which their concentration can be detected by virus-specific immunoglobulin detection through different techniques that are to be illustrated further in the practical part.

6 Immunization

Meanwhile immunity is the reaction of the host cell to produce antibodies to the specific antigens that invaded the host cell to become an infected cell. This process of immunity can be called as immunization because immunization can be illustrated as a process by which a person becomes protected against a disease through vaccination that is known as passive immunization [67].

However, vaccination is the process of inducing killed, attenuated, or nonpathogenic forms of the pathogenic agent or its antigens in order to generate the adaptive immune response by T-cells and memory B-cells [68]. In addition, immunization can be categorized into active and passive immunity that can be natural or acquired, wherein, plasma administration is included in passive immunization [69].

6.1.1 Active immunity

Naturally, when a person is exposed to a novel pathogen that causes specific viral, bacterial or Fungal diseases, the host organism triggers to induce an immune response through which results in creating antibodies that bind to the antigens in order to neutralize them [69]. This immunity lasts longer and creates a memory cells that could robust any future re-infection [70]. Otherwise, active immunity can be induced by a vaccination with death or weakened form of the disease agent in order to stimulate the immune response.

6.1.2 Passive immunity

Passive immunity has been used since centuries ago and has been implemented during outbreaks such as neutralizing diphtheria toxin by its anti-toxin that has been discovered from a recovered animal [71]. Therefore, when the host cell or the person who got infected is not capable enough of producing these specific antibodies and the antibodies are transfused into the host cell, it is known artificially that the host cell is passively immunized toward the specific antigen [72]. Also, it was used to treat toxin infections like botulism and tetanus back then when vaccinations were not available. This made passive immunity as first-line therapy for certain severe respiratory diseases [71].

As a result, interspecies have been such as a great laboratory research methodology in approaching a passive immunization advancement because they are used in producing polyclonal antibodies against specific viral infections including mice, birds, pigs or even rabbits [73]. This has led to the usage of immunoglobulin Y which is present in most of mammalian species and have shown a great rapid immune response in human.

During the influenza type A (H5N1 and H1N1), there was a systemic review on extracting IgY from hen egg yolk to be used as vaccination after a rapid dilution with water for most poultry in Vietnam because they have shown great outcomes on infected mice of H1N1 and H5N1 virus strains [74]. This technology has influenced more researcher in using IgY for further vaccinations development and it has been used previously in China, Middle East and Africa to fight the respiratory viral infections [75].

Naturally acquired passive immunity is recognized in the last trimester of pregnancy when antibodies and white cells are crossing the placenta contributing to the fetus development and after getting born by the mother's breast milk which is rich in antibodies [76]. It provides a protection of six months when the infants start developing their own immune system [77].

Some of the passive artificial immunity can be possible in form of such as monoclonal antibody (MoAb) production, hyperimmune globulin (HIG) generation, and convalescent plasma transfusion as potential treatments for a pandemic influenza [78]. Generally, convalescent plasma transfusion has been the suggested technique to fight severe patients of the novel epidemic SARS-CoV-2 that is globally known as COVID-19 [79]. The process of infusing antibodies can be broadly introduced as passive immunotherapy.

7 Antibody therapeutic approach

In general, viruses are known to be serious threatening pathogens to the host cell, and they are capable to infect severely, spread rapidly, replicate mercilessly, and mutate continuously [80]. The viral infection can be a global threat once the viral infection breaks out as pandemic or epidemic affecting the population health and the public health sector that is in charge of securing the safest efficient treatment for these outbreaks to preserve people's life and prolong their life expectancy through providing better innovative medical services or even top-advanced medications [81].

Thus, therapies are varied based on the pathogenic gene of a virus which needs to be neutralized by specific antibody eliminating the specific pathogen such as Influenza A (H1N1 and H5N1), MERS, SARS, Ebola virus, HIV, smallpox and the most recent pathogenic pandemic is SARS-CoV-2 (COVID-19) [82].

Antibody therapeutic means the technique, medication (drug), or vaccination that is responsible of killing the specific virus, reducing its severity or triggering more specific-antibody extraction by B-cells. The U.S. Food and Drug Administration (FDA) is the authority that authorizes the usage of new drugs, vaccinations or recommending therapeutic approach as it happened in May, 2020 when FDA has given the greenlight for remdesivir antiviral drug to be used to treat confirmed COVID-19 in adults and children hospitalized with severe disease [83]. Meanwhile, vaccinations are licensed to specific viral infection given in specific dose based on age and that is managed by the World Health Organization (WHO). Therefore, AUDENZ is the licensed trade name vaccination for Influenza A virus (H5N1) [84].

It applies to the antiviral techniques that concentrate further on the usage of specific antibodies and how that can impact the immune response inside the infected cell. Specific antibodies or immunoglobulin can be obtained by various techniques including human whole blood, human or animal plasma or serum, pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, high-titer human immunoglobulin for intravenous or intramuscular use from immunized or convalescing donors, and monoclonal antibodies [85]. Each technique has its advantages, disadvantages, and protocols on how to be used, collected, implemented or investigated for efficiency and effectiveness so it can be recognized as licensed antiviral treatment option.

However, using convalescent plasma as antiviral therapeutic approach is to be discussed further in details and when it has been used, how effective studies have shown its influence and feasibility in treating the infected patients, its adverse events as risks that could complicate the outcome results of using convalescent plasma and finally the technical part on its collection, separation, routine testing and transfusing protocol.

8 Convalescent plasma

Convalescent plasma (CP) therapy has been known as immunotherapy that can be described as passive immunity since the main principle of convalescent plasma is to infuse intravenously antibodies or immune fractionations existing in the blood plasma of patients who have recovered from specific viral infection into patients who have not yet developed their own antibodies to obtain immunity [86]. However, CP therapy is predominantly a protective mechanism of pathogen neutralization which is reflected into the viral load graph [87].

The viral load expresses the quantity load of a virus in a patient while the antibody titer is the quantitative concentration of antibodies in the plasma that could be higher or lower depending on the therapy protocol and the patient's need for the immunoglobulins as well as the amount of specific antibodies [88]. CP therapy has been widely used since ages and it has seen as first application during the Spanish influenza A (H1N1) pneumonia pandemic in 1918–1920 [89]. Since then, CP therapy has been diversely used in treating severe respiratory infections such as SARS, MERS, influenza A (H1N1), Ebola and nowadays SARS-CoV-2 (COVID-19) as is described respectively in more detail.

8.1 Severe Acute Respiratory Syndrome (SARS)

SARS is a severe viral respiratory infection caused by a coronavirus, called the SARS-CoV-associated coronavirus. SARS was first reported in Asia in February 2003 and the WHO has declared it as outbreak in 2003 and since 2004 there have been no indications of pandemic worldwide [90]. It spreads by direct contact and large droplet aerosolization and is spread globally by travelers [91]. It was diagnosed by symptoms and medical history because there were still serological tests with an incubation period of 1-10 days and started developing a dry cough, shortness of breath and hypoxemia [92].

CP therapeutic approach has shown a promising result among affected health-care worker in reducing the viral load and increasing the anti-SARS-CoV IgM and IgG [93] so this has led into lower mortality and shorter hospitalization based on a study of 80 SARS patients in 2005 who received convalescent plasma at Prince of Wales Hospital, Hong Kong [94].

8.2 Middle East Respiratory Syndrome (MERS)

MERS is a very fetal respiratory virus with a high mortality rate caused by the MERS-CoV coronavirus transferred to humans from infected dromedary camels because it is a zoonotic virus [95]. According to WHO, the first case of MERS was reported in 2012 in Jordan and globally reported in Saudi Arabia later in 2012 and had been an outbreak in 27 countries with 85% of confirmed cases only in Saudi Arabia [96].

Severe MERS is mostly notable in men with previous diseases including diabetes mellitus, cirrhosis and various lung, renal and cardiac conditions so diagnosis progresses into acute respiratory distress syndrome and multiorgan system failure with the suspicion of lower respiratory tract (LRT) involvement [97].

MERS is confirmed by RT-PCR assays of its sensitivity and specificity and another diagnostic laboratory approach is monoclonal antibody based on ELISA [98]. Based on feasible study in Saudi Arabia shows the possibility of using convalescent plasma as immunotherapy for the MERS infection.

The study proves that CP therapeutic approach is feasible with infusing sufficiently high anti-MERS-CoV immunoglobulins titer at early stage of diagnosing patient and collecting CP and testing the donated plasma by ELISA and indirect fluorescent antibody screening [99]. Therefore, there is not yet available vaccine for the MERS, but clinical trials are still running by various institutions [100].

8.3 Influenza A (H1N1)

Influenza virus is classified as a member of the family Orthomyxoviridae and it has 3 types (A, B, C) and each one has different strains and subtypes. Influenza virus has caused a couple of outbreaks since 1918 that resulted in around 500 million cases according to the CDC [101].

Influenza virus type A and B are more relevant to infect humans. In 2009, Influenza A (H1N1) emerged as a novel virus known as influenza A (H1N1) pdm09 virus called as swine flu and CDC reported an estimation of 60.8 million cases and later in 2010, WHO declared an end to the global 2009 H1N1 influenza pandemic [102].

Swine flu is caused by the influenza virus H1N1 mutation that made it easier to transfer from human-to-human with the symptoms of malaise and cough during the incubation period from 1-4 days and in some cases 1-7 days that made the contagious period within first day [103].

There were two developed vaccines which were recommended by the CDC to be distributed and given to above the age of 10 years old. They are known as A 2009 H1N1 "flu shot" and the 2009 H1N1 nasal spray flu vaccine [104].

In terms of using convalescent plasma as a therapeutic method for patients with severe influenza A 2009, it showed a reduction in respiratory tract viral load, serum cytokine response, and mortality based on a cohort study of 93 patients [105].

8.4 Ebola

Ebola virus is a fetal virus that was first identified in 1976 causing the first outbreak in Zaire known now as the Democratic Republic of Congo [106] and ending up with the largest Ebola outbreak in 2014 especially in West Africa infecting 7 countries including Guinea, Sierra Leone, and Liberia [107].

In terms of virology, Ebola virus is known as Ebola virus disease that causes Ebola hemorrhagic fever (EHF) and is considered a rare viral infection with high virulence and fatality rate of 50% [108]. It is a member of the Filoviridae family with five subtypes including Zaire (EOBV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV), and Reston (RESTV) [109]. The Zaire Ebola Virus is a zoonotic virus that is transferred by the blood or body fluids of animals to humans such as fruit bats, chimpanzees, gorillas, monkeys or forest antelope. Then, the transmission is done by direct contact from human-to-human [107].

After so many trials and studies, the FDA has approved two vaccines in late 2020 and they are named as Inmazeb and Ebanga. Their vaccine principle is based on the monoclonal antibodies that have shown a great result in reducing infections [110].

As a therapeutic method during the outbreak, the use of convalescent plasma was an option that has shown good outcomes in different studies and randomized or nonrandomized trails such as the Ebola-Tx trail in Conakry, Guinea, the Ebola-CP trail in Sierra Leone and the EVD001 trail in Liberia which all have given insights of safety, acceptability and feasibility of CP as EVD treatment [111].

Therefore, there is a randomized controlled trail that is based on comparing convalescent plasma with the normal plasma in treating the hemorrhagic fever which has resulted in indicating a mortality rate of 1.1% among these who received the CP and 16.55% among who received normal plasma with the same single unit of 500 ml plasma [112].

8.5 COVID-19

The COVID-19 pandemic is the recent infection in the world that has paralyzed the life and movement as a result of its rapid transmission along with the continuous mutation of new strains. COVID-19 was identified as a new virus in China later in 2019 as new beta-coronavirus, named the 2019 novel coronavirus (2019-nCoV), and at the beginning of 2020, WHO has declared the COVID-19 infection, which is characterized by acute pneumonia as severe acute respiratory syndrome (SARS-CoV-2) [113], is a global outbreak infecting an estimation of more than 120 million as of March 30,2021 [114].

The SARS-CoV-2 virus is a zoonotic because it was transferred from animals such as bats to humans and then transmission starts to airborne transmission from human-to-human by direct contact and droplet [115] with clinical manifestation including pyrexia, cough , fatigue/tiredness , sputum production, dyspnea , sore throat, and headache [116] during the incubation period of 9.7 -14.2 days which varies from person to person regarding their manifested symptoms and medical history based on systemic review and meta-analysis [117].

Since the SARS-CoV-2 virus is evolving every day, the Mayo Clinic is an open-access platform of trials, research, or even studies on the severity of COVID-19 and related impacting aspects. CDC has investigated and recommended a supportive care plan to treat the severity of COVID-19 with its fatality rate. It is divided into mild, critical or severe which indicates respiratory failure, septic shock or multiple organ dysfunction causing the death [118].

The COVID-19 therapeutic approach got very diverse worldwide in terms of the virus pathogenicity that has shown different results in randomized and non-randomized trials, in which have contributed to manufacturing the available used vaccines that have gone through the 1-3 phase clinical trials to be approved by the FDA, CDC, and ICP in order to validate its efficacy and adverse reactivity because the high-risk individual population is above the age of 60 [119].

These vaccines are strategized on diverse immunological and virologic approaches that could neutralize the SARS-CoV-2 antigen, that is identified as having Spike (S) protein on the surface [120]. Thus, two most used vaccines for COVID-19 are commercially known as Pfizer BioNTech and Moderna which are used in 61 and 27 countries respectively [121]. As it is shown in figure 6, an illustration of workflow for using convalescent plasma therapy as prophylaxis method in midst COVID-19 outbreak.

CP approach in term of prophylaxis COVID-19 has been reported to be an effective way in preliminary studies [123] and couple of randomized clinical trials has recommended that as well to be at earlies in order save lives [124]. Therefore, it has been part of so many clinical studies worldwide to observe and study the virus development and its viral load in relation to neutralizing specific antibodies IgM and IgG.

Based on a study at Bengbu Medical College Hospital, Republic of China, it has shown a long term presence of IgG antibody up to six months in the COVID-19 patients from 92.3% and it remained high while a decrease in IgM antibody from 90.4% to 22.73% [125].

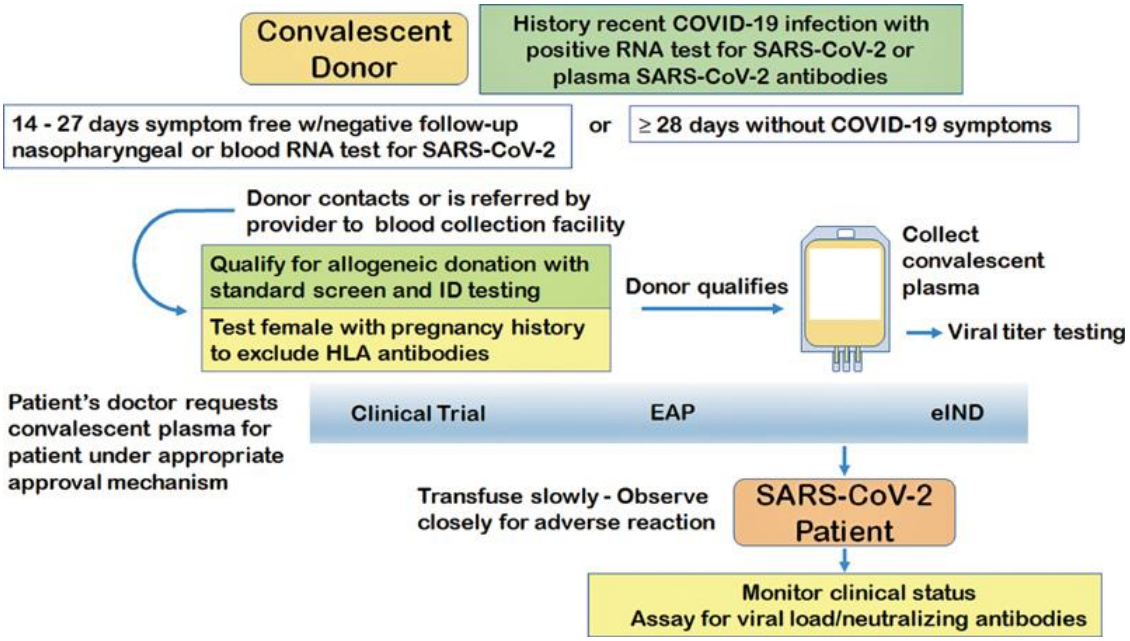

Figure 6: CP Workflow for COVID-19 *(122)*

As a result, a good potential outcome for implementing CP therapy is better when a patient has hyperimmunoglobulin, which is a high titer of virus-specific antibodies [122]. Another case study in China included 19 acute patients with COVID-19 and using the CP approach in their case has resulted in an improvement in oxygenation rate and a reducing inflammatory along with the viral load [126] since antibodies work to modify the inflammatory immune response in the innate stage, the passive antibody approach must be well-administered in term of the specific antibody titer given [127].

9 Convalescent Plasma Transfusion

Plasma transfusion is a crucial process since it performs different procedures, required criteria, clear protocols for donors, recipients and handling the samples, and specific tests including screening tests, antigen and antibody identifications [128].

Convalescent plasma is collected from recovered patients and then infused into recipients, who are in need of plasma components, mainly antibodies against viral diseases in this case. Donors are restricted by specific guidelines provided by the WHO and the European Medicines Agency (EMA).

This requires the donor to provide official identification in order to fill donor questionnaire that gives a deeper glance of the medical history and donation acceptance approval since plasma donation is done voluntarily [129]. Because through this process, it helps in selecting healthy donors and avoiding the spread of any transmissible infections that might put the donor's health in any risk [130]. After that, the donor is undergone a physical examination by general practitioner (GP) to measure blood pressure, temperature, weight and physique [131].

Also, the donor is subjected to blood sampling to identify the blood type and Rh-factor in order to validate any compatibility and cross-matching [132] between the donor and recipient avoiding any risks of posttransfusion since cross-matching identifies the antigens presence on the blood cells surface [133].

Because these preanalytical procedures play a great role in identifying the acceptability criteria for most donation and transfusion parameters [134]. Another preanalytical procedure is screening test for viral transmissible diseases including Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immune deficiency virus (HIV) and Syphilis [135].

9.1 Plasma Collection and Separation

Blood donation is generally a critical component of transfusion and manufacturing blood into its components that could be used sufficiently based on its need, requirement and urgency. Donated blood is collected on various volume and techniques based on what is needed to transfuse the recipient with because it is not limited to RBCs, PL, frozen plasma, or cryoprecipitate but also it could be manufactured into granulocytes, cryoprecipitate depleted plasma [136].

As a result, CP can be used as prophylaxis or treatment providing passive immunity by transfusing plasma which further manufactured to monoclonal or polyclonal hyperimmune immunoglobulin against specific pathogen [137].

Plasma can be collected by two clinical methods either from whole blood and then it is fractionated by sedimentation or centrifugation [138]. Second method is by plasmapheresis technique that needs to be available at the plasma collection laboratory and it can be principally performed by centrifugation or filtration under professional observation [139].

9.1.1 Whole Blood

Once the donor is set ready for the donation, phlebotomist starts sterilizing the drawing site and then does a venipuncture through which blood is drown from a vein into a collection plastic bag known as polyvinyl chloride (PVC) blood bags [140]. PVC bags come in various volumes, sizes and plasticizers which coordinate in flexibility, resistance and safety of collected or fractionated blood components such as di-ethylhexyl phthalate (DEHP) [141].

Also, PVC bags include anticoagulants to prevent any clotting or blood coagulation in the PVC bag and the most common used one is citrate phosphate dextrose adenine (CPDA) because citrate is an anticoagulant to chelate calcium, dextrose takes part in RBC metabolism, phosphate assists in acidity and pH and adenine is to improve the viability of RBCs [142].

Normally, whole blood is drawn into one-unit bag that volumes 350-450 ml capacity and the drawn amount is varied based on the age and weight of the donor. PVC bags can be available in four types. So, regarding PVC blood bags usage, they can be as single bag for whole blood collection, double bag for RBCs and plasma, triple bag for RBCs, plasma and platelets, and quadruple bag for RBCs, plasma, platelets, and cryoprecipitate [143]. The collect whole blood unit is stored at 22°C up to 24 hours for plasma separation by centrifugation [144]. The whole donation and collection process take up to 40-60 minutes.

Whole blood units are centrifugated in order to fractionate RBCs and plasma. Centrifugation principle is to use the centrifugal force separating and purifying the mixture of whole blood into plasma, platelets and red blood cells according to the particles size, density, viscosity of medium and rotor speed [145]. Then plasma is collected into sterilized VPC plasma bag of chosen volume and anticoagulant for further usage or fractionating plasma component. In term of this thesis, it is further manufactured for extracting antibodies for passive immunity.

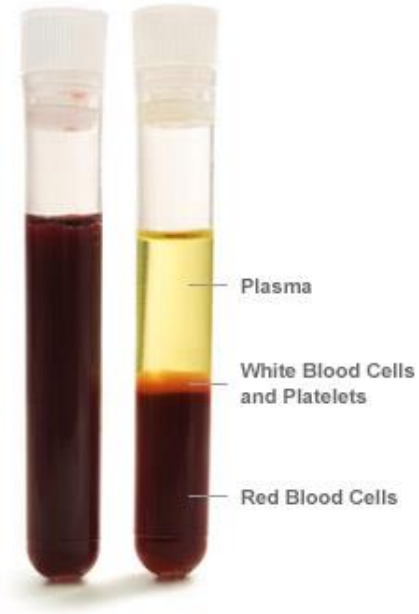

Figure 7: Plasma from Whole Blood after Centrifugation *[146]*

Figure 8: Plasmapheresis Separation *[147]*

9.1.2 Apheresis

Another technique for separating plasma is using apheresis that is known as plasmapheresis. Apheresis is the process of removing the blood, filtering, retaining its element, and then returning the blood to the body again [148] by using apheresis machine that replaces the drawn plasma with substitute solution like saline or albumin [149].

Basically, plasmapheresis selectively separates plasma from blood and returns the rest into the donor's body again using two separation techniques such as size-based separation using filtration or density-based separation using centrifugation principle [150]. The collected volume is done and monitored by a professional to ensure the process is going well and it can take up to 3-4 hours [151].

10 Laboratory Antibody Detection

Part of the diagnostic protocol for infectious diseases is to identify a specific antibody in plasma or serum to qualitatively analyze its presence or absence in the form of detectable / non-detectable or quantitatively measure the concentration in the sample that is given in the form of titer which is a ratio 1 to the dilution rate such as 1:60 or 1:360 [152]. Antibody identification testing is very significant in facilitating in determination the immune status. Identification methods vary depending on the antibody specificity in capturing a specific antigen agent in the testing technique.

These tests come mostly in various forms of diagnostic immunoassay including enzyme immunoassay (EIA) or known as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), agglutination reaction, and western blots (WB) [153]. Tests can be done directly and indirectly depending on for which specific antibody is being identified. As serological approach, these tests mostly identify the immune related immunoglobulins such as IgM and IgG which both indicate the immune system status against specific viral infections because positive IgM gives insight of initiating immune response meanwhile IgG gives indications of existing previous infections or late stage of infection [154].

The most common used antibody identification test is ELISA of its high sensitivity and specificity with implementing it directly, indirectly or sandwich ELISA [155]. Fundamentally, it is a technique that targets antibody or antigen capture in the given sample using specific antigen or antibody in order to indicate the quantitative titer of specific antibody in the sample because it has been proved in more than a trail demonstrating effectiveness and efficiency of passive immunization depending on antibody titer level [156].

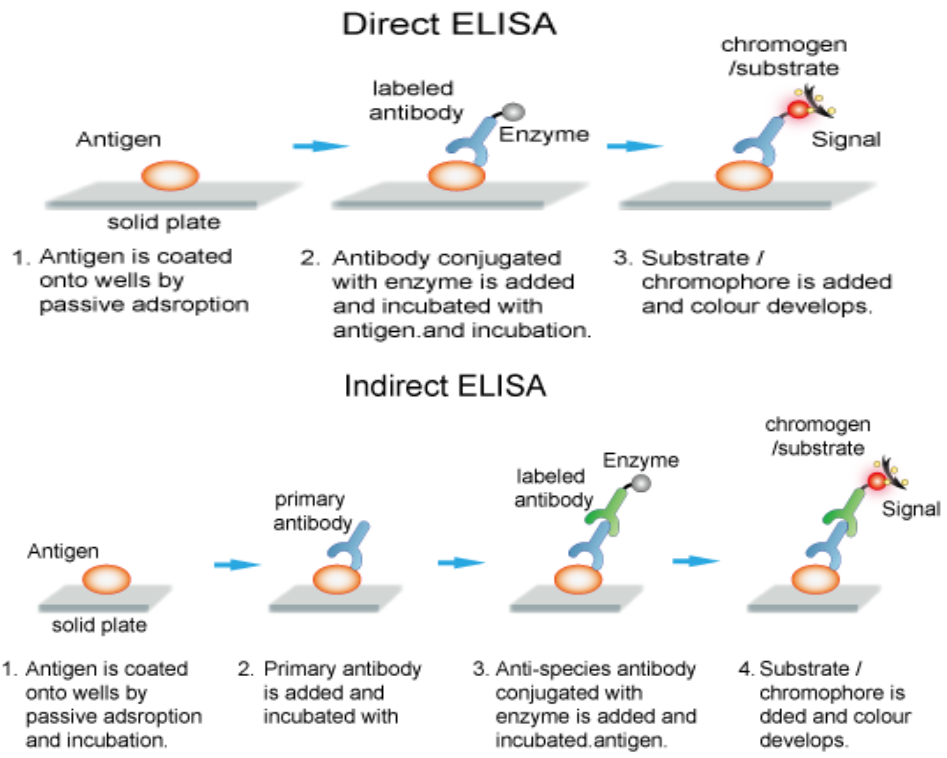

Figure 9: Principle of Direct and Indirect ELISA Assay *[157]*

Another confirmatory test is the neutralization antibody (NAb) assay which can be available as a cell-based, ligand binding and enzyme activity assay, but it requires an access to the biosafety level 3 (BSL3) laboratory [158]. Specifically, it is based on measuring the antigenicity and immunogenicity of neutralizing epitopes on a naive or recombinant antigen [159].

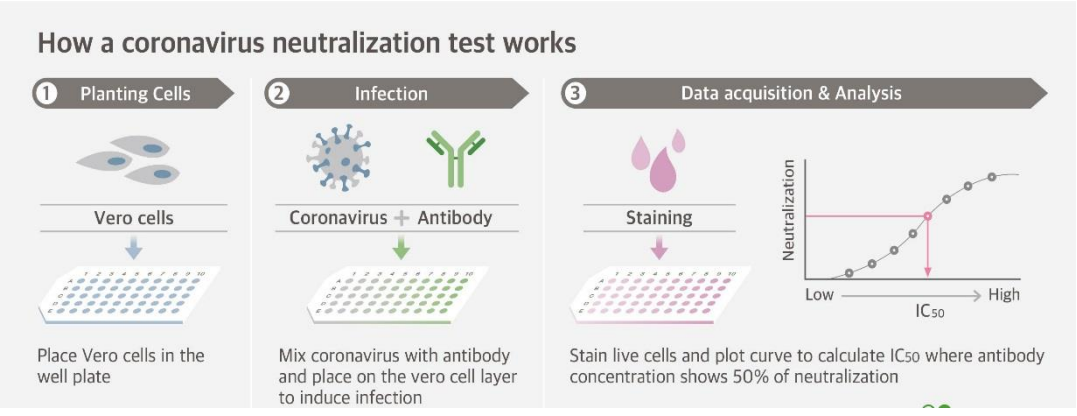

Figure 10: Cell-based Neutralizing Antibody Test *[160]*

10.1 SARS

Once the SARS-CoV epidemic broke out, laboratories and researchers all over the world began investigating, discovering and developing methods to identify the presence of antibody in plasma, which is produced against the pathogen by adaptive immune response. The main two antibody detection tests are the ELISA and NAb assay as a confirmatory test as this approach was recommended by various clinical studies and trails because they have shown effective outcomes determining the antibody titer in convalescent plasma [161]. SARS antibody is possibly responsive to be detected using ELISA technique against the receptor-binding domain (RBD) of SARS-CoV spike (S) protein [162] as a naive antigen. The RBD of SARS-CoV S protein is considered a highly immunogenic in humans that also has been used as mediate protective response to develop a vaccine and recommended for further clinical diagnostic investigation [163].

The RBD-based ELISA method can use either naive antigen associated with S protein or other recombinant proteins associated with S, E, M and N protein. Therefore, a study has shown antibodies are more detectable against anti-S and anti-N antibodies which made it a good proposed candidate as a subunit vaccine antigen [164] RBD linked to IgG1 has induced a higher titer of RBD-specific antibody in convalescent plasma [165] as result testing that on mice generated an effectively mouse antisera neutralizing infection by SARS-CoV of 1:15 and 1:360 neutralization titer [166]. One limitation of using ELISA with recombinant proteins is the need for biosafety (BSL) level 3 facility because it is needed to ensure the proper antigen inactivation process [167].

The advantage of using ELISA with recombinant protein is more reliable, specific and sensitive detecting IgM and IgG in the CP in contrast to using the immunofluorescence (IFA) technique [168]. The neutralization assay has proved its effectiveness in detecting antibodies with a high titer of 1:80 [169]. Today, researchers are still developing better fast and efficient methods against SARS-CoV.

10.2 MERS

Since MERS-CoV is one of beta-coronaviruses, it shares similar antibody detection methods with SARS-CoV. However, it was recommended by WHO to use a recombinant spike protein along to NAb assay as confirmatory test.

Therefore, sera were tested for identifying IgG antibodies against MERS-CoV using S1 antigen where RBD has shown a high specificity and NAb test using Erasmus MC isolate and bovine coronavirus against MERS-CoV detected a plaque reduction of 90 % [170].

In addition, a case study in the Middle East investigated same technique finding IgG titers peaked in 3rd week after the symptoms onset and IgM level constantly remained elevating [171].

Another serological analysis of sera collected from 17 patients used ELISA with recombinant S1 protein strained from EMC of its sufficient specificity in order to detect the IgG antibody [172]. This approach has contributed to understand the kinetic immune response of MERS-CoV antibody. S1-based ELISA comes in higher specificity and reliability according to qualified analysis in China against other antibody identification methods [173].

However, IFA and WB were used as confirmatory recombinant assays using Vero B4 cells and S protein with nucleocapsid (N) protein respectively [174]. Antibody identification against MERS-CoV has been varied depending on used technique and tested serum if it is a human serum or dromedary serum.

10.3 H1N1

The influenza virus has been around since centuries inducing outbreaks from time to time geographically varied. Thus, identifying its antibody depends on the type of virus in order to find an effective approach. Influenza type A (H1N1) commonly known as swine flu has caused a recent epidemic in 2009; different antibody detection approaches introduced but most common used one in hemagglutination inhibition (HI) assay besides to the ELISA, IFA and NAb assay [175]. Principally, HI assay is a specific antibody test based on the reaction of viral H1N1 hemagglutinins with the RBCs resulting in agglutinated lattice that mostly settles in testing tube or microtiter [176]. It means if the sample contains antibodies, an agglutination will not occur, and this is called hemagglutination inhibition [177].

HI test is relatively simple and inexpensive which made it widely used technique measuring the specific antibody against H1N1 virus type. HI test has been used in a study that revealed a great performance in distinguishing the antibody titer in acute and convalescent sera without showing cross-reactivity between the antibody against H1N1 [178].

Whereas in one of the trails on pigs, ELISA has shown low sensitivity to detect monoclonal antibody and it needed to use recombinant antigen to identify higher antibody titer [179]. Also, this technique has shown different results in various studies which made not well-recommended approach.

However, there is a great study that evaluated the comparison between using a commercial ELISA kit and HI test. The analysis was carried out on 1,086 collected sera, resulting in that HI test was a good detection test in early stage of infection meanwhile the ELISA was not, but it was a great subtype detection technique and HI was effective in detecting antibodies against naive swine flu virus [180]. However, producing vaccination against swine flu has benefited from HI assay testing approach.

10.4 Ebola

Ebola virus disease (EVD) is associated geographically to Africa since most of the outbreaks emerged there and its types are named after their located emergence. EVD is very fetal contiguous infection and has a very high mortality rate.

As result, diagnostic methods are continuously developed antibody detection approach. Highly specifically monoclonal antibody against EVD are glycoprotein (GP) and secreted glycoprotein (sGP) [181]. They have proved their effective induction of immune response against EVD using ELISA and NAb assay generating specific antibody which plays a big role in diagnostic Ebola [182].

Whereas specific monoclonal IgM and IgG antibody ELISA-based test have demonstrated a great level of specificity and sensitivity capturing IgM by Zaire ebolavirus antigen that has grown in Vero E6 cells within first 6 days of infection, while using detergent-extracted viral antigen in order to capture IgG which may last longer [183]. Basically, ELISA can be prepared using recombinant automatic antigen [184] in serological detection of specific-virus IgM and IgG faster and precious in contrary to what LFIs greater advantage is to identify antibody qualitatively during the recent outbreak [185].

A futuristic review has evaluated the detection of antibody using immunochromatographic strip and a smartphone reader which facilitates in semi-quantifying EVD convalescent specific antibodies.

Principally, it is monoplex glycoprotein based that has performed sensitivity of 100% and specificity of 100 % in comparison to standard whole antigen ELISA meanwhile it got validated to other fresh collected serum samples in other locations [186]. This study has introduced a fast, validated, digital friendly and effective testing technique in form of lateral immunochromatographic flow assay in order to reach out to as many patients as possible since most of EVD cases are found in rural areas.

Antibody detection methods are always on continuous development to meet up-to-date epidemiological data and contribute in quick epidemiological response to save the public's health and as well to prevent such fetal viral infections.

10.5 COVID-19

Currently, SARS-CoV-2 is considered the center of interest for so many preliminary studies worldwide, as the virus continues to mutate, new strains are emerging. Therefore, the use of convalescent plasma from recovered patients with SARS-CoV-2 has been associated with neutralizing antibody concentration [187].

As a result, serological testing methods have been developed and proposed using the SARS-CoV-2 S protein particularly trimeric spike protein or the N protein to measure the titer of the IgG, IgM or its qualitative presence along with a sensitivity of 91.6% and a specificity of 99.1% in the form of lateral flow assay test [188].

According to a good study case of evaluating CP efficacy using a commercially known NAb assay such as Ortho VITROS Anti-SARS-CoV-2 Total (CoV2T) based on signal-to-cutoff ratios (S/C) criterion since it was recommended by the FDA under emergency use authorization [189]. The VITROS CoV2T test is an automated antigen immunoassay that qualitatively detects total IgG and IgM antibody against SARS-CoV-2 in the form of reactive or nonreactive using recombinant antigen [190].

VITRO CoV2T has prioritized its very high specificity 99.7% and sensitivity 90.5 % due to the cut off total [191]. Another quantitative antibody detection test is the SARS-CoV-2 reporter viral particle neutralization (RVPN) assay.

The RVPN assay measures the direct neutralizing antibody against the pseudo-typed Ontoa rhabdovirus reporter virus [189]. However, as long as the COVID epidemic continues, the SARS-CoV-2 is a very demanding potential interest to be further studied, investigated, validated, and understanding it much better along with its correlations to other factors.

11 Feasibility and Efficacy of Convalescent Plasma

Passive immunization is not a total new therapeutic approach confronting infectious or bacterial diseases, but it is dated back to 1890s when the immunologist Emil Von Behring developed a cure against diphtheria and tetanus using antibodies that had been isolated from horse blood [192]. Later in 1901, Von Behring received the Nobel Prize and since then this therapeutic approach has been successfully feasible in treating most of world outbreaks. Immunology has explained the mechanism of these antibodies which facilitate in blocking viral surface receptors that leads into blocking the cell entry, promoting cell-mediated cytotoxicity by natural killer cells which destruct the infected cell and neutralizing the virus by activating complement cascade of reacting antigen-antibody [193,194].

Normally, these antibodies can be managed by human or animal plasma or serum and then it is pooled into recipient intravenously or intramuscularly as high titer from immunized or convalescent donor [195].

However, there have been couple licensed monoclonal and polyclonal antibodies depending on their usage either against bacterial or infectious diseases [196]. In this chapter, effectiveness of passive immunization is to be discussed regarding usage against SARS, MERS, Influenza H1N1, Ebola and COVID-19.

11.1 SARS

Severe Acute Respiratory Syndrome has a higher fatality rate ranging between 13.2% in patient younger than 60 and 43.3 % in patients aged 60 and above based on a Hong Kong study case of 1425 cases [197]. Here comes the efficacy of using CP in reducing the severity and mortality rate.

Therefore, using CP in infected healthcare workers (HCW) in a Taiwan hospital indicated a decrease in viral load from 495 × 103, 76 × 103 or 650 × 103 copies/ml almost to zero within one day of transfusing the convalescent plasma that was collected in 500 ml from three recovered patients and then infused in eight HCWs respectively varied with antibody titer ranging 160 to >640 [198].

This approach has resulted in recovery of these patients and one case got pregnant passing the anti-SARS-CoV antibody to the newborn as natural passive immunization because antibodies have been transferred through the placenta

Another meta-analysis of 32 studies has shown the effectiveness of administrating CP, serum or hyperimmune immunoglobulin in early stages resulting in a statistically significant mortality reduction and safety but also, it recommended a further clinical investigation [199].

Additionally, there has been a case of an old lady aged 57 who was diagnosed with positive SARS and she was treated successfully with using CP, ribavirin and corticosteroid as antiviral drugs even though she was in bad medical conditions that she almost suffered from multiorgan failure [200].

A very detailed summary of effectiveness comparison on using convalescent plasma against SARS-CoV and SARS-CoV-2 has been done by department microbiology and immunology at the Weill Cornell medicine presented by professor Per Johan Klasse [201] as it is referred in table 1.

Reference	Virus	Antibody source	Number of patients	Efficacy	Safety
Cheng et al., 2005	SARS-CoV-1	CP 160–640 ml Seropositive titer range: 160–2,560	80 patients with SARS	Better outcome with plasma before than after day 14	No immediate adverse effects
Yeh et al., 2005	SARS-CoV-1	CP 500 ml IF IgG titer >640	3 hospital workers with SARS	Drop within 24 hr in viral load from ~ 10^5 to < 1 RNA copies/ml	No significant side effects
Soo et al., 2004	SARS-CoV-1	CP Ab titers not measured	19 (plasma) vs. 21 (methylprednisolone) SARS patients	Faster release, lower mortality with plasma than comparator	No immediate adverse effects
Shen et al., 2020	SARS-CoV-2	CP 400 ml Ab binding >1000 NAb > 40	5 COVID-19 patients	Reduced viral load, clinical improvement Release of 3/5	None reported
Duan et al., 2020	SARS-CoV-2	CP 200 ml NAb > 640	10 COVID-19 patients	Virus undetectable in 7/10 Varying clinical, laboratory, radiological improvements	No adverse effects observed
Zhang et al., 2020	SARS-CoV-2	CP 200–2,400 ml Ab not measured	4 COVID-19 patients	Negative PCR Pulmonological improvements Discharge of 3/4	No adverse effects observed
Ahn et al., 2020	SARS-CoV-2	CP 2 × 250 ml Binding IgG detected by ELISA	2 COVID-19 patients	Reduced sputum viral load Radiological and clinical improvements	No adverse effects observed

Table 1: Efficacy comparison on using CP against SARS and COVID-19

11.2 MERS

Middle East respiratory syndrome is known for its high mortality rate of 32.7% based on in-depth analysis of the period 2012-2020 [202]. Therefore, a sufficient, effective and feasible therapeutic approach was very much needed during the outbreak in 2012, so passive immunization by using antibody immunotherapy has shown its clinical outcome against the MERS-CoV. It was mostly started with identifying the human monoclonal antibody against the MERS-CoV which had shown a good outcome in neutralizing the virus.

These three antibodies were identified as M366, M337 and M338 mostly targeting the virus receptor CD26 which binds to the MERS-CoV spike glycoprotein [203]. Meanwhile, M366 has proved an average of 50 % neutralization at 0.005 and 0.07 μg/ml.

Another study has demonstrated the feasibility of MERS-immune camel sera in showing clearance and severity reduction of infected lungs additionally to the proportional efficacy to MERS-CoV neutralizing antibody titer [204] because camels have high antibody titer. The low number of recovered patients has limited the human convalescent plasma usage because of its low neutralizing antibody titer and passive immunization requires high neutralizing antibody titer which proves the usage of dromedary camel serum [205].

A good experiment was done by transfusing the dromedary-immune serum into mice and then transferred into a patient containing high neutralizing antibody titer resulting in reducing viral load and lung pathology [204]. MERS outbreak has shown effective passive immunization using animal immune serum containing high neutralizing antibody titer of greater than 1:80 based on a study that was run in Korea evaluating the neutralizing antibodies by enzyme-linked immunosorbent assay (ELISA) capturing IgG and using immunofluorescence assay capturing IgM [206].

11.3 H1N1

Influenza type A (H1N1) or commonly known as swine flu is considered one of the early outbreaks that used passive immunization as therapeutic or prophylaxis approach. Throughout using convalescent plasma in previous influenza types has given deep insights of approaching passive immunization against swine flu. There was a cohort study including severe patients with H1N1 treated with CP of neutralizing antibody titer greater than 1:160 where the outcomes were compared to the ones who didn't receive CP.

The study conducted 93 patients requiring intensive care and mortality rate was decreased to 20 % while 54.8 % in these nontreated patients with CP and a significant lower rate of viral load, cytokine level, interleukin 6 and interleukin 10 within the first 7 days [207].

Another case study has investigated the efficacy of using hyperimmune intravenous immunoglobin with high antibody titers containing hemagglutinin and neuraminidase against the virus in SCID mice as a model. This resulted in revealing the efficacy of hyperimmune immunoglobulin with titer of 1:1,280 providing a complete protection meanwhile with titer 1:70 providing no significant outcomes [208].

Additionally, from a limited case reports and non-randomized studies showed data of RCT in severe H1N1 patients lowering viral load, mortality rate and clinical developments were reported within 5 days of symptoms inception after they were transfused with convalescent hyperimmune immunoglobulins [209]. Fundamentally, effectiveness of passive immunization showed a good potential in Influenza virus with sufficient neutralizing antibody titers [210].

11.4 Ebola

Throughout history Ebola has caused more than an outbreak and there have been different therapeutic approaches including passive immunization using neutralizing antibody that has shown different clinical effectiveness and improvement in patient's health condition. Since there has not been any licensed vaccine available besides to the existing experimental drugs, antibody-based treatment was a good recommendation but firstly it was done in non-human primates as model of EVD including using monoclonal and polyclonal antibody.

One review on passive immunization in non-human primates used purified IgG from convalescent serum of macaques that showed 100% protection with a plague reduction [211] and this gave predictive assumption value for human's trails because this has led to a dramatically lower viral load. However, it was recommended using higher dose of immunoglobulins to be effectives in human. Another nonrandomized trail of 99 patients revealed an average of 20% mortality reduction rate and shorter symptoms onset after infusing patients with 200-250 ml of compatible convalescent plasma [212]. Although no adverse reactions were observed associated with the use of CP.

In contrary, during 2013-2016 West African epidemic, another non-randomized trial on nine rhesus monkeys was conducted in order to experimentally evaluate the effectiveness of using Sudan Ebolavirus convalescent macaque sera resulting insufficiency of CP alone to provide 100% protection against ZEBOB infection [213].

Other promising trail was in Liberia demonstrating the possibility of reducing the viral load after administrating CP to patient through ensuring measurement of antibody titer by ELISA due to its sensitivity and specificity [214]. Recovered patients may show greater effect of lower viral load and clinical within infusing higher antibody titer against EVD.

11.5 COVID-19

SARS-CoV-2 is one of the main human coronaviruses of which all have caused outbreaks throughout history SARS, MARS and now COVID-19 with a higher transmission potential. Since SARS-CoV-2 shares genomic similarities of 79% with SARS-CoV and 50% with MERS-CoV [215], a passive immunotherapy approach was suggested in mid of 2020 as it has proved a great efficacy in treating other infectious diseases.

Therefore, there have been many nonrandomized and randomized trials investigating CP effectiveness. Thus, nonrandomized studies have demonstrated a benefit while randomized studies have not shown much of clinical outcome improvements [216]. One of the main three trails that have shown a great clinical improvement in patient is a trail of 5 patients. They were diagnosed with positive SARS-CoV2 with rapid progression, high viral load, severe pneumonia and they were intubated by mechanical ventilation.

They were transfused with SARS-CoV-2 specific antibody (IgG) of 1:1000 antibody titer and neutralizing antibody greater than 1:40. Within 3 days of CP transfusion, their body temperature normalized, decreased the viral load with 12 days, increased specific antibody and neutralizing antibody titer and eventually 3 patients out of the 5 were removed from the mechanical ventilation [217]. This has demonstrated promising clinical outcomes in severe cases administrating passive immunization.

Another trail was conducted on 10 patients with one dose (200 ml) of CP and its clinical outcomes rapidly were seen shorter than expected within 3 days and radiologically within 7 days [218]. This passive immunization approach has shown promising outcomes once 7 out of the 10 patients showed undetectable viral load. Last randomized trial included 4 patients.

The first patient was treated with arbidol, lopinavir-ritonavir and other supportive therapies but they didn’t show any improvement till the patient was transfused with a total of 900 mL compatible CP in varied doses and after first dose, the viral load decreased significantly from 55×105 copies/ml to 3.9×104 copies/ml after the last dose of 300 ml convalescent plasma. The patient was extubated and discharged within almost a month after screening negative SARS-CoV-2 so similar clinical outcomes were shown in the other patients [219].

All of these trails have shown a great efficacy of using passive immunization through approaching antibodies in convalescent plasma. However, it is needed to be further investigated in order to determine the final efficacy of promising immunotherapy approach using convalescent plasma in the future.

12 Adverse Effects of Convalescent Plasma

Convalescent plasma has shown great therapeutic potential against viral infections, although it carries some risks that can be eliminated, reduced or managed in order to not harm the recipient's life. Due to screening sample advancement, it has considerably reduced the risks of transmitting infections [220] since the screening tests are part of pre-analytical procedure of transfusion. Normally, adverse effects are varied depending on transfusion type, donor-recipient specific characteristics including significantly age and gender.

As a result, hemovigilance system is introduced as definite guideline that keeps control, observation and manual guidance regarding to the preparation, quality and use of blood component particularly intended to blood fractionations or even fresh frozen plasma and its purified fractionations [221]. In general, convalescent plasma has adverse effects similarly to fresh frozen plasma transfusion but CP is mostly associated with to transfusion-related acute lung (TRALI), transfusion-related circulatory overload, allergic or anaphylactic reactions, febrile nonhemolytic transfusion reaction [222].

Allergic reactions are some of common effects during transfusion and they could be mild and severe. Urticaria is a very mild form that is associated with fever, sickness and dizziness because it reacts due to the IgE mediation reaction between the recipient and donor leading to cause itching that may last for hours [223].

Meanwhile anaphylaxis is a rare severe form of allergic reaction associated with IgA deficiencies or pre-existing anti-IgA antibodies in the recipient [224]. CP feasibility has been varied during different outbreaks against infectious diseases and its adverse effects have varied as well depending on the patient's case, health condition and accepting the convalescent plasma in order to bust the immune response [225]. Therefore, adverse effects are still needed to be further investigated and larger scale studies are much needed especially during this time of using more immunotherapy as potential future therapeutic or prophylaxis as a passive immunization.

13 Conclusion

With today's medical and technological advancements, medical science has developed tremendously much better than it used to even in the last three decades. This thesis is part of this advancement in showing the potential interest in passive immunization, antibodies, and convalescent plasma to be a promising therapeutic approach in reducing epidemical health impact of infectious diseases.

Immunology has proved itself in understanding, illustrating and expanding its research inventory in most of the world's laboratories, clinical trials and research-based institutions. So does convalescent plasma in showing more efficiency and feasibility against most of viral infections. In other words, immunotherapy is currently the focus of most studies to deepen more discoveries and development of clinical outcomes to help humanity as well as to save millions of lives.

This literature has met its position and potential goal on how the convalescent plasma therapeutic approach has contributed throughout history and is still contributing to the use of immunoglobulin treatment as a therapeutic approach in confronting the current epidemic of SARS-CoV-2 (COVID-19) within the severe cases as it has done during the SARS, MERS, influenza type A, and Ebola outbreak.

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