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Early View

Editorial

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Elena Ortona, Walter Malorni

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EDITORIAL

Long COVID: to investigate immunological mechanisms and sex/gender related aspects as fundamental steps for a tailored therapy

Elena Ortona[1] and Walter Malorni[2]

1. Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome, Italy
2. Center for Global Health, Università Cattolica del Sacro Cuore, Rome, Italy

Correspondence to:

Prof. Walter Malorni
Center for Global Health
Università Cattolica del Sacro Cuore, Rome, Italy
E-mail: Walter.Malorni@unicatt.it

SHORT SENTENCE: *Long COVID was detected in both adults and children and is characterized by* immunological dysregulation. *Autoimmune reactions in adult patients and allergic reactions in children appear as critical factors.*

Around a quarter of people who have had COVID-19 experience symptoms that continue for at least a month but one in ten are still unwell after 12 weeks. This very debilitating condition has been defined by patient groups as "Long COVID" elsewhere called post-COVID, whereas the patients are frequently called COVID-19 long-haulers [1]. Long COVID has a serious impact on patient ability to go back to work or school, to have a social life and may have significant economic consequences for patients, their families and for society. The condition is characterized by long-term sequelae and can involve a range of about 200 different and overlapping symptoms such as persistent fatigue, chest and muscle pain, headache, shortness of breath, anosmia, muscle weakness, fever, cognitive

dysfunction (brain fog), tachycardia, intestinal disorders and skin manifestations. It can affect anyone but women appear to be twice as likely to develop Long COVID as men, but only until around age 60, when the risk level becomes similar [2-4]. Long COVID has also been described in paediatric age [5]. An Italian study reported that at least one symptom persisted 4 months after COVID-19 infection [6] whereas an Australian analysis suggested that only 8% of children had ongoing symptoms 3-6 months after mild SARS-Cov-2 infection [7]. No gender difference was observed in the prevalence of Long-COVID in this population [5].

Long COVID etiology. The Long COVID syndrome has a similarity to the post-infectious syndromes that followed the outbreaks of chikungunya [8] and Ebola [9]. What are the factors responsible for this syndrome? They could be many and different. Organ damage caused by an excessive inflammatory response activated by the virus, persistent reservoirs of SARS-CoV-2 in certain tissues that could trigger post-infection morbidity, re-activation of pathogens due to immune dysregulation, host microbiome alterations, clotting/coagulation issues and autoimmunity due to molecular mimicry between SARS-CoV-2 and self-proteins have been hypothesized to play a role [10]. Furthermore, it has also been suggested that Long COVID symptoms may not be a direct result of the SARS-CoV-2 infection but may be the consequence of COVID-19 inflammation-induced Epstein Bar virus reactivation [11]. The search for fine molecular mechanisms underlying Long COVID is also underway. Some insights derive from metabolic studies, e.g. an altered tryptophan absorption and metabolism that could underlie the post-infection disease [12], whereas further hypotheses derive from cytopathological studies that suggest that SARS-CoV-2 could be capable of hindering autophagic processes of host cells thus favouring the "journey" of viral particles inside the cell cytoplasm and their survival. As a consequence, it has been argued that modulators of cellular metabolism or agents bolstering autophagy could represent a therapeutic strategy against post-COVID sequelae helping to eliminate viral particles or fragments potentially immunogenic and active [13]. However, to date, the plethora of different Long COVID symptoms and disturbs seems to indicate that different concurrent mechanisms could be involved

(**Figure 1**) and that different therapeutic approaches should be settled in order to cure these patients.

Long COVID in children. As concerns paediatric population, clinical data on Long COVID-19 are limited. The most common complications were muscle and/or joint pain, headache, chest pain or a feeling of chest tightness, palpitations, respiratory problems and sleep disturbances [5, 6]. In their study, Osmanov and co-workers [5], thanks to a standardized follow-up data collection protocol developed by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) [https://isaric.org/research/covid-19-clinical-research- 498 resources/paediatric-follow-up/], observed that older age and allergic diseases were associated with higher risk of persistent symptoms at follow-up. Osmanov and colleagues suggested that, at least in children, immunological mechanisms may be responsible for an increased risk of long-term consequences of infection. In particular, COVID-19 sequelae could be linked with the mast cell activation syndrome and the Th-2 biased immunological response in children with allergic diseases [5]. Importantly, these findings are in line with a recent case report describing an adolescent with persisting symptoms with first documentation of ongoing immune dysfunction and lung perfusion defects after mild COVID-19 [14].

Gender disparity: a "Black hole" for Long COVID. As mentioned above, Long COVID and its symptoms reporting is more frequent by women than men [2]. This is both a gender and sex disparity that is being discussed and underscored from public health policy makers in different terms. One deals with the fact that women frequently pay more attention to their body and to its alterations. This often leads to a more rapid diagnostic and therapeutic intervention. Unfortunately, there is also "a school of thought" sustaining that the Long COVID gender skew could simply represent an artefact: it could just be conceived by "hysterical, middle-aged women". This sexist approach could certainly represent a strong bias from both an ethical and a clinical point of view and it could also impact the healthcare and working rights for female patients with Long COVID.

Actually, females have both innate and acquired immunological responses stronger than males and both genes and hormones are involved in this sex

difference [15, 16]. These sex-based immunological differences contribute to variations in the incidence of autoimmune diseases, higher in females than in males and in susceptibility to malignancies and infectious diseases, more frequent in males than in females, and probably represent the major cause of female prevalence of Long COVID in adults. Finally, as happens with Lyme disease in which the pathogen remains hided and generates higher levels of inflammatory cytokines in women than men [17], it can be hypothesized that fragments of SARS-Cov-2 could remain hidden on reservoirs such as kidney or brain igniting some chronic inflammation-associated cascade giving rise to symptoms such as pain or brain fog experienced by patients with Long COVID. Importantly, as mentioned above, no significant difference was reported to date between youngest male and female patients [5]. This could reinforce the hypothesis that sex hormones and their immunomodulating activity could play a role in adult Long COVID patients [18, 19].

Autoimmunity and Long COVID. It has been observed that, compared with uninfected controls and as for Epstein-Barr virus, cytomegalovirus and human immunodeficiency virus, SARS-Cov-2 infection is associated with the generation of a wide range autoantibodies that can attack tissues of infected subjects [20]. Some of the infected individuals had autoantibodies against proteins involved in several immunological activities including interferon responses, leukocyte trafficking, and lymphocyte function/activation [21]. Other autoantibodies are tissue-specific including autoantibodies specific to blood vessels, heart and brain. The propensity of patients to develop over 15 separate types of autoantibodies and above 10 distinct autoimmune diseases have been observed [22]. The main mechanisms that may contribute to the development of autoimmunity in COVID-19 are the following: i) the hyper-activation the immune system, ii) the induction of excessive neutrophil extracellular traps formation, and iii) SARS-CoV-2 cross-reaction with self-components of the host. In fact, SARS-CoV-2 has been shown to cross-react with gut, kidney, lung, heart, and brain antigens and SARS-CoV-2 proteins can share homology with some self-protein epitopes leading to molecular mimicry paths [22]. Furthermore, under conditions of inflammation, other organisms

of the microbiome/virome communities, that could vary widely between different patients, may also contribute to autoantibody production and cause the great variety of autoantibody reactivity. This complex scenario could explain the significant percentage of clinical variations detected in patients with Long COVID

Conclusions. Long COVID is characterized by specific long-lasting inflammatory/immunological dysregulation and can not be considered as a unique pathology but a huge series of different morbidity states. Hence, a better understanding of the heterogeneity of this pathology, assessing the appearance of autoantibodies in the serum of adult patients, in particular women, and evaluating the Th2 immune response and plasma IgE levels in children could be an important goal to begin to identify personalized and specific treatments for patients with Long COVID.

AUTHORS DECLARE NO CONFLICT OF INTEREST

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Figure 1. Possible concurrent mechanisms leading to Long Covid