Molecular Biological Mechanisms and Biomarkers of Multisystem Sequelae in Long COVID

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Abstract. With the prolonged evolution of SARS-CoV-2, the post-acute infection sequelae, widely recognized as Long COVID, has become a long-term challenge to global public health. Long COVID is a complex syndrome that affects multiple organs and systems; Its pathogenesis has not yet been fully clarified, and there is a lack of unified diagnostic and management strategies. This article provides a systematic review of the four core molecular biological mechanisms of Long COVID, and these include viral residuals, autoimmunity, microthrombosis, and reactivation of latent viruses. This review also looks into how these underlying molecular mechanisms are directly mapped to clinical multi-organ sequelae affecting the nervous, cardiovascular, and systemic and musculoskeletal. Building on this, the paper deeply summarizes the application value of cutting-edge biomarkers, for instance, plasma neurofilament light chain (NfL), thioflavin T fluorescence staining, and circulating cell-free mitochondrial DNA (ccf-mtDNA), and proposes a precision screening approach based on combined multi-biomarker testing. The significance of this study is to provide theoretical support for the public health system's transition from empirical prevention and control to molecularly precise interventions. Future research could focus on standardized validation of potential biomarkers to establish a more stable biomarker detection system, thereby providing a more reliable theoretical basis for the early screening and precise intervention of Long COVID.

Keywords: Long COVID, Molecular Mechanisms, Biomarkers, Precision Medicine.

1. Introduction

Long COVID, also known as Post-Acute Sequelae of SARS-CoV-2 Infection (PASC), is evolving into the most severe and insidious chronic disease crisis facing global public health systems in the 21st century. Although the rates of severity and mortality of acute SARS-CoV-2 infection have declined significantly with the establishment of herd immunity and the widespread use of multivalent vaccines, hundreds of millions of recovered patients continue to suffer from persistent multisystem symptoms months or even years after infection. According to statistics, at least 65 million people worldwide are affected by Long COVID [1]. At the macro-public health level, this chronic disease exhibits extremely high systemic complexity and population heterogeneity [2]. In the systemic and musculoskeletal systems, patients often report severe chronic fatigue and post-exertional malaise (PEM); in the central nervous system, symptoms commonly manifest as memory loss, difficulty concentrating, and cognitive impairment—commonly known as "brain fog." Epidemiological data also show that within one year of infection, recovered patients face a sharply increased risk of cardiovascular diseases such as myocarditis, thrombosis, and arrhythmias [3]. The emergence of this large-scale chronic disease population has not only led to a hidden reduction in the global workforce but also brought profound negative impacts on the allocation of healthcare resources and chronic disease prevention and control systems in various countries. Therefore, exploring the patterns of occurrence and development of Long COVID is a core issue that urgently needs to be addressed in the field of public health.

In recent years, independent research in the field of molecular biology has been springing up at a very fast pace, and a growing number of studies have tried to dig out the underlying biochemical explanations for these systemic clinical phenotypes. The scientific community has now gradually reached a consensus around several core biological hypotheses, which include viral residuals and persistent antigenic stimulation, autoimmune activation and immune exhaustion, latent microthrombosis and endothelial damage, as well as gut microbiota dysbiosis and barrier dysfunction

[1, 4, 5]. However, these scientific advances are currently highly fragmented, and many of the studies have only managed to clarify single molecular pathways. Current public health research still lacks a systematic summary that deeply integrates these molecular mechanisms with macroscopic population-specific precision screening. To fill this research gap, this review summarizes the macroscopic public health manifestations of Long COVID across multiple systems; it has also summarized four underlying biological hypotheses regarding Long COVID in a deeper way and has analyzed their microscopic molecular pathways. Building on this, the paper has moved on to discuss how these molecular mechanisms can be translated into objective clinical diagnostic markers, for instance, plasma neurofilament light chain (NfL) and the combination of thioflavin T fluorescence staining with flow cytometry, as well as other cutting-edge biomarkers [6, 7], with the aim of offering technical insights that can be used for large-scale public health screening. This will assist public health systems in conducting scientific population stratification of Long COVID patients, and in doing so it can help to optimize the allocation of medical resources and push forward a shift in Long COVID management. Make it go from the traditional "one-size-fits-all" approach to infectious disease control and toward precision public health. As a result, it can provide theoretical support for easing the global socioeconomic and healthcare burdens that Long COVID has brought about.

2. The Four Key Molecular and Biological Hypotheses Underlying Long COVID.

The pathophysiological basis of Long COVID is extremely complex. The current scientific consensus is that it is not driven by a single pathway. Rather, it is the result of the combined effects of multiple microscopic biological changes. This part will focus on the four core molecular and biological hypotheses underlying the development and progression of Long COVID.

2.1. Viral Persistence and Persistent Antigenic Stimulation

Viral persistence and persistent antigenic stimulation are seen as the root causes of the ongoing inflammation that happens in the host. After the acute phase of infection has passed, the virus is not completely cleared from the bodies of some patients. Anatomical evidence in humans has shown that SARS-CoV-2 can form long-term, trace residues in organs like the brain, intestines, and lymphoid tissues [4]. These leftover viral RNA and spike proteins act as persistent exogenous antigens, and they can be recognized by intracellular pattern recognition receptors (PRRs), which include the innate immune receptors TLR7/8 in endosomes and MDA5/RIG-1 in the cytoplasm. These receptors keep activating the type I interferon response, and at the same time they trigger NF-κB mediated pro-inflammatory cytokine expression, which eventually leads to a long-term state of low-level immune activation [8]. Persistent viral antigens can also cause the NLRP3 inflammasome to stay activated, and this mediates the maturation and release of IL-1β and IL-18 through caspase-1, which further amplifies local inflammation and pyroptosis. This whole process keeps the body in a chronic, unregulated state of systemic inflammation, and that forms the core molecular pathological basis of Long COVID [8].

2.2. Autoimmunity and Immune Exhaustion

The second major core mechanism has to do with the breakdown of host immune tolerance. On one hand, SARS-CoV-2 sequences show high levels of molecular mimicry with human self-antigens, and persistent viral antigens can be taken up by antigen-presenting cells (APCs) and then presented to $CD4^+$ T cells. After that, some of the activated $CD4^+$ T cells will differentiate into follicular helper T (Tfh) cells. Inside the germinal center, these Tfh cells make use of molecular signals like CD40L and IL-21 to stimulate the generation of autoantibodies, which can mistakenly go after the host's own immune system and healthy tissues [9]. This pathological process also triggers a drop in systemic cortisol levels at the same time [9]. On the other hand, long-term antigen exposure brings about sustained activation of T cells and B cells, and this comes with a significant increase in the expression

of the inhibitory receptors PD-1 and TIM-3 on the surface of lymphocytes, a situation that results in immune exhaustion. This does not just stop the body from completely clearing the leftover viruses, but it also causes prolonged damage to healthy tissues because the immune network gets disrupted [10].

2.3. Fibrinaloid Microclots and Endothelial Dysfunction

Endothelial damage and microcirculatory dysfunction are the physical and biochemical root causes of Long COVID. Studies have shown that free spike protein can directly act on vascular endothelial cells. This does not just lead to endothelial dysfunction, but it also stimulates the excessive expression of tissue factor (TF), and that speeds up thrombin production. These proteins can also form noncovalent bonds with fibrinogen and bring about fibrin deposition, which may be the key mechanism behind the formation of fibrinaloid microclots [11]. On top of that, endothelial cell activation comes with an increased release of von Willebrand factor (vWF), and this further worsens platelet adhesion and microclot formation [11]. The spatial conformation of these microclots shows a highly abnormal antiprotease structure, and that makes them very resistant to endogenous plasmin and extremely hard to break down on their own [11]. These circulating microclots can physically block the capillary network, and this leads to microcirculatory dysfunction in multiple organs and induces endotheliitis.

2.4. Gut Dysbiosis and Barrier Dysfunction

Multiple genomic sequencing studies have found that SARS-CoV-2 infection can disrupt the balance of the gut microbiota. This change is mainly reflected in a decrease in beneficial bacteria, an increase in opportunistic pathogens, and changes in microbial metabolic functions. Caliman-Sturdza et al. summarized that the abundance of commensal bacteria with immunomodulatory functions and the ability to produce short-chain fatty acids (SCFAs) continues to decline after infection. These bacteria include Faecalibacterium prausnitzii, Eubacterium rectale, and the genus Bifidobacterium [5]. As the abundance of these bacteria drops, the production of SCFAs also decreases. This weakens the gut barrier maintained by tight junction proteins and can lead to leaky gut [5]. After that, intestinal metabolites and the endotoxin lipopolysaccharide (LPS) can pass into the bloodstream, which increases chronic systemic immune activation. SARS-CoV-2 infection can also reactivate latent viruses that already exist in the body at the molecular level. One example is Epstein-Barr virus. The rapid expression of its lytic genes can trigger a strong immune response in the host, and this process may contribute to many symptoms of Long COVID [12].

3. Mapping Molecular Mechanisms to Clinical Phenotypes and Development of Diagnostic Biomarkers

Four core molecular mechanisms described above are linked to different multisystem clinical sequelae in various organ systems. Understanding how these molecular pathways lead to clinical manifestations can help identify more accurate diagnostic biomarkers for Long COVID.

3.1. Neurological Symptoms and Cognitive Impairment

Within the nervous system, the autoantibodies described in Part 3 can cross the blood-brain barrier. When these autoantibodies go after pericytes in the brain specifically, it causes localized microcirculatory dysfunction and neuroinflammation, and at the same time, systemic inflammation triggers abnormal activation of brain microglia and neuronal damage, which sets off a neuroinflammatory cascade [13,14]. At the macro level, this maps to the brain fog, cognitive impairment, and autonomic dysfunction like postural orthostatic tachycardia syndrome (POTS) that patients commonly experience [14].

This molecular pathway suggests that when developing neurological biomarkers for Long COVID, the key pathological processes above should be given priority. Neurofilament light chain (NfL),

which is a structural protein that gets released into the bloodstream after axonal damage, is currently one of the most sensitive markers of neuronal damage, while glial fibrillary acidic protein (GFAP), which mainly comes from activated astrocytes, reflects the glial response and neuroinflammation. Compared to nonspecific inflammatory markers like IL-6 and CRP, NfL and GFAP can reflect structural damage to the central nervous system in a more direct way, and that makes them more suitable as candidate biomarkers for the neurological form of Long COVID. Clinical diagnosis may be carried out objectively by detecting abnormally elevated levels of plasma NfL and GFAP [6].

3.2. Cardiovascular System Risks

Cardiovascular sequelae of Long COVID primarily result from the persistent presence of microthrombi, as discussed in Part 3, and endothelial dysfunction. When persistent fibrin-like microthrombi continues to obstruct myocardial capillaries and the Spike protein inhibits endothelial cells from producing nitric oxide (NO), this leads to microvascular dysregulation and tissue hypoxia [15]. The clinical phenotype subsequently maps to palpitations, persistent chest pain, and new-onset hypertension.

For this mechanism, candidate biomarkers should cover the three pathological stages of thrombus formation, endothelial injury, and endothelial activation. First, thioflavin T (ThT) fluorescence staining can specifically bind to abnormally folded structures in microthrombi and can be detected using fluorescence microscopy to enable quantitative assessment of microthrombus particles in peripheral blood. In recent years, research has further combined ThT fluorescence staining with imaging flow cytometry, providing an objective and high-throughput method for detecting microthrombi [7]. Second, elevated levels of circulating endothelial cells (CECs) indicate endothelial cell shedding and vascular damage; Soluble vascular cell adhesion molecule-1 (sVCAM-1), a classic marker of endothelial activation, can assess vascular dysfunction under persistent inflammatory conditions. Both two have the potential to serve as highly sensitive molecular markers for evaluating widespread vascular endothelial damage in patients with Long COVID [16,17].

3.3. Systemic and Musculoskeletal Manifestations

One of the most common manifestations of Long COVID is chronic fatigue and post-exertional malaise (PEM). Current evidence suggests that these symptoms may come from persistent cellular bioenergetic dysfunction. This is the result of multiple mechanisms acting together, and they include impaired mitochondrial energy metabolism, reduced efficiency of oxidative phosphorylation (OXPHOS), and diminished peripheral oxygen utilization [18]. Sustained immune activation and inflammation can throw off mitochondrial function, bring down the activity of the electron transport chain, and lead to insufficient ATP production [18]. At the same time, the reactivation of latent Epstein-Barr virus (EBV) has also been shown to have a connection with persistent fatigue [12,18].

To screen for and evaluate this type of chronic fatigue patient from a public health perspective, mitochondrial metabolites make an excellent choice of biomarkers. For one thing, a decrease in mitochondrial membrane potential ($\Delta\Psi m$) in peripheral blood mononuclear cells can directly reflect mitochondrial oxidative phosphorylation capacity, and that points to impaired mitochondrial function. For another, an increased plasma lactate/pyruvate ratio indicates a shift toward anaerobic metabolism and defective oxidative phosphorylation [19]. Circulating cell-free mitochondrial DNA (ccf-mtDNA) has also turned into a promising biomarker of mitochondrial damage and immune activation, although its diagnostic value still needs to be checked through further validation in large prospective cohorts [20]. At the same time, putting together a combined screening model that uses IFN-γ and a specific recombinant EBV early-antibody can help rapidly pick out Long COVID subtypes caused by latent viral activation in community health management, and this provides a molecular basis for subsequent targeted interventions. That said, these biomarkers are currently used mainly in the research phase, and their detection methods, reference ranges, and clinical diagnostic value still need to go through further validation by way of large-scale prospective studies.

The four core molecular mechanisms described in Part 3 above do not exist on their own, but rather they drive the multisystem clinical manifestations of Long COVID together through various processes. Different organ systems show distinct molecular pathological changes, and these line up with different potential biomarkers, which provides an important basis for the precise diagnosis and treatment of the disease. To more clearly show the connections between the molecular mechanisms, clinical phenotypes, and candidate biomarkers of Long COVID, Table 1 summarizes the main points.

Table 1. Representative molecular mechanisms, clinical phenotypes, and candidate biomarkers across major organ systems in Long COVID.

Clinical System	Key Molecular Mechanisms	Representative Clinical Phenotypes	Potential Molecular Biomarkers
Neurological	Autoantibody-mediated neural injury; Microglial activation; Neuronal damage	Brain fog; Cognitive impairment; POTS	NfL; GFAP
Cardiovascular	Fibrin amyloid microclots; Endothelial dysfunction	Palpitations; Chest pain; New onset hypertension	ThT + imaging flow cytometry; CECs; sVCAM-1
Systemic and Musculoskeletal	Cellular bioenergetic dysfunction; Possible EBV reactivation	PEM; ME/CFS-like fatigue	ΔΨm; Lactate/Pyruvate ratio; ccf-mtDNA

4. Conclusion

This review not only provides a detailed summary of the four major molecular and biological hypotheses underlying Long COVID but also builds a logical framework linking "molecular mechanisms—clinical manifestations—biomarkers", providing critical theoretical support for the transition of public health from empirical prevention and control to molecularly precise interventions. In response to the concerns in the introduction about the massive global patient population and the resulting loss of labor force, this review concludes that Long COVID is by no means a single-system disease, but a macroscopic reflection of disruptions in a multi-organ molecular pathological network, requiring early screening based on specific biomarkers. This conclusion provides a directional reference for the future development of multidisciplinary team (MDT) clinical guidelines and the establishment of multi-omics follow-up cohorts, thereby helping to optimize the precise allocation of public health and medical resources.

However, this study also has some limitations. The medical community has not yet arrived at unified diagnostic criteria for Long COVID biomarkers, and different mutants can bring about differences in molecular subtypes, and so this review relies mainly on pulling together the existing literature. In the follow-up research that lies ahead, it is urgent to carry out standardized verification of potential biomarkers, and to set up a more stable and sensitive combined marker detection system by bringing transcriptomics, proteomics and other multi-omics technologies into the picture. At the same time, more work needs to be done on the interactions that go on between different molecular mechanisms, so as to push forward the accurate classification of Long COVID patients and provide a scientific basis for individualized treatment and disease intervention.

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