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Review on Anemia as an Independent Risk Factor Associated with the Severe Illness of Covid-19.

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ABSTRACT

In the past two decades, the world has faced several infectious disease outbreaks. This virus had a massive global impact in terms of economic disruption, the strain on local and global public health. Most recently, the global outbreak of novel coronavirus 2019 (SARS-CoV-2) that causes COVID-19 is a newly discovered virus from the coronavirus family in Wuhan city, China, known to be a great threat to the public health systems. Anemia on the other side is a common nutritional deficiency disorder and global public health problem which affects both developing and developed countries with major consequences for human health and their social and economic development. Anemia commonly aggravates the severity of respiratory diseases, and several studies have suggested that the prevalence of anemia was associated with poor outcomes and increased mortality in patients with community-acquired pneumococcal pneumonia. This article aims to review the potential risk factor associated between anemia and the severity of COVID-19 infection, and their structural mechanism of action in the cell.

Keywords: COVID-19, Anemia, Outbreaks and Mechanism of Action.

INTRODUCTION

Since the end of December 2019, clusters of cases of unexplained pneumonia linked to seafood market exposure have been reported in Wuhan, China. A novel member of the coronavirus family was identified in samples of bronchoalveolar lavage fluid from patients in Wuhan Jinyintan Hospital, which was named severe acute respiratory syndrome coronavirus-2(SARS-CoV-2)[1].

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds [1]. In humans and birds, they cause respiratory tract infections that can range from mild to lethal. Based on next-generation sequencing data, it has been shown that SARS-CoV-2 is similar to the SARS-CoV and Middle East respiratory syndrome coronavirus, with 79% and 50% sequence identity, respectively [2]. Moreover, laboratory findings, together with the clinical manifestation of 2019 novel coronavirus-infected pneumonia, are analogous to what has been described in cases of SARS. Notably, person-to-person transmission was confirmed among close contacts [2]. Thus far, the outbreak has rapidly spread to over the world, and the number of confirmed cases continues to grow.

Anaemia is a common nutritional deficiency disorder and global public health problem which affects both developing and developed countries with major consequences for human health and their social and economic development [3]. According to WHO reports, one third of the global populations (over 2 billion) are anaemic due to imbalance in their nutritious food intake [3]. Anemia commonly aggravates the severity of respiratory diseases, and several studies have suggested that the prevalence of anemia was associated with poor outcomes and increased mortality in patients with community-acquired pneumococcal pneumonia [3]. In 99 coronavirus disease 2019 (COVID-19) patients transferred to Jinyintan Hospital, 51% of patients showed a decreasing tendency in hemoglobin levels [4]. In a study on 1099 laboratory-confirmed COVID-19 cases, it was shown that severe

patients had significantly lower hemoglobin levels than those diagnosed as nonsevere cases. It should be noted that the decline in hemoglobin was more pronounced in patients who reached to the composite endpoint incorporating admission to the intensive care units (ICUs), or mechanical ventilation, or death, thus indicating that low hemoglobin levels might be related to poor progression and prognosis [5]. Therefore, anemia could possibly be a risk factor for severe disease in COVID-19. WHO estimates that anaemia affects over half of the pregnant women in developing countries [5]. Recent estimates in the developingcountries including Nigeria put the prevalence at 60.0% in pregnancy and about 7.0% of the women are said to be severely anaemic [6]. In this review, we sought to reveal on anemia as an independent risk factor associated with the severe illness of COVID-19, with the aim of contributing to the early recognition of disease severity and to extend the understanding of anemia in COVID-19 patients.

TYPES OF ANEMIA

There are several types and classifications of anaemia. The occurrence of anaemia is due to the various red cell defects such as production defect (aplastic anaemia), maturation defect (megaloblastic anaemia), defects in haemoglobin synthesis (iron deficiency anaemia), genetic defects of haemoglobin maturation (thalassaemia) or due to the synthesis of abnormal haemoglobin (haemoglobinopathies, sickle cell anaemia and thalassaemia) and physical loss of red cells (haemolytic anaemias) [9]. Below are the following types of anemia:

Iron-Deficiency Anaemia

Iron is essential for the various activities of the human body especially in the haemoglobin synthesis. Iron deficiency anaemia is a condition in which the body has too little iron in the bloodstream. This form of anaemia is more common in adolescents and in women before menopause [10]. Blood loss from heavy periods, internal bleeding from the gastrointestinal tract, or donating too much blood can all contribute to this disease. A low level of iron, leading to anaemia, can result from various causes. The causes of iron-deficiency anaemia are pregnancy or childhood growth spurts, Heavy menstrual periods, Poor absorption of iron, Bleeding from the gut (intestines), dietary factors (iron poor or restricted diet), medication (aspirin ibuprofen, naproxen and diclofenac), Lack of certain vitamins (folic acid and vitamin B12), Bleeding from the kidney, Hookworm infection, Red blood cell problems, Bone marrow problems [8].

Pernicious Anaemia

Pernicious anaemia is the most common cause of Vitamin B12 deficiency Vitamin B12 is essential for life. It is needed to make new cells in the body such as the many new red blood cells which are made every day. Vitamin B12 is found in meat, fish, eggs, and milk. A lack of vitamin B12 leads to anaemia and sometimes to other problems [11]. A lack of vitamin B12 (B12 deficiency) is one cause of anaemia. Pernicious anaemia usually develops over the age of 50. Women are more commonly affected than men, and it tends to run in families. It occurs more commonly in people who have other autoimmune diseases. Certain medicines used also may affect the absorbtion of vitamin B12. The most common example is metformin, colchicine, neomycin, and some anticonvulsants used to treat epilepsy [9].

Haemolytic Anaemia

Haemolytic anaemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is up. Haemolytic anaemia can affect people of all ages, races and sexes. Haemolytic anaemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure [12]. Inherited haemolytic anaemias include Sickle cell anaemia, Thalassaemias, hereditary spherocytosis, hereditary elliptocytosis, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Pyruvate kinase

deficiency. Acquired haemolytic anaemias include Immune haemolytic anaemia, autoimmune haemolytic anaemia, Alloimmune haemolytic anaemia, Drug-induced haemolytic anaemia, Mechanical haemolytic anaemias, and Paroxysmal nocturnal haemoglobinuria [13]. Certain infections and substances can also damage red blood cells and lead to haemolytic anaemia.

Sickle cell anaemia

Anaemia in which the body makes sickle-shaped ("C"-shaped) red blood cells is called Sickle Cell anaemia. It contain abnormal haemoglobin which causes sickle shape and can't move easily through the blood vessels. The clumps of sickle cells block blood flows that lead to the limbs and organs [14]. Blocked blood vessels causes pain, serious infections, and organ damage. Sickle cells usually die after about 10 to 20 days and the body can't reproduce red blood cells fast enough to replace the dying ones, which causes anaemia.

Thalassaemia

Thalassaemia is an inherited blood disorder which causes the body to make fewer healthy red blood cells and less haemoglobin [15]. The two major types of thalassaemia are alpha- and beta thalassaemia. The most severe form of alpha thalassaemia is known as alpha thalassaemia major or hydrops fetalis, while the severe form of beta thalassaemia is known as thalassaemia major or Cooley's anaemia. Thalassaemias affect both males and females and occur most often in people of Italian, Greek, Middle Eastern, Asian, and African descent. Haemoglobin in red blood cells has two kinds of protein chains: alpha globin and beta globin. If your body doesn't make enough of these protein chains, red blood cells don't form properly and can't carry enough oxygen. Genes control how the body makes haemoglobin protein chains. When these genes are missing or altered, thalassaemias occur. Thalassaemias are passed on from parents to their children through genes [16].

Aplastic Anaemia

Aplastic anaemia is a blood disorder in which the body's bone marrow doesn't make enough new blood cells. This may result in a number of health problems including arrhythmias, an enlarged heart, heart failure, infections and bleeding. Damage to the bone marrow's stem cells causes aplastic anaemia [17]. A number of acquired diseases, conditions, and factors can cause aplastic anaemia including Toxins, such as pesticides, arsenic, and benzene, Radiation and chemotherapy, Medicines such as chloramphenicol, Infectious diseases such as hepatitis, Epstein-Barr virus, cytomegalovirus, parvovirus B19, and HIV, Autoimmune disorders such as lupus and rheumatoid arthritis [17]. Inherited conditions, such as Fanconi anaemia, Shwachman-Diamond syndrome, dyskeratosis and Diamond-Blackfan anaemia may also cause aplastic anaemia.

Mechanism of Anemia-Induced Organ Injuries

Based on a contrite meta-analysis of available data, [16] anemia seems to be associated with an enhanced risk of severe COVID-19 infection. The possible pathophysiological link between anemia and severe COVID-19 can be explained by these reasons. Patients with anemia will have low hemoglobin levels. In the circulation system, hemoglobin serves as a carrier for oxygen to target organs in the body [14]. When the concentration of the hemoglobin in the circulation is low, the transport of oxygen to several organs in the body will be disrupted, therefore causing hypoxia that will eventually result in multiple organ dysfunction, especially respiratory organ dysfunction [16]. Multiple organ dysfunction will contribute to the development of severe outcomes in COVID-19 infection. Moreover in COVID-19 infections, the state of anemia in the patients could be worsened. First, SARS-CoV-2 can interact with hemoglobin molecules on the erythrocyte through ACE2, CD147, and CD26 receptors. This viral-hemoglobin interaction will cause

the virus to attack the heme on the 1-beta chain of hemoglobin and causing hemolysis [18]. Second, SARS-CoV-2 may mimic the action of hepcidin which increases circulating and tissue ferritin (affecting liver, spleen, bone marrow, and muscles mainly), while inducing serum iron deficiency and lack of hemoglobin, by consequence. The resulting hyperferritinemia will give rise to ferroptosis, with high oxidative stress and lipoperoxidation that can precipitate the inflammatory/immune over-response (cytokine storm) and causing a severe outcome of the disease [16].

Mechanism of Action for Covid-19

Human have long been infected by coronavirus as it is one of those responsible for the common cold [7]. It is a contagious viral infection that can be spread through inhalation or ingestion of viral droplets as a result coughing and sneezing and touching infected surface are primary sources of infection. The coronavirus genome is comprised of 30000 nucleotides. It encodes four structural proteins, Nucleocapsid (N) protein, Membrane (M) protein, Spike (S) protein and Envelop (E) protein and several non-structural proteins (nsp) [12]. The capsid is the protein shell, inside the capsid, there is nuclear capsid or N-protein which is bound to the virus single positive strand RNA that allows the virus to hijack human cells and turn them into virus factories. The N protein coats the viral RNA genome which plays a vital role in its replication and transcription. The N-terminal of the N protein which is binding to genomic and sub-genomic RNAs in MHV and IBV virions and process the viral replication and transcription. This is one of the important open research problems the developing of an effective drug targeting to prevent the contacts between N-terminal of N-protein and single positive RNA strand which can stop viral replication and transcription. [13] reported that two important class of compounds, theophylline and pyrimidone drugs as possible inhibitors of RNA binding to the N terminal domain of N protein of coronavirus, thus opening new avenues for in vitro validations. The M-protein is most abundant in the viral surface and it is believed to be the central organizer for the coronavirus assembly. The S-protein is integrated over the surface of the virus, it mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell [12]. The E-protein is a small membrane protein composed of 76 to 109 amino-acid and minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell and virus host cell interaction. A lipid envelop encapsulates the genetic material. Hemagglutinin-esterase dimer (HE) has been located on the surface of the viral. The HE protein may be involved in virus entry, is not required for replication, but appears to be important for infection of the natural host-cell. State-of-the-art cryo-EM experiments have revealed the full structure of the Spike (S) protein in the close [pdb id: 6VXX] and open (prefusion) states [pdb id: 6VYB] [14]. Such glycoprotein is made of three identical chains with 1273 amino acid each and it is composed by two well-defined protein domain regions: S1 and S2 subunits which are associated to cell recognition and the fusion of viral and cellular membranes respectively. The latter process occurs through different protein conformational changes that remain still uncharacterized. The mechanism of viral entry and replication and RNA packing in the human cell is mapped in Figure 1 [17]. The coronavirus spike (S) protein attaches to angiotensin converting enzyme 2 (ACE2) receptors that is found on the surface of many human cells, including those in the lungs allowing virus entry [16]. The coronavirus S protein is subjected to proteolytic cleavages by host proteases (i.e. trypsin and furin), in two sites located at the boundary between the S1 and S2 subunits (S1/S2 site). In a later stage happens the cleavage of the S2 domain (S20 site) in order to release the fusion peptide. This event will trigger the activation of the

membrane fusion mechanism. Searching for antibodies can find support on molecular targeting which can utilize the structural information (aa sequence) of the binding region which is found in angiotensin-converting enzyme 2 receptor. In this way this protocol could device a treatment to block the viral entry. Typically, human cell ingests the virus in a process called endocytosis [17]. Once entered the cytoplasm, it has been suggested most likely that COVID-19 employs a unique three step method for membrane fusion, involving receptor-binding and induced conformational changes in Spike (S) glycoprotein followed by cathepsin L proteolysis through intracellular proteases and further activation of membrane fusion mechanism within endosomes. Then, the endosome opens to release virus to the cytoplasm, and uncoating of viral nucleocapsid (N) is started via proteasomes which typically can hydrolyse endogenous proteins, but they are also capable of degrading exogenous proteins such as the SARS nucleocapsid protein. A different two-step mechanism has been suggested and in this case the virion binds to a receptor on the target host cell surface through its S1 subunit and the Spike is cleaved by host proteases and then it is expected the fusion at low pH between viral and host target membranes via S2 subunit [20]. Finally, the viral genetic material a single stranded RNA is fully released into the cytoplasm. There takes place the replication and transcription processes which are mediated by the so-called replication/ transcription complex (RTC). Such complex is encoded in the viral genome and it is made of non-structural proteins (nsp). The RTC is believed to induced double-membrane structures in the cytoplasm of the infected cell [21]. Following the positive RNA genome is translated to generate replicase proteins from open reading frame 1a/b (ORF 1a/b) [12]. These proteins use the genome as a template to generated full-length negative sense RNAs, which subsequently serve as templates in generating addition full-length genomes. Structural viral proteins, M, S and E are synthesized in the cytoplasm and then inserted into the endoplasmic reticulum (ER), and transfer to endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Also, in the cytoplasm nucleocapsids are formed from the encapsidation of replicated genomes by N protein, and as a result they coalesce within the ERGIC membrane in order to self-assembly into new virions. Finally, novel virions are exported from infected cells by transport to the cell membrane in smooth walled vesicles and then secreted via a process called exocytosis, so that can infect other cells. In the meantime, the stress of viral production on the endoplasmic reticulum eventually leads to cell death [21].

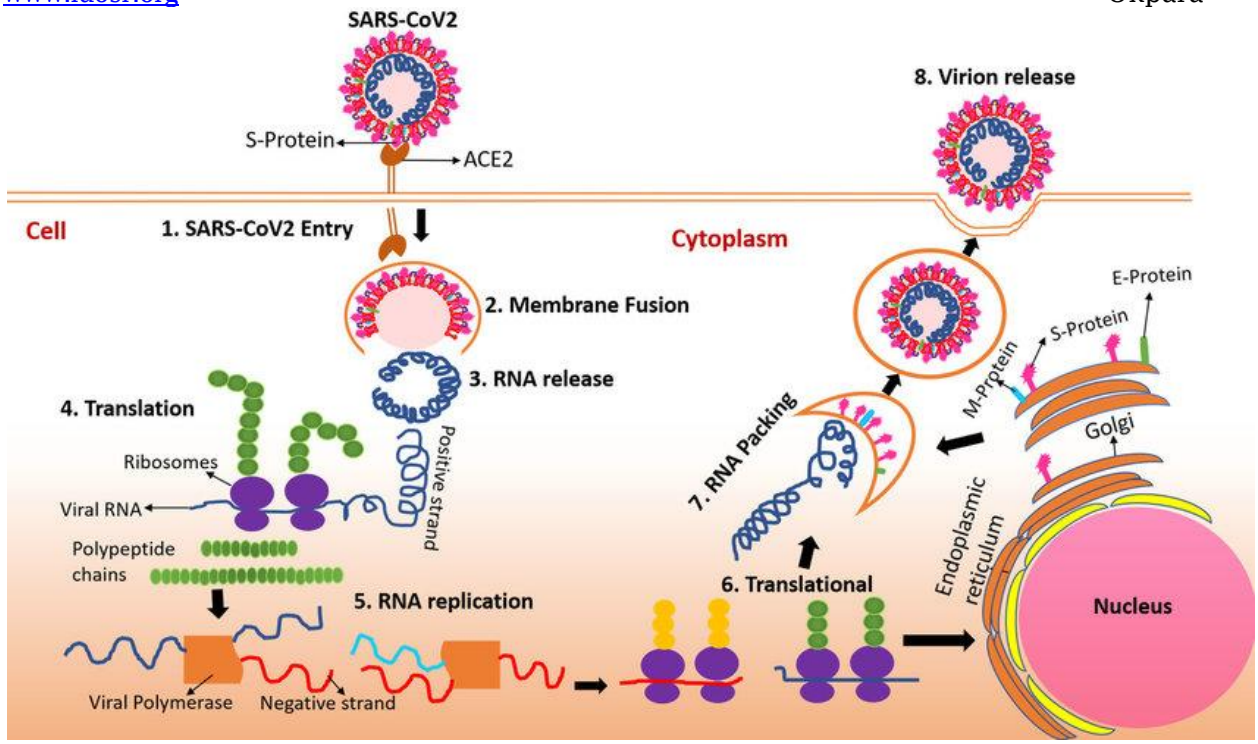

Figure 1: Diagram showing the mechanism of COVID-19 entry and viral replication and viral RNA packing in the human cell [17].

Anemia and severe illness of COVID-19

To assess whether anaemia is a risk factors for the severe illness of COVID-19, logistic regression analysis was performed in various literatures [12]. 222 patients with COVID-19 were used in their analysis. During the study, they mainly identified that COVID-19 patients with anaemia were more likely to develop severe conditions and had a higher mortality. Comorbidities were more commonly seen in patients with potential anaemia. In addition, anemic patients were older and had a higher risk of severe inflammatory responses and organ injuries. Moreover, the severity of anemia was positively and strongly associated with more serious inflammatory responses. Anemia is common among patients suffering from pneumonia, with nearly 7% to 12% in community-acquired pneumonia and 31.8% in severe influenza A [12]. Anemia commonly aggravates the severity of respiratory diseases, and it has been documented that respiratory diseases combined with anemia are associated with poor outcomes and increased mortality [22]. Hitherto, little research has noted the clinical characteristics of COVID-19 patients with anemia as well as the direct correlation between anemia and disease severity in patients with COVID-19. Previous investigations have revealed that anemic patients had poorer lung function than nonanaemic patients [23]. Additionally, it is well acknowledged that anemia and low hemoglobin could decrease oxygen delivery. Therefore, it is plausible to speculate that COVID-19 patients with anemia were more susceptible to severe illness due to worse pulmonary function and poor tissue oxygenation [26].

CONCLUSION

Anemia has been an independent risk factor for adverse outcomes in various diseases, including pneumonia, stroke, and heart failure. Previous investigations have revealed that anemic patients had poorer lung function than nonanaemic patients. Additionally, it is well acknowledged in the present research that anemia and low hemoglobin could decrease oxygen delivery. Therefore, it is

plausible to speculate that COVID-19 patients with anemia were more susceptible to severe illness due to worse pulmonary function and poor tissue oxygenation. Patients with anemia should hence be advised to take extra precautions to minimize risk exposure to the virus. Physicians should be engaged in close monitoring of anemic patients with suspected COVID-19, for timely detecting signs of disease progression. Finally, the presence of anemia shall be regarded as an important factor in future risk stratification models for COVID-19.

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