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SARS-CoV-2 Genetic Diversity and Lineage Dynamics in Egypt and Potential Risk Factors That May Increase the Disease Severity

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Abstract

The velocity of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic spread, and the variable severity of the disease course have forced scientists to search for potential predictors of the disease outcome. COVID-19 was first detected in Egypt in February 2020. From one wave to the next, dominant strains have been observed to be replaced by other dominant strains. Here we describe the genomic epidemiology of SARS-CoV-2 in Egypt, with a focus on the potential risk factors that may worsen the clinical outcome of the disease.

Keywords: SARS-CoV-2, Mutation, Variants, clinical outcome

Full-length article **Corresponding Author*, e-mail: *dr.raghda.shady.2@gmail.com*

1. Introduction

COVID-19 is a life-threatening infectious disease sustained by SARS-CoV-2; the virus first identified in the Chinese town of Wuhan in November 2019. Thereafter, it spread rapidly worldwide, and the World Health Organization (WHO) finally classified it as a pandemic disease in March 2020 [1]. Resembling the neighboring countries, the Ministry of Health in Egypt declared a state of health emergency and enforced precautionary public health actions that included the mandatory use of masks, social distancing, and quarantine for suspected patients [2]. The spectrum of clinical manifestations of SARS-CoV-2 is wide and ranges from asymptomatic or mild cases, characterized by fever and malaise, up to severe and complicated cases, such as acute respiratory distress-like syndrome (ARD-LS), that may lead to death in some cases [3]. Although Egypt is the largest connecting point between Europe, Asia, and Africa with a remarkable number of intercontinental airlines directly linked to Europe, data about the prevalence rates of the SARS-CoV-2 lineages and sublineages including the VOIs, VOCs, and VUMs are relatively scarce. Here we describe the genomic epidemiology of SARS-CoV-2 in Egypt, highlighting the prevalence of certain mutations and their cross-reactivity, in addition to discussing the Potential risk factors for increasing the disease severity.

2. SARS-CoV-2 genetic diversity and lineage dynamics in Egypt

Egypt confirmed its first case of COVID-19 on February 14, 2020, as the first African country to report a confirmed case. From February 14, 2020, to November 25, 2021, 516,023 laboratory-confirmed infections (Fig. 1), including 24,830 deaths (4.8%) by SARS-CoV-2 infection (Fig. 2), were reported to WHO and recorded according to the official website of the Egyptian MOH (https://www.care.gov.eg/EgyptCare/index.aspx, accessed November 25, 2023), specialized for the news of the COVID-19 outbreak in Egypt. Mutations in the SARS-CoV-2 virus, as an RNA virus, arise spontaneously during replication due to the lack of RNA polymerase proofreading activity and thus make far more mistakes. Hundreds of cumulative mutations have existed since the virus's emergence [4].

Over 6 million genome sequences are shared in the online database of the Global Initiative on Sharing Avian Influenza Data (GISAID). The international dissemination of SARS-CoV-2 sequences was used for contact tracing and outbreak control, enabling the discovery of variants of concern (VOCs) or other lineages of virological or epidemiological interest [5]. The World Health Organization (WHO) names new coronavirus variants by using Greek alphabet letters; the alpha variant (B.1.1.7) first variant of concern described in the United Kingdom (UK) in late December 2020, beta variant (B.1.351) was the first reported in South Africa in December 2020, gamma variant (P.1) first reported in Brazil in early January 2021, delta variant

(B.1.617.2) first reported in India in December 2020, Lambda (C.37) first reported in Peru in August 2020, Mu (B.1.621) reported in Colombia in January 2021, and Omicron (B.1.1.529) first reported in South Africa in November 2021 [6].

Emerging variants that show increased transmissibility and/or immune evasion are classified as VOCs. Several other variants of unknown risks have been detected globally and therefore designated as Variants Under Monitoring (VUMs) [7]. Omicron was recognized as a fifth VOC, as within a few months, it became the dominant SARS-CoV-2 strain in South Africa and elsewhere, displacing the delta variant that had led to a devastating surge in cases, hospitalizations, and deaths [8]. Genetic analysis of the omicron variant showed higher mutation rates in the spike protein, representing a distinct evolutionary lineage that deviated from the mainstream of the evolving SARS-CoV-2 detected in mid-2020 [9]. Egypt experienced five waves of COVID-19. The first wave began in April 2020 and receded by August 2020. It was associated with the religious and cultural celebrations of the holy month of Ramadan as well as the yearly wedding celebration season. The second wave coincided with the onset of winter weather. The third reported wave followed the mass gatherings associated with the start of the following year's religious and cultural celebrations in 2021. By the beginning of the fifth COVID-19 wave, Omicron was the dominant coronavirus variant in Egypt representing 20.95% of all sequences obtained from Egypt [10].

The variant B.1 was the most dominant variant in the first wave, and C.36 was identified first in Egypt in March 2020 and after May 2021, and was largely detected during the second and third waves. According to a study done in Egypt analyzing the genomic variation of COVID-19 cases, the beta variant was not detected in Egypt, and the number of alpha variants was limited [11]. Variant C.36.3 acquired several mutations including the S12F, W152R, R346S, L452R, D614G, Q677H, A899S substitutions, and 69–70 deletion (Del 69–70) in spike protein [10]. R346S, L452R, and Q677H mutations were associated with better receptor binding affinities and consequently increased transmissibility and reduced ability to neutralize convalescent or vaccinated sera [9], and W152R and ΔH69-V70 mutations are associated with lower sensitivity to monoclonal antibodies (mAbs) and vaccine sera [10]. The D614G mutation, the replacement of aspartic acid by glycine at position 614 of the spike glycoprotein, was also associated with increased SARS-CoV-2 transmissibility [11].

Analysis of the spike (S) glycoproteins of Egyptian SARS CoV-2 viruses indicated that 94.9% of all Egyptian sequences have D614G forms. In Egypt, the D614G variant is the most dominant [12]. T851, F307F, 15907G, P323L, Q57H, Q57L, Q822K, V5F, G15S, T148I, G212V, K2798R, and T5020I are examples of mutations reported in Egypt [10]. D614G and P4715L mutations are linked to transmissibility, regardless of symptom variability, while Nsp6-L3606fs, spike-glycoprotein-V6fs, and nsp13-S5398L variants may be linked to the worsening of clinical symptoms. The E3909G-nsp7 variant was shown to be more frequent in children and could explain why children recover so quickly [13].

3. Viral Structure

Coronaviruses are enveloped, medium-sized spheroidal viruses with a diameter of 80–220 nm and whose genome is +ve single-stranded large RNA with variable open reading frames (ORFs) [4]. The Coronavirus genome size ranges from 26 to 32 kb. It consists of a structural gene unit encoding 9680 amino acids (6–11 ORFs that encode S, E, M, and N proteins) and two large ORF genes (ORF1a and ORF1b) that encode 16 non-structural proteins (NSP), including RNA-dependent RNA polymerase (RdRp). The remaining ORFs encode structural and accessory proteins of SARS-CoV-2 [14]. SARS-CoV-2 surface M (membrane) proteins, S (spike), and E (envelope) are implanted in the lipid bilayer membrane enveloped by the helical nucleocapsid (N) coating viral RNA. Membrane proteins found in the virus assist viral assembly. The hemagglutinin-esterase glycoprotein, which is found in beta-coronavirus, enhances viral uptake into mucosal cells [15]. Coronaviruses were termed so due to having spikes that look like crowns. These glycoprotein spikes allow it to attach to human cell receptors, permitting its entry into the cells [16].

Each Coronavirus, on average, has 74 surface spikes, which are the most distinguishing feature of the virus particle. S protein is 20 nm long and composed of 2 subunits, named S1 and S2 [17]. The S1 subunit is a class I fusion protein composing the head of the spike. It mediates receptor binding and membrane fusion between the virus and the host cell. It is divided into the receptor binding domain that recognizes its specific receptor (ACE II) and the N-terminal domain (NTD), which facilitates viral entry into the host cell and serves as a potential target for neutralization in response to antisera or vaccines [4]. The S2 subunit forms the stem of the spike, which anchors the spike in the viral envelope and enables fusion on protease activity [14].

4. Viral entry and pathogenesis

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE-2) receptor in the body cells; primarily, it is found abundantly on the respiratory epithelium (type II alveolar epithelial cells in the lung and bronchial epithelia), in addition to its presence in other organs such as the upper esophagus, enterocytes from the ileum, myocardial cells, proximal tubular cells of the kidney, and urothelial cells of the bladder [18]. SARS-CoV-2 uses the S protein (the viral structural proteins, spike shape) for binding (fig. 4), dependent on transmembrane proteinase-serine 2 (TMPRSS2), facilitating the entry of viral particles, and the endosomal cysteine proteases cathepsin B and L (CatB/L) for viral spike (S) protein priming [16]. On the other hand, SARS-CoV-2 cannot enter cells expressing either dipeptidyl peptidase 4 or aminopeptidase N, the entry receptors for MERS-CoV and HCoV-229E, respectively [18].

TMPRSS2 is necessary for cleaving the viral envelope located trimeric S protein at the S1/S2 and the S2' sites, leading to the fusion of the viral and cellular membranes mediated by the S2 subunit of S protein after the engagement of the S1 subunit to the cell surface receptor and for the subsequent viral internalization in the pulmonary epithelium [19]. Following the infection of SARS-CoV-2 in patients, symptoms occur within 5–6 days when the viral load reaches a peak, and 97.5% of symptomatic patients further develop COVID-19 within 2 weeks [20]. Once SARS-CoVs enter the host via the respiratory tract, airway, and alveolar epithelial cells, vascular endothelial cells and alveolar macrophages are among their first targets of viral entry [21]. Viral replication releases a large number of virions, leading to infection of neighboring target cells and viremia, which then causes an

exaggerated pulmonary and systemic inflammatory response, respectively. This explains the clinical presentation of severe COVID-19, which is predominated by ARDS, shock, and coagulopathy [22]. Such severe clinical presentations provided logistic challenges for healthcare facilities, forcing them to increase their ICU capacities [23]. Therefore, the prediction of clinical outcomes and the determination of patients who are at risk of developing severe symptoms requiring ICU admission will result in more accurate scenario planning. Even more, the determination of disease severity and prognosis facilitates prioritizing vaccination plans for high-risk populations [24]. The velocity of the COVID-19 pandemic spread and the variable severity of the disease course have forced scientists to search for potential predictors of the disease outcome [25].

5. Potential risk factors for increasing the disease severity

Recent studies suggested some risk factors influencing the risk of severe infection and death from COVID-19, and the determination of such risk factors helps to identify the high-risk population and facilitates providing timely management. There are conflicting reports regarding the specific risk factors for severe COVID-19 infection in different populations [26]. A recent meta-analysis study demonstrated that male gender, hypertension, smoking history, diabetes, and myalgia are predictors of COVID-19 severity [27]. However, factors including vomiting, diarrhea, fever, and cough were not related to the disease severity [26]. A possible explanation for the mild gastrointestinal manifestation is the attenuation of SARS-CoV-2 virulence after swallowing by the effect of digestive enzymes, which cause its degradation [28].

Recent reports have revealed that obesity causes a waning of the immune system and increases, thereby, the vulnerability to infectious diseases. Obesity with excess adiposity is evidenced to induce an interruption of the equilibrium between pro-inflammatory and anti-inflammatory immune cells, with a predominance of the former [29]. This disturbance induces a chronic low-grade inflammatory milieu, which is exaggerated by an intense inflammatory storm arising from the COVID-19 infection itself, resulting in more severe disease pathology and worse sequelae [2].

Age also plays an important role in disease prognosis, classifying elderly people as high-risk groups for COVID-19, where older patients have been reported to have higher morbidity and mortality, more comorbidities and complications, and a poor prognosis [30]. A study done in Egypt reported that the mortality risk appears to be significantly increased by age and comorbidities (cardiovascular diseases, cancers, diabetes mellitus, and chronic lung diseases) [31]. This was also supported by another Egyptian study, which reported that patients with one or more comorbidities had a worse survival rate [32]. It was stated that people aged 46 years and older represented 91.5% of coronavirus-related deaths in Egypt [33]. This could be explained by the age-dependent decline in cell-mediated immune function and reduced humoral immune function [17].

It is evident that COVID-19 is not a localized "respiratory infection" but a "multisystem disease" caused by a diffuse systemic process involving a complex interplay of the immunological, inflammatory, and coagulative cascades (fig. 5). Genetic and acquired differences in the host immune system further complicate the host repertoire, leading to wide heterogeneity in the clinical picture, course, and outcome [34].

6. Host immune response against SARS-CoV-2

The recent pandemic of SARS-CoV-2 has once again reminded us of the significance of host immune responses and the consequential havoc of immune dysregulation, as differences in the outcome of an infected host with COVID-19 are closely related to host innate and acquired immunity. Innate immunity rapidly recognizes infection and triggers adaptive immunity. Acquired immunity includes humoral and cellular immunity, both of which work together to defend against viruses and protect the body from damage by destroying viruses in the circulatory system and killing viruses in cells [35]. Upon viral infection, alveolar epithelial cells, macrophages, and blood-circulating monocytes are activated via toll-like receptors as pattern recognition receptors (PRRs) by the virus products and produce a robust amount of inflammatory cytokines and chemokines, which attract more immune cells, in particular monocytes and T cells, resulting in widespread lung inflammation. The postmortem pathology of COVID-19 patients shows interstitial mononuclear inflammatory infiltrates dominated by lymphocytes in the lung and severe 19 lymphopenia with hyperactivated T cells in the peripheral blood [36].

Many reports concluded that in COVID-19, macrophages act as the first antigen-presenting cell (APC) responder to viral invasion, eliciting innate and adaptive immune responses [37]. It generates macrophage inflammatory protein 1 (MIP1) and type I interferons (IFNs), which activate T-cell responses [38]. In addition, macrophages produce interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-), and interleukin-1 beta (IL-1β) [39]. In response to macrophage alarming and macrophage-T cell interaction, T cell subset differentiation occurs quickly, resulting in the proliferation of T helper 1 (Th1), cytotoxic T cells (CTLs), and Th17 [38]. Specific cellular immunity, including both $CD4^+$ and $CD8^+$ T-cells, is crucial for achieving control over viral infections. Although the immune system is important for disease elimination, its over-activation may cause significant harm and contribute to disease pathogenesis. Differences in immune profiles can help to better understand the pathogenesis and clinical expression of COVID-19 [40].

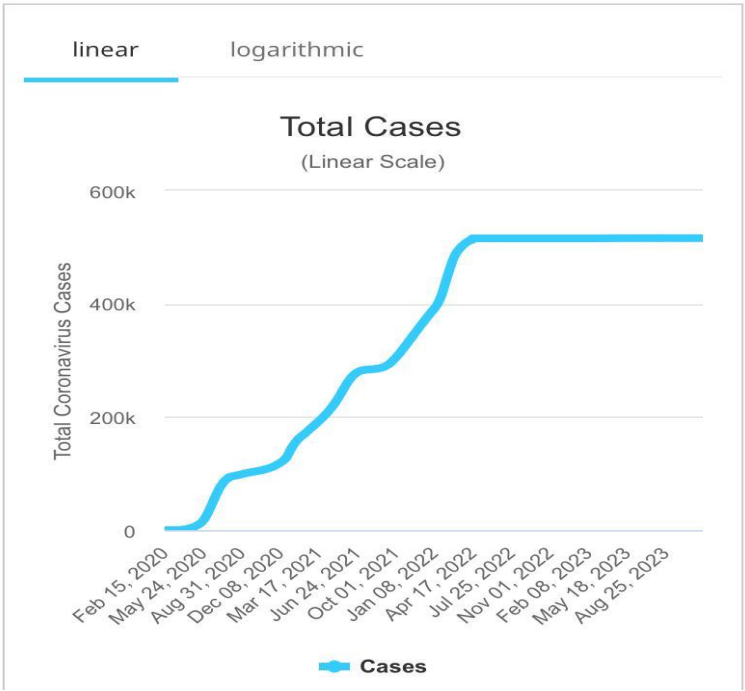

Figure 1: Total Coronavirus cases in Egypt till November,2023

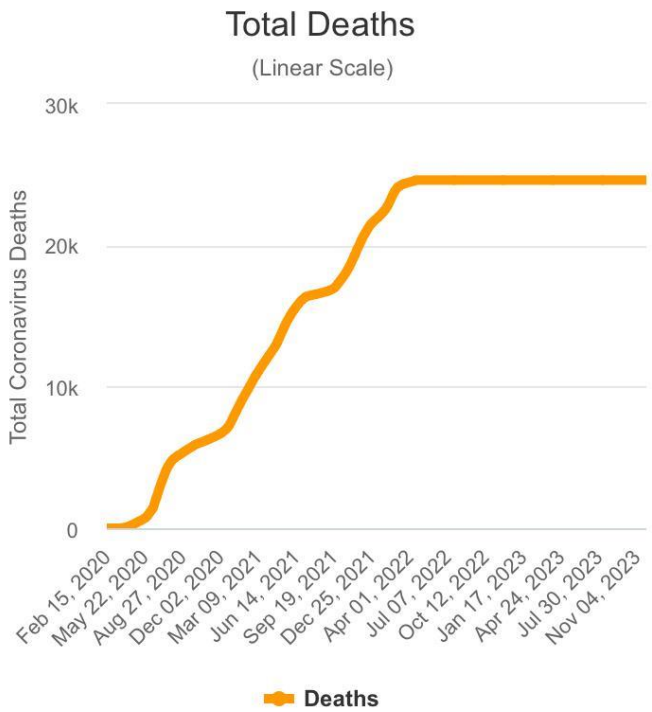

Figure 2: Total Coronavirus deaths in Egypt till November,2023

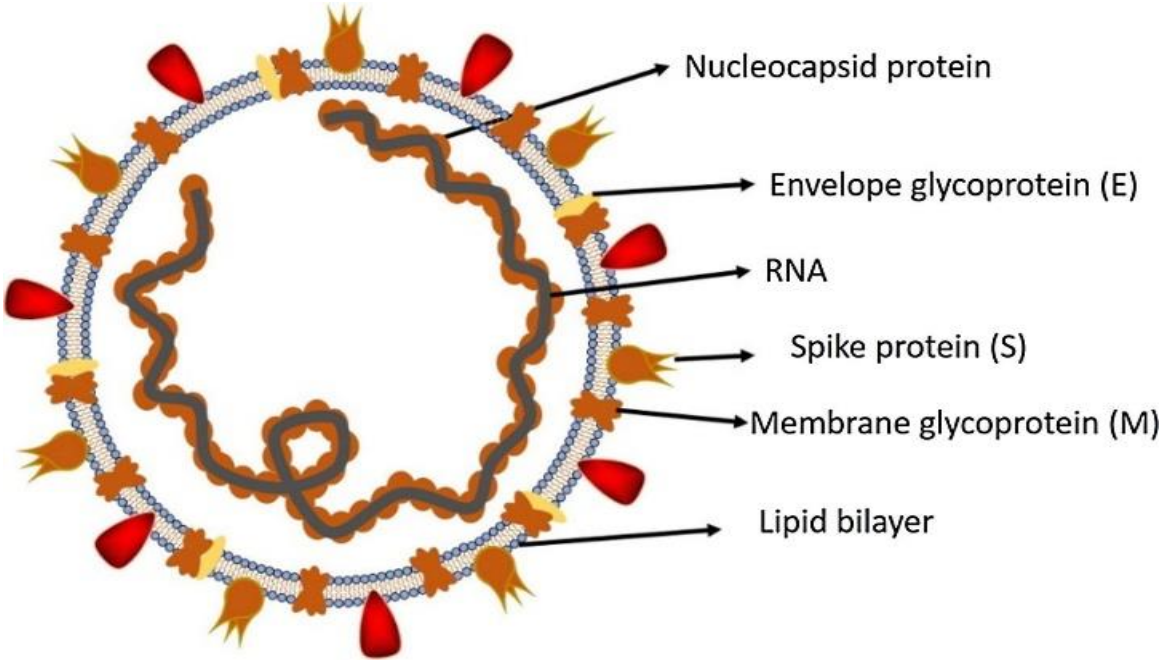

Figure 3: The structure of Coronavirus

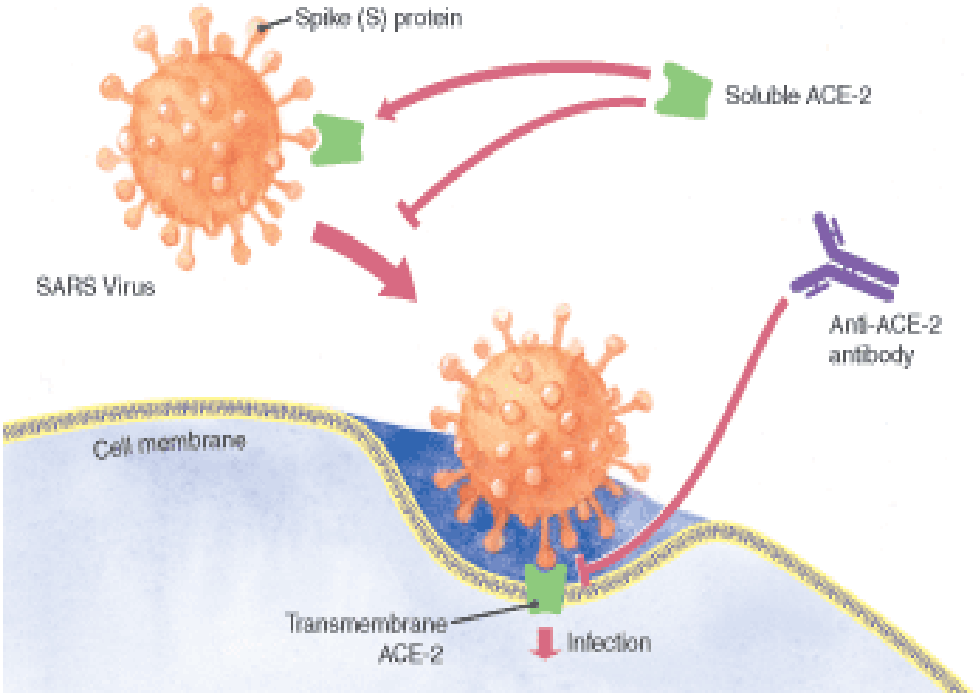

Figure 4: SARS-CoV-2 binding to angiotensin-converting enzyme 2 (ACE-2) receptor in the body cells

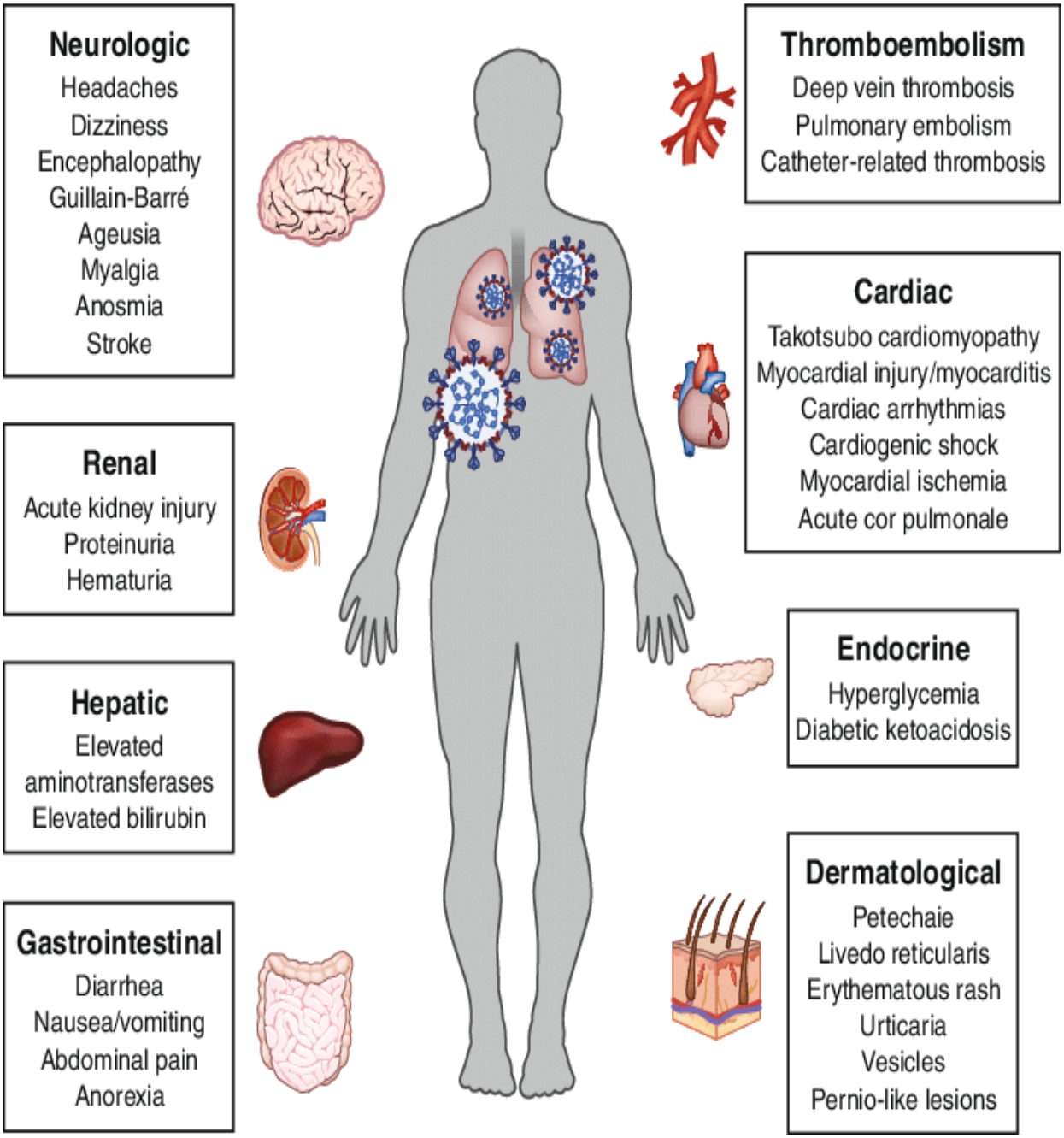

Figure 5: Extrapulmonary manifestation of COVID-19

Table 1: Common inflammatory mediators and immune cells involved in cytokine storm [41].

	origin	Action
IL-1	Macrophages, epithelial cells; pyroptotic cells	Pro-inflammatory alarmin cytokine; pyrogenic activity, activates macrophage and Th17 cell
IL-2	T-cells	Effector T-cell and regulatory T-cell growth factor
IL-6	Macrophages, T-cells and endothelial cells	Pyrogenic cytokine with pro-inflammatory activity, stimulate acute-phase reactions and antibody production
IL-9	Th9 cells	Defense against helminth infections, activates mast cells, association with type I interferon in COVID-19
IL-10	Regulator t cells, Th9 cells	Anti-inflammatory cytokine; inhibit Th1 and cytokine release
IL-12	Dendritic cells and macrophages	Th1 pathway activation; induce Th1 cells for INF-γ release; activate CTLs and NK cells; show synergism with IL- 18
IL-17	Th17 cells, NK cells, group 3 innate lymphoid cells	Activate and propagate neutrophilic inflammation; protect against infections
IL-18	Monocytes, macrophages, dendritic cells	Alarmin cytokine with pro-inflammatory function; activation of Th1 pathway, exhibit synergism with IL-12
IL-33	Macrophages, dendritic cells, mast cells, epithelial cells	Pro-inflammatory cytokine with alarmin function; potentiates Th1 and Th2 cells, NK cells, CTLs, and mast cells
INF-γ	Th1, CTLs, group 1 innate lymphoid and NK cells	Pro-inflammatory cytokine; macrophages stimulation
TNF	Macrophages, T cells, NK cells, mast cells	Pro-inflammatory cytokine with pyrogenic function, increase vascular permeability
GM-CSF VEGF	Th17 cells, Macrophages	Pro-inflammatory cytokine Promotes angiogenesis
Chemokines IL-8 (CXCL8)	Macrophages, epithelial cells	Chemotactic agent of neutrophils
CRP	Hepatocytes	Monomeric CRP increases IL-8 and MCP-1 production; IL-6 induced upregulated expression of CRP
Complement	Hepatocytes, other cells	Amplify tissue damage in cytokine storm; suppression of complement system abrogates pathophysiological effects of cytokine storm
Iron	Ubiquitous	Primary intracellular storage site of iron

Regarding biochemical parameters, COVID-19 patients present an elevation of proinflammatory cytokines like interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), C-X-C motif chemokine ligand 10 (CXCL10), and monocyte chemoattractant protein-1 (MCP-1), which can lead in certain cases to a cytokine storm [34].

Therefore, circulating biomarkers such as neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), hemoglobin, albumin, lymphocyte, and platelet score (HALP), which represent inflammatory and immune status, may be potential biomarkers for the severity assessment and prognosis of COVID-19 patients. At present, there have been studies on NLR, MLR, and PLR to predict the severity of COVID-19 patients [42]. Humoral immunity can be acquired by natural infection and/or vaccination, with evidence reporting optimal protection against previous infection combined with 1–3 doses of COVID-19 vaccines [43]. Severe inflammation leads to the weakening of the adaptive immune response, which leads to an imbalance in the immune response [44].

In conclusion, the evolution of C.36 into various sub-lineages, some of global interest, reiterates a single important message: that no SARS-CoV-2 lineages should be ignored. Particularly, we have shown that if not controlled, non-VOC lineages can evolve rapidly within a single country to acquire adaptive mutations necessary to sustain community transmission and affect the prognosis of SARS-CoV-2 infections. This message is reinforced with the emergence of the Omicron variant suspected to have emerged from the cryptic circulation of B.1.1. lineage viruses from late 2020 in Southern Africa.

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