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**UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA**

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**Avaliação da resposta inflamatória de
pacientes que desenvolveram COVID
longa.**

Dissertação apresentada ao Programa de Pós-Graduação em Patologia, da Faculdade de Medicina, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Botucatu, como pré-requisito para obtenção do título de Mestre em Ciências, área de concentração em Patologia.

Orientadora: Profa. Dra. Luciane Alarcão Dias-Melicio

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1. Enzima de Conversão de Angiotensina. 2. Síndrome Pós-COVID-19 Aguda. 3. Citocinas. 4. SARS-CoV-2. 5. Receptor 4 Toll-Like.

Palavras-chave: ACE2; COVID longa; Citocinas; SARS-CoV-2; TLR4.

Impacto potencial desta pesquisa

Impacto Científico: A geração de conhecimento na área de COVID Longa auxiliará nos avanços em pesquisa para que haja melhora na capacidade de tratamento e atenção aos pacientes. Encontrar um possível foco terapêutico à partir do entendimento da resposta de pacientes com COVID longa, é a proposta da pesquisa. Por conta do pouco conhecimento nesse campo, não se sabe ainda como tratar as sequelas que a COVID pode deixar, causando impactos de saúde no mundo todo.

Impacto Social: A pesquisa contribui com o bem-estar social, auxiliando no atendimento de saúde de qualidade, visando a diminuição da morbidade causada pela COVID e pelas suas sequelas na população. Abrir o precedente necessário para o teste de um imunobiológico específico pode avançar as condutas que visam melhoras dos sintomas dos pacientes.

Impacto Econômico: Apesar de não gerar renda direta, o avanço na área do tratamento da COVID Longa economizará grandes valores aos cofres dos sistemas de saúde. Isso aliviaria o uso de recursos, que assim podem ser dirigidos à outras causas.

Potential Impact of this Research

Scientific Impact: The generation of knowledge in the field of Long COVID will assist in advancements in research to improve the treatment capabilities and patient care. Identifying a possible therapeutic focus is the primary aim of this research. Due to the limited understanding in this field, it is still unknown how to effectively address the lingering effects that COVID can leave behind, causing health impacts worldwide.

Social Impact: The research contributes to social well-being by aiding in the provision of quality healthcare, aiming to reduce the morbidity caused by COVID and its lingering effects in the population. Creating the necessary precedent for testing a specific immunobiological could advance approaches that target symptom improvement in patients.

Economic Impact: While not directly generating income, progress in the field of Long COVID treatment could save significant amounts for healthcare systems. This would alleviate the burden on resources, which could then be directed towards other causes.

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À minha família: mãe, pai, irmão e esposa. Sem vocês eu não teria tido pelo que continuar.

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RESUMO

A COVID longa é uma doença debilitante, que ocorre em pelo menos 10% das pessoas infectadas pelo SARS-CoV-2. Muitos sintomas diferentes já foram identificados, mas a natureza da doença, sua frequência e principalmente sua etiologia e patogênese ainda não foram bem esclarecidos. Os estudos tem encontrado bastante heterogeneidade sobre os padrões de resposta e possíveis alterações encontradas na imunidade desses pacientes. Parte do problema se dá por conta da difícil seleção desses indivíduos, já que a sobreposição de outras doenças e de sequelas físicas pode dificultar e muito o diagnóstico correto da COVID longa. Por conta dessa dificuldade diagnóstica todos os pacientes do estudo tiveram casos leves e moderados de infecção inicial. Dessa forma, o presente trabalho visa esclarecer se existe diferenças nos padrões de resposta inflamatória desses pacientes, focando na produção de citocinas como IL-2, CXCL10 (IP-10), IL-1 β , TNF- α , CCL2 (MCP-1), IL-17A, IL-6, IL-10, IL-4, IFN- γ , IL-12p70, CXCL8 (IL-8), TGF- β 1; além de buscar identificar a frequência de subpopulações de leucócitos do sangue periférico e avaliação da expressão dos receptores ACE2 e TLR4 em monócitos, neutrófilos, linfócitos TCD4/CD8 e linfócitos B. Nossos resultados mostraram que os pacientes, além de terem aumento na expressão de TLR4 nos linfócitos B, também cursam com uma diminuição de pelo menos metade da população de linfócitos T CD4+ e aumento na população dos linfócitos T CD8+. Encontramos também aumento absoluto na população de monócitos não clássicos redução na expressão de ACE2 na membrana dos monócitos clássicos. Além disso caracterizamos nos pacientes, aumento da IL-6 e da IL17A e valores menores de IL-4, IL-2, IL-1 β , TNF- α , IL-10 e IL-12p70. Sendo assim há uma indicação compatível com a literatura atual, mostrando uma possível resposta inflamatória crônica, com diminuição dos CD4+ e aumento dos monócitos não clássicos. Esse tipo de resposta com IL17A e IL-6 elevada é diferente do padrão aceito atualmente de IL-1 β , IL-6 e TNF- α , mostrando que possivelmente pacientes com gravidades diferentes de infecção inicial podem ter diferentes padrões de citocinas quando cursam com sequelas.

Palavras-chave: SARS-CoV-2; COVID longa; citocinas; ACE2; TLR4.

ABSTRACT

Long COVID is a debilitating disease that occurs in at least 10% of individuals infected with SARS-CoV-2. Many different symptoms have been identified, but the nature of the disease, its frequency, and, most importantly, its etiology and pathogenesis have not yet been well elucidated. Studies have found significant heterogeneity in response patterns and potential alterations in the immunity of these patients. Part of the problem lies in the difficult selection of these individuals, as the overlap with other diseases and physical sequelae can greatly hinder the correct diagnosis of Long COVID. Due to this diagnostic challenge, all patients in the study had mild to moderate cases of initial infection. Thus, the present work aims to clarify whether there are differences in the inflammatory response patterns of these patients, focusing on the production of cytokines such as IL-2, CXCL10 (IP-10), IL-1 β , TNF- α , CCL2 (MCP-1), IL-17A, IL-6, IL-10, IL-4, IFN- γ , IL-12p70, CXCL8 (IL-8), TGF- β 1; as well as to identify the frequency of peripheral blood leukocyte subpopulations and evaluate the expression of ACE2 and TLR4 receptors in monocytes, neutrophils, TCD4/CD8 lymphocytes, and B lymphocytes. Our results showed that patients, in addition to an increase in TLR4 expression in B lymphocytes, also have a decrease of at least half of the CD4+ T lymphocyte population and an increase in the CD8+ T lymphocyte population. We also found an absolute increase in the non-classical monocyte population and a reduction in ACE2 expression on the membrane of classical monocytes. Furthermore, we characterized an increase in IL-6 and IL-17A and lower values of IL-4, IL-2, IL-1 β , TNF- α , IL-10, and IL-12p70 in patients. Thus, there is an indication consistent with the current literature, showing a possible chronic inflammatory response, with a decrease in CD4+ and an increase in non-classical monocytes. This type of response with elevated IL-17A and IL-6 is different from the currently accepted pattern of IL-1 β , IL-6, and TNF- α , indicating that patients with different severities of initial infection may have different cytokine patterns when experiencing sequelae.

Keywords: SARS-CoV-2; long COVID; cytokines; ACE2; TLR4.

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