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Interestingly, at least some of the proposed mechanisms of LC development overlap with mechanisms of Autonomic Nervous System (ANS) imbalance, previously detailed in the framework of the aging process. ANS imbalance is characterized by a proinflammatory sympathetic overdrive, and a concomitant decreased anti-inflammatory vagal parasympathetic activity, associated with reduced anti-inflammatory effects of the HPA axis and cholinergic anti-inflammatory pathway (CAP). These neuro-immune-endocrine system imbalanced activities fuel the vicious circle of chronic inflammation, *i.e.* inflammaging.

Here, we refine our original hypothesis that ANS dysfunction fuels inflammaging and propose that biomarkers of ANS imbalance could also be considered biomarkers of inflammaging, recognized as the main risk factor for developing age-related diseases and the sequelae of viral infections, *i.e.* LC.

Key Words: Autonomic dysfunction, Long Covid, inflammaging, Anti-Inflammaging, cortisol, serotonin

Figure 1

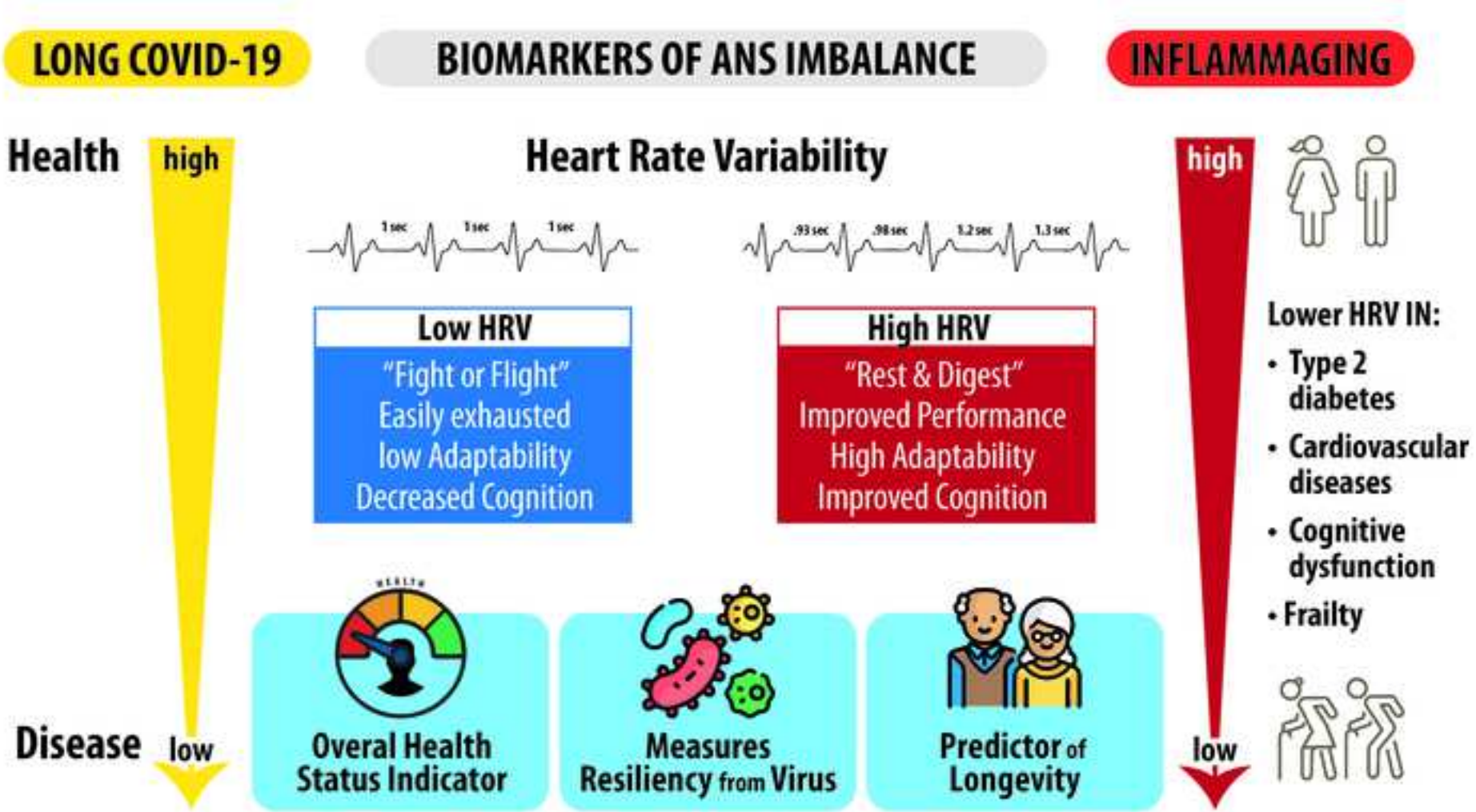
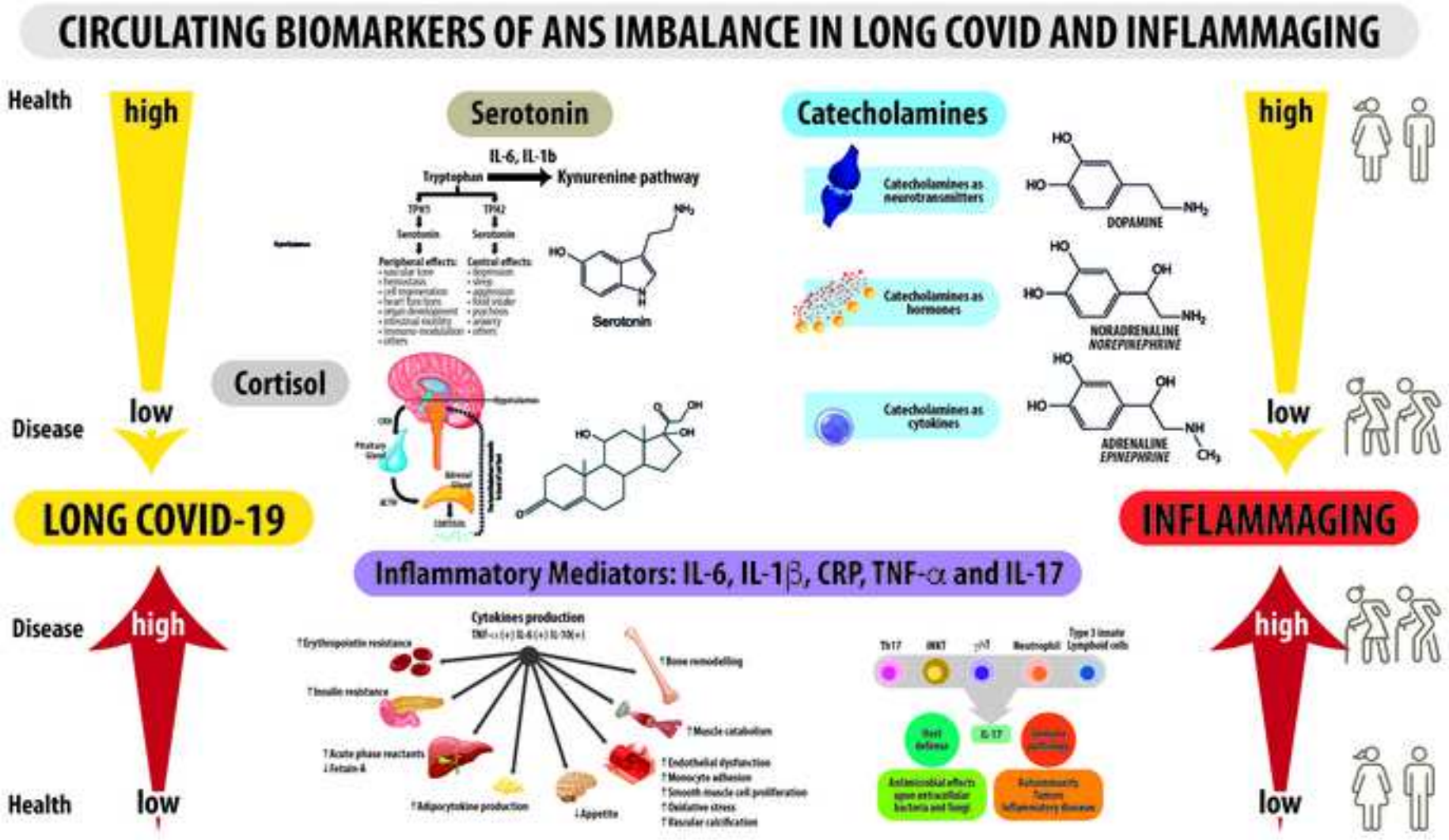


Figure 2



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Sergio Giunta[1], Chiara Giordani[2], Maria De Luca[3], Fabiola Olivieri [2,4]

[1] Casa di Cura Prof. Nobili (Gruppo Garofalo (GHC) Castiglione dei Pepoli -Bologna) Italy.

[2] Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy

[3] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.

[4]Department of Clinical and Molecular Sciences, DISCLIMO, Università Politecnica delle Marche, Ancona, Italy.

Corresponding author: Giordani Chiara

Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy

e-mail: c.giordani@inrca.it

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1. Introduction

Post-Acute Sequelae of SARS-CoV-2 (PASC), known colloquially as Long COVID (LC), encompasses a plethora of debilitating symptoms that can occur in people with varying degrees of COVID-19 severity. Even if an elevated prevalence of physical symptoms was observed particularly among individuals suffering a severe acute illness **(Shen et al., 2023)**, also patients who have had mild or asymptomatic SARS-CoV-2 infection can develop LC **(Brightling et al., 2022; Nasserieet al., 2021)**. These symptoms, which include fatigue, breathlessness, heart palpitations, dizziness, orthostatic intolerance, and syncope, could signal a dysfunction of the autonomic nervous system (ANS), a condition defined as dysautonomia **(Larsen et al., 2022; Jammoul et al., 2023).** The ANS is the involuntary branch of the peripheral and central nervous system, consisting of anatomically distinct divisions, including the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), acting typically in opposition to regulate automatic physiological functions, such as breathing, heartbeat, blood pressure, respiratory rate, and digestion (**LeBouef et al., 2023)**. The enteric nervous system (ENS), innervating the gastrointestinal tract, is considered a separate branch of the ANS (**Valdetaro et al., 2023**).

Direct and long-lasting effects in tissue and organs elicited by the ANS are achieved through the action of neurotransmitters, including catecholamines, like dopamine, norepinephrine, and epinephrine (**Gómez-Moyano et al., 2023)**. Additionally, the ANS neurotransmitters control the organisms' physiological response to acute and chronic stress in parallel with the hypothalamic-pituitary-adrenal (HPA) axis, whose activation results in increased cortisol plasma levels **(Ulrich-Lai et al., 2009)**. The dysfunction of both the ANS and the HPA axis is conducive to the development of several symptoms observed in most LC patients, in association with changes in catecholamines and cortisol circulating levels **(Yavropoulou et al., 2022)**. In fact, the inability of the HPA axis to recover after acute COVID-19, likely due to protracted stress in individuals predisposed to increased vulnerability to stress, was associated with hypocortisolemia and has been

suggested as a potential mechanism behind the LC-associated clinical and immunological manifestations (**Yavropoulou et al., 2022**).

Several hypotheses have been formulated to explain the autonomic dysfunction in LC syndrome pathophysiology, including persistent virus or viral remnants, latent viral reactivation, autoimmunity, dysbiosis, and unrepaired tissue damage (**Goldstein, 2020)**. Although the biological mechanisms remain largely unclear, a growing body of evidence suggests that chronic, systemic, and age-related inflammation, *i.e.* inflammaging, might contribute to the development and persistence of LC symptoms, as well as the LC relationship with ANS dysautonomia **(Giunta et al., 2023; Zazzara et al., 2022)**. It is well-established that both aging and the presence of comorbidities, such as hypertension, dyslipidemia, cardiovascular disease, metabolic and endocrine dysregulation, and type 2 diabetes mellitus (T2DM), are major risk factors for developing COVID-19 severe outcomes, and LC syndrome **(Mansell et al., 2022)**. As such, we previously proposed that ANS imbalance can contribute to fueling a vicious circle resulting in increased inflammaging levels **(Giunta et al., 2023)**. In this review, we will draw attention to the growing literature supporting the idea that inflammaging can be regarded as the culprit behind the most severe outcomes of age-related diseases **(Fulop et al., 2018)** and acute COVID-19 infection **(Bonafè et al., 2020)**, and we will hypothesize that it also plays a role in LC development. In this integrated view, biomarkers of ANS imbalance associated with LC onset could also be considered biomarkers of inflammaging and their monitoring could contribute not only to the diagnosis of LC but also to estimating the risk of an unsuccessful aging trajectory.

2. Inflammaging and Long-COVID

A healthy longevity is characterized by a balance between pro-inflammatory and anti-inflammatory strategies (**Franceschi et al., 2007**). A pro-inflammatory tendency can confer high resistance against infectious diseases but, on the other hand, may increase susceptibility to inflammation-based diseases throughout life. An anti-inflammatory trend, instead, may cause an increased susceptibility

to infections in pre-reproducing life, but it is associated with a reduced risk of developing the most common age-related diseases later in life.

The age-related pro-inflammatory state, called 'inflammaging' is characterized by a chronic and systemic age-related increase in plasma levels of pro-inflammatory mediators **(Franceschi et al., 2000**). Slightly altered cytokine levels by innate immunity are known to be associated with homeostasis imbalances **(Giunta et al., 2022).**

Macrophages are intimately involved in initiating and resolving inflammation. Among the age-related changes that occur in macrophages are a heightened state of basal inflammation and diminished or hyperactive inflammatory responses, which are driven by metabolic-dependent epigenetic changes **(Yarbro et al., 2020; van Beek et al., 2019).**

Macrophage chronic activation is a primary contributor to inflammaging, and it has been observed in the most common age-related diseases **(Prattichizzo et al., 2016; Yarbro et al., 2020)**.

A strong monocyte activation has also been observed in LC patients and it has been found associated with the severity of LC-related fatigue **(Berentschot et al., 2023)**. Furthermore**,** it has been reported that SARS-CoV-2 infection leads to a prolonged increase in the pro-inflammatory transcriptional status of circulating immune cells, including monocytes, with the persistent activation of NF-κB playing a vital role in the transcriptional reprogramming **(J. Y. Zhang et al., 2023)**.

In the largest COVID-19 post-hospital cohort with systemic inflammatory profiling to date, inflammatory mediators consistent with persistent lung and systemic inflammation were increased in patients affected by severe and moderate LC symptoms **(Sykes et al., 2023**). The persistence of a systemic, low-level inflammatory profile in patients affected by LC resembles “inflammaging”.

It is important to highlight that the anti-inflammatory effects of the cholinergic anti-inflammatory pathway (CAP), which is involved in the control of peripheral immunity by the brain, rely upon the activation of the vagus nerve, the main mediator of the PNS **(Rosas-Ballina et al., 2015)**. The acetylcholine released by the vagus nerve endings binds to α7 nicotinic receptors expressed on

cytokine-producing macrophages and elicits signaling pathways that downregulate their inflammatory actions by controlling the activity of the transcription factor NF-κB **(Rosas-Ballina et al., 2015)**.

Together, these observations suggest that insufficient vagal activity related to viral infection sequelae, could contribute to both inflammaging **(Jiang et al., 2022)** and LC symptoms **(Queiroz et al., 2022)**.

Most LC patients are younger to middle-aged women (70-80%), suggesting that viral infections can fuel inflammaging in the setting of young subjects, especially in women, characterized by reduced basal levels of inflammaging (**Olivieri et al., 2023**). Notably, women have higher total life expectancies than men, but they also have an increased risk of living an unhealthy longevity. Intriguingly, sex-dependent differences in inflammaging were highlighted, suggesting sex differences in immune responses and cellular senescence, and the complex effects of such mechanisms on total life expectancy and disability-free life expectancy **(Olivieri et al., 2023)**.

This complex reasoning leads to the hypothesis that this setting population, young women affected by LC, should be extensively monitored for the development of the most common age-related diseases later in life. In this framework, the biomarkers of ANS imbalance associated with LC onset could be considered biomarkers of inflammaging, and their monitoring could contribute to estimating the risk of an unsuccessful aging trajectory.

3. Long-COVID and autonomic nervous system dysfunction: a complex neuro-immune-endocrine scenario

There is now increasing evidence that autonomic-immune-endocrine dysregulations are involved in the clinical manifestations of LC **(Mehandru and Merad, 2022)**. Although this area of research is still evolving, focusing on the multifaceted relationship of LC with the PNS and SNS and its effects on the anti- and pro-inflammatory states will provide valuable insights into the pathogenesis of LC **(Jammoul et al., 2023)**. Further research will pave the way for not only characterizing the

autonomic-immune-endocrine profile associated with LC but also for identifying relevant biomarkers (Choutka et al., 2022). A bi-directional relationship exists between the neuro-immune-endocrine system and LC so that many of the generalized LC symptoms overlap with: i) symptoms of postural orthostatic tachycardia syndrome (POTS), a variety of dysautonomia, which is characterized by chronic symptoms that occur with standing and a sustained increase in heart rate, without orthostatic hypotension **(Reis Carneiro et al., 2023)**; notably, a potential POTS association with COVID-19 vaccination was also observed, even if it seems weaker than with COVID-19 infection **(Fanciulli et al., 2023)**; ii) HPA-insufficiency, *i.e*. low-levels cortisol **(Yavropoulou et al., 2022)**; iii) CAP impairment, *i.e.* reduced anti-inflammatory endogenous strategies with consequent inflammation overdrive **(Giunta et al., 2023)**.

Numerous pathophysiological mechanisms have been proposed for autonomic impairment caused by SARS-CoV-2: direct tissue damage, immune dysregulation, hormonal disturbances, persistent low-grade infection, invasion of the virus into the brain, elevated cytokine levels due to immune reactions leading to chronic inflammation **(Gómez-Moyano et al., 2023)**. All the proposed mechanisms can converge on vagus nerve-reduced activity, the branch of ANS with anti-inflammatory abilities **(Giunta et al., 2023)**. SARS-CoV-2 RNA has been detected in the vagus nerve together with inflammatory cell infiltration, composed primarily of monocytes, suggesting that SARS-CoV-2 can fuel inflammation and impair autonomic function **(Woo et al., 2023)**. In this regard, a recent histopathological study showed that the known SARS-CoV-2 entry receptors on host cells, angiotensin-converting enzyme 2 (ACE2), neuropilin 1 (NRP1), and transmembrane protease serine subtype 2 (TMPRSS2) are expressed on the vagus nerve **(Gómez-Moyano et al., 2023; Vitale-Cross et al., 2022)**. SARS-CoV-2 RNA has been identified at the entry site of the vagus nerve into the brainstem of COVID-19 patients, which implies that SARS-CoV RNA is detectable along the entire route of the vagus nerve **(Radke et al., 2023; Vitale-Cross et al., 2022)**. Overall, it has been hypothesized that SARS-CoV-2 infects the vagus nerve resulting in an

inflammatory response that might subsequently lead to dysautonomia during acute COVID-19 and LC **(Andersson and Tracey, 2023)**.

Increased levels of biomarkers of axonal damage, *i.e.* neurofilament light (NfL), were observed in acute COVID-19 patients **(Meinhardt et al., 2021).** A recent systematic review focused on blood biomarkers of LC highlighted that LC patients with neurological symptoms exhibited higher levels of NfL chain and glial fibrillary acidic protein, suggestive of persistent axonal damages in LC **(Marchegiani et al., 2023)**.

4. Clinical tools for the diagnosis of ANS imbalance in Long COVID-19 in the framework of inflammaging

There are several diagnostic tools to monitor ANS dysfunction, especially in the cardiovascular field, such as active standing test, tilt testing, deep breathing, Valsalva test, 24-hour blood pressure monitoring, and prolonged ECG monitoring including heart rate variability (HRV), to name the most important ones, and some composite test scores, *i.e.* the Composite Autonomic Symptom Score **(Anaya et al., 2021; Lai et al., 2023; Xie et al., 2022)**. We will focus our discussion on HRV, since, even if a relationship between inflammation and HRV was highlighted, the hypothesis of HRV as a biomarker of inflammaging was not extensively investigated. Even if HRV has the potential to aid in early inflammatory response detection and tracking, studies evaluating inflammatory markers and HRV are still scarce and should be encouraged.

Our analysis will be focused also on some blood biomarkers, such as catecholamines, serotonin, and cortisol, that can be considered as biomarkers of impaired ANS in LC, and remarkably they are all associated with inflammatory conditions **(Hüfner et al., 2023)**.

4.1 Heart rate variability as a biomarker of ANS imbalance in Long COVID-19 in the framework of inflammaging

HRV, which is the measurement of the fluctuations in time intervals between successive heartbeats **(Thomas et al., 2019)**, is a well-established marker of ANS function, both sympathetic and parasympathetic, and relevant correlations with the modulation of inflammatory conditions have been highlighted **(Giunta et al., 2023)**.

The HRV measurement approach includes over 60 metrics as well as the application of machine learning, possibly deep learning, to detect a signature of chronic or past inflammation **(Frasch, 2022)**. In a metanalysis on HRV and inflammation in human studies, the standard deviation of R-R intervals and power in the high-frequency band of HRV (HF-HRV) showed the strongest and most robust associations with inflammatory markers compared to other time- and frequency-domain measures of HRV **(Williams et al., 2019)**. Lower HRV, particularly vagal HRV, was associated with higher levels of inflammation **(Williams et al., 2019)**. Reduced HRV, which reflects sympatho-vagal imbalance was associated with cardiovascular risk factors, such as physical inactivity, hypertension, diabetes **(Soares-Miranda et al., 2014)** and cardiovascular disease (CVD), as well as neurological conditions **(Arakaki et al., 2023; Soares-Miranda et al., 2014; Yugar et al., 2023)**.

Recently, HRV was proposed as a suitable tool for diagnosing post-COVID dysautonomia. The main advantages of HRV monitoring are that it generates a quantitative score, it is non-invasive, its application is independent of consciousness or cognitive function, it does not rely on patient recall or honesty, and it has been robustly validated in clinical practice as an indicator of autonomic function **(Faust et al., 2022)**. A pilot study has validated HRV analysis as a predictor for the inflammatory and autonomic state of post-COVID patients by using short ECG recordings and AI processing, making it a potentially powerful tool for diagnosing long-COVID dysautonomia and predicting related cardiovascular dysfunction **(Shah et al., 2022)**. Long COVID results in lower HRV in association with higher IL-6 circulating levels, the key circulating mediator of inflammaging **(Marques et al., 2023).** Reduced HRV and reduced HR changes in response to daily activities were reported also in frail subjects **(Arantes et al., 2022).**

Figure 1 summarizes the potential relationship between reduced HRV and both LC and increased inflammaging.

Overall, subjects affected by autonomic imbalance are at increased risk of developing ARDs, severe outcomes of COVID-19 acute infection, as well as COVID-19 sequelae *i.e.* LC, supporting our hypothesis that HRV could be a useful biomarker of inflammaging.

Considering that: i) T2DM is associated with an overall decrease in the HRV (**Ceriello and Prattichizzo, 2021**), ii) T2DM patients are also characterized by a more pervasive status of inflammaging (**Prattichizzo et al., 2020);** iii) COVID-19 increases the risk of developing T2DM (**Xie and Al-Aly, 2022; Rathmann et al., 2022**), a result confirmed by recent systematic reviews (**Bellia et al., 2023; Harding et al., 2022**), and iv) LC syndrome has a high incidence in patients with T2DM **(Mittal et al., 2021);** T2DM could be highlighted as a prototypical condition that could eventually benefit by the use of ANS imbalance markers to predict the LC course or other complications related to COVID-19 disease. Some emerging evidence shows that vagal nerve activation may reduce biomarkers and processes related to diabetes (**reviewed in Sorski and Gidron, 2023),** thus supporting our hypothesis.

4.2 Circulating biomarkers of ANS imbalance in Long COVID-19 in the framework of inflammaging

It has been recently postulated that alterations in both dopamine and serotonin levels might be involved in COVID-19 pathophysiology, thus supporting the hypothesis that short- and long-term sequelae in COVID-19 patients could be explained, at least in part, by dysregulation of neurotransmitters **(Attademo L, 2021)**.

4.2.1 Catecholamines

Catecholamines, such as dopamine, adrenaline (epinephrine), and noradrenaline (norepinephrine), are neurotransmitters in the central and peripheral nervous systems as well as hormones in the

endocrine system, involved in the "fight or flight" response. The adrenal medulla and the post-ganglionic fibers of the SNS are the main sites of production, storage, and release of catecholamines **(Flierl et al., 2007)**.

Catecholamines can lead to the release of cytokines and *vice versa* and a marked increase in serum catecholamines levels was observed during the acute phase of critically COVID-19, in association with increased inflammatory parameters **(Dammassa et al., 2023)**. This is not unexpected since catecholamines are produced in abundance when the body is under severe stress, *i.e.* sepsis. In recent years, the role of dopamine in inflammatory diseases has been extensively studied, focusing mainly on immune cells, the NLRP3 inflammasome, and the NF-κB pathway **(Feng and Lu, 2021)**. Catecholamines have been shown to modulate immune cell responses leading to increased anti-inflammatory and blunting of pro-inflammatory effects (**Thoppil et al., 2023**).

Recently, it was reported that SARS-CoV-2-dependent inflammation in LC is associated with changes in the activity of the kynurenine and catecholamines pathways, and these changes may lead to defective systemic availability of both dopamine and serotonin **(Hüfner et al., 2023)**. Preliminary *in vitro* observations suggest that the dopamine metabolism and production in iPSC-derived dopaminergic neurons are reduced by SARS-CoV-2 infection, partially explaining some of the neurological symptoms manifested **(Cappelletti et al., 2023).**

Dopaminergic activity decreases in normal aging, with an exacerbated decrease observed in Parkinson's disease (PD) **(Luo and Rhot, 2000; Krashia et al., 2019; Warren et al., 2017)**. Oxidative stress and neuroinflammation, including activation of NADPH-dependent oxidases, play a major role in the progression of dopaminergic cell death, thus contributing to reduced dopamine metabolism and production **(Labandeira-Garcia et al., 2012)**.

Interestingly, elevated levels of circulating syndecan have been recently associated with increased blood pressure and 24-hour urine levels of noradrenaline and dopamine in healthy women, suggesting that syndecan could have a role in sympathoadrenal activation **(De Luca et al., 2021)**. A wealth of evidence revealed that members of the syndecan family of cell surface heparan sulfate

proteoglycans contribute to the cellular entry of SARS-CoV-2 in cooperation with ACE2 **(Bermejo- Jambrina et al., 2021; Hudák et al., 2021)**. Accordingly, serum syndecan was reported to correlate with increased mortality in COVID-19 patients **(Q. Zhang et al., 2023)**.

4.2.2 Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter that is biosynthesized from the essential amino acid L-tryptophan primarily in the enterochromaffin cells of the intestinal mucosa and in neurons of the hindbrain raphe area **(Layunta et al., 2022; Raghupathi et al., 2013)**. Its main receptors and their subtypes are expressed in many tissues and organs throughout the body; therefore, 5-HT plays a vital physiological role in humans by regulating numerous biological processes, including behavior, mood, memory, and gastrointestinal homeostasis **(Berger et al., 2009)**. Additionally, within the central nervous system, 5-HT holds an integrative role in the behavioral and neuroendocrine stress response **(Puglisi-Allegra and Andolina, 2015)**. The possible involvement of the neurotransmitters, serotonin and dopamine, in COVID-19 is suggested by a significant link between Angiotensin II Converting Enzyme 2 (ACE2), the main receptor to SARS-CoV-2, and Dopa Decarboxylase (DDC), the enzyme that catalyzes the biosynthesis of dopamine, serotonin, and histamine. It was hypothesized that a SARS-CoV-2-induced defective expression of ACE2 might be paralleled by a DDC dysfunction, with consequent potentially altered levels of neurotransmitters in COVID-19 patients **(Berger et al., 2009)**.

Some studies recently confirmed this hypothesis providing evidence of the regulation of catecholamine-signaling by cytokines released during SARS-CoV-2 infection and by neuroinflammation associated with LC **(Mancini et al., 2023)**. Reduced circulating serotonin levels were recently observed in LC patients in association with inflammatory conditions **(Wong et al., 2023)**. In LC patients, it was also observed an increased innate cell activation, with myeloid cells displaying an activated phenotype, as well as an increased production of type I and type III interferons **(Phetsouphanh et al., 2022)**. Persistent interferon abnormalities are perhaps not

surprising, given the key role played by dysfunctional interferon responses in the development of severe acute COVID-19 **(Bastard et al., 2021; Smith et al., 2022)**. Viral infection and type I interferon-driven inflammation could contribute to reducing serotonin through different mechanisms, such as diminished intestinal absorption of tryptophan and enhanced monoamine oxidase (MAO)-mediated serotonin turnover **(Wong et al., 2023)**. Intriguingly, peripheral serotonin reduction, in turn, can impede the activity of the vagus nerve impairing hippocampal responses, memory, and anti-inflammaging strategies, such as the activation of CAP. These findings provide a possible explanation for the neurocognitive symptoms associated with LC and suggest that at least a subset of LC patients might respond to therapies that activate the serotonin pathway.

A recent trial on LC patients treated with selective serotonin reuptake inhibitors (SSRIs), demonstrated that two-thirds of patients had a reasonably good to strong response to SSRIs, over a quarter of patients had a moderate response, while 10% reported no response **(Rus et al., 2023)**. Overall, patients experienced substantially improved well-being, since brain fog and sensory overload decreased most, followed by chronic fatigue and dysautonomia **(Rus et al., 2023)**.

Both dopamine and serotonin are associated with the modulation of inflammatory conditions. The main link between serotonin and inflammation appears to be the kynurenine pathway which interacts extensively with the immune system **(Tsuji et al., 2023).** The catabolism of tryptophan has two major enzymatic pathways. The first pathway is the serotonin pathway, which is mediated by tryptophan hydroxylase and results in the generation of serotonin or 5-hydroxytryptamine (5-HT), a precursor of melatonin. In the second pathway, tryptophan is catabolized to kynurenine, subsequently metabolized in the neuroprotective kynurenic acid, and in the neurotoxic quinolinic acid (QA) (**Tsuji et al., 2023**). Inflammation shifts the metabolism of tryptophan from serotonin to the kynurenine pathway, a mechanism that plays a key role in inflammaging, **(Sorgdrager et al., 2019)**. Interestingly, the kynurenine pathway is overactive in LC patients; thus, sustaining inflammation and leading to low tryptophan levels.

4.2.3 Cortisol

Of all the factors associated with the risk of LC, abnormally low cortisol levels were highlighted as the biomarker that truly can contribute to differentiating the LC patients from the healthy controls, even if the levels of its trigger, ACTH, could be normal **(Yavropoulou et al., 2022)**. Notably, ACTH has an extremely short half-life in plasma, which may impair change detection accuracy. An earlier study on survivors of SARS-CoV infection showed evidence of hypocortisolemia and blunted ACTH responses three months after acute disease **(Harris et al., 2019)**. Furthermore, decreased cortisol levels during the early phases of COVID-19 were associated with the development of respiratory LC symptoms **(Su et al., 2022)**. It was hypothesized that the inability of the HPA axis to recover after the acute illness, perhaps due to protracted stress in predisposed individuals, may represent the pathogenetic basis of the LC-associated clinical and immunological manifestations **(Klein et al., 2021; Yavropoulou et al., 2022)**. Different mechanisms potentially involved in HPA axis dysfunction in survivors of SARS-CoV infection can be highlighted, such as critical illness-related corticosteroid insufficiency, immune-mediated inflammations, the resistance of cortisol receptors, impaired post-receptor signaling, as well as the direct cytopathic impacts of SARS-CoV-2 infection on the adrenals, pituitary, and hypothalamus **(Jensterle et al., 2022)**. The adrenal cortex produces ACE2 receptors and the binding of SarsCov-2 to the adrenal ACE2 receptors may lead to direct damage to cells within the adrenal cortex, resulting in reduced cortisol production. Autopsy studies in patients with COVID-19 revealed necrosis of the adrenal cortical cells and identified the virus in the adrenal glands **(Salzano et al., 2021)**. As cortisol is central to a variety of homeostatic and stress responses, the current finding of persistently lower cortisol levels in those with LC more than a year after acute infection warrants further investigations **(Husebye et al., 2021)**.

Inflammaging, characterized by elevated cytokine production, is associated with dysfunctions of the HPA axis and diminished serum corticosteroid levels, both in animal models and humans

(Valbuena Perez et al., 2020). The reduced circulating cortisol levels might contribute to the imbalance between pro- and anti-inflammatory signaling in aged macrophages, thus promoting inflammaging. Increased fatigue severity associated with stronger signs of monocyte activation in LC patients suggested monocyte-endothelial interaction **(Berentschot et al., 2023)**.

4.2.4 Inflammatory mediators

The importance of measuring inflammatory biomarkers in patients hospitalized with COVID- 19 is well-established **(Zeng et al., 2020)**. Many serum biomarkers have been studied in relation to COVID- 19, primarily interleukin- 6 (IL- 6), a pro- inflammatory cytokine produced by macrophages that has been shown to play a role in the pathophysiology of COVID- 19, particularly in severe cases **(Bonafè et al., 2020; Coomes and Haghbayan, 2020)**.

However, there is a relative dearth of studies examining the association of admission inflammatory profiles with long- term COVID-19 symptoms. A recent systematic review on blood biomarkers associated with LC symptoms, identified up-regulated IL-6, CRP, and TNF-alpha as the potential diagnostic biomarkers for LC **(Marchegiani et al., 2023; Zazzara et al., 2022)**. Notably, IL-6 is the most prominent cytokine that is shared across age-related disease conditions, so its serum levels predict incident disability and frailty **(Maggio et al., 2006)**. As such, IL-6 is a hallmark of chronic morbidity and a commonly used biomarker of inflammaging **(Franceschi et al., 2007)**.

Some studies highlighted some other inflammatory circulating mediators deregulated in LC patients, suggesting that elevated serum levels of IL-17 and IL-2 and low levels of IL-4 and IL-10 could be the signature of LC **(Queiroz et al., 2022)**.

Different studies confirmed that increased IL-17 levels, and cytokine playing roles in human autoimmune and neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson’s disease, multiple sclerosis, and amyotrophic lateral sclerosis, are associated with complicated LC evolution **(López-Hernández et al., 2023)**. Although the specific mechanism of IL-17 in neurodegenerative diseases is still controversial, it is generally accepted that this cytokine can cause

diseases by activating glial cells. Notably, IL-17 antibodies reduced neuroinflammation and behavioral symptoms induced by Aβ in animal models, suggesting a possible future therapeutic approach in patients with AD **(Cristiano et al., 2019)**.

A single study on a limited number of LC patients reported a significant reduction in circulating levels of different cytokines, thus suggesting immune exhaustion as the driver of LC, with the complete absence of IFNγ and IL-8 preventing the lungs and other organs from healing after acute infection, and reducing the ability to fight off subsequent infections, both contributing to the myriad of symptoms suffered by those with long-COVID **(Williams et al., 2022)**.

Overall, despite the ongoing debate on the involvement of inflammatory/vascular processes with prolonged COVID- 19 symptoms, IL-6, CRP, TNF-alpha, and IL-17 circulating levels could be potential biomarkers of LC, associated with inflammaging levels.

Figure 2 summarizes the potential relationships between circulating levels of serotonin, catecholamines, cortisol, and inflammatory cytokines and both LC and increased inflammaging.

5. Conclusion

A dynamic interplay exists between inflammaging and SARS-CoV-2 infection that involves both acute inflammation and low-grade chronic inflammation, thus predisposing patients not only old but also younger and middle-aged, to severe COVID-19 outcomes and sequelae. In the post-acute phase, some patients show an accelerated decline in physical, cognitive, and functional abilities that resemble ANS imbalance. Evidence suggests that cytokine levels are still increased in patients with long COVID and the persistence of chronic inflammation, *i.e.* an increased level of inflammaging, could be one of the mechanisms that might explain symptom persistence in patients.

In this very complex scenario, we suggest that biomarkers of ANS imbalance associated with the onset of post-COVID sequelae like LC, such as low cortisol levels, low serotonin levels, and reduced HRV, could be considered also biomarkers of inflammaging. In this framework, their monitoring could contribute to estimating not only the risk of LC but, more in general, the risk of

trajectories of unsuccessful aging. The diagnostic utility of the selected biomarkers should be evaluated in longitudinal studies.

Figures legends

Figure 1 summarizes the potential relevance of ANS imbalance both in LC and inflammaging, focusing on the reduced HRV.

Inflammaging is the chronic, proinflammatory, systemic and subclinical condition that underpins the most common age-diseases and conditions, such as cardiovascular diseases, type 2 diabetes, neurodegenerative diseases, cancer, and frailty.

Reduced HRV is inversely associated with increased plasmatic IL-6 levels, the key cytokine of inflammaging. Reduced HRV could be proposed as a minimally invasive biomarker of inflammaging.

HRV: heart rate variability; LC: long Covid-19.

Figure 2 summarizes the potential relevance of some circulating biomarkers of ANS imbalance in Long COVID and inflammaging.

LC and inflammaging are both characterized by: dysfunctional HPA axis activities associated with diminished serum corticosteroid levels; shift of tryptophan metabolism from serotonin to kynurenine pathway, thus resulting in lower serotonin levels; decreased dopaminergic activity and increased levels of circulating inflammatory mediators, such as IL-6, IL-1β, CRP, TNF-α and IL-17. Reduced circulating levels of cortisol, serotonin, and catecholamines associated with increased levels of circulating inflammatory parameters can be proposed as minimally invasive biomarkers of inflammaging,

The selected circulating biomarkers are all associated with autonomic dysfunction and with anti-inflammatory impairment (anti-inflammaging), related to reduced HPA axis and reduced CAP activities.

ANS: autonomic nervus system; LC: long Covid-19; CRP: C reactive protein; HPA axis: hypothalamic-pituitary-adrenal axis; CAP: cholinergic anti-inflammatory pathway.

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Declaration of Competing Interest

The authors state no conflict of interest.

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Long-COVID-19 autonomic dysfunction: an integrated view in the framework of inflammaging

Sergio Giunta[1], Chiara Giordani[2], Maria De Luca[3], Fabiola Olivieri [2,4]

[1] Casa di Cura Prof. Nobili (Gruppo Garofalo (GHC) Castiglione dei Pepoli -Bologna) Italy.

[2] Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy

[3] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.

[4]Department of Clinical and Molecular Sciences, DISCLIMO, Università Politecnica delle Marche, Ancona, Italy.

Corresponding author: Giordani Chiara

Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy

e-mail: c.giordani@inrca.it