JANAINA NICOLAU DE OLIVEIRA

ANÁLISE DAS VARIANTES rs1800896, rs1800871 E rs1800872 E HAPLÓTIPOS DO GENE *IL10*, E SUA ASSOCIAÇÃO COM A GRAVIDADE E DESFECHO DA COVID-19

Londrina
2025

JANAINA NICOLAU DE OLIVEIRA

ANÁLISE DAS VARIANTES rs1800896, rs1800871 E rs1800872 E HAPLÓTIPOS DO GENE *IL10*, E SUA ASSOCIAÇÃO COM A GRAVIDADE E DESFECHO DA COVID-19

Tese apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina, como requisito parcial para obtenção do título de Doutora em Patologia Experimental.

Área de Concentração: Imunologia, da Universidade Estadual de Londrina

Orientadora: Prof.ª Dr.ª Karen Brajão de Oliveira

Londrina
2025

JANAINA NICOLAU DE OLIVEIRA

ANÁLISE DAS VARIANTES rs1800896, rs1800871 E rs1800872 E HAPLÓTIPOS DO GENE *IL10*, E SUA ASSOCIAÇÃO COM A GRAVIDADE E DESFECHO DA COVID-19

Tese apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina, como requisito parcial para obtenção do título de Doutora em Patologia Experimental.

Área de Concentração: Imunologia, da Universidade Estadual de Londrina

BANCA EXAMINADORA

Orientadora: Prof. ª Dr. ª Karen Brajão de Oliveira
Universidade Estadual de Londrina – UEL

Prof. Dr. Emerson José Venâncio
Universidade Estadual de Londrina - UEL

Profª. Dra. Roberta Losi Guembarovski
Universidade Estadual de Londrina – UEL

Profª. Dra. Ligia Carla Faccin Galhardi
Universidade Estadual de Londrina – UEL

Profª. Dra. Juliana Mara Serpeloni
Universidade Estadual de Londrina - UEL

Londrina, 17 de março de 2025.

Dedico este trabalho à minha amada mãe, Sueli.

AGRADECIMENTOS

Agradeço imensamente à minha orientadora, Profª. Drª. Karen Brajão de Oliveira, pelas palavras de conforto e compreensão nos momentos difíceis durante esta jornada e por todo o apoio e motivação que recebi desde o momento em que decidi realizar o doutorado, até hoje. Agradeço por ter acreditado em mim, mesmo quando eu mesma não acreditava.

Aos professores do Programa de Pós-Graduação em Patologia Experimental, por compartilhar suas experiências profissionais e conhecimento, e em especial ao Professor Dr. Emerson José Venâncio pelo suporte e incentivo durante minha trajetória acadêmica.

Aos professores que generosamente aceitaram contribuir com esta pesquisa compondo a banca examinadora desta defesa, Dr. Emerson José Venâncio, Dra. Roberta Losi Guembarovski, Dra. Ligia Carla Faccin Galhardi e Dra. Juliana Mara Serpeloni.

Aos colegas do Laboratório de Genética Molecular e Imunologia, Thaílla Pacheco, Sarah Lott Moretto, Rafaela Roberta de Jaime Curti, Caroline Yukari Motoori Fernandes, Bianca Pacheco, Sara Mataroli de Godoy e Wilson Frantine pelo suporte e amizade. À Eliza Pizarro Castilha, pelo companheirismo, amizade e apoio sempre presente nesta trajetória. Às colegas Mariane Ricciardi da Silva, Giulia Mariane Fortunato, Pamella Rodrigues da Silva e Maylla Cardoso de Oliveira pelo comprometimento, amizade e participação primordial no desenvolvimento desta pesquisa. À técnica do Laboratório de Estudos e Aplicações de Polimorfismos e Imunologia, Vânia Darc de Castro, pela amizade e suporte.

Ao HU/UEL e aos professores Andrea Name Colado Simão e Marcell Alysson Batisti Lozovoy pelo auxílio na obtenção das amostras. A todos os participantes que, voluntariamente, se disponibilizaram a integrar esta pesquisa. Aos órgão de fomento, CAPES e CNPq, e a PROPPG-UEL pelo suporte financeiro que permitiram a realização deste projeto.

À minha mãe, Sueli Aparecida Nicolau Pereira, por toda educação, amor, cuidado e sacrifício, que me fortaleceram e me permitiram desfrutar da minha existência e alcançar meus objetivos. À minha avó, Luzia de

Souza Nicolau, que é minha segunda mãe, e preencheu minha infância de carinho. A todos os meus familiares que me apoiam e incentivam sempre, muito obrigada.

Ao meu marido e companheiro de jornada existencial, Lucas Germani Wendt, pelo incentivo, amorosidade e apoio imensuráveis, que fizeram com que esta jornada fosse mais leve e produtiva.

Enfim, à todas as pessoas que por descuido não tenham sido mencionadas, mas que fizeram parte da realização deste projeto.

RESUMO

OLIVEIRA, Janaina Nicolau de. **Análise das variantes rs1800896, rs1800871 e rs1800872 e haplótipos do gene *IL10*, e sua associação com a gravidade e desfecho da COVID-19**. 2025. 95 f. Tese (Pós-Graduação em Patologia Experimental) – Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, 2025.

Gravidade e letalidade são manifestações observadas em parte dos casos de doença do coronavírus-19 (COVID-19), e estão ligadas a fatores como hipercitocinemia, linfopenia e Síndrome Respiratória Aguda Grave (SRAG). Nesse contexto, a IL-10, uma citocina com função imunorreguladora, surgiu como um potencial marcador de gravidade da doença, sendo observada em concentrações elevadas em pacientes com COVID-19. Variações genéticas na região proximal do gene, particularmente variantes de nucleotídeo único (SNVs) como rs1800896 (–1082A>G), rs1800871 (–819C>T) e rs1800872 (–592C>A), juntamente com seus haplótipos GCC, ACC e ATA, estão associadas a diferenças em níveis de IL-10 e suscetibilidade a doenças virais. Embora diversos estudos tenham explorado a relação entre essas variações genéticas e doenças virais, incluindo a COVID-19, os resultados são contraditórios. Desta forma, parte deste estudo foi dedicado a revisar dados da literatura afim de compreender de forma mais abrangente os efeitos destas SNVs e seus haplótipos na produção de IL-10 e em associação com doenças virais. E ainda, este estudo teve como objetivo avaliar a associação desses SNVs e seus haplótipos em pacientes com diagnóstico de COVID-19 em Londrina, Paraná, com a gravidade e os desfechos da doença, visto que nenhum estudo investigou tais associações entre as SNVs e a COVID-19 na população brasileira. Desta forma, este estudo envolveu 367 pacientes selecionados do Hospital Universitário da Universidade Estadual de Londrina (HU-UEL). Os pacientes foram categorizados em grupos denominados leve (n=165), moderado (n=72) e grave (n=130), de acordo com as diretrizes da Organização Mundial da Saúde (OMS). O grupo grave foi ainda classificado nos subgrupos recuperado (n=64) e óbito (n=66). A genotipagem dos SNVs foi feita por reação em cadeia da polimerase (PCR) em tempo real com sondas TaqMan. Os haplótipos foram inferidos pelo software PHASE e as análises de associação foram realizadas com teste Qui-quadrado ou regressão logística multinomial. O genótipo GG (1082 A>G) foi independentemente associado aos casos graves de COVID-19 (P=0,038, OR= 2,522, IC 95% 1,053 – 6,038), enquanto o haplótipo GCC em homozigose também foi associado aos casos graves (P=0,037, OR 2,767, IC 95% 1,065 – 7,191). Portanto, este estudo demonstrou que a presença do genótipo GG da SNV 1082 A>G (rs1800896) ou do haplótipo GCC está associada à gravidade da COVID-19 em uma amostragem brasileira.

Palavras-chave: Coronavírus Relacionado à Síndrome Respiratória Aguda Grave; Polimorfismo de Nucleotídeo Único; Interleucina-10; Haplótipos; Suscetibilidade a infecção viral.

ABSTRACT

OLIVEIRA, Janaina Nicolau de. **Analysis of *IL*10 gene polymorphisms and haplotypes and its association with the severity and outcome of COVID-19.** 2025. 95 p. Thesis (Postgraduate in Experimental Pathology) – Center for Biological Sciences, State University of Londrina, Londrina, 2025.

Severity and lethality are manifestations observed in some cases of coronavirus disease-19 (COVID-19), and are linked to factors such as hypercytokinemia, lymphopenia and Severe Acute Respiratory Syndrome (SARS). In this context, IL-10, a cytokine with immunoregulatory function, has emerged as a potential marker of disease severity, being observed in high concentrations in patients with COVID-19. Genetic variations in the proximal region of the gene, particularly single nucleotide variants (SNVs) such as rs1800896 (–1082A>G), rs1800871 (–819C>T) and rs1800872 (–592C>A), together with their haplotypes GCC, ACC and ATA, are associated with differences in IL-10 levels and susceptibility to viral diseases. Although several studies have explored the relationship between these genetic variations and viral diseases, including COVID-19, the results are contradictory. Therefore, part of this study was dedicated to reviewing data from the literature in order to more comprehensively understand the effects of these SNVs and their haplotypes on IL-10 production and in association with viral diseases. Furthermore, this study aimed to evaluate the association of these SNVs and their haplotypes in patients diagnosed with COVID-19 in Londrina, Paraná, with the severity and outcomes of the disease, since no study has investigated such associations between SNVs and COVID-19 in the Brazilian population. Thus, the study involved 367 patients selected from the University Hospital of the State University of Londrina (HU-UEL). Patients were categorized into groups called mild (n=165), moderate (n=72) and severe (n=130), according to the guidelines of the World Health Organization (WHO). The severe group was further classified into the recovered (n=64) and death (n=66) subgroups. SNV genotyping was performed by real-time polymerase chain reaction (PCR) with TaqMan probes. Haplotypes were inferred by PHASE software and association analyses were performed using the chi-square test or multinomial logistic regression. Demographic factors such as male sex (p=0.002), advanced age (p<0.001) and comorbidities such as systemic arterial hypertension, diabetes mellitus, chronic kidney disease, heart disease (p<0.001) and chronic obstructive pulmonary disease (p=0.007) were associated with COVID-19 severity. Additionally, the GG genotype (1082 A>G) was independently associated with severe cases of COVID-19 (P=0.038, OR 2.522, 95% CI 1.053–6.038). In agreement, the GCC haplotype in homozygosity was also associated with severe cases (P=0.037, OR 2.767, 95% CI 1.065–7.191). Therefore, this study demonstrated that the presence of the GG genotype of SNV 1082 A>G (rs1800896) or the GCC haplotype is associated with the severity of COVID-19 in a Brazilian sample.

Key-words: Severe acute respiratory syndrome-related coronavirus; Polymorphism, Single Nucleotide; Interleukin-10; Haplotype; Viral infection susceptibility.

LISTA DE ABREVIATURAS E SIGLAS

AP-1	*Activator protein-1*
APC	*Antigen- presenting cell*
Blimp-1	*B-lymphocyte-induced maturation protein 1*
BMI	*Body mass index*
c-MAF	*Cellular musculoaponeurotic fibrosarcoma*
COVID-19	*Coronavirus disease-19*
CSIF	*Cytokine synthesis inhibitor factor*
CTL $CD8^+$	*Cytotoxic T lymphocyte*
DM	Diabetes Mellitus
DNA	*Deoxyribonucleic acid*
dsRNA	*Double-strand RNA*
E	Proteína E (envelope viral do SARS-CoV-2)
ECA2	Enzima conversora de angiotensina - 2
EDTA	*Ethylenediamine tetraacetic acid*
FP	*Fusion peptide (SARS-CoV-2)*
GATA-3	GATA *binding protein 3*
HAS	Hipertensão Arterial Sistêmica
HU-UEL	Hospital Universitário da Universidade Estadual de Londrina
HWE	*Hardy-Weinberg Equilibrium*
ICTV	*International Committee on Taxonomy of Viruses*
IFN-I	Interferon do tipo I
IFN-III	Interferon do tipo III
IFN-α	Interferon-alfa
IFN-β	Interferon-beta
IFN-γ	Interferon-gama
IFN-λ	Interferon-lâmbda
IL10	Gene da interleucina-10
IL-6	Interleucina-6
IL-10	Interleucina-10
IL-10Rα	Subunidade alfa do receptor de interleucina-10
IL-10Rβ	Subunidade beta do receptor de interleucina-10
JAK1	*Janus kinase 1*
LES	Lupus Eritematoso Sistêmico
M	Proteína M de membrana (SARS-CoV-2)
MERS	*Middle East Respiratory Syndrome*
MHC	*Major Histocompatibility Complex*
N	Proteína N do nucleocapsídeo (SARS-CoV-2)
NCBI	*National Center for Biotechnology Information*
NFkB	*Nuclear factor kappa-light-chain-enhancer of activated B cells*
NK	*Natural killer cells*
NSP	*Non-structural proteins (SARS-CoV-2)*
NTC	*No template control*
OMS	Organização Mundial da Saúde
OR	*Odds Ratio*
PCR	*Polymerase Chain Reaction*

PKR	*Protein kinase R*
RBD	*Receptor binding domain*
RIG-I	*Retinoic acid-inducible gene 1*
RNA	*Ribonucleic acid*
S	*Spike protein (SARS-CoV-2)*
SARS-CoV	*Severe acute respiratory syndrome associated coronavirus*
SARS-CoV-2	*Severe acute respiratory syndrome coronavírus 2*
SNV	*Single nucleotide variant*
STAT3	*Signal transducer and activator of transcription*
SRAG	Síndrome Respiratória Aguda Grave
Th1	*T helper cell 1*
Th2	*T helper cell 2*
TLR4	*Toll-like receptor 4*
TMRPSS2	*Transmembrane serine protease 2*
TNF-α	*Tumor necrosis factor*
X^2	Teste do Qui-quadrado

SUMÁRIO

Introdução

1 INTRODUÇÃO

Doença do Coronavírus (COVID-19, do inglês *coronavírus disease -19*) foi a denominação determinada pela Organização Mundial da Saúde (OMS) em março de 2020 para a doença causada pelo novo coronavírus, SARS-CoV-2 (do inglês *Severe Acute Respiratory Syndrome coronavirus-2*), inicialmente observada como casos de pneumonia de origem desconhecida, em dezembro de 2019 (Li, J. Y *et al.*, 2020; OMS, 2020a). Com a ampla transmissão do vírus, observou-se que a sintomatologia da doença pode ser numerosa, podendo abranger manifestações de anosmia, ageusia, cefaleia, febre, tosse, dispneia e sintomas gastrointestinais (Huang *et al.*, 2020; Li, L-q. *et al.*, 2020).

Diferentes quadros clínicos decorrentes da COVID-19 foram observados, incluindo casos assintomáticos, de gravidade leve e casos moderados que juntos normalmente contabilizam 81% dos casos, e quadros graves e críticos, que podem atingir 14% e 5% dos indivíduos infectados, respectivamente (Wu, McGoogan, 2020). Os casos de maior gravidade foram associados à idade elevada, ao sexo masculino e à presença de determinadas comorbidades, em especial a hipertensão arterial sistêmica (HAS) e a diabetes *mellitus* (DM) que parecem predispor o indivíduo ao pior prognóstico (Richardson, *et al.*, 2020).

Nos casos graves e críticos pode haver o desenvolvimento de Síndrome Respiratória Aguda Grave (SRAG), choque séptico, disfunção coagulatória e falência múltipla dos órgãos, o que eventualmente culmina no óbito do paciente (*Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment*, 2020). Uma resposta imune equilibrada e eficaz na eliminação viral parece ser de suma importância para a resolução do quadro. Dentre as alterações observadas nos casos graves da COVID-19, a progressão para SRAG é associada ao quadro de hipercitocinemia ou “tempestade de citocinas” (Ye *et al.*, 2020). Em pacientes com COVID-19, Diao *et al.* (2020) observaram uma alta concentração plasmática de citocinas como interferon-gama (IFN-γ), interleucina-6 (IL-6), fator de necrose tumoral-alfa (TNF-α) e interleucina-10 (IL-10) em pacientes com progressão severa da doença. Neste contexto, Han *et al.* (2020) e Zhao *et al.* (2020) sugerem que IL-6 e IL-10 podem servir como marcadores de prognóstico de gravidade, pois seus níveis plasmáticos estão elevados já nos estágios iniciais da doença, indicando sua relevância como preditores de progressão da enfermidade.

A interleucina-10 possui atividade imunorregulatória, promovendo, por exemplo, a redução na síntese de citocinas pró-inflamatórias. Pode, ainda, apresentar função imunoestimuladora, principalmente de linfócitos T $CD8^+$ (Saxton *et al.*, 2021; Saraiva *et al.*, 2020). Porém, na fisiopatologia da COVID-19, o papel da IL-10 ainda não está completamente elucidado. A variabilidade nos níveis de IL-10 já foi associada à suscetibilidade às doenças virais e ao desenvolvimento de alguns tipos de câncer, tornando essencial a compreensão desta variabilidade (Torres-Poveda *et al.*, 2012; Helminem *et al.*, 2001; Berti *et al.*, 2017).

A variabilidade genética entre indivíduos pode influenciar a produção da IL-10. A presença de variantes de nucleotídeo único (SNV, do inglês *single nucleotide variants*) como rs1800896 (g.–1082A>G), rs1800871 (c.–819C>T) e rs1800872 (c.–592C>A) na região à montante do gene já foi associada a alterações nos níveis sistêmicos, cervicais e *in vitro* da citocina, bem como à suscetibilidade à infecções virais (Torres-Poveda *et al.*, 2012; Turner *et al.*, 1997).

Desta forma, em acordo com a importância epidemiológica do SARS-CoV-2 e a necessidade de maior compreensão de possíveis fatores de risco para maior gravidade da doença, torna-se importante considerar a avaliação da associação de SNVs do gene *IL10* aos casos agravados da COVID-19.

2 DOENÇA DO CORONAVÍRUS (COVID-19)

2.1 EPIDEMIOLOGIA

Os coronavírus são vírus pertencentes à família *Coronaviridae.* São constituídos de RNA (ácido ribonucleico, do inglês *ribonucleic acid*) de senso-positivo, envelopados e esféricos (Burrel, Howard & Murphy, 2017) sendo assim nomeados devido à presença de proteínas *spike* em sua superfície, cuja forma assemelha-se à uma coroa (Yang *et al.*, 2020). Podem causar doenças em animais domésticos, selvagens e em seres humanos, sendo que nestes últimos, normalmente manifesta-se como uma gripe comum (Weiss & Leibowitz, 2011). Recentemente, porém, alguns destes vírus vêm causando grande preocupação para a saúde pública global. Em 2002, o coronavírus associado à Síndrome Respiratória Aguda Grave (SARS-CoV, do inglês *Severe Acute Respiratory*

Syndrome associated coronavirus) foi o agente causador de surtos de infecção em 32 países, com 8.422 casos confirmados da doença, no período de novembro de 2002 a agosto de 2003. Já em 2012, um novo coronavírus foi observado como agente causador da Síndrome Respiratória do Oriente Médio (MERS, do inglês *Middle East Respiratory Syndrome*), sendo responsável por 2.494 casos da doença em 27 estados do Oriente Médio, deixando 868 mortes, contabilizadas entre 2012 e 2019 (Meo, *et al.*, 2020). Em 31 de dezembro de 2019, o primeiro novo caso de pneumonia de agente etiológico desconhecido foi relatado na cidade de Wuhan, China (Li, J. Y. *et al.*, 2020), sendo posteriormente identificado e nomeado como Síndrome Respiratória Aguda Grave do coronavírus-2 ou SARS-CoV-2 pelo Comitê Internacional de Taxonomia de Vírus (ICTV, do inglês *International Committee on Taxonomy of Viruses),* no dia 11 de fevereiro de 2020. Neste mesmo dia, a Organização Mundial de Saúde divulgou como doença do coronavírus (COVID-19), a denominação para a doença causada pelo SARS-CoV-2 (OMS, 2020a). Com a disseminação acelerada da doença, no dia 11 de março de 2020, mais de 118.000 casos em 114 países já haviam sido relatados, levando à declaração pela OMS (2020b), de que a COVID-19 caracterizava-se como uma pandemia. Atualmente, em meados de 2025, já são contabilizados 771.720.205 casos da doença, com 7.094.447 sendo fatais, distribuídos em 216 países (OMS, 2025c).

Em um esforço admirável da comunidade científica mundial, diversas vacinas foram disponibilizadas para uso e, atualmente, mais de 120 já foram ou estão sendo testadas em ensaios clínicos em diferentes países (OMS, 2023d). Segundo dados da Organização Mundial da Saúde (2023c) mais de 13 bilhões de doses já foram administradas ao redor do mundo, posicionando a vacinação como uma estratégia essencial para reduzir a carga mundial da COVID-19 (Shao, *et al.*, 2022). Em confirmação, diversos estudos foram delineados para avaliar a efetividade desta estratégia. Um estudo realizado ainda em 2021, nos Estados Unidos, permitiu observar que a taxa de infecção foi 4,9 vezes maior nos pacientes que não se vacinaram, e a taxa de hospitalização foi 29,2 vezes maior do que nos indivíduos vacinados (Griffin, *et al.*, 2021). Em estudos subsequentes foi relatada e confirmada a segurança e efetividade da vacinação em prevenir quadros graves da doença, hospitalização e morte frente às variantes virais Alpha, Beta, Gamma e Delta (Fiolet, *et al.*, 2022). E, por fim, Gao

e colaboradores (2022) demonstram também que indivíduos vacinados apresentam um risco 29% menor de desenvolver a síndrome da “COVID-19 Longa”, uma condição na qual sintomas da infecção persistem por um longo período mesmo após resolução da doença.

Apesar da importante contribuição da vacinação para reduzir a gravidade epidemiológica da COVID-19, existem ainda lacunas a respeito da compreensão de mecanismos patológicos da doença, mantendo assim a necessidade de desenvolvimento de novos estudos, a fim de amplificar o conhecimento acerca da COVID-19.

2.2 ETIOLOGIA, INFECTIVIDADE E TRANSMISSÃO DO SARS-CoV-2

O agente etiológico da COVID-19, o SARS-CoV-2, é um vírus envelopado e constituído de RNA de senso-positivo. Possui quatro proteínas estruturais, denominadas de proteína *spike* (S), proteína de envelope (E), de membrana (M) e de nucleocapsídeo (N), bem como dezesseis proteínas não-estruturais (NSP 1 – 16, do inglês *non-structural protein*) (Jackson, *et al.*, 2023; Wang, M. *et al.*, 2020). A proteína N participa do encapsulamento do vírus, e juntamente das proteínas NSP, contribui com os processos de replicação e transcrição, bem como montagem do genoma viral. A proteína M auxilia na manutenção da morfologia viral, enquanto a proteína E desempenha papel na montagem do vírus. A proteína S, uma glicoproteína que se configura como um homotrímero, é subdividida em subunidade S1 e subunidade S2, e participa da entrada do vírus nas células-alvo (Samavati, *et al.*, 2020; Marcink, *et al.*, 2022).

Para que o vírus possa infectar a célula-alvo, a presença do receptor denominado enzima conversora de angiotensina 2 (ECA2) é essencial, visto que a proteína viral S possui um domínio de ligação ao receptor (RBD, do inglês *receptor binding domain)* com afinidade para ECA2, permitindo a interação do vírus com a célula-alvo (Samavati, *et al.*, 2020; Wang, M. *et al.*, 2020; Letko, *et al.*, 2020). Esta enzima está presente especialmente na membrana de células epiteliais alveolares do tipo II nos pulmões, células epiteliais da mucosa oral, nasal e nasofaringe, enterócitos no intestino delgado e células endoteliais dos vasos sanguíneos (Hamming, *et al.*, 2004). Ela participa do sistema renina-angiotensina-aldosterona, convertendo angiotensina II, que em células epiteliais

pulmonares é pró-apoptótica, em angiotensina 1-7, que possui efeitos protetores pois antagoniza a angiotensina II (Samavati, *et al.*, 2020). Além da presença da ECA2, também é necessário que a proteína S sofra clivagens promovendo mudanças conformacionais na subunidade S2, o que permite a exposição do peptídeo de fusão de membrana (FP, do inglês *fusion peptid*) que, por sua vez, atua na fusão das membranas virais e celulares e permite a consequente liberação do RNA viral no citoplasma celular. A primeira clivagem ocorre ainda durante a maturação do vírus na célula hospedeira, enquanto a segunda ocorre na superfície da célula-alvo mediante a presença de serina protease transmembrana 2 (TMRPSS2) ou na via endossomal mediante atividade de catepsinas endossomais (Jackson, *et al.*, 2023). Desta forma, de acordo com a disponibilidade de TMRPSS2 ou clatrina na membrana das células-alvo e o encontro destas com o complexo ECA2-proteína S viral, a entrada do vírus na célula pode ocorrer por duas vias distintas. Inicialmente, a interação da proteína S com o receptor ECA2 provoca a mudança conformacional da subunidade S1, expondo o sítio de clivagem S2'. Em presença da TMRPSS2, há a clivagem do sítio S2' ainda na superfície celular, e a exposição do FP e consequente propulsão deste na membrana alvo, promovendo a formação de um poro pelo qual o RNA viral é liberado no citoplasma da célula. Caso não haja o encontro do complexo proteína S- ECA2 com TMRPSS2, o complexo é internalizado por endocitose mediada por clatrina e, nos endolisossomos, o sítio S2' é clivado por catepsinas, com consequente exposição do peptídeo de fusão e liberação do RNA viral da via endossomal para o citoplasma. Em ambos os casos o nucleocapsídeo é então desconstituído, e o processo de replicação viral pode ser iniciado (Jackson, *et al.*, 2023; Takeda, 2022).

A infecção de células epiteliais da mucosa das vias respiratórias e consequente replicação viral promove a liberação de vírions no líquido extracelular do trato respiratório superior favorecendo a secreção de gotículas e aerossóis através de espirros, tosse ou mesmo da fala de indivíduos infectados, levando à transmissão do vírus (Geng, *et al.*, 2023). De fato, observou-se que a principal via de transmissão do SARS-CoV-2 ocorre por aerossóis ou por gotículas provenientes das vias respiratórias de indivíduos contaminados que, após liberadas no meio, permanecem suspensas no ar, permitindo que outros indivíduos entrem em contato direto com essas partículas. Adicionalmente, a

transmissão por contato direto também foi observada, na qual um aperto de mão, por exemplo, poderia transmitir gotículas presentes na pele do indivíduo infectado, provenientes das vias respiratórias, a outro indivíduo (Zhang, *et al.*, 2020). No entanto, a transmissão indireta, ou seja, através de fômites e superfícies contaminadas, permanece em discussão (Lewis, 2021).

A liberação de vírions do SARS-CoV-2 através das vias respiratórias pode ter início ainda na fase assintomática, com pico observado na primeira semana da doença (He, *et al.*, 2020; Cevik, *et al.*, 2021), o que provavelmente contribuiu para a rápida disseminação da COVID-19, visto que indivíduos em fase assintomática são difíceis de serem identificados (Johansson, *et al.*, 2021; Geng, *et al.*, 2023).

2.3 FISIOPATOLOGIA

As manifestações clínicas da COVID-19 vêm sendo descritas por diversos autores que relatam majoritariamente febre, tosse e dispneia como os principais sinais observados (Wang, F. *et al.*, 2020; LI, L-q. *et al.*, 2020). Porém, a doença na população pode se manifestar desde casos assintomáticos (Kim *et al.*, 2020) a casos graves e críticos que, de acordo com o Guia de Orientações Clínicas Chinesa para Diagnóstico e Tratamento da COVID-19 (*Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment*, 2020) pode manifestar-se com o desenvolvimento de SRAG, choque séptico, disfunção da coagulação e falência múltipla dos órgãos. Diversos fatores de risco para pior gravidade da COVID-19 já foram descritos como, idade avançada e presença de determinadas comorbidades, em destaque para HAS e DM (Richardson *et al.*, 2020). No entanto, os mecanismos moleculares que induzem a progressão para casos agravados da doença em parte dos indivíduos infectados por SARS-CoV-2 ainda estão sendo elucidados e podem incluir fatores predisponentes individuais e uma resposta imune ineficaz na eliminação do vírus, concomitante à expressão exacerbada de citocinas, que culmina em lesão tecidual (Perico *et al.*, 2021).

Nos casos leves e assintomáticos, o controle inicial da infecção é possivelmente efetuado pelo sistema imune inato que atua prontamente ao desafio imunológico sem depender do contato prévio com o vírus (Boechat *et al.*,

2021). Esta resposta é normalmente caracterizada pelo reconhecimento de RNA viral por sensores citoplasmáticos, como por exemplo, RIG-I (do inglês, *retinoic acid-inducible gene 1*), no citoplasma de células infectadas. Quando engajados, estes sensores induzem a síntese e liberação de interferons do tipo I (IFN-I), como IFN-α ou IFN-β, que atuam de modo autócrino ou parácrino, contribuindo para o estabelecimento do "estado antiviral" (Abbas *et al.*, 2019). Este estado é assim denominado pois as células sinalizadas por IFN-I sintetizam enzimas que bloqueiam a replicação viral, reduzindo a capacidade proliferativa do vírus. Ademais, estimulam a expressão de MHC de classe I (complexo de histocompatibilidade principal de classe I, do inglês *major histocompatibility complex*) em todas as células, o que favorece o reconhecimento das células infectadas por linfócitos citotóxicos (CTLs $CD8^+$, do inglês *cytotoxic T lymphocyte*), um importante componente da resposta imune adaptativa contra infecções virais. No entanto, no início da infecção, a célula NK (célula *natural killer*), também favorecida por moléculas de IFN do tipo I, atua de forma importante no *killing* das células infectadas. E, por fim, além de promover efetiva resposta antiviral, o sistema imune inato também atua na ativação da resposta adaptativa, que pode reforçar a eliminação viral e gerar componentes de memória imunológica (Murphy, 2008). No contexto da COVID-19, pacientes assintomáticos ou que desenvolveram quadros leves apresentaram redução no número de células NK, porém esta redução foi ainda mais significativa nos pacientes que desenvolveram o quadro grave, sendo que a maior prevalência de células NK demonstrou-se associada às infecções assintomáticas (Carsetti *et al.*, 2020). Adicionalmente, o perfil de expressão gênica de biópsias pulmonares *post-mortem* de pacientes infectados por SARS-CoV-2 demonstrou o aumento na expressão de citocinas e quimiocinas pró-inflamatórias, porém com baixa expressão de interferons antivirais como IFN-I e IFN-III (interferon do tipo III) (Blanco-Melo *et al.*, 2020). Nos casos leves, além de maior prevalência de células NK, também foi observada menor frequência de monócitos, e a razão monócito/NK foi menor do que nos pacientes agravados (Carsetti *et al.*, 2020). Na imunidade adaptativa, número mais elevados de linfócitos T foram observados neste grupo quando comparados aos pacientes agravados (Yin *et al.*, 2021). E, ainda, Rodda e colaboradores (2021) demonstraram que pacientes com quadros leves da doença foram capazes de desenvolver e manter resposta

imune de memória por pelo menos três meses após recuperação. Desta forma, estas evidências contribuem para a compreensão de que uma resposta imune eficaz no início do quadro seja essencial para a resolução da infecção.

Nos casos graves da doença, diversas alterações no perfil de resposta imunológica foram observadas, como redução na frequência de células NK, aumento de monócitos e neutrófilos circulantes (Carsetti *et al.*, 2020) e intensa e persistente linfopenia (Yin *et al.*, 2021). De acordo com Soy e colaboradores (2020), a linfopenia grave é um sinal precoce da doença, que tende a normalizar com a recuperação do paciente, sendo, portanto, um dos critérios diagnósticos utilizados na China. Liu *et al.* (2020) relataram observar a presença de linfopenia em 44,4% dos casos leves da doença, e em 84,6% dos casos graves. Resultado semelhante foi relatado por Wang *et al.* (2020), que observaram uma redução significativa na contagem de linfócitos de pacientes com quadro crítico em relação aos casos moderados, e uma redução gradual no número absoluto de linfócitos T $CD3^+$, T $CD4^+$, T $CD8^+$ e linfócitos B com a progressão grave da doença. Evidências sugerem que a linfopenia seja decorrente de apoptose devido à verificação da atividade de caspases em linfócitos T (André *et al.*, 2022). Como discutido por Wang e colaboradores (2022) estudos sugerem que o vírus SARS-CoV-2 seja capaz de invadir estas células, causando lesão direta, e, ainda, o excesso de citocinas e mediadores pró-inflamatórios com estimulação intensa da resposta imune possa ser uma explicação para suprimir a expansão e a resposta de linfócitos T (Wang *et al.*, 2022; Pontelli *et al.,* 2022; Shen *et al.,* 2022).

A gravidade da doença COVID-19 e progressão para SRAG é principalmente associada ao quadro de hipercitocinemia ou “tempestade de citocinas” (Ye *et al.*, 2020). A liberação exacerbada de citocinas pró-inflamatórias em infecções pulmonares virais, pode causar, além de SRAG, apoptose de células endoteliais, com consequente edema tecidual e hipóxia, desregulação na homeostase tecidual, e ainda, prejuízo da resposta imune de linfócitos T (Lucena *et al.*, 2020). Neste contexto, Diao e colaboradores (2020) observaram aumento expressivo de citocinas como IFN-γ, IL-6, TNF-α e IL-10 em pacientes com quadro moderado, e um aumento ainda maior nos níveis destas citocinas em pacientes com progressão grave da doença. Desta forma, segundo Kuppalli & Rasmussen (2020), a elevada concentração de IL-6 e IL-10 com concomitante

linfopenia, podem indicar que a hipercitocinemia aliada à supressão de células T são fatores envolvidos na patogenia da COVID-19.

Como revisado por Meyer e colaboradores (2021), de modo geral, o excesso de citocinas, a persistência da inflamação e a própria lesão tecidual pulmonar podem constituir estímulos capazes de promover a ativação endotelial local resultando no aumento de permeabilidade e edema. Este edema pulmonar bilateral decorrente do aumento da permeabilidade dos capilares alveolares caracteriza a SRAG, observada nos casos de COVID-19. E, ainda, a lesão endotelial contribui para exposição de fatores pró-coagulantes que podem direcionar a ativação da cascata da coagulação levando a eventos de trombose microvascular, como observado em pacientes com COVID-19 (Perico *et al.*, 2021).

3 INTERLEUCINA-10

3.1 DESCRIÇÃO

Citocinas são moléculas do sistema imune que permeiam a sinalização entre células imunológicas e que podem, por exemplo, mediar o recrutamento e ativação celular, estimular a síntese de mediadores imunológicos ou ainda causar supressão da resposta (Abbas *et al.*, 2019). A interleucina-10 é uma citocina que classicamente atua de forma imunorregulatória, compondo um mecanismo de contrabalancear a resposta imune, para que o desenvolvimento de possíveis lesões resultantes da resposta seja mínimo, enquanto se mantém uma resposta efetiva (Saraiva, Vieira, O'Garra, 2020).

A IL-10 foi descrita por Fiorentino e colaboradores (1989), sendo inicialmente denominada de Fator Inibidor da Síntese de Citocinas (CSIF do inglês, *cytokine synthesis inhibitor factor*) devido à observação de que esta proteína era capaz de inibir a síntese de citocinas de perfil Th1 (do inglês, *T helper* 1), especialmente IFN-y, sendo liberada por linfócitos de perfil Th2 (do inglês, *T helper 2*) (Fiorentino, Bond, Mosmann, 1989). É constituída por 178 resíduos de aminoácidos formando um homodímero (Saxton *et al.*, 2021) e, como revisado por Ouyang e O'Garra (2019), faz parte de uma família de citocinas que são agrupadas devido à semelhança em sua estrutura e em vias de transdução de sinal utilizadas, que inclui as interleucinas -19, IL-20, IL-22, IL-

24, IL-26 e interleucinas interferon do tipo III, denominadas IFN-λ_1, IFN-λ_2, IFN-λ_3 ou IL-29, IL-28A e IL-28B, respectivamente (Ouyang, O'Garra, 2019).

Apesar de sua síntese ter sido inicialmente associada aos linfócitos de perfil Th2 (Fiorentino, Bond, Mosmann, 1989), sua liberação por outras células do sistema imune foi sendo detectada em trabalhos subsequentes, nos quais se observou que as células produtoras desta citocina podem ser de origem mieloide e linfoide como neutrófilos, eosinófilos, mastócitos, células dendríticas, monócitos e macrófagos, incluindo os residentes teciduais, linfócitos $CD4^+$, $CD8^+$, células B e ainda células não imunes, como as células epiteliais (Saraiva, O'Garra, 2010; Ouyang, O'Garra, 2019). Sua síntese pode ser modulada por diferentes vias a depender da célula a ser estimulada. Em macrófagos e células dendríticas, o engajamento de receptores de reconhecimento de padrão como TLR4 (do inglês, *Toll-like receptor 4*) resulta na ativação de fatores de transcrição da família NFkB (fator nuclear kB, do inglês *nuclear fator* kB) capazes de interagir com a região gênica da IL-10 promovendo sua transcrição. Em macrófagos derivados do baço, a proteína quinase R (PKR, *protein kinase R*) capaz de reconhecer RNA dupla fita (dsRNA, *double-strand* RNA) também demonstrou mediar a transcrição de IL-10 através da ativação de NFkB (Chakrabarti *et al.*, 2008). Em linfócitos, a IL-10 pode ser sintetizada por qualquer subtipo celular. O engajamento do receptor de célula T (TCR, do inglês *T cell receptor*), por exemplo, culmina na ativação do fator de transcrição AP-1 (proteína ativadora, do inglês *activator protein-1*), capaz de ativar a transcrição de IL-10, no entanto, outros fatores de transcrição também podem atuar modulando a transcrição desta citocina em linfócitos, como Blimp-1 (proteína 1 de maturação induzida por linfócitos B, do inglês *B-lymphocyte-induced maturation protein 1*) e c-MAF (fator de transcrição fibrossarcoma músculo-aponeurótico celular, do inglês *cellular musculoaponeurotic fibrosarcoma*) (Jones, Flavell, 2005; Saraiva, Vieira, O'Garra, 2020; Neumann *et al.*, 2014).

Após secretada, a interleucina-10 pode atuar em macrófagos, células dendríticas, linfócitos e em células não imunes, como adipócitos (Rajbhandari *et al.* 2018; Saraiva, Vieira, O'Garra, 2020). Seu receptor é formado por uma subunidade IL10Rα, de alta afinidade, e uma subunidade IL10Rβ, comum à outras citocinas da mesma família. Após a ligação da IL-10 ao seu receptor, este se torna oligomerizado, aproximando as enzimas JAK1 (do inglês, *Janus kinase*)

e TyK2 (*Tyrosine kinase*) presentes próximas às caudas citoplasmáticas de respectivas subunidades, IL10Rα e IL10Rβ. Após fosforilação se tornam ativas, sendo capazes de recrutar a proteína transdutora de sinal e ativadora de transcrição 3 (STAT3, do inglês *signal transducer and activator of transcription*). Este fator se transloca para o núcleo e leva à expressão de genes envolvidos nas funções da IL-10, estimulando a expressão de moléculas que inibem a síntese de proteínas pró-inflamatórias (Saxton *et al.,* 2021), e, desta forma, contribuindo para amenizar os efeitos da inflamação. De fato, foi demonstrado que em camundongos *knock-out* para o gene *IL10 (IL10$^{-/-}$),* observou-se o desenvolvimento de doença inflamatória intestinal crônica visto que a reação inflamatória à microbiota não era efetivamente controlada na ausência de IL-10 (Kuhn *et al.,* 1993).

Além de reduzir a transcrição e produção de citocinas pró-inflamatórias em células como neutrófilos e macrófagos, a IL-10 pode modular a apresentação de antígenos. Em monócitos, a IL-10 pode suprimir a maturação e capacidade de apresentar antígenos às células do sistema imune adaptativo, pois provoca redução da expressão de moléculas de MHCII e moléculas coestimuladoras (de Waal Malefyt *et al.*, 1991). Em células TCD4^{+}, foi observado que a IL-10 atua diretamente causando anergia (Groux *et al.,* 1996).

No entanto, alguns autores discutem que pode não ser adequado classificar a IL-10 como citocina imunorreguladora em vista de sua atuação também em funções estimuladoras (Mocellin *et al.,* 2003). Em células NK, a IL-10 parece estimular sua atividade induzindo a expressão de receptores de ativação (Lauw *et al.*, 2000; Parato *et al.*, 2002) contribuindo para a eliminação do patógeno. Adicionalmente, a administração de IL-10 intravenosa foi capaz de aumentar a produção de IFN-γ, granzimas e atividade de CTLs CD8+ (Saraiva *et al.*, 2020; Lauw *et al.*, 2000). Desta forma, esta citocina parece apresentar um efeito ambíguo, contribuindo para a atividade de células NK e CTLs CD8^{+} que cumprem mecanismos efetores na eliminação de patógenos. E, ainda, pode suprimir a inflamação através do estímulo à redução da síntese de citocinas pró-inflamatórias, bem como redução da atividade de apresentação de antígenos das APCs (célula apresentadora de antígeno, do inglês *antigen-presenting cell*), potencialmente reduzindo a ativação de linfócitos e imunidade adaptativa.

3.2 INTERLEUCINA-10 NA COVID-19

A SRAG, juntamente com a tempestade de citocinas, são características comuns nos casos graves de COVID-19 bem como de infecções por outros coronavírus. No entanto, a elevação dos níveis de IL-10 nos quadros agravados é um aspecto especial aos casos de infecção por SARS-CoV-2, não tendo sido observado em infecções por SARS-CoV-1 (Han *et al.*, 2020; Huang *et al.*, 2005).

Diversos autores relataram um aumento sustentado no nível sérico desta citocina, bem como da IL-6 nos casos graves em relação aos casos moderados da doença (Liu *et al.*, 2020; Chen *et al.*, 2020; Wang *et al.*, 2020). Han *et al.* (2020) e Zhao *et al.* (2020), por sua vez, apontam que as citocinas IL-6 e IL-10, poderiam ser utilizadas como marcadores prognósticos de gravidade, visto que suas concentrações plasmáticas se encontram elevadas já nos estágios iniciais da doença. Enquanto a IL-6 atua como uma citocina pró-inflamatória no processo imunológico, promovendo por exemplo, a diferenciação de monócitos em macrófagos (Gubernatorova *et al.*, 2020), a IL-10 constitui importante citocina imunorregulatória, contribuindo para a inibição da apresentação de antígenos por macrófagos e supressão de células T (Pestka *et al.*, 2004). Porém, na fisiopatologia da COVID-19, o papel da IL-10 ainda não está completamente elucidado.

Considerando seu papel regulatório canônico, uma das hipóteses sugeridas na literatura é a de que a IL-10 é liberada no início do quadro clínico, em estímulo à inflamação que começa a ser estabelecida, e, portanto, aos mediadores inflamatórios presentes, como um mecanismo de contrabalancear o processo inflamatório que se estabelece, e suprimir eventuais lesões teciduais causadas pela resposta imune (Carlini *et al.*, 2023). No entanto, o persistente aumento de sua concentração nos casos graves foi observado concomitantemente à presença de marcadores de exaustão de células T, sugerindo que esta citocina inibitória possa estar envolvida nesta alteração (Diao *et al.*, 2020). Além disto, o nível elevado de IL-10, como já discutido, foi associado aos casos graves e à tempestade de citocinas com aumento de citocinas pró-inflamatórias, o que indica que possivelmente a IL-10 falha em regular a produção destes mediadores (Carlini *et al.*, 2023; Islam *et al.*, 2021). Esta falha, em tese, é descrita como uma possível resistência à IL-10 em monócitos e

macrófagos, reduzindo sua capacidade de inibir a produção de citocinas pró-inflamatórias por estas células da imunidade inata (Carlini *et al.*, 2023; Islam *et al.*, 2021). Esta resistência foi observada em condições de hiperglicemia *in vitro* e *in vivo* em pacientes com diabetes *mellitus* tipo 2 (Barry *et al.*, 2016), sendo que esta condição clínica foi associada com os casos graves da COVID-19.

Alternativamente, elaborou-se a hipótese de que a IL-10, normalmente regulatória, pode, na verdade, assumir papéis pró-inflamatórios no contexto da tempestade de citocinas (Islam *et al.*, 2021). Como discutido por Islam e colaboradores (2021), o balanço entre a atuação regulatória ou pró-inflamatória da IL-10 parece ser dependente de contexto. Atividade pró-inflamatória já foi observada mediante a administração de IL-10 recombinante em voluntários saudáveis e, em outro estudo, em pacientes oncogênicos, nos quais foram detectados níveis elevados de IFN-y e ativação de linfócitos $CD8^+$ (Lauw *et al.*, 2000; Naing *et al.*, 2018). Desta forma, o aumento sustentado na concentração da IL-10 poderia contribuir para manutenção do estado hiperinflamatório observado nos casos graves de COVID-19, levando à hiperativação de células T $CD8^+$, o que poderia contribuir para a exaustão destes linfócitos observada nos pacientes agravados (Islam *et al.*, 2021).

Contudo, o papel exato desempenhado por esta citocina na patogenia da COVID-19 continua em discussão. No entanto, a elevação nos níveis de IL-10 observado desde o início do quadro nos pacientes que tendem a desenvolver COVID-19 compõe, em parte, o desequilíbrio na resposta imune e inflamatória observada na tempestade de citocinas, sendo, portanto, importante compreender a variabilidade interindividual na expressão de IL-10.

3.3 POLIMORFISMOS DE IL-10

A produção de IL-10 ocorre de forma diferencial entre indivíduos e pode ser influenciada por fatores ambientais e genéticos, sendo estes últimos associados às variações presentes no gene *IL10*. Com a finalidade de avaliar a extensão da influência genética na produção desta citocina, Westendorp e colaboradores (1997) analisaram e compararam amostras de irmãos gêmeos. A partir deste delineamento experimental puderam observar que esta variabilidade está predominantemente associada à fatores genéticos, sendo este fator,

responsável por até 75% das diferenças individuais na produção de IL-10. Neste mesmo esforço, Reuss e colaboradores (2002) investigaram a expressão gênica de *IL10* em linhagem celular monocítica e células totais do sangue de gêmeos homozigóticos e dizigóticos. Adicionalmente, fatores ambientais foram avaliados como possíveis fontes de influência para produção de IL-10. Desta forma, em parcial concordância com os dados de Westendorp e colaboradores (1997), os autores puderam observar que ao menos 50% da variabilidade entre indivíduos na produção de IL-10 pode ser explicada pela composição genética individual, e ainda que, fatores ambientais também podem influenciar esta variabilidade. Além da variabilidade genética, fatores como, sexo masculino e maiores índices de massa corporal foram associados à maior produção de IL-10 e o hábito tabagista à menor produção (Reuss *et al.*, 2002).

Considerando que ao menos 50% da variabilidade na produção de IL-10 pode ser influenciada pela composição genética, torna-se importante salientar que a região gênica da IL-10 é considerada altamente polimórfica. Desta forma, já foram identificados microssatélites (IL-10.R/IL-10.G) no gene *IL10* (Eskdale *et al.*, 1995; Eskdale *et al.*, 1996) e SNVs. Dentre as SNVs identificadas, destacam-se -3575 T>A, -2849 G>A, -2763 C>A na região 5' distal do gene (Gibson *et al.*, 2001) e rs1800896 (g.–1082A>G), rs1800871 (c.–819C>T) e rs1800872 (c.–592C>A) na região proximal do gene (Turner *et al.*, 1997). As SNVs localizadas na região proximal do gene são mais frequentemente estudadas (Hedrich *et al.*, 2011), e formam blocos de haplótipos, sendo os mais comuns descritos por Turner *et al.* (1997) como GCC, ACC e ATA. A presença destes polimorfismos e seus haplótipos está associada a diferenças nos níveis sistêmicos (Torres-Poveda *et al.*, 2012; Helminem *et al.*, 2001), cervicais (Berti *et al.*, 2017) e *in vitro* de IL-10 (Turner *et al.*, 1997).

A substituição da base C (citosina) por uma A (adenina) na posição -592C>A (rs1800872) está associada com a produção diferencial de IL-10. Torres-Poveda *et al.* (2012) observaram aumento nos níveis plasmáticos de IL-10 e maior suscetibilidade ao desenvolvimento de lesão cervical pelo papilomavírus humano em mulheres portadoras do alelo A, enquanto Helminen *et al.* (2001), Pereira *et al.* (2015) e Temple *et al.* (2003) observaram níveis elevados de IL-10 em portadores do haplótipo ATA (rs1800896, rs1800871 e rs1800872). No entanto, outros autores apontam dados nos quais observa-se efeito contraditório,

principalmente quando este SNV é avaliado conjuntamente às SNVs rs1800871 (C>T) e rs1800896 (A>G) formando haplótipos.

Neste contexto, o haplótipo completo ATA ou haplótipo AA, formado apenas pelos SNVs rs1800872 e rs1800896, encontram-se normalmente associados a baixos níveis de IL-10 enquanto o haplótipo GCC, que inclui o alelo C do SNV rs1800872 e o alelo G de rs1800896, associa-se a níveis mais elevados de IL-10 (Crawley *et al.*, 1999; Hulkkonen *et al.,* 2001; Chen *et al.,* 2012). Adicionalmente, Reuss (2002) e Turner (1997) descrevem ter observado *in vitro* que o haplótipo GCC associa-se a maior produção de IL-10 enquanto os haplótipos ACC e ATA demonstram redução na atividade transcricional. Neste contexto observa-se também, que apenas o alelo A do SNV rs1800896 tem efeito independentemente significativo em reduzir a produção da citocina, enquanto o alelo G associa-se ao aumento (Reuss *et al.*, 2002). A presença do alelo A (rs1800896) confere maior afinidade de ligação do fator de transcrição PU.1 à região promotora do gene *IL10.* Este fator atua reduzindo a transcrição gênica, e, desta forma, em presença do alelo A, liga-se com mais afinidade à região promotora contribuindo para a redução da atividade transcricional do gene (Reuss *et al.*, 2002; Capasso *et al.*, 2007).

Além de serem encontradas associações destas SNVs à produção diferencial de IL-10, também são relatadas associações destes polimorfismos a diversos contextos patológicos, incluindo quadros infecciosos (Alagarasu *et al.*, 2021), autoimunes (Mohammadi *et al.*, 2019; Braga *et al.,* 2021) e de desenvolvimento tumoral (Bai *et al.,* 2016; Dhouioui *et al.,* 2024). No contexto de Lúpus Eritematoso Sistêmico (LES) em iranianos, o genótipo AA (rs1800872) foi mais frequentemente observado nos indivíduos do grupo controle, sendo também associado a menores níveis de IL-10 em relação ao genótipo CC, e, portanto, foi associado ao risco reduzido de LES. O genótipo CC (rs1800871) demonstrou-se associado ao grupo de casos, porém sem associar-se aos níveis da citocina. O genótipo heterozigoto GA (rs1800896) também foi associado com um maior risco de LES, enquanto o alelo G foi observado em associação a níveis elevados de IL-10 (Mohammadi *et al.*, 2019). Em uma população brasileira, a espondilite anquilosante foi associada ao alelo G e modelo dominante (AG+GG) da SNV rs1800896, e, apesar de não se apresentar associada aos níveis de IL-10, os pacientes do grupo caso apresentaram níveis elevados desta citocina

(Braga *et al.*, 2021). No contexto das infecções virais, Alagarasu e colaboradores (2021) relataram que o haplótipo GA (rs1800896 e rs1800872) foi observado em associação com casos fatais de influenza A (H1N1) em população indiana, apesar de não detectarem associação dos genótipos com os níveis da citocina.

Recentemente, alguns grupos de pesquisa avaliaram a possível associação dos genótipos das SNVs rs1800872, rs1800871 e rs1800896 com a gravidade e ou desfecho da COVID-19 em diferentes grupos étnicos, incluindo a população mexicana (Avendano-Félix *et al.*, 2021), cazaque (Yessenbayeva *et al.*, 2023), italiana (Balzanelli *et al.*, 2022), indiana (Rizvi *et al.*, 2022) e iraniana (Abbood *et al.*, 2023). Dentre esses grupos populacionais, observou-se a associação dos genótipos AA, CC e GG (rs1800872, rs1800871 e rs1800896, respectivamente) com o desfecho óbito em decorrência da COVID-19 apenas na população iraniana, a qual não foi avaliada quanto à associação com a gravidade da doença. Considerando as diferenças entre estes achados, torna-se importante que novos estudos avaliem esta associação para melhor compreender o efeito destes polimorfismos da região promotora do gene *IL10* na gravidade da COVID-19.

Até o presente momento, não existem estudos direcionados a avaliar se a presença destes polimorfismos (rs1800896, A>G, rs1800871, C>T e rs1800872, C>A) e seus haplótipos GCC, ACC e ATA está relacionada à gravidade da doença COVID-19 na população brasileira. Portanto, considerando a importância epidemiológica do SARS-CoV-2 e a escassez de informações quanto a esta associação, torna-se importante esta avaliação.

Objetivos

4 OBJETIVOS

4.1 GERAL

Avaliar o efeito da presença dos SNVs g.–1082A>G (rs1800896), c.–819C>T (rs1800871), c.-592C>A (rs1800872), e seus haplótipos (GCC, ACC e ATA) no contexto da COVID-19 por meio da revisão de literatura e da condução de estudo observacional. Analisar a possível associação destas SNVs com as manifestações de quadro leve, moderado e grave da doença, bem como com o desfecho óbito em pacientes diagnosticados com COVID-19, na cidade de Londrina, no Paraná.

4.2 ESPECÍFICOS

- Realizar uma revisão narrativa acerca do efeito das SNVs localizadas na região proximal do gene *IL10* em infecções virais, com ênfase na infecção por SARS-CoV-2;
- Determinar o perfil sociodemográfico (idade, sexo, etnia e hábito tabagista) e clínico (presença de comorbidades) dos participantes do estudo observacional;
- Avaliar a frequência dos SNVs g.–1082A>G (rs1800896), c.–819C>T (rs1800871) e c.-592C>A (rs1800872), em pacientes com detecção positiva para SARS-CoV-2;
- Realizar a inferência da frequência dos haplótipos encontrados na população amostrada;
- Verificar se há associação dos genótipos, alelos e haplótipos das SNVs investigadas aos quadros leve, moderado e grave da COVID-19;
- Verificar se há associação dos genótipos, alelos e haplótipos das SNVs investigadas ao desfecho óbito dentre os pacientes classificados como grupo grave.

Produção Científica

Human Immunology 86 (2025) 111261

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Research article

Association of *IL10* gene SNVs rs1800896 (A > G), rs1800871 (C > T), rs1800872 (C > A) and haplotypes with COVID-19 severity and outcome in the Brazilian population

Janaina Nicolau de Oliveira [a], Caroline Yukari Motoori Fernandes [a], Sara Mataroli de Godoy [b], Wilson Frantine-Silva [b], Pedro Luis Candido de Souza Cassela [c], Guilherme Lerner Trigo [c], Marcell Alysson Batisti Lozovoy [c], Zuleica Naomi Tano [d], Andrea Name Colado Simão [c], Karen Brajão de Oliveira [a,b,*]

[a] *Laboratory of Molecular Genetics and Immunology, Department of Immunology, Parasitology and General Pathology, Center of Biological Sciences, State University of Londrina, Pr 445 km 380 Celso Garcia Cid Highway 86.057-970 PR, Brazil*

[b] *Laboratory for Studies and Analysis of Polymorphisms, Department of Immunology, Parasitology and General Pathology, Center of Biological Sciences, State University of Londrina 86.057-970 PR, Brazil*

[c] *Department of Applied Pathology, Clinical and Toxicological Analysis, State University of Londrina 86.057-970 PR, Brazil*

[d] *Department of Clinical Medicine, University of Londrina, Londrina, PR, Brazil*

ARTICLE INFO

Keywords:
Rs1800896 (−1082 A > G)
Rs1800871 (−819 C > T)
Rs1800872 (−592 C > A)
Severe acute respiratory syndrome-related coronavirus
Polymorphism
Single nucleotide
Interleukin-10

ABSTRACT

Background: Elevated concentrations of IL-10 have been detected in coronavirus disease (COVID-19) patients and are a possible disease severity marker. Single nucleotide variants (SNVs) and their haplotypes can be associated with differences in IL-10 levels and with viral disease susceptibility.

Aim: Evaluate the associations of SNVs and their haplotypes in Brazilian patients with COVID-19 severity and outcome.

Methods: In this cross-sectional and case-control study, the patients were selected from the University Hospital of State University of Londrina (HU-UEL) (n = 367) and were subdivided into mild (n = 165), moderate (n = 72) and severe (n = 130) groups. The DNA samples of the participants were subjected to real-time PCR for the detection of rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) genotypes. The haplotypes were inferred with PHASE v2.1.1.

Results: The severe cases of COVID-19 were independently associated with the GG genotype (rs1800896) (P = 0.038, OR 2.522, 95 % CI 1.053–6.038) as well as with the GCC haplotype in homozygosity (P = 0.037, OR 2.767, 95 % CI 1.065–7.191).

Conclusion: These results showed that the GG genotype of rs1800896 or the GCC haplotype are associated with COVID-19 severity in Brazilian patients.

1. Introduction

The clinical manifestations of coronavirus disease (COVID-19), a pandemic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are diverse, ranging from asymptomatic to critical [1]. Severe progression is marked by the presence of hypercytokinaemia, or a “cytokine storm”, which consists of an exacerbated release of cytokines and can contribute to the pathophysiology of the disease, resulting in an impaired immune response and progression to severe acute respiratory syndrome (SARS) [2]. In this context, high plasma concentrations of cytokines such as interferon–gamma (IFN-γ), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) are consistently observed in patients with severe disease [3]. Additionally, it is suggested that IL-10 could be used as a

* Corresponding author at: Laboratory of Molecular Genetics and Immunology, Department of Immunology, Parasitology and General Pathology, State University of Londrina, Pr 445 km 380 Celso Garcia Cid Highway, 86.057-970 Londrina, PR, Brazil.
E-mail address: karen.brajao@uel.br (K.B. de Oliveira).

https://doi.org/10.1016/j.humimm.2025.111261
Received 13 August 2024; Revised 30 January 2025; Accepted 3 February 2025

prognostic severity marker since high levels of this cytokine are observed in early days post-admission in patients who progress to severe disease [4–6].

Interleukin-10, a pleiotropic cytokine, is released by several cells of the immune system and acts by reducing the immune response. IL-10 causes the suppression of monocyte and macrophage functions related to the antigen presentation and consequently adaptive immunity activation and stimulates the reduction of the expression of pro-inflammatory cytokines [7,8]. However, a possible pro-inflammatory effect of IL-10 was also observed, in which the administration of recombinant IL-10 was shown to stimulate cytotoxic lymphocyte function (T CD8 +) [9,10].

In this context, altered and high levels of IL-10 have previously been associated with susceptibility to certain viral diseases [11], such as viral hepatitis [12] and fatal cases of influenza A (H1N1) infection [13]. In COVID-19, the role of IL-10 is not yet established. One of the hypotheses discussed by Islam and collaborators [14] and Lu and colleagues [15] is the possibility that the early elevation of IL-10 peripheral levels could be an attempt to regulate the immune response in a negative feedback loop but the persistent exacerbated levels ends up resulting in an opposite direction, where IL-10 can lead to a pro-inflammatory role through the hyperactivation of CD8 + T lymphocytes, which could provoke an increase in IFN-γ levels, worsening the inflammatory state. This overstimulation of CD8 + T lymphocytes over time could even contribute to the exhaustion of these cells and impairment of antiviral response. Therefore, Lu and colleagues [15] indicate that IL-10 contributes to COVID-19 pathophysiology and that perhaps it could be beneficial to inhibit IL-10 activity while also regulating other pro-inflammatory cytokines activity in patients.

The variability of IL-10 expression may be associated with the presence of genetic variants in regulatory regions of the gene distributed throughout the population [16], and, in hypothesis, the presence of certain variants could be associated with the susceptibility to COVID-19 worse prognostics. To date, progression to severe disease is known to be associated with factors such as advanced age and the presence of certain preexisting comorbidities. However, considering the role of cytokine storm in severe cases of this disease, other individual factors such as genetic variability in immune system genes, could contribute to the diverse clinical manifestations observed in COVID-19 patients.

Many polymorphisms have been identified in the interleukin-10 gene (*IL10*) flanking region, including the microsatellites IL10.R and IL10.G [17,18] and the single nucleotide variants (SNVs) 3575 (T > A), −2849 (G > A), −2763 (C > A) [19] but the most frequently studied of those polymorphisms are three SNVs in the proximal region of the gene, rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A). The presence of these proximal SNVs and their haplotypes has already been associated with differences in IL-10 levels, especially the GCC haplotype and G allele, which were associated with increased levels of this cytokine in the majority of the research already published [16,20–27]. Additionally, these SNVs were also associated with susceptibility to viral infections [13,28], which strengthens the need to evaluate its possible association with COVID-19 clinical context, a viral disease whose severe cases have been associated with increased levels of IL-10. Therefore, recently, research groups [29–33] have set out to evaluate whether these SNVs are associated with severity, or the outcome of death caused by COVID-19; however, only one study, carried out with a population from Iran, detected this association [30].

Considering that such work [29–33] was carried out in different ethnicities and possibly different population genetic architectures and that, to date, there are no studies aimed at evaluating this association in the Brazilian population, the objective of this work was to evaluate and confirm whether the presence of these SNVs, rs1800896 (−1082 A > G), rs1800871 (−819C > T), rs1800872 (−592C > A) and their most frequent haplotypes, could be related to the severity or worse outcome of COVID-19 in this population.

In this scenario, the detection of SNVs that could associate with COVID-19 severe progression could strengthen the prognostic value of IL-10 peripheral dosage and reinforce its potential as a biomarker for disease worsening progression.

2. Materials and methods

2.1. Sample characterization

This cross-sectional and case-control study was approved by the Human Research Ethics Committee of Londrina State University, protocol n° 4.204.004, CAAE: 36247920.1.0000.5231, and was carried out in accordance with the Declaration of Helsinki.

Unrelated patients (n = 367) diagnosed with COVID-19 were selected from the University Hospital of the State University of Londrina (HU-UEL), Paraná, Brazil. The selection and inclusion criteria consisted of the detection of SARS-CoV-2 viral RNA via RT–PCR (reverse-transcriptase polymerase chain reaction), using the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Taewon Bldg., 91 Ogeum-ro, Songpa-gu, Seoul, Republic of Korea), and the CFX96™ Real-time PCR detection system, the availability of sociodemographic, diagnostic and clinical data in medical record; age ≥ 18 years; and the availability to participate by signing of an informed consent form. Ethnicity was self-declared.

The classification of disease severity as mild (n = 165), moderate (n = 72) or severe (n = 130) was determined by health professionals based on the definitions of the World Health Organization [34]. Mild conditions were defined as patients who presented mild clinical symptoms without evidence of pneumonia or hypoxia. Patients in moderate condition presented signs of nonsevere pneumonia, including cough, fever, dyspnea and oxygen saturation ≥ 90 % on room air. Severe conditions included patients who developed severe pneumonia characterized by O_2 saturation less than 90 % in room air or a respiratory rate greater than 30 breaths per minute, in addition to symptoms such as cough, dyspnea and fever. Critical patients characterized by worsening severe pneumonia and the presence of SARS were included in the severe group.

The mild group was considered as the control group, whereas the moderate and severe groups were defined as the case groups. A second classification was carried out exclusively for patients in the severe group, which considered the outcome, death (n = 66) as the case group and recovery (n = 64) as the control group.

2.2. Sample collection and DNA extraction

Peripheral blood samples were collected in sterile syringes containing EDTA between March and October 2020 and subjected to extraction of genetic material using a resin column (Biopur, Biometrix, Curitiba, Brazil) according to the manufacturer's instructions. The DNA concentration was measured by spectrophotometry using a NanoDrop 2000c® instrument (Thermo Fisher Scientific) at 260 nm, and the purity was assessed using the 260/280 ratio; the DNA was stored at −20 °C until use. Genotyping and haplotype analysis were performed between 2022 and 2023 as described below.

2.3. IL10 SNVs genotyping and haplotype inference and inheritance models definition

Genotyping of *IL10* SNVs was performed by real-time PCR using on-demand assays with TaqMan probes (C_1747363_10, C_1747362_10 and C_1747360_10) and MasterMix from Applied Biosystems, Thermo Fisher Scientific, using StepOne equipment from the same company. PCR was performed as described in the manufac-

turer's manual. Allele calling for the SNV rs1800896 (NG_012088.1: g.3943 A > G), rs1800871 (NG_012088.1: g.4206 T > C) and rs1800872 (NG_012088.1: g.4433 A > C) was performed automatically, plate by plate, using Step-One software version 2.1. No template control (NTC) was used in all the reactions. Throughout the analysis, randomly chosen samples were subjected to repeated genotyping to verify the reproducibility of the assay.

The inference of haplotypes formed between the alleles of *IL10* SNVs was performed using PHASE software version 2.1.1 [35,36]. The linkage disequilibrium (LD) analysis between IL10 SNVs was performed using Haploview software version 4.2 [37], calculating D', r2, and LOD scores to quantify the strength of LD. The definition of the inheritance models for statistical analysis was done considering the variant allele of each SNV. The allele considered in rs1800896 (A > G) was the G allele, whereas the T and A alleles of rs1800871 (C > T) and rs1800872 (C > A), respectively, were used for analysis.

2.4. Statistical analysis

The Hardy–Weinberg equilibrium (HWE) of all groups was assessed using Pearson's chi-square test. The Pearson chi-square test ($\chi 2$) was also performed to verify differences in the distribution of frequencies of genotypes, haplotypes and sociodemographic characteristics between the groups and to evaluate possible associations between variables and the severity or outcome of COVID-19. When the expected frequency was less than 5, Fisher's exact test was used instead of Pearson's chi-square test. The Bonferroni correction was used as a post hoc test to avoid false-positive findings (type I error) resulting from multiple comparisons.

Multivariate logistic regression (forced entry method) was then used to control confounding factors and to predict independent associations between genotype or haplotype models (explanatory variables) and groups of cases (dependent variables) through the estimation of adjusted odds ratios (ORs) and 95 % confidence intervals (CIs). Using the “common cause” method of selection of covariates, sociodemographic and clinical data previously associated in the scientific literature with COVID-19 severity such as age, sex and presence of comorbidities (systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease) [38,39] were considered as a possible source of bias and therefore treated as confounder factors in logistic regression analysis.

Categorical variables are expressed as the absolute number (n) and percentage (%), and all tests were two-tailed, with a p value (P) < 0.05 considered to indicate statistical significance. All the above statistical analyses were performed using SPSS Statistics 22.0 software (SPSS, Inc., Chicago, IL, USA).

The statistical power of the genetic associations evaluated was calculated using the online Genetic Association Study (GAS) Power Calculator (Sponsored by University of Michigan School of Public Health Department of Biostatistics Center for Statistical Genetics), considering number of cases and controls, significance level (0.05), disease model (dominant or recessive), disease prevalence of 0.03 for Brazilian population of Paraná State at 2020 [40], disease allele frequency of the SNV evaluated and genotype relative risk.

The reporting of this study was based on the guideline STrengthening the REporting of Genetic Association studies (STREGA) [41].

3. Results

3.1. Sociodemographic and clinical characteristics of COVID-19 patients

The 367 study participants were classified into three groups, namely, mild (n = 165), moderate (n = 72) or severe (n = 130), according to the clinical condition of the disease. In this study, the frequency of male participants (51 %) was similar to that of female participants (49 %). Patients who declared themselves to be Caucasian were the most common participants in the present study (77.8 %), while the other declared ethnicities accounted for 22.2 % of the participants. When evaluating the distribution of participants among the different clinical conditions, no significant difference was observed (p > 0.05), indicating a homogeneous distribution of different ethnicities among the groups. There was no significant difference in the frequency of smokers or ex-smokers between the groups evaluated (p > 0.05) (Supplementary Table 1).

To assess whether the factors mentioned above are independently associated with severe cases of the disease, the adjusted odds ratio was calculated for confounding factors, including sex, age and the presence of comorbidities such as systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease. The results in Supplementary Table 2 demonstrate that the chances of developing severe COVID-19 increase, independent of other factors, with increasing age. In the population evaluated, patients aged between 50 and 59 years and individuals aged 80 years old or higher are approximately 6.65 times and 35.33 times, respectively, more likely to develop severe COVID-19 than individuals aged up to 39 years (p < 0.001). Male patients were 2.02 times more likely to suffer from the moderate form of the disease than female patients were (p = 0.030); however, when the odds were adjusted, the regression analysis did not reveal a significant difference in the group of severe patients, despite demonstrating this tendency (p = 0.055). Among the comorbidities associated with severe cases in this study, diabetes mellitus was independently associated with severity (p = 0.006), as was chronic kidney disease (p = 0.046). However, the presence of systemic arterial hypertension was not found to be an independent factor (p > 0.05).

3.2. IL10 SNVs and COVID-19 severity

The genotyping of the evaluated *loci* was successful in 99.45 % (n = 365) of the samples. Considering this number of samples, a statistical power of 80 % was observed for the inheritance models tested for rs1800872 (C > A) and rs1800871 (C > T). Regarding rs1800896 (A > G), a number of samples of approximately 370 would reach 80 % of statistical power for the models tested.

The distributions of the frequencies of the *IL10* SNVs rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) followed the Hardy–Weinberg equilibrium (HWE) in all groups (p > 0.05), as can be seen in Supplementary Table 3. The rs1800872 (C > A) and rs1800871 (C > T) SNVs presented an identical frequency distribution of alleles and genotypes, which is consistent with the results of linkage disequilibrium analysis (D′ = 1.0, r2 = 1.0, LOD = 159.38; Table S4, Fig. S1), indicating 100 % linkage disequilibrium.

The distributions of the frequencies of the alleles and genotypes are shown in Supplementary Table 5, and when compared between groups, no association with disease severity was found (p > 0.05) for any of the SNVs evaluated. However, adjustment for confounding factors was performed to evaluate the independent effect of genotype on the severity and outcome of the disease, since other factors inherent to the sample could interfere with the visualization of this effect. The factors considered for adjustment were age, sex and the presence of comorbidities such as systemic arterial hypertension, diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease and heart disease. As shown in Table 1, with the adjusted data, the GG genotype, in the recessive model of the SNV rs1800896 (A > G), was shown to be associated with the group of individuals who developed severe COVID-19 and may contribute to increasing the chance of developing severe disease by 2.5 times on average (*P* = 0.038, OR 2.522, 95 % CI 1.053–6.038).

Table 1
Association of *IL*10 rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) models and genotypes with COVID-19 severity through adjusted multinomial logistic regression.

	COVID-19			
	Moderate		Severe	
Genotypes *IL10*	Adj OR (CI95 %)	Adj *P*-value	Adj OR (CI95 %)	Adj *P*-value
rs1800896				
Additive				
AA	Reference		Reference	
AG	1.039 (0.526–2.054)	0.912	0.895 (0.479–1.671)	0.727
GG	1.684 (0.603–4.703)	0.320	2.374 (0.929–6.064)	0.071
Dominant				
AA	Reference		Reference	
AG + GG	1.142 (0.596–2.189)	0.689	1.099 (0.609–1.985)	0.753
Recessive				
AA + AG	Reference		Reference	
GG	1.648 (0.637–4.267)	0.303	2.522 (1.053–6.038)	**0.038**
rs1800871				
Additive				
CC	Reference		reference	
CT	1.215 (0.617–2.390)	0.573	1.241 (0.667–2.310)	0.496
TT	1.349 (0.500–3.639)	0.554	1.386 (0.565–3.400)	0.475
Dominant				
CC	Reference		reference	
CT + TT	1.243 (0.653–2.364)	0.507	1.272 (0.706–2.293)	0.423
Recessive				
CC + CT	Reference		reference	
TT	1.213 (0.484–3.038)	0.681	1.230 (0.538–2.814)	0.623
rs1800872				
Additive				
CC	Reference		reference	
CA	1.239 (0.630–2.435)	0.535	1.261 (0.678–2.345)	0.464
AA	1.377 (0.511–3.713)	0.527	1.410 (0.575–3.457)	0.453
Dominant				
CC	Reference		reference	
CA + AA	1.268 (0.667–2.409)	0.469	1.293 (0.718–2.329)	0.392
Recessive				
CC + CA	Reference		reference	
AA	1.225 (0.489–3.071)	0.665	1.241 (0.542–2.840)	0.610

Adjusted multinomial logistic regression analysis for: age, sex, systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease. Reference: mild group. Significance level adopted of $p < 0.05$. OR = Odds Ratio; CI = confidence interval; adj = adjusted. (SPSS Inc., Chicago, Illinois, USA).

3.3. IL10 SNVs haplotype and COVID-19 severity

Haplotype information was inferred from the observed genotypes of the SNVs rs1800896 (−1082 A > G), rs1800871 (−819C > T) and rs1800872 (−592C > A), resulting in three haplotypes common to this population, ATA, GCC and ACC, as can be seen in Supplementary Table 5. When comparing the distribution of haplotype frequencies between case and control groups, no significant association was found ($p > 0.05$).

However, Table 2 demonstrates that when evaluating possible models of haplotype inheritance in the multinomial regression adjusted for confounding factors, the GCC haplotype, in homozygosity, was shown to be associated with aggravated cases of the disease and may contribute to increase the chance of developing severe COVID-19 by approximately 2.8 times compared to haplotype combinations formed only by ATA or ACC (P = 0.037, OR 2.767, 95 % CI 1.065–7.191).

3.4. Association of IL10 SNVs genotypes and haplotypes and COVID-19 outcome

The associations of the SNVs were also evaluated with disease outcome including patients with severe disease who were subdivided according to the occurrence of death (n = 66) or recovery (n = 64); the latter subgroup included those patients who were discharged and therefore was used as the reference group in logistic regression analysis (Table 3). Tables 3 and Supplementary Table 5 show that none of the *IL10* SNVs analyzed regarding the distribution of alleles, genotypes or haplotypes were associated with the outcome of death in patients with COVID-19 ($p > 0.05$), even when adjusted analysis was performed.

4. Discussion

We proposed to evaluate whether the single nucleotide variants rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) of *IL10* are associated with severe cases of COVID-19 or the worst outcome, death, in the Paraná population, south of Brazil. An admixture population is found in Brazil, being the south region composed predominantly of European genomic ancestry [42]. To the best of our knowledge, this is the first study to investigate this association in a Brazilian sample, and we observed that the GG genotype of rs1800896 (1082 A > G) and the GCC haplotype are independently associated with severe cases of this disease.

In this work, the observed frequency of alleles and genotypes of both SNVs rs1800872 (C > A) and rs1800871 (C > T) were identical, indicating complete linkage disequilibrium between these *loci,* which was confirmed by the linkage disequilibrium analysis. This disequilibrium was also found in other clinical contexts in Caucasian populations from different countries [43]. Therefore, these SNVs together with

Table 2
Association of haplotype models of *IL*10 rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) SNVs with COVID-19 severity.

	COVID-19									
	Mild		Moderate				Severe			
Haplotypes *IL10*	N	%	N	%	Adj OR (CI95 %)	Adj *P*-value	N	%	Adj OR (CI95 %)	Adj *P*-value
ATA										
Others	71	43.3	28	38.9	Reference		47	36.4	Reference	
ATA/ATA	23	14	10	14.1	1.377 (0.511–3.713)	0.527	20	15.5	1.410 (0.575–3.457)	0.453
ATA/GCC + ACC	70	42.7	34	47.2	1.239 (0.630–2.435)	0.535	62	48.1	1.261 (0.678–2.345)	0.464
GCC										
Others	68	41.2	27	38	Reference		55	42.3	Reference	
GCC/GCC	15	9.1	10	14.1	1.879 (0.664–5.315)	0.234	21	16.2	2.767 (1.065–7.191)	**0.037**
GCC/ACC + ATA	82	49.7	34	47.9	1.022 (0.517–2.020)	0.950	54	41.5	0.883 (0.472–1.651)	0.697
ACC										
Others	78	47.3	41	56.9	Reference		78	60.0	Reference	
ACC/ACC	15	9.1	5	6.9	0.378 (0.109–1.303)	0.123	11	8.5	0.392 (0.137–1.122)	0.081
ACC/GCC + ATA	72	43.6	26	36.1	0.690 (0.354–1.343)	0.274	41	31.5	0.515 (0.276–0.960)	**0.037**

Adjusted multinomial logistic regression analysis for: age, sex, systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease. Reference: mild group. Significance level adopted of $p < 0.05$, represented by values in bold. OR = Odds Ratio; CI = confidence interval; adj = adjusted. (SPSS Inc., Chicago, Illinois, USA).

rs1800896 (A > G) form three main haplotypes in the Caucasian population, which were predominant in this study: GCC, ACC and ATA [16].

The frequency of the A allele of SNV rs1800872 (C > A) and the T allele of rs1800871 (C > T) was 37.1 %, which agrees with the frequency reported for the Latin American population 2 in the National Center for Biotechnology Information database (NCBI, A = 0.36 and T = 0.37). No significant associations were found between alleles or genotypes of either SNV and severe cases of COVID-19 or the worst outcome of the disease. This result is consistent with the findings of Avendaño-Felix and collaborators [33], who evaluated the association between the rs1800872 (C > A) and rs1800871 (C > T) SNVs and the severity or outcome of COVID-19 in the Mexican population (Latin American) and did not observe statistical significance for either of the SNVs.

These SNVs, rs1800872 (C > A) and rs1800871 (C > T), were already associated with other viral diseases as fatal cases of influenza A (H1N1) virus (GC haplotype $p = 0.041$; GA haplotype $p = 0.005$) [13], HPV infection (rs1800872 A allele, $p < 0.001$) [28] and even with a greater risk of death in Iranian COVID-19 patients (rs1800872 AA genotype and rs1800871 CC genotype, $p < 0.0001$) [30]. However, this association with severity or outcome was not found in our work.

This contradiction, especially when comparing our results with Iranian COVID-19 patients [30], may arise from factors such as differences in the experimental design of each analysis, in the definition of groups, in the sample size and in the presence of differences in the genetic architecture existing between populations of diverse ethnicities and, consequently, from differences in the allelic distribution of these loci [44].

Additionally, it is known that the *IL10* gene expression is a complex regulated process which involves different pathways and transcription factors according to the IL-10 producing cell type and stimulus [45] and the detection of the effects of these SNVs, especially regarding its association with lower or higher gene transcription, can vary according to the cell type evaluated and even the stimulus used [46]. Although we did not measure *IL10* expression in this research, it could be possible to hypothesize that this variation in the observed effects of the SNVs in *IL10* gene could also be seen in different clinical contexts, with different etiological agents and pathophysiological background.

Results of *in vitro* studies with monocytic cells, designed to investigate the influence of the SNVs rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) on the IL-10 production, demonstrated a significant effect on altering gene expression only by rs1800896 (A > G), when the presence of the G allele in the haplotype GCC increased the transcription activity of the gene compared with ACC ($p = 0.042$) and ATA ($p = 0.0026$) haplotypes [16]. In agreement, the presence of the GCC haplotype and G allele was observed to be associated with high levels of IL-10 in most of the research that analyzed these SNVs [16,20–27].

Considering the rs1800896 (A > G) SNV, it was expected that the homozygous GG genotype or G allele would be associated with severe cases of COVID-19 disease. In line with this expectation, in this study, the GG genotype was observed more frequently in the severe and moderate groups than in the mild group, with 16.2 %, 13.9 % and 9.2 %, respectively. However, no significant differences were initially found using the chi-square test when the alleles and genotypes in the different additive, dominant and recessive models were evaluated for their association with severe cases of the disease or outcome. In contrast, when possible confounding factors were considered and adjusted, through multinomial logistic regression, it was possible to observe an association of the GG genotype in the recessive model with the severe group of the disease, whose presence could contribute to an average increase of 2.5 times the chance of this genotype carriers developing severe COVID-19.

Although the haplotype frequency distribution analysis did not reveal a significant difference between the groups, when evaluated for possible inheritance models adjusted for confounding factors, individuals with the GCC haplotype in homozygosity, that is, in the presence of two G alleles, were more strongly associated with severe cases than those who do not had any GCC haplotype. In the same direction as our findings, in the Iranian population, it was observed that among all patients with COVID-19 evaluated, those carrying the GG genotype had a greater risk of death [30]. In this sense, it is possible to explain these results considering the work of Reuss and collaborators [16], who observed an association between the GCC haplotype and a higher transcriptional level of IL-10, in detriment of the ACC and ATA haplotypes. Importantly, the authors detected that environmental factors such as sex, smoking habit and body mass index (BMI) also affect the production of IL-10, demonstrating that the effect of rs1800896 (A > G) may be camouflaged or superimposed by other environmental factors that may also influence cytokine levels. Although we did not measure cytokine levels, we could observe that the association between the GG genotype and the GCC haplotype for severe cases of COVID-19 was observed in this work only after statistical corrections were made for possible confounding environmental factors, such as sex, age and comorbidities.

Table 3
Association of *IL*10 models and genotypes of rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A) with the outcome of COVID-19 using adjusted binary logistic regression.

	Outcome	
	Death	
Genotypes *IL10*	**Adj OR (CI95 %)**	**Adj *P*-value**
rs1800896		
Additive		
AA	Reference	
AG	0.958 (0.409–2.243)	0.922
GG	0.666 (0.192–2.309)	0.522
Dominant		
AA	Reference	
AG + GG	0.883 (0.394–1.978)	0.762
Recessive		
AA + AG	Reference	
GG	0.685 (0.214–2.166)	0.516
rs1800871		
Additive		
CC	Reference	
CT	1.712 (0.728–4.028)	0.218
TT	1.179 (0.358–3.885)	0.787
Dominant		
CC	Reference	
CT + TT	1.564 (0.699–3.501)	0.277
Recessive		
CC + CT	Reference	
TT	0.875 (0.293–2.616)	0.811
rs1800872		
Additive		
CC	Reference	
CA	1.712 (0.728–4.028)	0.218
AA	1.179 (0.358–3.885)	0.787
Dominant		
CC	Reference	
CA + AA	1.564 (0.699–3.501)	0.277
Recessive		
CC + CA	Reference	
AA	0.875 (0.293–2.616)	0.811
Haplotype *IL10*		
ATA		
Others	Reference	
ATA/ATA	1.179 (0.358–3.885)	0.787
ATA/GCC + ACC	1.712 (0.728–4.028)	0.218
GCC		
Others	Reference	
GCC/GCC	0.681 (0.196–2.361)	0.544
GCC/ACC + ATA	0.997 (0.424–2.342)	0.994
ACC		
Others	Reference	
ACC/ACC	0.330 (0.070–1.547)	0.159
ACC/GCC + ATA	2.254 (0.922–5.510)	0.075

Adjusted binary logistic regression analysis for: age, sex, systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease. Reference: recovered group. Significance level adopted of $p < 0.05$, represented by values in bold. OR= Odds Ratio; CI = confidence interval; adj = adjusted. (SPSS Inc., Chicago, Illinois, USA).

IL-10 production is normally stimulated after immune system activation by infective agents in a regulatory effort to prevent tissue lesions derived from the immune response [8]. However, in severe cases of COVID-19, this release is exacerbated and accompanied by a cytokine storm, likely contributing to an impaired immune response, as it is known that IL-10 can reduce the activity of antigen-presenting cells (APCs) by downregulating the expression of costimulatory proteins and MHC, both of which are important components of lymphocyte activation and the adaptive immune response [47]. As discussed above, the G allele of rs1800896 (−1082 A > G) SNV was previously established to increase the expression of IL-10. This could explain the association between the GG genotype of the rs1800896 (−1082 A > G) SNV and the GCC haplotype and the severe cases of COVID-19 found in this work.

This research has limitations such as the lack of cytokine dosage and lack of a healthy uninfected group. Regarding the first issue, although the IL-10 protein levels could potentially enhance understanding of the functional impact of the variants evaluated here, the findings presented provide valuable insights into the genetic predisposition to severe COVID-19 and complement the broader understanding of its role in COVID-19 pathophysiology. It is important to note that elevated IL-10 levels in COVID-19 patients and their association with disease severity have already been reported in the literature [5,48,49]. However, correlating IL-10 levels with SNVs and disease severity would be an important addition to the scientific literature and should be considered in future studies. The lack of a healthy uninfected or exposure-resistant control group is explained considering our study's focus on disease severity rather than infection susceptibility. Our reference group consisted of infected individuals with mild symptoms, compared to those who developed moderate or severe disease. The inclusion of an exposure-resistant control group would not align with our study's primary aim and would require a different research design.

Nevertheless, the present study has several strengths, such as the haplotype analysis including three important SNVs from the proximal flanking region, a statistical power of 80 % and a robust statistical analysis adjusted for confounding factors, which allowed us to observe rs1800896 (A > G) GG and the GCC effect. Furthermore, to the best of our knowledge, this is the first reported work evaluating these SNVs in a Brazilian sample. Therefore, considering the prominence of IL-10, which is a prognostic marker for the severity of COVID-19, this work is important because of the influence of variants in the proximal flanking region of the *IL10* gene, rs1800896 (A > G), rs1800871 (C > T) and rs1800872 (C > A), as well as their haplotypes, on the severity and outcome of COVID-19.

Overall, the results of this study demonstrated that, in the Brazilian population, the IL-10 SNVs rs1800872 (C > A) and rs1800871 (C > T) are not associated with severe cases or death from COVID-19. However, the GG genotype of rs1800896 (A > G) and the homozygous GCC haplotype were associated with severe cases of the disease after confounding factors such as sex, age and the presence of comorbidities were considered in the analysis. These results could reinforce the use of IL-10 as a prognostic marker for disease worsening progression in COVID-19 patients, enabling the detection of rs1800896 (A > G) SNV together with IL-10 cytokine dosage to a more accurate prognostic.

CRediT authorship contribution statement

Janaina Nicolau de Oliveira: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Caroline Yukari Motoori Fernandes:** Writing – review & editing, Methodology. **Sara Mataroli de Godoy:** Writing – review & editing, Formal analysis. **Wilson Frantine-Silva:** Writing – review & editing, Formal analysis. **Pedro Luis Candido de Souza Cassela:** Writing – review & editing, Data curation. **Guilherme Lerner Trigo:** Writing – review & editing, Methodology. **Marcell Alysson Batisti Lozovoy:** Writing – review & editing, Methodology. **Zuleica Naomi Tano:** Writing – review & editing, Methodology, Data curation. **Andrea Name Colado Simão:** Writing – review & editing, Methodology, Data curation. **Karen Brajão de Oliveira:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The research leading to these results received funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Nível Superior (CAPES) under Grant Agreement No. 001, with no role in study design. The authors declare that they have no financial interests or non-financial interests.

Acknowledgement

The authors would like to acknowledge the patients who agreed to participate in this research, as well as the HU-UEL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2025.111261.

References

[1] G.U. Kim, M.J. Kim, S.H. Ra, J. Lee, S. Bae, J. Jung, S.H. Kim, Clinical characteristics of asymptomatic and symptomatic patients with mild COVID-19, Clin. Microbiol. Infect. 26 (2020) 948.e1–948.e3, https://doi.org/10.1016/j.cmi.2020.04.040.

[2] Q. Ye, B. Wang, J. Mao, The pathogenesis and treatment of the 'Cytokine Storm'' in COVID-19,, J. Infect. 80 (2020) 607–613, https://doi.org/10.1016/j.jinf.2020.03.037.

[3] B. Diao, C. Wang, Y. Tan, X. Chen, Y. Liu, L. Ning, L. Chen, M. Li, Y. Liu, G. Wang, Z. Yuan, Z. Feng, Y. Zhang, Y. Wu, Y. Chen, Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19), Front. Immunol. 11 (2020) 827, https://doi.org/10.3389/fimmu.2020.00827.

[4] M. Ben Azaiz, A. Ben Jemaa, W. Sellami, C. Romdhani, R. Ouslati, H. Gharsallah, E. Ghazouani, M. Ferjani, Deciphering the balance of IL-6/IL-10 cytokines in severe to critical COVID-19 patients, Immunobiology 227 (2022), https://doi.org/10.1016/j.imbio.2022.152236 152236.

[5] H. Han, Q. Ma, C. Li, R. Liu, L. Zhao, W. Wang, P. Zhang, X. Liu, G. Gao, F. Liu, Y. Jiang, X. Cheng, C. Zhu, Y. Xia, Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors, Emerg. Microbes Infect. 9 (2020) 1123–1130, https://doi.org/10.1080/22221751.2020.1770129.

[6] Y. Zhao, L. Qin, P. Zhang, K. Li, L. Liang, J. Sun, B. Xu, Y. Dai, X. Li, C. Zhang, Y. Peng, Y. Feng, A. Li, Z. Hu, H. Xiang, G. Ogg, L.P. Ho, A. McMichael, R. Jin, J.C. Knight, T. Dong, Y. Zhang, Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease, JCI Insight 5 (2020), https://doi.org/10.1172/jci.insight.139834. e139834.

[7] R.A. Saxton, N. Tsutsumi, L.L. Su, G.C. Abhiraman, K. Mohan, L.T. Henneberg, N.G. Aduri, C. Gati, K.C. Garcia, Structure-based decoupling of the pro- And anti-inflammatory functions of interleukin-10, Science 371 (2021), https://doi.org/10.1126/science.abc8433 eabc8433.

[8] M. Saraiva, P. Vieira, A. O'Garra, Biology and therapeutic potential of interleukin-10, J. Exp. Med. 217 (2020), https://doi.org/10.1084/jem.20190418. e20190418.

[9] I.H. Chan, V. Wu, M. Bilardello, E. Mar, M. Oft, P. van Vlasselaer, J.B. Mumm, The potentiation of IFN-γ and induction of cytotoxic proteins by pegylated IL-10 in human CD8 T cells, J. Interferon Cytokine Res. 36 (2015) 948–955, https://doi.org/10.1089/jir.2014.0221.

[10] A. Naing, J.R. Infante, K.P. Papadopoulos, I.H. Chan, C. Shen, N.P. Ratti, B. Rojo, K. A. Autio, D.J. Wong, M.R. Patel, P.A. Ott, G.S. Falchook, S. Pant, A. Hung, K.L. Pekarek, V. Wu, M. Adamow, S. McCauley, J.B. Mumm, P. Wong, P. Van Vlasselaer, J. Leveque, N.M. Tannir, M. Oft, PEGylated IL-10 (Pegilodecakin) induces systemic immune activation, CD8+ T cell invigoration and polyclonal T cell expansion in cancer patients, Cancer Cell 34 (2018) 775–791.e3, https://doi.org/10.1016/j.ccell.2018.10.007.

[11] J.M. Rojas, M. Avila, V. Martin, N. Sevilla, IL-10: A multifunctional cytokine in viral infections, J. Immunol. Res. 2017 (2017), https://doi.org/10.1155/2017/6104054 6104054.

[12] K. Dimitriadis, S. Katelani, M. Pappa, G.E. Fragkoulis, T. Androutsakos, The role of interleukins in HBV infection: a narrative review, J. Pers. Med. 13 (2023) 1675, https://doi.org/10.3390/jpm13121675.

[13] K. Alagarasu, H. Kaushal, P. Shinde, M. Kakade, U. Chaudhary, V. Padbidri, S.A. Sangle, S. Salvi, A.R. Bavdekar, P. D'costa, M.L. Choudhary, Tnfa and il10 polymorphisms and il-6 and il-10 levels influence disease severity in influenza a (H1n1)pdm09 virus infected patients, Genes 12 (2021) 1914, https://doi.org/10.3390/genes12121914.

[14] H. Islam, T.C. Chamberlain, A.L. Mui, J.P. Little, Elevated interleukin-10 levels in COVID-19: potentiation of pro-inflammatory responses or impaired anti-inflammatory action?, Front Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.677008 677008.

[15] L. Lu, H. Zhang, D.J. Dauphars, Y.-W. He, A potential role of interleukin 10 in COVID-19 pathogenesis, Trends Immunol. 42 (2021) 3–5, https://doi.org/10.1016/j.it.2020.10.012.

[16] E. Reuss, R. Fimmers, A. Kruger, C. Becker, C. Rittner, T. Höhler, Differential regulation of interleukin-10 production by genetic and environmental factors - A twin study, Genes Immun. 3 (2002) 407–413, https://doi.org/10.1038/sj.gene.6363920.

[17] J. Eskdale, G. Gallagher, A polymorphic dinucleotide repeat in the human IL-IO promoter, Immunogenetics 42 (1995) 444–445, https://doi.org/10.1007/BF00179416.

[18] J. Eskdale, D. Kube, G. Gallagher, A second polymorphic dinucleotide repeat in the 5' flanking region of the human IL-10 gene, Immunogenetics 45 (1996) 82–83, https://doi.org/10.1007/s002510050174.

[19] A.W. Gibson, J.C. Edberg, J. Wu, R.G.J. Westendorp, T.W.J. Huizinga, R.P. Kimberly, Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus, J. Immunol. 166 (2001) 3915–3922, https://doi.org/10.4049/jimmunol.166.6.3915.

[20] R. Tesse, G.C. Del Vecchio, D. De Mattia, M. Sangerardi, F. Valente, P. Giordano, Association of interleukin-(IL)10 haplotypes and serum IL-10 levels in the progression of childhood immune thrombocytopenic purpura, Gene 505 (2012) 53–56, https://doi.org/10.1016/j.gene.2012.05.050.

[21] J. Hernández-Bello, E. Oregón-Romero, M. Vázquez-Villamar, S. García-Arellano, Y. Valle, J.R. Padilla-Gutiérrez, I.V. Román-Fernández, C.A. Palafox-Sánchez, G.E. Martínez-Bonilla, J.F. Muñoz-Valle, Aberrant expression of interleukin-10 in rheumatoid arthritis: Relationship with IL10 haplotypes and autoantibodies, Cytokine 95 (2017) 88–96, https://doi.org/10.1016/j.cyto.2017.02.022.

[22] S. Assis, C.R. Marques, T.M. Silva, R.S. Costa, N.M. Alcantara-Neves, M.L. Barreto, K.C. Barnes, C.A. Figueiredo, IL10 Single Nucleotide Polymorphisms are related to upregulation of constitutive IL-10 production and susceptibility to Helicobacter pylori infection, Helicobacter 19 (2014) 168–173, https://doi.org/10.1111/hel.12119.

[23] B.J. Świątek, Is interleukin-10 gene polymorphism a predictive marker in HCV infection?, Cytokine Growth Factor Rev 23 (2012) 47–59, https://doi.org/10.1016/j.cytogfr.2012.01.005.

[24] T. Dhaouadi, A. Riahi, T.B. Abdallah, Y. Gorgi, I. Sfar, Impact of IL-10 gene promoter polymorphisms on treatment response in HCV patients: A systematic review, a meta-analysis, and a meta-regression, Int. J. Immunopathol. Pharmacol. 38 (2024) 1–18, https://doi.org/10.1177/03946320241240705.

[25] K. Matsumoto, A. Oki, T. Satoh, S. Okada, T. Minaguchi, M. Onuki, H. Ochi, S. Nakao, M. Sakurai, A. Abe, H. Hamada, H. Yoshikawa, Interleukin-10 – 1082 gene polymorphism and susceptibility to cervical cancer among Japanese women, Jpn. J. Clin. Oncol. 40 (2010) 1113–1116, https://doi.org/10.1093/jjco/hyq094.

[26] L. Miteva, S. Stanilova, The combined effect of interleukin (IL)-10 and IL-12 polymorphisms on induced cytokine production, Hum. Immunol. 69 (2008) 562–566, https://doi.org/10.1016/j.humimm.2008.07.008.

[27] T.K. Chen, J.H. Lee, H.H. Yu, Y.H. Yang, L.C. Wang, Y.T. Lin, B.L. Chiang, Association between human IL-10 gene polymorphisms and serum IL-10 level in patients with food allergy, J. Formos. Med. Assoc. 111 (2012) 686–692, https://doi.org/10.1016/j.jfma.2011.11.027.

[28] F.C.B. Berti, A.P.L. Pereira, K.P. Trugilo, G.C.M. Cebinelli, L.F. da R.S. Silva, M.A.B. Lozovoy, A.N.C. Simão, M.A.E. Watanabe, K.B. de Oliveira, IL-10 gene polymorphism c.-592C > A increases HPV infection susceptibility and influences IL-10 levels in HPV infected women, Infect. Genet. Evol. 53 (2017) 128–134, https://doi.org/10.1016/j.meegid.2017.05.020.

[29] M.G. Balzanelli, P. Distratis, R. Lazzaro, V.H. Pham, T.C. Tran, G. Dipalma, A. Bianco, E.M. Serlenga, S.K. Aityan, V. Pierangeli, K.C.D. Nguyen, F. Inchingolo, D. Tomassone, C. Gargiulo Isacco, Analysis of gene single nucleotide polymorphisms in COVID-19 disease highlighting the susceptibility and the severity towards the infection, Diagnostics 12 (2022) 2824, https://doi.org/10.3390/diagnostics12112824.

[30] S.J.A. Abbood, E. Anvari, A. Fateh, Association between interleukin-10 gene polymorphisms (rs1800871, rs1800872, and rs1800896) and severity of infection in different SARS-CoV-2 variants, Hum. Genomics 17 (2023) 19, https://doi.org/10.1186/s40246-023-00468-6.

[31] A. Yessenbayeva, B. Apsalikov, M. Massabayeva, M. Kazymov, A. Shakhanova, Z. Mussazhanova, I. Kadyrova, N. Aukenov, N. Shaimardanov, Biomarkers of immunothrombosis and polymorphisms of IL2, IL6, and IL10 genes as predictors of the severity of COVID-19 in a Kazakh population, PLoS One 18 (2023), https://doi.org/10.1371/journal.pone.0288139. e0288139.

[32] S. Rizvi, S.M.S. Rizvi, S.T. Raza, M. Abbas, K. Fatima, Z.H. Zaidi, F. Mahdi, Implication of single nucleotide polymorphisms in Interleukin-10 gene (rs1800896 and rs1800872) with severity of COVID-19, Egypt. J. Med. Human Genet. 23 (2022) 145, https://doi.org/10.1186/s43042-022-00344-3.

[33] M. Avendaño-Félix, L.A. Ochoa-Ramírez, R. Ramos-Payán, M. Aguilar-Medina, A. Ayala-Ham, H. Rendón-Aguilar, E. Lizárraga-Verdugo, F. Peraza-Garay, J.J. Ríos-Tostado, J.S. Velarde-Félix, Lack of effects of the genetic polymorphisms of

interleukin-10 in clinical outcomes of COVID-19, Viral Immunol. 34 (2021) 567–572, https://doi.org/10.1089/vim.2021.0022.
[34] World Health Organization, Clinical Management of COVID-19: Interim Guidance. https://iris.who.int/handle/10665/332196?locale-attribute=pt&show=full, 2020 (accessed 9 October 2023).
[35] M. Stephens, N.J. Smith, P. Donnelly, A new statistical method for haplotype reconstruction from population data, Am. J. Hum. Genet. 68 (2001) 978–989, https://doi.org/10.1086/319501.
[36] M. Stephens, P. Donnelly, A comparison of Bayesian methods for haplotype reconstruction from population genotype data, Am. J. Hum. Genet. 73 (2003) 1162–1169, https://doi.org/10.1086/379378.
[37] J.C. Barrett, B. Fry, J. Maller, M.J. Daly, Haploview: analysis and visualization of LD and haplotype maps, Bioinformatics 21 (2005) 263–265, https://doi.org/10.1093/bioinformatics/bth457.
[38] S. Richardson, J.S. Hirsch, M. Narasimhan, J.M. Crawford, T. McGinn, K.W. Davidson, D.P. Barnaby, L.B. Becker, J.D. Chelico, S.L. Cohen, J. Cookingham, K. Coppa, M.A. Diefenbach, A.J. Dominello, J. Duer-Hefele, L. Falzon, J. Gitlin, N. Hajizadeh, T.G. Harvin, D.A. Hirschwerk, E.J. Kim, Z.M. Kozel, L.M. Marrast, J.N. Mogavero, G.A. Osorio, M. Qiu, T.P. Zanos, Presenting characteristics, comorbidities, and outcomes among 5700 Patients hospitalized with COVID-19 in the New York City Area, JAMA – J. American Med. Assoc. 323 (2020) 2052–2059, https://doi.org/10.1001/jama.2020.6775.
[39] R. Silaghi-Dumitrescu, I. Patrascu, M. Lehene, I. Bercea, Comorbidities of COVID-19 patients, Medicina 59 (2023) 1393, https://doi.org/10.3390/medicina59081393.
[40] Departamento de Monitoramento, Avaliação e Disseminação de Informações Estratégicas em Saúde (DEMAS) da Secretaria da Informação e Saúde Digital (SEIDIGI) do Ministério da Saúde – Brazil, COVID-19 no Brasil. https://infoms.saude.gov.br/Extensions/Covid-19_html/Covid-19_html.Html, 2020 (accessed 9 October 2023).
[41] J. Little, J.P.T. Higgins, J.P.A. Ioannidis, D. Moher, F. Gagnon, E. Von Elm, M.J. Khoury, B. Cohen, G. Davey-Smith, J. Grimshaw, P. Scheet, M. Gwinn, R.E. Williamson, G.Y. Zou, K. Hutchings, C.Y. Johnson, V. Tait, M. Wiens, J. Golding, C. Van Duijn, J. McLaughlin, A. Paterson, G. Wells, I. Fortier, M. Freedman, M. Zecevic, R. King, C. Infante-Rivard, A. Stewart, N. Birkett, STrengthening the REporting of genetic association studies (STREGA)- An extension of the STROBE statement, Genet. Epidemiol. 33 (2009) 581–598, https://doi.org/10.1002/gepi.20410.
[42] S.D.J. Pena, F.R. Santos, E. Tarazona-Santos, Genetic admixture in Brazil, Am. J. Med. Genet. C Semin. Med. Genet. 184 (2020) 928–938, https://doi.org/10.1002/ajmg.c.31853.
[43] A. Vilkeviciute, D. Cebatoriene, L. Kriauciuniene, R. Zemaitiene, R. Liutkeviciene, IL-9 and IL-10 single-nucleotide variants and serum levels in age-related macular degeneration in the Caucasian population, Mediators Inflamm. (2021), https://doi.org/10.1155/2021/6622934 6622934.
[44] S. Dattani, D.M. Howard, C.M. Lewis, P.C. Sham, Clarifying the causes of consistent and inconsistent findings in genetics, Genet. Epidemiol. 46 (2022) 372–389, https://doi.org/10.1002/gepi.22459.
[45] H. Zhang, V. Kuchroo, Epigenetic and transcriptional mechanisms for the regulation of IL-10, Semin. Immunol. 44 (2019), https://doi.org/10.1016/j.smim.2019.101324 101324.
[46] M. Mörmann, H. Rieth, T.D. Hua, C. Assohou, M. Roupelieva, S.L. Hu, P.G. Kremsner, A.J. Luty, D. Kube, Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used, Genes Immun. 5 (2004) 246–255. http://doi:10.1038/sj.gene.6364073.
[47] A.K. Mittal, P.A. Roche, Suppression of antigen presentation by IL-10, Curr. Opin. Immunol. 34 (2015) 22–27, https://doi.org/10.1016/j.coi.2014.12.009.
[48] J. Liu, S. Li, J. Liu, B. Liang, X. Wang, H. Wang, W. Li, Q. Tong, J. Yi, L. Zhao, L. Xiong, C. Guo, J. Tian, J. Luo, J. Yao, R. Pang, H. Shen, C. Peng, T. Liu, Q. Zhang, J. Wu, L. Xu, S. Lu, B. Wang, Z. Weng, C. Han, H. Zhu, R. Zhou, H. Zhou, X. Chen, P. Ye, B. Zhu, L. Wang, W. Zhou, S. He, Y. He, S. Jie, P. Wei, J. Zhang, Y. Lu, W. Wang, L. Zhang, L. Li, F. Zhou, J. Wang, U. Dittmer, M. Lu, Y. Hu, D. Yang, X. Zheng, Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients, EBioMedicine 55 (2020), https://doi.org/10.1016/j.ebiom.2020.102763 102763.
[49] G. Chen, D. Wu, W. Guo, Y. Cao, D. Huang, H. Wang, T. Wang, X. Zhang, H. Chen, H. Yu, X. Zhang, M. Zhang, S. Wu, J. Song, T. Chen, M. Han, S. Li, X. Luo, J. Zhao, Q. Ning, Clinical and immunological features of severe and moderate coronavirus disease 2019, J. Clin. Investig. 130 (2020) 2620–2629, https://doi.org/10.1172/JCI137244.

Supplementary material

Supplementary Table 1. Sociodemographic parameters and smoking habits of patients with COVID-19

Variable		COVID-19							Outcome				
		Mild (n=165)		Moderate (n=72)		Severe (n=130)		*p*-value	Recovered (n=64)		Death (n=66)		p-value
		N	%	N	%	N	%		N	%	N	%	
Sex	Female	98	59.4	28	38.9	54	41.5	**0.002**	28	43.8	26	39.4	0.614
	Male	67	40.6	44	61.1	76	**58.5**		36	56.3	40	60.6	
Age	18 to 39	82	49.7	8	11.1	7	5.4	**<0.001**	7	11.5	0	0.0	**0.001**
	40 to 49	27	16.4	16	22.2	11	8.5		9	14.8	2	3.0	
	50 to 59	26	15.8	14	19.4	22	16.9		11	18.0	10	15.2	
	60 to 69	13	7.9	11	15.3	25	**19.2**		13	21.3	12	18.2	
	70 to 79	10	6.1	11	15.3	29	**22.3**		12	19.7	17	25.8	
	≥80	7	4.2	12	16.7	36	**27.7**		9	14.8	25	**37.9**	
Ethnicity	Caucasian	131	79.4	56	80.0	96	74.4	0.525	48	75	49	74.2	0.921
	No caucasian	34	20.6	14	20.0	33	25.6		16	25	17	25.8	
	Yes	3	1.8	6	8.3	4	3.1	0.054*	4	6.3	0	0.0	0.056*
Smoking habit	No	162	98.2	66	91.7	126	96.9		60	93.8	66	100	
	Ex-smoker	8	4.8	7	9.7	12	9.2	0.249	7	10.9	5	7.6	0.508

Analysis using the two-tailed Chi-square test (χ^2) or Fisher's Exact Test*. P<0.05 was adopted as the level of significance, represented by values in bold (SPSS Inc., Chicago, Illinois, USA).

Supplementary Table 2. Strength of association of demographic parameters and presence of comorbidities with COVID-19 severity

	COVID-19					
	Moderate			Severe		
Demographic characteristics and comorbidities	Wald	adj OR (CI95%)	adj p-value	Wald	adj OR (CI95%)	adj p-value
Age (years)						
18 to 39		Reference			reference	
40 to 49	9.964	5.053 (1.848 – 13.818)	**0.002**	5.818	3.795 (1.284 – 11.215)	**0.016**
50 to 59	6.424	3.796 (1.353 – 10.650)	**0.011**	13.714	6.654 (2.440 – 18.142)	**<0.001**
60 to 69	6.817	4.831 (1.481 – 15.757)	**0.009**	20.592	12.521 (4.203 – 37.299)	**<0.001**
70 to 79	8.020	5.899 (1.727 – 20.148)	**0.005**	26.692	19.060 (6.230 – 58.313)	**<0.001**
≥80	11.574	9.554 (2.603 – 35.069)	**0.001**	33.914	35.327 (10.643 – 117.254)	**<0.001**
Sex						
Female		Reference			reference	
Male	4.684	2.024 (1.069 – 3.833)	**0.030**	3.671	1.765 (0.987 – 3.156)	0.055
Comorbidities						
SAH						
No		Reference			reference	
Yes	3.684	2.081 (0.985 – 4.399)	0.055	0.502	1.280 (0.647 – 2.534)	0.479
DM						
No		Reference			reference	
Yes	1.052	1.581 (0.659 – 3.796)	0.305	7.572	2.964 (1.367 – 6.426)	**0.006**
COPD						
No		Reference			reference	
Yes	-	-	-	-	-	-
CKD						
No		Reference			reference	
Yes	6.879	6.330 (1.594 – 25.136)	**0.009**	3.974	3.932 (1.023 – 15.107)	**0.046**
Heart Diseases						
No		Reference			reference	
Yes	3.867	0.336 (0.114 – 0.996)	**0.049**	0.005	0.970 (0.427 – 2.207)	0.943

Adjusted multinomial logistic regression analysis for: age, sex, systemic arterial hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease and heart disease. Reference: mild group. Significance level adopted of $p<0.05$, represented by values in bold. OR= Odds Ratio; CI= confidence interval; adj=adjusted. (SPSS Inc., Chicago, Illinois, USA). SAH= Systemic Arterial Hypertension; DM= Diabetes *Mellitus*; COPD= Chronic Obstructive Pulmonary Disease; CKD= Chronic Kidney Disease.

Supplementary Table 3. Hardy-Weinberg Equilibrium analysis of the SNVs rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A) of *IL*10 according with the severity and outcome of COVID-19

	COVID-19						Outcome			
SNV *IL10*	Mild		Moderate		Severe		Recovered		Death	
	X^2	*P - value*	X^2	*P - value*	X^2	*P - value*	X^2	*P - value*	X^2	*P - value*
rs1800896	0,856	0,651	0	1	0,812	0,666	1,19	0,550	0,033	0,983
rs1800871	0,327	0,849	0	1	0	1	0,582	0,748	0,512	0,774
rs1800872	0,327	0,849	0	1	0	1	0,582	0,748	0,512	0,774

Analysis using the two-tailed Chi-square test (X^2). P<0.05 was adopted as the level of significance, represented by values in bold (SPSS Inc., Chicago, Illinois, USA).

Supplementary Table 4. Summary of linkage disequilibrium (LD) metrics.

L1	L2	*D'*	LOD	r^2	CI-low	CI-high	Dist (bp)	T-int
rs1800872	rs1800871	1.0	159.38	1.0	0.99	1.0	227	200.34
rs1800872	s1800896	1.0	40.96	0.327	0.95	1.0	490	-
rs1800871	s1800896	1.0	40.53	0.327	0.95	1.0	263	81.49

Analysis of linkage disequilibrium (LD). D' (measure of LD completeness), LOD score (logarithm of odds for LD significance), r^2 (correlation measure), CI-low and CI-high (lower and upper bounds of the confidence intervals for *D'*, physical distance between SNPs (in base pairs), and transmission intensity (T-int).

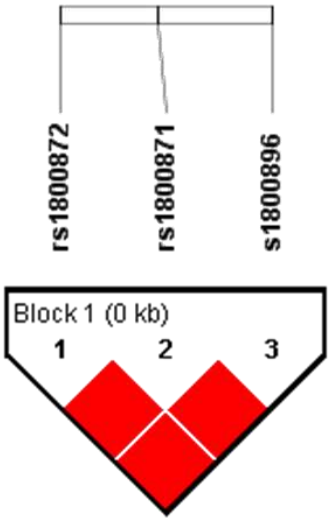


Figure S1. Linkage disequilibrium (LD) plot among the three SNPs analyzed (rs1800872, rs1800871, and rs1800896). The plot was generated using Haploview (Barrett *et al.*, 2005). SNPs are represented at the top, and the red diamonds indicate LD metrics between pairs of loci. High D' values and strong LD are indicated by darker red shades.

Supplementary Table 5. Association of genotypes and haplotypes of the SNVs rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A) of *IL*10 with the severity and outcome of COVID-19

Genotype *IL10*		COVID-19						*P*-value	Outcome				*P*-value
		Mild (n=163)		Moderate (n=72)		Severe (n=130)			Recovered (n=64)		Death (n=66)		
		N	%	N	%	N	%		N	%	N	%	
rs1800896													
Codominant	AA	68	41.7	28	38.9	56	43.1	0.377	25	39.1	31	47.0	
	AG	80	49.1	34	47.2	53	40.8		25	39.1	28	42.4	0.211
	GG	15	9.2	10	13.9	21	16.2		14	21.9	7	10.3	
Dominant	AA	68	41.7	28	38.7	56	43.1	0.846	25	39.1	31	47.0	0.363
	GG+AG	95	58.3	44	61.3	74	56.9		39	60.9	35	53.0	
Recessive	AA+AG	148	90.8	62	86.1	109	83.8	0.191	50	78.1	59	89.4	0.081
	GG	15	9.2	10	13.9	21	16.2		14	21.9	7	10.6	
Alleles	A	216	66.3	90	62.5	165	63.5	0.665	75	58.6	90	68.2	0.108
	G	110	33.7	54	37.5	95	36.5		53	41.4	42	31.8	
rs1800871													
Codominant	CC	70	42.9	28	38.9	48	36.9	0.877	28	43.8	20	30.3	0.233
	CT	70	42.9	34	47.2	62	47.7		26	40.6	36	54.5	
	TT	23	14.1	10	13.9	20	15.4		10	15.6	10	15.2	
Dominant	CC	70	42.9	28	38.9	48	36.9	0.566	28	43.8	20	30.3	0.112
	CT+TT	93	57.1	44	61.1	82	63.1		36	56.3	46	69.7	
Recessive	CC+CT	140	85.9	62	86.1	110	84.6	0.940	54	84.4	56	84.8	0.940
	TT	23	14.1	10	13.9	20	15.4		10	15.6	10	15.2	
Alleles	C	214	64.9	90	62.5	158	60.8	0.590	82	64.0	76	57.6	0.284
	T	116	35.1	54	37.5	102	39.2		46	36.0	56	42.4	
rs1800872													
Codominant	CC	70	42.9	28	38.9	48	36.9	0.877	28	43.8	20	30.3	0.233
	CA	70	42.9	34	47.2	62	47.7		26	40.6	36	54.5	
	AA	23	14.1	10	13.9	20	15.4		10	15.6	10	15.2	
Dominant	CC	70	42.9	28	38.9	48	36.9	0.566	28	43.8	20	30.3	0.112
	AA+CA	93	57.1	44	61.1	82	63.1		36	56.3	46	69.7	
Recessive	CC+CA	140	85.9	62	86.1	110	84.6	0.940	54	84.4	56	84.8	0.940

	AA	23	14.1	10	13.9	20	15.4		10	15.6	10	15.2	
Alleles	C	214	64.9	90	62.5	158	60.8	0.590	82	64.0	76	57.6	0.284
	A	116	35.1	54	37.5	102	39.2		46	36.0	56	42.4	
Haplotypes *IL10*													
	GCC	113	34.2	54	37.5	95	36.3		53	41.4	42	31.8	
	ACC	101	30.6	36	25.0	64	24.4	0.488	29	22.7	34	25.8	0.273
	ATA	116	35.2	54	37.5	103	39.3		46	35.9	56	42.4	

Analysis carried out using the two-tailed Chi-square test (χ^2), with $p<0.05$ being adopted as the level of significance (SPSS Inc., Chicago, Illinois, USA).

Revisão narrativa

IL10 GENE UPSTREAM VARIANTS: CONTRIBUTION TO DIFFERENTIAL GENE EXPRESSION AND VIRAL DISEASE ASSOCIATION

Janaina Nicolau de Oliveira[1], Maylla Cardoso de Oliveira[1], Giulia Mariane Fortunato[1], Mariane Ricciardi da Silva[1], Pamella Rodrigues da Silva[1], Karen Brajão de Oliveira[1]

1 Department of Immunology, Parasitology and General Pathology, Biological Sciences Center, State University of Londrina, Londrina 86057-970, PR, Brazil

* Correspondence: karen.brajao@uel.br; Tel.: +55 43 33715629

Abstract

Interleukin-10 (IL-10) is considered a regulatory cytokine because it can suppress or stimulate the activity of several types of immune cells. In this context, disturbances in the regulation of this cytokine may be related to several pathological contexts. Thus, genetic variants that regulate the expression of this gene may also be associated with various clinical conditions. SNVs rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A), of the upstream region of the *IL10* gene have been extensively studied regarding their possible contribution and association with viral diseases. However, some results in genetic association studies have proven contradictory in the literature, making it necessary to synthesize the knowledge generated to date in this field of knowledge. Although promising, the use of these SNVs as biomarkers should be preceded by further studies, including larger haplotypes and systematic reviews, deepening the understanding about its effects in each viral disease, including COVID-19.

Key-words: COVID-19; rs1800890; IL10.G; IL10.R; viral infections

1. Introduction

Genetic variants are modifications in the deoxyribonucleic acid (DNA) sequence compared to a reference sequence (Den Dunnen *et al.*, 2016) Among the types of existing variants, the single nucleotide variations (SNV) are the most frequent in the human genome. Depending on where this change occurs, different molecular consequences are triggered. The presence of variants in coding regions of the gene can lead to changes in the codon in the transcribed messenger ribonucleic acid (mRNA) molecule, resulting in the exchange of an

amino acid in the peptide chain (missense variant), or in the maintenance of the same amino acid (synonymous variant), in the premature stop of protein synthesis (nonsense variant) or in the alteration of the codon reading frame (frameshift variant). Variants in regulatory *loci* or promoter regions do not lead to changes in the peptide chain sequence but can lead to increased or reduced gene expression and therefore, possibly, the amount of protein synthesized (Marian, 2020).

Several variants have been identified in the *IL10* gene, including variants in intronic regions and in the 3' untranslated region (3'UTR). Other variants have been identified upstream of the gene, in potentially regulatory regions, which could therefore interfere with the binding of transcription factors (TF) to DNA, and consequently, with gene expression (Lambert *et al.*, 2018; Marian, 2020).

Interleukin-10 (IL-10) is considered a regulatory cytokine because it can suppress or stimulate the activity of several types of immune cells. It can be secreted by almost all cells of the immune system (Ouyang; O'Garra, 2019), and can act on dendritic cells and macrophages by reducing the expression of major histocompatibility complex (MHC) molecules, costimulatory molecules and pro-inflammatory cytokines, generally reducing the activation of the adaptive immune system and the inflammatory response (Saraiva; Vieira; O'Garra, 2020).

On the other hand, this cytokine can stimulate the activity of CD8+ T cells and Natural Killer (NK) cells, essential components of the antiviral immune response (Saraiva; Vieira; O'Garra, 2020). Given the importance of this cytokine in the immune response, changes in the levels of this protein have already been associated with several clinical contexts (Alagarasu *et al.*, 2015; Berti *et al.*, 2017; Freitas *et al.*, 2024; Helminen; Lahdenpohja; Hurme, 1999; Li *et al.*, 2013; Naicker *et al.*, 2012; Nedelkopoulou *et al.*, 2021; Schuurhof *et al.*, 2011; Singhal *et al.*, 2015; Torres-Poveda *et al.*, 2012; Wu *et al.*, 2015; Zhang *et al.*, 2018; Zhao *et al.*, 2017).

Similarly, variants located upstream of the gene have also been the target of extensive scientific research, in which, mainly the SNVs rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A), were associated with several clinical contexts such as cancers, sepsis, spontaneous abortion, autoimmune diseases, infections by protozoa, bacteria and several viruses (Bai *et al.*, 2016; Berti *et al.*, 2017; Dhouioui *et al.*, 2024; Mohammadi *et al.*, 2019; Schotte *et al.*, 2015; Singhal

et al., 2015; Torres-Poveda *et al.*, 2012; Vakili *et al.*, 2024; Yu *et al.*, 2013). However, some results in genetic association studies have proven contradictory in the literature (Eskdale *et al.*, 1999; Reuss *et al.*, 2002), making it necessary to synthesize the knowledge generated to date in this field of knowledge, to enable the design of new studies aimed at understanding the role of variants located upstream of the gene in viral diseases.

2. Gene structure and regulation of *IL10* transcription

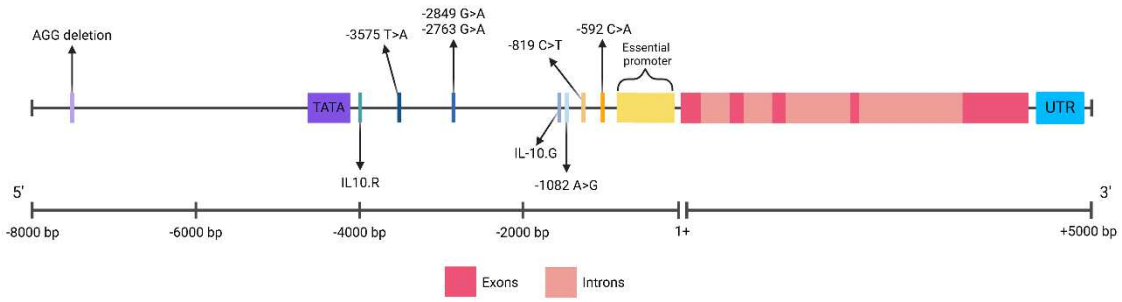
The interleukin-10 gene (*IL10)* is located on chromosome 1, in the 1q32.1 region (Eskdale *et al.*, 1997). It extends for approximately 5kb and is composed of five exons and four introns (Figure 1). It is preceded by a 5´ flanking region, characterized as proximal. This region contains the promoter (141/+27), which includes the transcription start site (+1), a TATA-box segment (-77) and the upstream sequence containing approximately 80 to 140 bases. In the proximal region of the gene, positive (-1000/-800) and negative (-750/-350) regulatory regions were also identified (Eskdale *et al.*, 1997; Kube *et al.*, 1995). Located between bases -9296 and -4021 upstream of the proximal region, potentially functional and regulatory motifs were also identified, including a TATA-box region at position -4021 (Kube *et al.*, 2001). Among the potentially functional motifs identified upstream of the gene, several regions were detected that could allow the binding of transcription factors, such as AP-1, EBNA2-like, PEA1, Pu-box, ETS-1, ETS-1, SP1, YY1, NF-kB, NFAT, CREB, among others (Kube *et al.*, 1995, 2001). Although the regulation of *IL10* gene transcription has not yet been completely elucidated, it is known that this process can vary according to the cell type involved and the stimulus received.

It was reviewed that in myeloid lineage cells such as macrophages and conventional dendritic cells, the main pathways and transcription factors involved are ERK, NF-kB, mTOR and p38, resulting from the engagement of pattern recognition receptors (PRR) such as toll-like receptors (TLR), and also interferon type I (IFN I) receptor. In $CD4^+$ T lymphocytes, T-cell receptor (TCR) activation induces *IL10* transcription via the nuclear factor of activated T cells (NFAT), nuclear factor kappa B (NF-kB) and extracellular signal-regulated kinase (ERK) pathways, while cytokine receptors engage GATA binding protein 3 (GATA-3) and

signal transducer and activator of transcription (STAT1, STAT3 and STAT4), which in turn positively regulates *IL10* expression (Gabrysová *et al.*, 2014, Mackenzie; Pattinson; Arthur, 2014).

In addition to the transcriptional regulation of *IL10*, protein levels are also regulated through chromatin remodeling and post-transcriptional control mechanisms, as reviewed in other studies (Gabrysová *et al.*, 2014; Zhang; Kuchroo, 2019). In this complex regulatory network, it is known that IL-10 levels can still vary substantially between different individuals, with this intervariability being associated with certain environmental factors such as sex, body mass index and smoking habits and, mainly, with the presence of genetic variants in the *IL10* gene region, which can contribute up to 50% of the variability found between individuals (Reuss *et al.*, 2002; Westendorp *et al.*, 1997).

Figure 1. *IL10* gene and main upstream variants



Source: the author himself.

3. *IL10* gene proximal and distal variants

Traditionally, genetic polymorphisms were defined as the presence of two or more allelic forms at a given DNA *locus* in at least 1% of the population (Karki *et al.*, 2015). Currently, however, it is proposed to call “variants” instead of polymorphisms the alterations in a gene sequence that differ from the reference sequence (Den Dunnen *et al.*, 2016). Variants that involve only one nucleotide, such as substitutions and occasionally insertions, deletions and duplications, are called single nucleotide variants (SNV), as recommended by the Human Genome Variation Society (HGVS) Nomenclature. Variants positioned contiguously on the same chromosome can form blocks or haplotypes, and those in linkage disequilibrium (LD) tend to be inherited together (Salim; Xavier, 2014).

The *IL10* gene has a 5' flanking region in which several variants have been identified, which can be accessed through the National Center for Biotechnology Information (NCBI) dbSNP platform (Phan et al., 2025) and in GenBank under code X78437. In the proximal 5' region, the biallelic SNVs rs1800896 (-1082 A>G), rs1800871 (-819 C>T), rs1800872 (-592 C>A) and the microsatellite IL10.G (-1.1 kb), stand out. This microsatellite has 16 possible alleles which were identified in a European population and are characterized by the presence of the CA dinucleotide, repeated 16 to 28 times in tandem (Crawley et al., 1999; Eskdale; Gallagher, 1995; Kube et al., 1995; Turner et al., 1997). Among the variants identified in the distal 5' flanking region, the following stands out: SNVs rs1800890 (-3575 T>A), rs6703630 (-2849 G>A), rs6693899 (-2763 C>A), a 3-bp deletion (AGG) at position -7400, 4 Alu-repetitive regions and the microsatellite IL10.R (-4.0kb), which consists of the repetition of the CA dinucleotide, in five different alleles (Eskdale; Kube; Gallagher, 1996; Gibson et al., 2001; Kube et al., 2001) (Figure 1).

The SNVs of the proximal 5' flanking region mainly form the GCC, ACC and ATA haplotypes in Caucasian populations, in which the -819C and -592C alleles are in strong linkage disequilibrium and can form blocks with the -1082G or -1082A alleles (Oliveira *et al.*, 2025). The GTA haplotype, rarely found in Caucasians (Chiu Mok *et al.*, 1998; Minnicelli, 2009), was found in Chinese individuals. Larger haplotypes, formed by alleles of the IL10.R and GCC, ACC and ATA haplotypes, were also identified, allowing the characterization of four haplotype families in European individuals. The families IL10.01, R3-(IL10.G)-G-C-C, and IL10.04, R2/IL10.G/A-T-A, were most frequently found in association with the G9 allele of the IL10.G, while the families IL10.02, R2-(IL10.G)-A-C-C, and IL10.03, R2-(IL10.G)-G-C-C, were most frequently associated with the IL10.G13 allele (Eskdale et al., 1999).

The presence of these genetic variants and their haplotypes has been associated with differential production of the IL-10 protein and with different diseases, such as autoimmune diseases, cancer, and infections. However, the effect or association of the variants with the expression of *IL10* is not yet completely established and presents controversies in the literature, according to different factors such as the cell type evaluated and molecular stimulus used in

in vitro studies, population ethnicity and pathological context, suggesting the need for a thorough look at such associations.

4. Variants contribution to *IL10* gene expression

IL10 gene expression is a complex process that appears to involve several transcription factors depending on the cell type and triggering stimulus (Mörmann *et al.*, 2004; Rees *et al.*, 2002). These transcription factors fulfill their function by stimulating or repressing gene expression through their interaction with specific DNA segments to which they have chemical affinity, and which are in promoter and regulatory regions. In the presence of genetic variants in these regions, this molecular interaction may have increased or decreased affinity, resulting in an increase or reduction in the function of a given transcription factor at a given *locus*, reflecting changes in gene expression (Duttke *et al.*, 2024).

It is also useful to investigate the presence of haplotypes formed by the variants, since the individual effect of these may be overlapped or overshadowed by other nearby variants. In this way, haplotype analysis can add a new perspective on the combined effect of all variants that can be inherited together and perhaps provide a new understanding of genetic variation over large regions (Carlson *et al.*, 2004; Rao *et al.*, 2007).

4.1 rs1800871 (-819 C>T) and rs1800872 (-592 C>A)

The proximal 5' flanking region contains potentially regulatory segments, such as a possibly positive regulatory region (-1000/-800), in which SNV rs1800871 (-819 C>T) is inserted, and a negative regulatory region (-750/-350), in which SNV rs1800872 (-592 C>A) is observed (Eskdale *et al.*, 1997; Kube *et al.*, 1995). In Caucasians, these SNVs presents complete linkage disequilibrium, and the variant alleles are inherited together (TA) (Oliveira *et al.*, 2025; Reuss *et al.*, 2002; Vilkeviciute *et al.*, 2021).

In cultures of cells isolated from the peripheral blood of individuals homozygous for the C allele or the A allele (rs1800872), it was observed that after stimulation mediated by lipopolysaccharide (LPS), a molecule commonly found in the cell wall of gram-negative bacteria, B lymphocytes and monocytes carrying

the CC genotype secreted higher levels of IL-10 than those with the AA genotype. In the same study, the total population of $CD4^+$ T lymphocytes and regulatory $CD4^+25^+$ T lymphocytes cell cultures did not show differences in IL-10 levels after stimulation with concanavalin A (Con A) or with PMA/ionomycin (Steinke; Culp, 2007).

When evaluated in the serum of Chinese patients, the A allele was also associated with the reduction of IL-10 levels (Zhao *et al.*, 2017). In contrast, no association was observed between serum IL-10 levels and the SNV rs1800872 (-592 C>A) in the studies of Alagarasu *et al.*, (2015) and Schuurhof *et al.*, (2011). The A allele was associated with elevated levels of protein in serum and cervical secretion in other clinical contexts (Berti *et al.*, 2017; Torres-Poveda *et al.*, 2012; Wu *et al.*, 2015). Considering the apparently contradictory results, it is possible that the -592 (C>A) region contributes to the increase and decrease of *IL10* transcription, depending on the cell type and stimulus present.

This SNV is flanked upstream by a binding site for the Sp1/Sp3 TFs, and the binding of these molecules to the DNA results in the repression of *IL10* gene expression. In B lymphocytes, it was observed that rs1800872 reduces the binding capacity of Sp1/Sp3, which therefore stops the suppression effect on gene expression, resulting in increased gene transcription and protein production in the presence of the A allele (Steinke *et al.*, 2004).

Although Sp1/Sp3 has been identified as a transcription factor capable of binding to the proximal region of the *IL10* gene, and in B lymphocytes its chemical affinity has been influenced by the SNV rs1800872 (-592 C>A), it is not guaranteed that these same TFs are essential for gene expression in other cell types. Depending on the stimulus received by the cell, it is possible that a different molecular signal transduction pathway is activated, culminating in the activation of different transcription factors, which may also have the capacity to regulate the expression of the *IL10* gene.

4.2 rs1800896 (-1082 A>G)

Among the variants already associated with changes in *IL10* expression, SNV rs1800896 (-1082 A>G) is one of the most studied. This SNV consists of the substitution of an adenine (A) for a guanine (G) at position c.-1082 of the proximal 5' flanking region, resulting in increased gene expression (Reuss *et al.*, 2002).

In vitro studies have associated the presence of the G allele with increased production of IL-10 from monocytic lineage (THP1) (Reuss *et al.*, 2002), B lymphocytes (Larsson *et al.*, 2009) and peripheral blood mononuclear cells (PBMC) (Mörmann *et al.*, 2004), resulting from stimulation with LPS. This same association was observed in T lymphocytes, after stimulation with Con A (Turner *et al.*, 1997).

In contrast, the evaluation of B cells transformed by Epstein-Baar virus (EBV) in another study, demonstrated the association of the G allele with low levels of IL-10, while the A allele was associated with high levels of the protein (Rees *et al.*, 2002). In patients with food allergy, the AA genotype was associated with high serum levels of IL-10 (Nedelkopoulou *et al.*, 2021). No association with changes in gene expression was observed after molecular stimulation with db-cAMP (Mörmann *et al.*, 2004), indicating that the observable effect of the presence of this variant also seems to change according to the stimulus applied or cell type evaluated, as happens with SNV rs1800872 (C>A).

However, in most published studies, the G allele of this variant was associated with higher gene expression, while the A allele was associated with lower expression (Assis *et al.*, 2014; Chen *et al.*, 2012; Dhaouadi *et al.*, 2024; Hernández-Bello *et al.*, 2017; Matsumoto *et al.*, 2010; Miteva; Stanilova, 2008; Reuss *et al.*, 2002; Światek-Koscielna *et al.*, 2017; Tesse *et al.*, 2012). The contribution of SNV rs1800896 (-1082 A>G) to increased gene expression may occur due to its location in a *locus* that binds to the transcription factors PU.1, Spi-B and Sp1 (Larsson *et al.*, 2009).

In B lymphocytes, the nuclear factor Spi-B can interact with both the A and G alleles, possibly undergoing little or no functional change upon the presence of SNV -1082 A>G. The transcription factor PU.1, in turn, has a repressor function and, when interacting with the flanking region of the gene, suppresses its expression. Despite interacting with DNA containing both the A and G alleles, the PU.1 factor was described as having a higher binding affinity to the A allele. Thus, in the presence of the A allele, the PU.1 factor would bind with greater affinity, suppressing gene expression (Capasso *et al.*, 2007; Reuss *et al.*, 2002). In contrast, the Sp1 factor acts by stimulating gene expression and interacts with greater affinity to the proximal region in the presence of the G allele, thus stimulating gene transcription (Larsson *et al.*, 2009).

Although Sp1 was observed to have a repressive function in the expression of the *IL10* gene in the study of Steinke *et al.*, (2004) in Larsson and colleagues' study (Larsson *et al.*, 2009), also done with B lymphocytes, it was observed that the binding of Sp1 to position -1082bp, instead of -592bp, stimulated its transcription. Sp1 is ubiquitously expressed in most cells of the organism, and it is known to be one of the first TF to bind to DNA allowing the transcription machinery to function, activating gene expression. Sp1 can also suppress it, through the interaction with epigenetic modifiers (O'Connor; Gilmour; Bonifer, 2016).

It is also important to consider that TFs can interact and bind together to activate or suppress the transcription machinery and a group of molecules are necessary for this biological process (Lambert *et al.*, 2018). Therefore, considering that the cell type, stimulus used and even the pathological context could influence the TFs activated in the cell (Lambert *et al.*, 2018), these differences, which underlies these genetic associations studies, could perhaps explain, at least in part, the contradictions found in the SNVs association with *IL10* expression. A summary of the studies found in literature which evaluated single variants effects on IL-10 levels are described in Table 1.

Table 1. Association of *IL10* variants with changes in IL-10 levels

Changes in gene expression and IL-10 levels	**Country**	**Reference**
rs1800896, -1082 A>G		
G allele ↑ production of IL-10	Finland	HELMINEN; LAHDENPOHJA; HURME, 1999
	South Africa	NAICKER *et al.*, 2012
AA genotype ↑ levels of IL-10	Greece	NEDELKOPOULOU *et al.*, 2021
No association	Mexico	TORRES- POVEDA *et al.*, 2012
	India	ALAGARASU *et al.*, 2021
rs1800872, -592 C>A		

A allele ↑ levels of IL-10	China	WU *et al.*, 2015
	Brazil	BERTI *et al.*, 2017
	Mexico	TORRES- POVEDA *et al.*, 2012
A allele ↓ levels of IL-10	China	ZHAO *et al.*, 2017
No association	The Netherlands	SCHUURHOF *et al.*, 2011
	South Africa	NAICKER *et al.*, 2012
	India	ALAGARASU *et al.*, 2021
rs1800890, -3575 T>A		
No association	China	(ZHANG *et al.*, 2018)
TT genotype ↑ levels of IL-10	Brazil	(FREITAS *et al.*, 2024)

Source: the author himself.

4.3 Haplotypes contribution to IL10 expression

Haplotypes are neighboring segments of DNA, on the same chromosome, that form blocks inherited together and that, therefore, do not undergo independent segregation. Thus, these DNA *loci* are said to be in linkage disequilibrium (LD) (Crawford; Nickerson, 2005; Zhao; Pfeiffer; Gail, 2003). As an example, hypothetically, a variant allele T could be frequently inherited together with a variant allele, A, of another closely located SNV, thus integrating the haplotype TA.

The haplotypes identified in the upstream region of the *IL10* gene, GCC, ACC and ATA, formed by the proximal SNVs (rs1800896, rs1800871 and rs1800872), appear to have a similar effect to that observed by the individual SNVs. In whole blood cell cultures stimulated with LPS, ATA in homozygosity reduced production of IL-10, compared with haplotypes containing GCC or ACC (Crawley *et al.*, 1999). In lymphocytes stimulated with concanavalin A, anti-CD3+ and anti-CD28+, the haplotypes GCC/GCC were identified as high producers of IL-10, GCC/ATA and GCC/ACC as intermediate producers and those haplotypes without the presence of the G allele of SNV rs1800896 (A>G), ACC/ACC and

ATA/ATA, as low producers (Hoffmann *et al.*, 2001). Furthermore, in monocytic lineage, the GCC haplotype was also associated with high levels of IL-10 after LPS (Reuss *et al.*, 2002). However, in other studies, no effect of any of the haplotypes on *IL10* expression or protein dosage was observed, either in memory T cells (Kremer; Kumar; Hedin, 2007) or in whole blood (Reuss *et al.*, 2002).

When considering SNV rs1800896 (A>G) in haplotype with the IL10.G microsatellite, it was identified that the presence of the G allele of the SNV, together with the IL10.G10, IL10.G12 and IL10.G14 alleles contributed to greater protein production, after stimulation with LPS (Mörmann *et al.*, 2004). In confirmation, when evaluating the functionality of the microsatellites, the IL10.G14 allele was associated with increased IL-10 levels both independently and forming a haplotype with the IL10.R2 microsatellite. In contrast, the IL10.G7 and IL10.R13 alleles, individually and in haplotype, were associated with reduced protein levels (Eskdale *et al.*, 1998).

Haplotype families were delineated in a European population, in which the microsatellites IL10.R and IL10.G were considered, as well as the three main proximal SNVs, rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A). The family called IL10.01, associated with low IL-10 production, consists of the R3 allele, any IL10.G alleles, with G9 being the most frequent, and the proximal haplotype GCC, represented as R3-(IL10.G)-G-C-C. The IL10.02 family, R2-(IL10.G, frequently G13)-A-C-C, was associated with high production. The remaining haplotype families were delineated as family IL10.03, R2-(IL10.G, frequently containing the G13 allele)-G-C-C, and family IL10.04, R2-(IL10.G)-A-T-A, which is characterized by containing, more frequently, the G9 allele (Eskdale *et al.*, 1999).

In expanded haplotypes, additionally containing the proximal SNV rs1800893 (-1330 G>A) and three common distal SNVs, rs1800890 (-3575 T>A), rs6703630 (-2849 G>A), rs6693899 (-2763 C>A), profiles of low and high IL-10 production were traced according to mRNA expression and protein secretion. Constitutive mRNA production did not differ between groups, however, upon inflammatory stimulation with LPS, individuals carrying the distal haplotype TGC, regardless of the proximal haplotype, were associated with high IL-10 production, while the distal haplotypes AAA and AGA, inherited together with the proximal

haplotype GCC, were associated with low protein levels (Gibson *et al.*, 2001) (Table 2).

Table 2. Identified haplotypes and IL-10 production profile

IL-10 production	Haplotypes	Reference
IL10.R, IL10.G, rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A)		
↓	R3- (IL10.G9) – GCC	ESKDALE *et al.*, 1999
↑	R2- (IL10.G13) – ACC	
rs1800890 (T>A), rs6703630 (G>A), rs6693899 (C>A), rs1800893 (G>A), rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A)		
↓	AAA – AGCC	GIBSON *et al.*, 2001
↓	AGA – AGCC	
↑	TGC -	
	TGC – GATA	
No diference	TGC – GACC	
	TGC – AGCC	
IL10.G and rs1800896 (A>G)		
	G10 – G	MÖRMANN *et al.*, 2004
↑	G12 – G	
	G14 – G	
rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A)		
↑	GTC	SINGHAL *et al.*, 2015
↓	ATA	CRAWLEY *et al.*, 1999
↑	GCC in homozigozity	HOFFMAN *et al.*, 2001
±	GCC in heterozigozity	
↓	ACC or ATA in homozigozity	
↑	GCC	REUSS *et al.*, 2002

Source: the author himself.

5. *IL10* upstream variants and viral diseases

In viral infections IL-10 plays an essential role in the balance between suppressing the exacerbated immune response without, however, in the beginning, reduce the activity of $CD8^+$ T cells, important components of the adaptive antiviral response. It was also identified that this cytokine could stimulate these cells activity. However, changes in the levels of this cytokine may contribute to viral persistence and stimulate early suppression of the immune response reviewed by Rojas *et al.* (2017).

As variants upstream of the gene appear to influence protein production, many authors have sought to evaluate its effect on viral diseases as well, given

the importance of this cytokine in the balance between an adequate or inadequate antiviral response. SNVs rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A), have been extensively studied regarding their possible contribution and association with viral diseases, as shown in Table 3.

Besides the contradictory results between the different research groups analyzing the same diseases, the C allele of rs1800871 (C>T) seems to be associated with increased infection risk to hepatitis B virus (HBV) (Ren; Zhang; Hu, 2015; Ye *et al.*, 2020) and the AA genotype of rs1800896 (A>G) in Caucasians and AA genotype of rs1800872 (C>A) were associated with human immunodeficiency virus (HIV-1) infection risk (Fu *et al.*, 2020).

Table 3. Studies on the association between *IL10* variants and haplotypes with viral diseases

Disease/Virus	Variant	Sample size	Association	Country	Reference
Respiratory infections					
SARS-CoV	-1082 A>G (rs1800896) and -592 C>A (1800872)	Cases (n=476) and controls (n=449)	No association	China	(CHONG *et al.*, 2006)
RSV	-592 C>A (rs1800872)	Cases (n=235) and controls (n=1,008)	No association	The Netherlands	(SCHUURHOF *et al.*, 2011)
IAV/H3N2	-592 C>A (rs1800872)	Influenza A/H3N2 (n=96), influenza like illness (n=114) and asymptomatic healthy contacts (n=147)	G allele was associated with an increased risk of infection by the influenza A/H3N2 virus	Iran	(ROGO *et al.*, 2016)
	-1082 A>G (rs1800896) and -592 C>A (rs1800872)	Mild disease (n=129) and severe disease (n=117)	CA genotype (-1082 A>G) was associated with a fatal outcome	India	(CHOUDHARY *et al.*, 2018)
IAV/H1N1	-1082 A>G (rs1800896) and -592 C>A (rs1800872)	Mild disease (n=293) and severe disease (n=86)	GC haplotype (-1082G and -592C) protected against fatal outcomes, while the GA	India	(ALAGARASU *et al.*, 2021)

			haplotype (-1082G and -592A) was a risk factor		
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=49) and controls (n=44)	No association	Iran	(MEHRBOD *et al.*, 2021)
IAV/IBV	-592 C>A (rs1800872)	Influenza A (n=50), influenza B (n=30) and influenza-like illness (n=96)	No association	Iran	(KESHAVARZ *et al.*, 2019)
Rhinovirus bronchiolitis	-3575 A>T (rs1800890), -1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=135) and controls (n=378)	G (-1082 A>G) and T alleles (-3575 A>T) were considered protective factors	Finland	(HOLSTER *et al.*, 2018)
Hepatitis viral infections					
	-1082 A>G (rs1800896)	Chronic cases (n=224) and recovery controls (n=389)	No association	United States	(TRUELOVE *et al.*, 2008)
	-819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=115) and controls (n=110)	No association	Japan	(KOMATSU *et al.*, 2014)
HBV	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Patients (n=2.306) and controls (n=1.376)	CC+CT of the -819 C>T SNV increased HBV risk of infection and ATA and ACC haplotypes were associated with disease progression	Asians	(REN; ZHANG; HU, 2015)
	-819 C>T (rs1800871)	-	C allele and CC genotype were associated with risk of	Various ethnicities (subgroups)	(YE *et al.*, 2020)

			infection		
	-1082 A>G (rs1800896) and -592 C>A (rs1800872)	HBV+ (n=287)	No association	Indonesia	(TURYADI *et al.*, 2022)
	1082 A>G (rs1800896) and 592 C>A (1800872)	Cases (n=75) and controls (n=50)	No association	Turkey	(TEMEL *et al.*, 2023)
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	HCV infected group (n=196)	C allele (-592 C>A) was associated with mild liver inflammation	Poland	(ŚWIĄTEK-KOŚCIELNA *et al.*, 2017)
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=202) and controls (n=100)	Significant difference in the distribution of genotypes of -1082 A>G	Pakistan	(NAEEMI *et al.*, 2018)
	-592 C>A (rs1800872)	Cases (n=144) and controls (n=221)	AA genotype was associated with susceptibility	Russia	(BARKHASH *et al.*, 2018)
HCV	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=198) and controls (n=142)	No association	China	(JING *et al.*, 2020)
	-592 C>A (1800872)	HCV+ (n=384)	No association	Pakistan	(JUNAID *et al.*, 2021)
	-1082 A>G (rs1800896) and -819 C>T (rs1800871)	Cases (n=76) and controls (n=40)	Significant differences in the frequencies of the -819 C>T genotypes and -1082 A>G alleles	Malaysia	(NOH *et al.*, 2021)
	IL-10.G and IL-10.R	HCV+ (n=659)	IL-10R.2 conferred susceptibility to the disease, whereas IL-10R.3 and IL-10.G13 were	United Kingdom and European countries	(KNAPP *et al.*, 2003)

			protective		
	-1082 A>G (rs1800896)	Chronic HCV (n=50) and resolved HCV patients (n=50)	No association	Egypt	(HELAL *et al.*, 2014)
Human immunodeficiency virus					
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	-1082 A>G (1,278 cases and 1,858); -819 (440 cases and 565 controls) -592 C>A (1,405 cases and 1,842 controls)	AA genotype of -1082 (A>G) and AA genotype of -592 (C>A) were associated with HIV-1 infection risk	Caucasian and global (various ethnicities), respectively	(FU *et al.*, 2020)
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and 592 C>A (1800872)	Sero-prevalent (n=968), sero-negative (n=449), and sero-prevalent patients (n=745)	ATA haplotype (-1082A, -819T and -592A) was associated with accelerated progression to AIDS	United States	(OLEKSYK *et al.*, 2009)
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=298) and controls (n=288)	G allele (-1082 A>G) was a protective factor	Poland	(BRATOSIEWICZ-WĄSIK; WĄSIK, 2024)
	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Cases (n=71) and controls (n=88)	No association	Mali	(DABITAO *et al.*, 2021)
Other infections					
Dengue	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	Controls (n=120), dengue fever (n=86), and dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) patients (n=196).	GTA and GCC haplotypes (-1082 A>G, -819 C>T and -592 C>A) were associated with reduced risk of DHF/DSS.	Malaysia, China, India, and others	(SAM *et al.*, 2015)

	-819 C>T (rs1800871)	Cases (n=132) and controls (n=108)	CT genotype was considered protective against dengue hemorrhagic fever	India	(ALAGARASU *et al.*, 2015)
CZS	-1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872)	CZS+ (n=76)	No association	Brazil	(SANTOS *et al.*, 2023)
CMV	-592 C>A (1800872) and -819 C>T (rs1800871)	Prophylaxis (n=57) and preemptive patients (n=59)	No association	Germany and Austria	(MAZZOLA *et al.*, 2021)

AIDS (acquired immunodeficiency syndrome); HIV (Human immunodeficiency virus); HBV (hepatitis B virus); HCV (hepatitis C virus); CZS (congenital Zika syndrome); CMV (cytomegalovirus); RSV (respiratory syncytial virus); EBV (Epstein-Baar virus); HDV (hepatitis D virus).

5.1 Association of IL10 variants and haplotypes with COVID-19

Coronavirus disease 2019 (COVID-19) is caused by the etiological agent SARS-CoV-2 and is characterized by a clinical condition that varies in quantity and severity of symptoms. While cases considered mild include flu-like and gastrointestinal symptoms, severe cases may include severe acute respiratory syndrome (SARS), shock, coagulation disorders, multiple organ failure, and death (Huang *et al.*, 2020; Li *et al.*, 2020).

The cytokine IL-10 has been indicated as a possible early biomarker of disease severity, as elevated levels are observed in the first days following hospitalization in those patients who tend to progress to severe outcomes, indicating a poorer prognosis (Han *et al.*, 2020; Zhao *et al.*, 2020).

The early increase in IL-10 levels, as well as its role in the pathophysiology of COVID-19, are still unclear. It is argued that, in principle, its release would have a physiological role in reducing tissue damage resulting from the inflammatory response. However, in this hypothesis, in which patients IL-10 levels are exacerbated and sustained, this cytokine could cause hyperactivation of CD8+ T lymphocytes, exhaustion of these cells and impairment of the antiviral response (Islam *et al.*, 2021; Lu *et al.*, 2021).

In this context, the variants upstream of the *IL10* gene have been tested for their association with the severity and mortality of COVID-19, in the search for

prognostic biomarkers. To date, as can be seen in Table 4, research has been developed in several ethnicities, but using only the proximal SNVs, rs1800896 (A>G), rs1800871 (C>T) and rs1800872 (C>A). Most results suggest that the G allele or GG genotype of rs1800896 (A>G) are associated with infection, severity or mortality in COVID-19 cases (Abbood; Anvari; Fateh, 2023; Alsayed *et al.*, 2024; Oliveira *et al.*, 2025; Eldesouki *et al.*, 2024). The A allele of SNV rs1800872 (C>A) is also associated with aggravated cases (Abbood; Avari; Fateh, 2023; Eldesouki *et al.*, 2024; Yessenbayeva *et al.*, 2023). However, some authors did not observe any association or had contradictory results compared to those described above (Avendaño-Félix *et al.*, 2021; Azadinejad *et al.*, 2024; Balzanelli *et al.*, 2022; Rizvi *et al.*, 2022).

Table 4. Studies on the association between *IL10* single nucleotide variants and haplotypes with COVID-19

Variant	Sample size	Country	Reference
No association			
rs1800871 and 1800872	Moderate (n=102) and severe (n=86)	Mexico	(AVENDAÑO-FÉLIX *et al.*, 2021)
rs1800896, rs1800871 and 1800872	Mild (n=150) and severe COVID-19 (n=143)	Iran	(AZADINEJAD *et al.*, 2024)
Protection against disease severity			
AG genotype (rs1800896)	Cases (n=41) and controls (n=43)	Italy	(BALZANELLI *et al.*, 2022)
CC genotype (1800872)	160 patients with mild (n=85) and severe (n=75) conditions	India	(RIZVI *et al.*, 2022)
C allele (1800872)	Mild (n=159) and severe (n=142)	Kazakhstan	(YESSENBAYEVA *et al.*, 2023)
Risk of infection or severity progression			

A allele (rs1800872)	Cases (n=110) and controls (n=110)	Egypt	(ELDESOUKI *et al.*, 2024)
AA genotype (rs1800896)	Cases (n=41) and controls (n=43)	Italy	(BALZANELLI *et al.*, 2022)
A allele (1800872)	Mild (n=159) and severe (n=142)	Kazakhstan	(YESSENBAYEVA *et al.*, 2023)
AG and GG genotypes (rs1800896)	Cases (n=115) and controls (n=115)	Saudi Arabia	(ALSAYED *et al.*, 2024)
G allele (rs1800896)	Cases (n=110) and controls (n=110)	Egypt	(ELDESOUKI *et al.*, 2024)
GG genotype and GCC haplotype (rs1800896, rs1800871 and rs1800872)	Mild (n=163), moderate (n=72) and severe (n=130) COVID-19	Brazil	(DE OLIVEIRA *et al.*, 2025)
Risk of outcome mortality			
G (rs1800896), C (rs1800871) and A allele (1800872)	Recovered (n=1,734) and deceased patients (n=1,450)	Iran	(ABBOOD; ANVARI; FATEH, 2023)

Source: the author himself.

6 CONCLUSION

Most of the studies available in the literature sought to analyze the effect of variants located upstream of the *IL10* gene, both in relation to their effect on gene expression and their association with viral diseases, focused on the proximal SNVs -1082 A>G (rs1800896), -819 C>T (rs1800871) and -592 C>A (1800872). Although these SNVs are associated with differences in gene expression and protein levels, as well as with several viral diseases and clinical conditions, these results are often contradictory. This fact may demonstrate the low penetrance of these variants and demand increasingly complete studies,

including a larger DNA region, considering the expanded haplotype with others SNVs in the 5' flanking region.

Furthermore, it is important to ensure that each research reached a sufficient sample size for analysis, as well as reported the statistical power achieved. The execution of systematic reviews could also better elucidate the viability of this marker, considering separately, the different ethnic populations in the meta-analysis.

Although promising, the use of these SNVs as biomarkers should be preceded by further studies, deepening the understanding about its effects in each viral disease, including COVID-19.

REFERENCE

ABBOOD, S. J. A.; ANVARI, E.; FATEH, A. Association between interleukin-10 gene polymorphisms (rs1800871, rs1800872, and rs1800896) and severity of infection in different SARS-CoV-2 variants. **Human Genomics**, v. 17, n. 1, p. 19, 2023.

ALAGARASU, K. *et al.* Association of combinations of interleukin-10 and pro-inflammatory cytokine gene polymorphisms with dengue hemorrhagic fever. **Cytokine**, v. 74, n. 1, p. 130–136, 2015.

ALAGARASU, K. *et al.* TNFA and IL10 Polymorphisms and IL-6 and IL-10 Levels Influence Disease Severity in Influenza A(H1N1) pdm09 Virus Infected Patients. **Genes**, v. 12, n. 12, p. 1914, 2021.

ALSAYED, B. A. *et al.* Molecular Determination of Tumor Necrosis Factor-alpha, Interleukin-8, Interleukin-10, and C-X-C Chemokine Receptor-2 Genetic Variations and their Association with Disease Susceptibility and Mortality in COVID-19 Patients. **Current Genomics**, v. 25, n. 1, p. 12–25, 2024.

ASSIS, S. *et al.* IL10 Single Nucleotide Polymorphisms are Related to Upregulation of Constitutive IL-10 Production and Susceptibility to Helicobacter pylori Infection. **Helicobacter**, v. 19, n. 3, p. 168–173, 2014.

AVENDAÑO-FÉLIX, M. *et al.* Lack of Effects of the Genetic Polymorphisms of Interleukin-10 in Clinical Outcomes of COVID-19. **Viral Immunology**, v. 34, n. 8, p. 567–572, 2021.

AZADINEJAD, H. *et al.* Relationship Between IL-10 Single Nucleotide Polymorphisms (rs1800871, rs1800872, and rs1800896) and the Severity of COVID-19. **Genetic Testing and Molecular Biomarkers**, v. 28, n. 11, p. 438–444, 2024.

BAI, C. Y. *et al.* Association between IL-10 genetic variations and cervical cancer susceptibility in a Chinese population. **Genetics and Molecular Research**, v. 15, n. 3, 5 ago. 2016.

BALZANELLI, M. *et al.* Analysis of Gene Single Nucleotide Polymorphisms in COVID-19 Disease Highlighting the Susceptibility and the Severity towards the Infection. **Diagnostics**, v. 12, n. 11, p. 2824, 2022.

BARKHASH, A. V. *et al.* Single nucleotide polymorphism rs1800872 in the promoter region of the IL10 gene is associated with predisposition to chronic hepatitis C in Russian population. **Microbes and Infection**, v. 20, n. 3, p. 212–216, 2018.

BERTI, F. C. B. *et al.* IL-10 gene polymorphism c.-592C> A increases HPV infection susceptibility and influences IL-10 levels in HPV infected women. **Infection, Genetics and Evolution**, v. 53, p. 128–134, 2017.

BRATOSIEWICZ-WĄSIK, J.; WĄSIK, T. J. Genetic variants of IL-10 promoter influence susceptibility to HIV-1 infection and disease progression in the Polish population. **Human Immunology**, v. 85, n. 5, p. 111086, 2024.

CAPASSO, M. *et al.* Cytokine Gene Polymorphisms in Italian Preterm Infants: Association Between Interleukin-10 –1082 G/A Polymorphism and Respiratory Distress Syndrome. **Pediatric Research**, v. 61, n. 3, p. 313–317, 2007.

CARLSON, C. S. *et al.* Selecting a Maximally Informative Set of Single-Nucleotide Polymorphisms for Association Analyses Using Linkage Disequilibrium. **Am. J. Hum. Genet**., v. 74, n. 1, p. 106-120, 2004.

CHEN, T. K. *et al.* Association between human IL-10 gene polymorphisms and serum IL-10 level in patients with food allergy. **Journal of the Formosan Medical Association**, v. 111, n. 12, p. 686–692, dez. 2012.

CHIU MOK, C. *et al.* Interleukin-10 Promoter Polymorphisms In Southern Chinese Patients With Systemic Lupus Erythematosus, **Arthritis Reum.**, v.41, n. 6, p. 1090-1095, 1998.

CHONG, W. P. *et al.* The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. **BMC Infectious Diseases**, v. 6, n. 1, p. 82, 2006.

CHOUDHARY, M. L. *et al.* Association of Single Nucleotide Polymorphisms in *TNFA* and *IL10* Genes with Disease Severity in Influenza A/H1N1pdm09 Virus Infections: A Study from Western India. **Viral Immunology**, v. 31, n. 10, p. 683–688, 2018.

CRAWFORD, D. C.; NICKERSON, D. A. Definition and Clinical Importance of Haplotypes. **Annual Review of Medicine**, v. 56, n. 1, p. 303–320, 2005.

CRAWLEY, E. *et al.* Polymorphic haplotypes of the interleukin-10 5? flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. **Arthritis & Rheumatism**, v. 42, n. 6, p. 1101–1108, 1999.

DABITAO, D. *et al.* Short Communication: Genetic Variation in Human *IL10* Proximal Promoter and Susceptibility to HIV-1 Infection in Mali, West Africa. **AIDS Research and Human Retroviruses**, v. 37, n. 1, p. 57–61, 2021.

DE OLIVEIRA, J. N. *et al.* Association of IL10 gene SNVs rs1800896 (A>G), rs1800871 (C>T), rs1800872 (C>A) and haplotypes with COVID-19 severity and outcome in the Brazilian population. **Human Immunology**, v. 86, n. 2, p. 111261, 2025.

DEN DUNNEN, J. T. *et al.* HGVS Recommendations for the Description of Sequence Variants: 2016 Update. **Human Mutation**, v. 37, n. 6, p. 564–569, 2016.

DHAOUADI, T. *et al.* Impact of IL-10 gene promoter polymorphisms on treatment response in HCV patients: A systematic review, a meta-analysis, and a meta-regression. **International Journal of Immunopathology and Pharmacology**, v. 38, 2024.

DHOUIOUI, S. *et al.* IL-10 polymorphism genotypes, haplotypes, and diplotypes are associated with colorectal cancer predisposition and outcome in Tunisian population. **Heliyon**, v. 10, n. 15, 2024.

DUTTKE, S. H. *et al.* Position-dependent function of human sequence-specific transcription factors. **Nature**, v. 631, n. 8022, p. 891–898, 2024.

ELDESOUKI, R. E. *et al.* Association of IL-10–592 C>A /-1082 A.G and the TNFα -308 G>A with susceptibility to COVID-19 and clinical outcomes. **BMC Medical Genomics**, v. 17, n. 1, p. 40, 2024.

ESKDALE, J. *et al.* Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. **Immunogenetics**, v. 46, n. 2, p. 120–128, 1997.

ESKDALE, J. *et al.* Interleukin 10 secretion in relation to human IL-10 locus haplotypes. **Proc Natl Acad Sci U S A**, v. 95, n. 16, p. 9465, 1998.

ESKDALE, J. *et al.* Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. **Genes Immun.**, v. 1, n. 2, p. 151 – 155, 1999.

ESKDALE, J.; GALLAGHER, G. A polymorphic dinucleotide repeat in the human IL-10 promoter. **Immunogenetics**, v. 42, n. 5, p. 444 – 445, 1995.

ESKDALE, J.; KUBE, D.; GALLAGHER, G. A second polymorphic dinucleotide repeat in the 5′ flanking region of the human IL10 gene. **Immunogenetics**, v. 45, n. 1, p. 82–83, 1996.

FREITAS, R. DE S. *et al.* IL-10 and IL-1β Serum Levels, Genetic Variants, and Metabolic Syndrome: Insights into Older Adults' Clinical Characteristics. **Nutrients**, v. 16, n. 8, 2024.

FU, D.-H. *et al.* Association between polymorphisms in the interleukin-10 gene and susceptibility to human immunodeficiency virus-1 infection. **Medicine**, v. 99, n. 48, 2020.

GABRYŠOVÁ, L. *et al.* The Regulation of IL-10 Expression. **Current topics in microbiology and immunology**, v. 380, p. 157–190, 2014.

GAO, L. *et al.* Association of IL-10 polymorphisms with hepatitis B virus infection and outcome in Han population. **European Journal of Medical Research**, v. 21, n. 1, p. 23, 2016.

GIBSON, A. W. *et al.* Novel Single Nucleotide Polymorphisms in the Distal IL-10 Promoter Affect IL-10 Production and Enhance the Risk of Systemic Lupus Erythematosus. **The Journal of Immunology**, v. 166, n. 6, p. 3915–3922, 2001.

HAN, H. *et al.* Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. **Emerging Microbes & Infections**, v. 9, n. 1, p. 1123–1130, 2020.

HELAL, S. F. *et al.* Impact of IL-10 (-1082) Promoter–Single Nucleotide Polymorphism on the Outcome of Hepatitis C Virus Genotype 4 Infection. **Clinical Medicine Insights: Gastroenterology**, v. 7, 2014.

HELMINEN, M.; LAHDENPOHJA, N.; HURME, M. Polymorphism of the Interleukin-10 Gene Is Associated with Susceptibility to Epstein-Barr Virus Infection. **The Journal of Infectious Diseases**, v. 180, n. 2, p. 496–499, 1999.

HERNÁNDEZ-BELLO, J. *et al.* Aberrant expression of interleukin-10 in rheumatoid arthritis: Relationship with IL10 haplotypes and autoantibodies. **Cytokine**, v. 95, p. 88–96, 1 jul. 2017.

HOFFMANN, S. C. *et al.* Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes 1. **Transplantation**, v. 72, n. 8, p. 1444–1450, 2001.

HOLSTER, A. *et al.* Polymorphisms in the promoter region of IL10 gene are associated with virus etiology of infant bronchiolitis. **World Journal of Pediatrics**, v. 14, n. 6, p. 594–600, 2018.

HUANG, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. **The Lancet**, v. 395, n. 10223, p. 497–506, 2020.

ISLAM, H. *et al.* Elevated Interleukin-10 Levels in COVID-19: Potentiation of Pro-Inflammatory Responses or Impaired Anti-Inflammatory Action? **Frontiers in immunology**, v. 12, 2021.

JING, J.-S. *et al.* Association of cytokine gene polymorphisms with chronic hepatitis C virus genotype 1b infection in Chinese Han population. **Medicine**, v. 99, n. 38, 2020.

JUNAID, K. *et al.* Association of IL28 B and IL10 Polymorphism with HCV Infection and Direct Antiviral Treatment. **Annals of clinical and laboratory science**, v. 51, n. 4, p. 512–520, 2021.

KARATAYLI, S. C. *et al.* Tumour necrosis factor-alpha, interleukin-10, interferon-gamma and vitamin D receptor gene polymorphisms in patients with chronic hepatitis delta. **Journal of Viral Hepatitis**, v. 21, n. 4, p. 297–304, 2014.

KARKI, R. *et al.* Defining “mutation” and “polymorphism” in the era of personal genomics. **BMC Medical Genomics**, v. 8, n. 1, p. 37, 2015.

KESHAVARZ, M. *et al.* Association of polymorphisms in inflammatory cytokines encoding genes with severe cases of influenza A/H1N1 and B in an Iranian population. **Virology Journal**, v. 16, n. 1, p. 79, 2019.

KNAPP, S. *et al.* Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. **Immunogenetics**, v. 55, n. 6, p. 362–369, 2003.

KOMATSU, H. *et al.* Association between single-nucleotide polymorphisms and early spontaneous hepatitis B virus e antigen seroconversion in children. **BMC Research Notes**, v. 7, n. 1, p. 789, 2014.

KREMER, K. N.; KUMAR, A.; HEDIN, K. E. Haplotype-independent co-stimulation of IL-10 secretion by SDF-1/CXCL12 proceeds via AP-1 binding to the human IL-10 promoter 1. **J Immunol.,** v.178, n. 3, p. 1581 – 1588, 2007.

KUBE, D. *et al.* Isolation of the Human Interleukin 10 Promoter. Characterization of the Promoter Activity in Burkitt's Lymphoma Cell Lines. **Cytokine**, v. 7, n. 1, p. 1–7, 1995.

KUBE, D. *et al.* Structural characterisation of the distal 5′ flanking region of the human interleukin-10 gene. **Genes & Immunity**, v. 2, n. 4, p. 181–190, 2001.

LAMBERT, S. A. *et al.* The Human Transcription Factors. **Cell**, v. 172, n. 4, p. 650–665, 2018.

LARSSON, L. *et al.* The Sp1 transcription factor binds to the G-allele of the –1087 IL-10 gene polymorphism and enhances transcriptional activation. **Genes & Immunity**, v. 10, n. 3, p. 280–284, 2009.

LI, J. *et al.* Three polymorphisms in the IL-10 gene and the risk of HCV infection: a meta-analysis plus a Chinese Association Study involving 1140 subjects. **Epidemiology and Infection**, v. 141, n. 5, p. 893–904, 2013.

LI, L. *et al.* COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. **Journal of Medical Virology**, v. 92, n. 6, p. 577–583, 2020.

LI, L. *et al.* Association of Interleukin-10 Polymorphism (rs1800896, rs1800871, and rs1800872) With Breast Cancer Risk: An Updated Meta-Analysis Based on Different Ethnic Groups. **Frontiers in Genetics**, v. 13, 4 fev. 2022.

LU, L. *et al.* A Potential Role of Interleukin 10 in COVID-19 Pathogenesis. **Trends in Immunology**, 2021.

MACKENZIE, K. F.; PATTISON, M. J.; ARTHUR, J. S. C. Transcriptional Regulation of IL-10 and Its Cell-Specific Role In Vivo. **Critical Reviews in Immunology**, v. 34, n. 4, p. 315–345, 2014.

MARIAN, A. J. Clinical Interpretation and Management of Genetic Variants. **JACC. Basic to translational science**, v. 5, n. 10, p. 1029–1042, 2020.

MATSUMOTO, K. *et al.* Interleukin-10 21082 gene polymorphism and susceptibility to cervical cancer among Japanese women. **Japanese Journal of Clinical Oncology**, v. 40, n. 11, p. 1113–1116, 2010.

MAZZOLA, P. *et al.* No association of genetic variants in TLR4, TNF-α, IL10, IFN-γ, and IL37 in cytomegalovirus-positive renal allograft recipients with active CMV infection—Subanalysis of the prospective randomised VIPP study. **PLOS ONE**, v. 16, n. 4, 2021.

MEHRBOD, P. *et al.* Association of the host genetic factors, hypercholesterolemia and diabetes with mild influenza in an Iranian population. **Virology Journal**, v. 18, n. 1, p. 64, 2021.

MINNICELLI, C. F. **O papel dos polimorfismos do promotor proximal do gene da Interleucina-10 na susceptibilidade e resposta terapêutica do Linfoma de Burkitt pediátrico em uma região de associação intermediária com o vírus Epstein-Barr.** 2009. 149 f. Dissertação (Mestrado). Instituto Nacional do Câncer. Programa de Pós-graduação em Oncologia do INCA, Rio de Janeiro, 2009.

MITEVA, L.; STANILOVA, S. The combined effect of interleukin (IL)-10 and IL-12 polymorphisms on induced cytokine production. **Human Immunology**, v. 69, n. 9, p. 562–566, 2008.

MOHAMMADI, S. *et al.* Interleukin-10 gene promoter polymorphisms (rs1800896, rs1800871 and rs1800872) and haplotypes are associated with the activity of systemic lupus erythematosus and IL10 levels in an Iranian population. **International Journal of Immunogenetics**, v. 46, n. 1, p. 20–30, 2019.

MÖRMANN, M. *et al.* Mosaics of gene variations in the interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. **Genes and Immunity**, 2004.

MOUDI, B. *et al.* Association Between IL-10 Gene Promoter Polymorphisms (-592 A/C, -819 T/C, -1082 A/G) and Susceptibility to HBV Infection in an Iranian Population. **Hepatitis Monthly**, v. 16, n. 2, 2016.

NAEEMI, H. *et al.* Distribution of IL28B and IL10 polymorphisms as genetic predictors of treatment response in Pakistani HCV genotype 3 patients. **Archives of Virology**, v. 163, n. 4, p. 997–1008, 2018.

NAICKER, D. D. *et al.* Association of IL-10-Promoter Genetic Variants With the Rate of CD4 T-Cell Loss, IL-10 Plasma Levels, and Breadth of Cytotoxic T-Cell Lymphocyte Response During Chronic HIV-1 Infection. **Clinical Infectious Diseases**, v. 54, n. 2, p. 294–302, 2012.

NEDELKOPOULOU, N. *et al.* Association of IL-10 gene promoter polymorphisms with food allergy susceptibility and serum IL-10 level in a pediatric Caucasian population. **Pediatric Allergy and Immunology**, v. 32, n. 3, p. 552–559, 2021.

NOH, I. C. *et al.* Cytokine (IL-10, IL-6, TNF-α and TGF-β1) Gene Polymorphisms in Chronic Hepatitis C Virus Infection among Malay Male Drug Abusers. **Biomedicines**, v. 9, n. 9, p. 1115, 2021.

O'CONNOR, L.; GILMOUR, J.; BONIFER, C. The Role of the Ubiquitously Expressed Transcription Factor Sp1 in Tissue-specific Transcriptional Regulation and in Disease. **Yale J Biol Med.,** v. 89, n. 4, p. 513-525, 2016.

OLEKSYK, T. K. *et al.* Extended IL10 haplotypes and their association with HIV progression to AIDS. **Genes & Immunity**, v. 10, n. 4, p. 309–322, 2009.

OUYANG, W.; O'GARRA, A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. **Immunity**, v. 50, n. 4, p. 871–891, 2019.

PHAN, L. *et al.* The evolution of dbSNP: 25 years of impact in genomic research. **Nucleic Acids Research**, v. 53, 2025.

RAO, M. *et al.* **Cytokine gene polymorphism and progression of renal and cardiovascular diseases**. **Kidney International**, 2007.

REES, L. E. N. *et al.* The interleukin-10-1082 G/A polymorphism: allele frequency in different populations and functional significance. **Cell. Mol. Life Sci**., v. 59, n. 3, p. 560-9, 2002.

REN, H.; ZHANG, T. T.; HU, W. L. A -819 C/T polymorphism in the interleukin-10 promoter is associated with persistent HBV infection, but -1082 A/G and -592A/C polymorphisms are not: a meta-analysis. **Archives of Virology**, v. 160, n. 3, p. 747–756, 2015.

REUSS, E. *et al.* Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. **Genes & Immunity**, v. 3, n. 7, p. 407–413, 2002.

RIZVI, S. *et al.* Implication of single nucleotide polymorphisms in Interleukin-10 gene (rs1800896 and rs1800872) with severity of COVID-19. **Egyptian Journal of Medical Human Genetics**, v. 23, n. 1, p. 145, 2022.

ROGO, L. D. *et al.* Seasonal influenza A/H3N2 virus infection and IL-1B, IL-10, IL-17, and IL-28 polymorphisms in Iranian population. **Journal of Medical Virology**, v. 88, n. 12, p. 2078–2084, 2016.

ROJAS, J. M. *et al.* IL-10: A multifunctional cytokine in viral infections. **Journal of Immunology Research,** 2017.

SALIM, P. H.; XAVIER, R. M. Influência dos polimorfismos genéticos (IL10/CXCL8/CXCR2/ NFκB) na susceptibilidade das doenças reumatológicas autoimunes. **Revista Brasileira de Reumatologia**, v. 54, n. 4, p. 301–310, 2014.

SAM, S.-S. *et al.* High Producing Tumor Necrosis Factor Alpha Gene Alleles in Protection against Severe Manifestations of Dengue. **International Journal of Medical Sciences**, v. 12, n. 2, p. 177–186, 2015.

SANTOS, C. N. O. *et al.* Association between genetic variants in TREM1, CXCL10, IL4, CXCL8 and TLR7 genes with the occurrence of congenital Zika syndrome and severe microcephaly. **Scientific Reports**, v. 13, n. 1, p. 3466, 2023.

SARAIVA, M.; VIEIRA, P.; O'GARRA, A. Biology and therapeutic potential of interleukin-10. **Journal of Experimental Medicine**, v. 217, n. 1, 2020.

SCHOTTE, H. *et al.* Putative IL-10 low producer genotypes are associated with a favourable etanercept response in patients with rheumatoid arthritis. **PLoS ONE**, v. 10, n. 6, 24 jun. 2015.

SCHUURHOF, A. *et al.* Local interleukin-10 production during respiratory syncytial virus bronchiolitis is associated with post-bronchiolitis wheeze. **Respiratory Research**, v. 12, n. 1, p. 121, 2011.

SINGHAL, P. *et al.* Association of IL-10 GTC haplotype with serum level and HPV infection in the development of cervical carcinoma. **Tumor Biology**, v. 36, n. 4, p. 2287–2298, 2015.

STEINKE, J. W. *et al.* Functional Analysis of −571 IL-10 Promoter Polymorphism Reveals a Repressor Element Controlled by Sp1. **The Journal of Immunology**, v. 173, n. 5, p. 3215–3222, 2004.

STEINKE, J. W.; CULP, J. A. Leukotriene Synthesis Inhibitors Versus Antagonists: The Pros and Cons. **Current Allergy and Asthma Reports**, v. 7, p. 126–133, 2007.

ŚWIĄTEK-KOŚCIELNA, B. *et al.* Interleukin 10 gene single nucleotide polymorphisms in Polish patients with chronic hepatitis C: Analysis of association with severity of disease and treatment outcome. **Human Immunology**, v. 78, n. 2, p. 192–200, 2017.

TEMEL, E. N. *et al.* Relationship between IL-17, TNF-α, IL-10, IFN-γ, and IL-18 polymorphisms with the outcome of hepatitis B virus infection in the Turkish population. **Revista da Associação Médica Brasileira**, v. 69, n. 8, 2023.

TESSE, R. *et al.* Association of interleukin-(IL)10 haplotypes and serum IL-10 levels in the progression of childhood immune thrombocytopenic purpura. **Gene**, v. 505, n. 1, p. 53–56, 2012.

TORRES-POVEDA, K. *et al.* A functional role of the SNP -592 of human IL10 gene regulatory region is associated with an increased IL-10 expression and risk for human papillomavirus cervical lesion and cervical cancer development. **BMC Proceedings**, v. 6, n. S6, p. P27, 2012.

TRUELOVE, A. L. *et al.* Evaluation of IL10, IL19 and IL20 gene polymorphisms and chronic hepatitis B infection outcome. **International Journal of Immunogenetics**, v. 35, n. 3, p. 255–264, 2008.

TURNER, D. M. *et al.* An investigation of polymorphism in the interleukin-10 gene promoter. **European Journal of Immunogenetics**, v. 24, n. 1, p. 1–8, 1997.

TURYADI, W. B. *et al.* Host Factors in the Natural History of Chronic Hepatitis B: Role of Genetic Determinants. **International Journal of Hepatology**, v. 2022, p. 1–10, 2022.

VAKILI, M. *et al.* Correlation between rs1800871, rs1800872 and rs1800896 Polymorphisms at IL-10 Gene and Lung Cancer Risk. **Asian Pacific Journal of Cancer Prevention**, v. 25, n. 1, p. 287–298, 2024.

VILKEVICIUTE, A. *et al.* IL-9 and IL-10 Single-Nucleotide Variants and Serum Levels in Age-Related Macular Degeneration in the Caucasian Population. **Mediators of Inflammation**, v. 2021, p. 1–13, 2021.

WANG, G. H.; ZUO, T.; ZUO, Z. C. Impact of IL-10 gene polymorphisms and its interaction with environment on susceptibility to systemic lupus erythematosus. **International Journal of Immunopathology and Pharmacology**, v. 34, 2020.

WESTENDORP, R. G. *et al.* Genetic influence on cytokine production and fatal meningococcal disease. **The Lancet**, v. 349, n. 9046, p. 170–173, 1997.

WU, J.-F. *et al.* The Effects of Cytokines on Spontaneous Hepatitis B Surface Antigen Seroconversion in Chronic Hepatitis B Virus Infection. **The Journal of Immunology**, v. 194, n. 2, p. 690–696, 2015.

YE, S. *et al.* Association of TLR3 (rs3775291) and IL-10 (rs1800871) gene polymorphisms with susceptibility to Hepatitis B infection: A meta-analysis. **Epidemiology and Infection**, v. 148, 2020.

YESSENBAYEVA, A. *et al.* Biomarkers of immunothrombosis and polymorphisms of IL2, IL6, and IL10 genes as predictors of the severity of COVID-19 in a Kazakh population. **PLOS ONE**, v. 18, n. 6, 2023.

YU, Y. *et al.* Cytokines Interleukin 4 (IL-4) and Interleukin 10 (IL-10) Gene Polymorphisms as Potential Host Susceptibility Factors in Virus-Induced Encephalitis. **Medical Science Monitor**, v. 23, p. 4541–4548, 2017.

YU, Z. *et al.* The interleukin 10-819C/T polymorphism and cancer risk: A HuGE review and meta-analysis of 73 studies including 15,942 cases and 22,336 controls. **OMICS A Journal of Integrative Biology**, 2013.

ZHANG, H.; KUCHROO, V. Epigenetic and transcriptional mechanisms for the regulation of IL-10. **Seminars in Immunology**, v. 44, p. 101324, 2019.

ZHANG, T. P. *et al.* Association of interleukin-10 gene single nucleotide polymorphisms with rheumatoid arthritis in a Chinese population. **Postgraduate Medical Journal**, v. 94, n. 1111, p. 284–288, 2018.

ZHAO, H.; PFEIFFER, R.; GAIL, M. H. Haplotype analysis in population genetics and association studies. **Pharmacogenomics**, v. 4, n. 2, p. 171–178, 2003.

ZHAO, N. *et al.* IL-10-592 polymorphism is associated with IL-10 expression and severity of enterovirus 71 infection in Chinese children. **Journal of Clinical Virology**, v. 95, p. 42–46, 2017.

ZHAO, Y. *et al.* Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. **JCI Insight**, v. 5, n. 13, 2020.

Conclusão

6 CONCLUSÃO

- A genotipagem das SNVs –1082A>G (rs1800896), –819C>T (rs1800871) e -592C>A (rs1800872), foi realizada com amostras de pacientes com detecção positiva para SARS-CoV-2 do HU-UEL;
- Nesta população não foi detectada associação dos genótipos ou alelos dos SNVs rs1800872 (-592C>A) e rs1800871 (–819C>T) com os diferentes quadros clínicos da COVID-19 ou com o pior desfecho, óbito (p>0,05);
- Não foi encontrada associação entre os haplótipos inferidos desta população (GCC, ACC ou ATA) com os desfechos da COVID-19;
- O genótipo GG do SNV rs1800896 (–1082A>G) foi observado em associação aos casos graves da COVID-19 quando o modelo recessivo foi analisado em regressão logística multinomial ajustada para fatores confundidores, apresentando 2,5 vezes mais chance de ser observado nestes pacientes do que nos pacientes de quadro leve (OR:2,5; IC: 1,053 – 6,038; p=0,038);
- O haplótipo GCC, em homozigose, foi observado em associação ao quadro grave da COVID-19 quando comparado à ausência deste haplótipo (OR: 2,767; IC: 1,065 – 7,191; p=0,037);
- A presença do haplótipo ACC em heterozigose reduz a chance de gravidade, quando comparado a não ter nenhum haplótipo ACC (OR: 0,515; IC: 0,276 – 0,960; p=0,037);
- A revisão narrativa apontou que a maior parte dos estudos de associação genética que buscam avaliar as variantes à montante do gene *IL10* consideram apenas as SNVs proximais, -1082 A>G (rs1800896), -819 C>T (rs1800871) e -592 C>A (1800872), no entanto, outras variantes desta região também foram associadas com diferenças na expressão do gene ou em associação com doenças virais, o que justificaria a demanda por estudos cada vez mais completos, incluindo outras variantes e uma maior região 5’ flanqueadora do gene.

Considerações Finais

7 CONSIDERAÇÕES FINAIS

Variações em genes de proteínas do sistema imune, como citocinas, que são essenciais na comunicação e coordenação da resposta imunológica, podem desempenhar um papel importante na determinação da suscetibilidade ou gravidade de doenças, sendo que a identificação de indivíduos portadores destas variantes pode auxiliar no reconhecimento de suscetibilidade à determinadas enfermidades.

Nos casos graves de COVID-19 a interleucina-10 é observada em níveis elevados desde o início da doença, e, apesar da contribuição da IL-10 na fisiopatologia da doença não estar esclarecida, esta citocina vem sendo indicada como possível marcador de gravidade e pior prognóstico.

Neste estudo, o genótipo GG da SNV rs1800896 de *IL10* foi associada aos casos graves da COVID-19, bem como o haplótipo GCC em homozigose (rs1800896, rs1800871 e rs1800872). Adicionalmente, o haplótipo ACC em heterozigose foi associado aos casos leves. Estes resultados remetem a um possível aumento nas chances de pior prognóstico para indivíduos portadores da SNV rs1800896.

Em uma pesquisa de revisão subsequente, a qual abrangeu outras doenças virais além da COVID-19, observou-se que a maior parte dos estudos buscam avaliar majoritariamente o efeito das SNVs rs1800896 (A>G), rs1800871 (C>T) e rs1800872 (C>A), seja na produção de IL-10 ou no risco e proteção a determinadas condições clínicas. No entanto, outras variantes presentes à montante do gene, pouco avaliadas, similarmente foram associadas a expressão gênica diferencial. Este fato justificaria a demanda por estudos cada vez mais completos, incluindo outras variantes e uma maior região 5’ flanqueadora do gene.

A associação aqui observada do genótipo GG e haplótipo GCC à gravidade da COVID-19, associada a estudos futuros incluindo outras variantes à montante do gene *IL10*, poderá auxiliar na identificação de um perfil de suscetibilidade de indivíduos contaminados com o SARS-CoV-2, o que poderia permitir um manejo e acompanhamento diferencial nestes casos.

REFERÊNCIAS

ABBAS, A. K.; PILLAI, S.; LICHTMAN, A. H. Imunologia celular e molecular. 9º ed. Rio de Janeiro: Guanabara Koogan, 2019.

ABBOOD; *et al.* Association between interleukin-10 gene polymorphisms (rs1800871, rs1800872, and rs1800896) and severity of infection in different SARS-CoV-2 variants. **Human Genomics,** v. 17, n. 19, 2023.

ALAGARASU, K.; *et al.* TNFA and IL10 polymorphisms and IL-6 and IL-10 Levels Influence Disease Severity in Influenza A(H1N1) pdm09 Virus Infected Patients. **Genes**, v. 12, 2021.

ANDRÉ, S.; *et al.* T cell apoptosis characterizes severe Covid-19 disease. **Cell Death Differ**, v. 29, n. 8, p. 1486-1499, 2022.

AVENDANO-FELIX; *et al.* Lack of Effects of the Genetic Polymorphisms of Interleukin-10 in Clinical Outcomes of COVID-19. **Viral Immunology**, p. 1-6, 2021.

BALZANELLI, M.G.; *et al.* Analysis of Gene Single Nucleotide Polymorphisms in COVID-19 Disease Highlighting the Susceptibility and the Severity towards the Infection. **Diagnostics**, 2022.

BARRY, J. C.; *et al.* Hyporesponsiveness to the Anti-Inflammatory Action of Interleukin-10 in Type 2 Diabetes. **Sci Rep,** 2016.

BERTI, F. C. B., *et al.* IL-10 gene polymorphism c.-592C/A increases HPV infection susceptibility and influences IL-10 levels in HPV infected women. **Infection, Genetics and Evolution***,* v. 53, p. 128-134, 2017.

BLANCO-MELO D.; *et al.* Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. **Cell**, v. 181, n. 5, p. 1036-1045, 2020.

BOECHAT, J. L.; *et al.* The immune response to SARS-CoV-2 and COVID-19 immunopathology - Current perspectives**. Pulmonology,** v. 5, p. 423-437, 2021.

BRAGA, M.; *et al.* Influence of *IL10* (rs1800896) Polymorphism and TNF-α, IL-10, IL-17A, and IL-17F Serum Levels in Ankylosing Spondylitis. **Front Immunol**, v.12, 2021.

BURREL, C. J., HOWARD, C. R., MURPHY, F. A. Chapter 31: Coronaviruses. **Fenner and White's Medical Virology**. 5º edição, p. 437-446, 2017.

CAPASSO, M.; *et al.* Cytokine Gene Polymorphisms in Italian Preterm Infants: Association Between Interleukin-10 –1082 G/A Polymorphism and Respiratory Distress Syndrome. **Pediatric Research**, v. 61, No. 3, p. 313- 317, 2007.

CARLINI, V.; *et al.* The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. **Front Immunol**, v. 14, 2023.

CARSETTI, R.; *et al.* Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases. **Front Immunol**, v. 11, 2020.

CEVIK, M.; *et al.* SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. **Lancet Microbe**, v. 2, n. 1, 2021.

CHAKRABARTI, A.; *et al.* Protein kinase R-dependent regulation of interleukin-10 in response to double-stranded RNA**. J Biol Chem**, v. 283, n. 37, p. 25132-25139, 2008.

CHEN, G., *et al.* Clinical and immunological features of severe and moderate coronavirus disease 2019. **Journal of Clinical Investigation**, v. 130, n. 5, p. 2620-2629, 2020.

CHEN, T-K., *et al.* Association between human IL-10 gene polymorphisms and serum IL-10 level in patients with food allergy. **Journal of the Formosan Medical Association**, v. 111, p. 686-692, 2012.

Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment, 7ºed., 2020. Disponível em: http://kjfy.meetingchina.org/msite/news/show/cn/3337.html. Acesso em: 17, Agosto, 2020.

CRAWLEY, E.; *et al.* Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. **Arthritis Rheum**, v. 42, n. 6, p. 1101-8, 1999.

DIAO, B.; *et al.* Reduction and function exhaustion of T cells in patients with Coronavirus disease 2019 (COVID-19). **Frontiers in Immunology**, v. 11, p. 827, 2020.

ESKDALE, J., KUBE, J. D., GALLAGHER, G. A second polymorphic dinucleotide repeat in the 5◆ flanking region of the human IL-10 gene. **Immunogenetics***,* v. 45, 1996.

ESKDALE, J.; GALLAGHER, G. A polymorphic dinucleotide repeat in the human IL-10 promoter. **Immunogenetics***,* v. 42, 1995.

FIOLET, T.; *et al.* Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. **Clin Microbiol Infect**, v. 28, n. 2, p. 202-221, 2022.

FIORENTINO, D. F.; BOND, M. W.; MOSMANN, T. R. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. **J Exp Med**, v. 170, n. 6, p. 2081-95, 1989.

GAO, P, LIU, J, LIU, M. Effect of COVID-19 Vaccines on Reducing the Risk of Long COVID in the Real World: A Systematic Review and Meta-Analysis**. Int J Environ Res Public Health**, v. 19, n. 19, 2022.

GENG, Y, WANG, Y. Stability and transmissibility of SARS-CoV-2 in the environment. **J Med Virol**, v. 95, n. 1, 2023.

GIBSON, A. W., *et al.* Novel Single Nucleotide Polymorphisms in the Distal IL-10 Promoter Affect IL-10 Production and Enhance the Risk of Systemic Lupus Erythematosus. **The Journal of Immunology***,* p. 3915-3922, 2001.

GRIFFIN, JB.; *et al.* SARS-CoV-2 Infections and Hospitalizations Among Persons Aged ≥16 Years, by Vaccination Status - Los Angeles County, California, May 1-July 25, 2021. **MMWR Morb Mortal Wkly Rep**, v. 70, n. 34, p. 1170-1176, 2021.

GROUX, H.; *et al.* Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+T cells. **J. Exp. Med**, v. 184, p. 19–29, 1996.

GUBERNATOROVA, E. O.; *et al.* IL-6: Relevance for immunopathology of SARS-CoV-2. **Cytokine and Growth Factor Reviews**, v. 53, p. 13–24, 2020.

HAMMING, I.; *et al.* Tissue distribution of ECA2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. **J Pathol**, v. 203, n. 2, p. 631-7, 2004.

HAN, H.; *et al.* Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. **Emerging microbes and infections**, v. 9:1, p. 1123-1130, 2020.

HE, X.; *et al.* Temporal dynamics in viral shedding and transmissibility of COVID-19. **Nat Med**, v. 26, n. 5, p. 672-675, 2020.

HEDRICH, C., M.; BREAM, J., H. Cell type-specific regulation of IL-10 expression in inflammation and disease. **Immunologic Research***,* v. 47, p. 185 – 206, 2010.

HELMINEN, M. E.; *et al.* Susceptibility to Primary Epstein-Barr Virus Infection Is Associated with Interleukin-10 Gene Promoter Polymorphism. **The Journal of Infectious Diseases**, v. 184, p. 777-780, 2001.

HUANG, C.; *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. **Lancet**, v.15, n.395, p. 497-506, 2020.

HUANG, K. J.; *et al.* An interferon-gamma-related cytokine storm in SARS patients. **J Med Virol**, v. 75, n. 2, p. 185-94, 2005.

HULKKONEN, J.; *et al.* Genetic association between interleukin-10 promoter region polymorphisms and primary Sjögren's syndrome. **Arthritis Rheum**, v. 44, n. 1, p. 176-9, 2001.

ISLAM, H.; *et al.* Elevated Interleukin-10 Levels in COVID-19: Potentiation of Pro-Inflammatory Responses or Impaired Anti-Inflammatory Action**? Front Immunol,** v. 12, 2021.

JACKSON, C. B.; *et al.* Mechanisms of SARS-CoV-2 entry into cells. **Nat Rev Mol Cell Biol,** v. 23, n. 1, p. 3-20, 2022.

JOHANSSON, M. A.; *et al.* SARS-CoV-2 Transmission From People Without COVID-19 Symptoms. **JAMA Netw Open**, v. 4, n. 1, 2021.

JONES, E.A., R.A. FLAVELL. Distal enhancer elements transcribe intergenic RNA in the IL-10 family gene cluster. **J. Immunol**, v. 175, p. 7437–7446, 2005.

KIM, G-U.; *et al.* Clinical characteristics of asymptomatic and symptomatic patients with mild COVID-19. **Clinical Microbiology and Infection***,* v. 26, p. 948_{e1} – 984_{e3}, 2020.

KUHN, R.; *et al.* Interleukin-10-deficient mice develop chronic enterocolitis. **Cell, v.** 75, p. 263–274, 1993.

KUPPALLI, K., RASMUSSEN, A. L. A glimpse into the eye of COVID-19 cytokine storm. **EbioMedicine***,* v. 55, 2020.

LAUW, F. N.; *et al.* Proinflammatory Effects of IL-10 During Human Endotoxemia. **J Immunol,** v. 165, p. 2783–9, 2000.

LEWIS, D. COVID-19 rarely spreads through surfaces. So why are we still deep cleaning. **Nature**, v. 590, n. 7844, p. 26-28, 2021.

LI, J. Y.; *et al.* The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future. **Microbes and Infection***,* v. 22, p. 80 – 85, 2020.

LI, L-q.; *et al.* COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. **Journal of Medical Virology***,* p. 1-7, 2020.

LIU, J.; *et al.* Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. **Ebio Medicine**, v. 55, 2020.

LUCENA, T. M. C.; *et al.* Mechanism of inflammatory response in associated comorbidities in COVID-19. **Diabetes & Metabolic Syndrome: Clinical Research & Reviews***,* v. 14, p. 597-600, 2020.

MARCINK, T. C.; *et al.* Intermediates in SARS-CoV-2 spike-mediated cell entry. **Sci Adv**, v. 8, n. 33, 2022.

MEO, S. A.; *et al.* Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. **European Review for Medical and Pharmacological Sciences,** v. 24, p. 2012-2019, 2020.

MEYER, N. J.; GATTINONI L.; CALFEE C. S. Acute respiratory distress syndrome. **Lancet**, v. 398, n. 10300, p. 622-637, 2021.

MOCELLIN, S.; *et al.* The dual role of IL-10. Trends Immunol, v. 24, n. 1, p. 36-43, 2003.

MOHAMMADI, S.; *et al.* Interleukin 10 gene promoter polymorphisms (rs1800896, rs1800871 and rs1800872) and haplotypes are associated with the activity of systemic lupus erythematosus and IL10 levels in an Iranian population**. Int J Immunogenet**, v. 46, n. 1, p. 20-30, 2019.

MURPHY, K. **Imunobiologia de Janeway**. 8. ed. Porto Alegre: Artmed, 2014.

NAING A.; *et al.* Pegylated IL-10 (Pegilodecakin) Induces Systemic Immune Activation, CD8+ T Cell Invigoration and Polyclonal T Cell Expansion in Cancer Patients. **Cancer Cell,** v. 34, 2018.

NEUMANN, C.; *et al.* Role of Blimp-1 in programing Th effector cells into IL-10 producers. **J. Exp. Med**, v. 211, p. 1807–1819, 2014.

OUYANG, W.; O'GARRA A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. **Immunity,** v. 50, n. 4, p. 871-891, 2019.

PARATO, K. G.; *et al.* Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. **AIDS**, v. 16, n. 9, p. 1251-6, 2002.

PERICO, L.; *et al.* Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. **Nat Rev Nephrol**, v. 17, n. 1, p. 46-64, 2021.

PESTKA, S.; *et al.* Interleukin-10 and related cytokines and receptors. **Annual Review of Immunology**, v. 22, p. 929-979, 2004.

PONTELLI, M.C.; *et al.* SARS-CoV-2 productively infects primary human immune system cells in vitro and in COVID-19 patients. **J. Mol. Cell Biol,** 2022.

RAJBHANDARI, P.; *et al.* IL-10 Signaling Remodels Adipose Chromatin Architecture to Limit Thermogenesis and Energy Expenditure. **Cell**, v. 172, 2018.

REUSS, E.*; et al.* Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. **Genes and Immunity**, n. 3, p. 407–413, 2002.

RICHARDSON, S.; *et al.* Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. **JAMA**, v. 323, n. 20, p. 2052-2059, 2020.

RIZVI.; *et al.* Implication of single nucleotide polymorphisms in *Interleukin-10* gene (*rs1800896* and *rs1800872*) with severity of COVID-19*.* **Egyptian Journal of Medical Human Genetics,** *v. 23, 2022.*

RODDA, L. B.; *et al.* Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19. **Cell,** v. 184, n. 1, p. 169-183, 2021.

SAMAVATI, L.; UHAL, B. D. ECA2. Much More Than Just a Receptor for SARS-COV-2. **Front Cell Infect Microbiol**, v. 10, n. 317, 2020.

SARAIVA, M, O'GARRA A. The regulation of IL-10 production by immune cells. **Nat Rev Immunol**, v. 10, n. 3, p. 170-81, 2010.

SARAIVA, M.; VIEIRA, P.; O'GARRA, A. Biology and therapeutic potential of interleukin-10. **J Exp Med**, v. 217, n. 1, 2020.

SAXTON, RA.; *et al.* Structure-based decoupling of the pro- and anti-inflammatory functions of interleukin-10. **Science**, v. 371, n. 6535, 2021.

SHAO, W.; *et al.* Effectiveness of COVID-19 vaccines against SARS-CoV-2 variants of concern in real-world: a literature review and meta-analysis. **Emerg Microbes Infect**, v. 11, n. 1, p. 2383-2392, 2022.

SHEN, X.R.; *et al.* ECA2-independent infection of T lymphocytes by SARS-CoV-2. **Signal Transduct. Target. Ther***,* 2022.

SOY, M.; *et al.* Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. **Clinical Rheumatology,** 2020**.**

TAKEDA, M. Proteolytic activation of SARS-CoV-2 spike protein. **Microbiol Immunol**, v. 66, n. 1, p. 15-23, 2022.

TEMPLE, S. E. L.; *et al.* Alleles carried at positions -819 and -592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with Streptococcus pneumoniae. **Immunogenetics**, v. 55, p. 629-632, 2003.

TORRES-POVEDA.; *et al.* The SNP at −592 of human IL-10 gene is associated with serum IL-10 levels and increased risk for human papillomavirus cervical lesion development. **Infectious Agents and Cancer**, v. 32, n. 7, 2012.

TURNER, D. M.; *et al.* An investigation of polymorphism in the interleukin-10 gene promoter. **European Journal of Immunogenetics**, v. 24, 1997.

WANG, M. Y.; *et al.* SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. **Front Cell Infect Microbiol**, v. 10, 2020.

WANG, X.; LIU, Z.; LU, L.; JIANG, S. The putative mechanism of lymphopenia in COVID-19 patients. **J Mol Cell Biol**, v.14, n. 5, 2022.

WEISS, S. R., LEIBOWITZ, J. L. Coronavirus Pathogenesis. **Advances in Virus Research***,* v. 81, p. 85 – 164, 2011.

WESTENDORP, R. G.; *et al.* Genetic influence on cytokine production and fatal meningococcal disease. **Lancet***,* v. 349, n. 170, 1997.

WORLD HEALTH ORGANIZATION. Naming the coronavirus disease (COVID-19) and the virus that causes it. Disponível em: who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it. Genebra, 2020. Acesso em: 21 jul, 2020a.

WORLD HEALTH ORGANIZATION. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Disponível em: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. Genebra, 11 march, 2020. Acesso em: 21 jul, 2020b.

WORLD HEALTH ORGANIZATION. WHO Coronavirus DASHBOARD. Disponível em: https://covid19.who.int/?adgroupsurvey={adgroupsurvey}&gclid=Cj0KCQjw1aOpBhCOARIsACXYv-fD3DzS38SZWlwBsTaOOQY_wYWTH8zGzJNtbv4Cbt8Z8f0gCUIhCgAaAu29EALw_wcB. Genebra, março, 2025. Acesso em: março, 2025c.

WORLD HEALTH ORGANIZATION. COVID-19 vaccine tracker and landscape. Disponível em: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. Genebra, outubro, 2023. Acesso em: outubro, 2023d.

WU, Z, MCGOOGAN, J. M. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases from the Chinese Center for Disease Control and Prevention. **JAMA,** v. 323, n.13, p. 1239–1242, 2020.

YANG, Y.; *et al.* The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. **Journal of Autoimmunity***,* v. 109, 2020.

YE, Q.; WANG, B.; MAO, J. The pathogenesis and treatment of the ‘Cytokine Storm’ in COVID-19. **Journal of Infection***,* v. 80, p. 607-613, 2020.

YESSENBAYEVA, A.; *et al.* Biomarkers of immunothrombosis and polymorphisms of *IL2*, *IL6*, and *IL10* genes as predictors of the severity of COVID-19 in a Kazakh population. **PLoS ONE, v.** 18, n. 6, 2023.

YIN, S. W.; *et al.* Viral loads, lymphocyte subsets and cytokines in asymptomatic, mildly and critical symptomatic patients with SARS-CoV-2 infection: a retrospective study**. Virol J,** v. 18, n. 1, 2021.

ZHANG, R.; *et al.* Identifying airborne transmission as the dominant route for the spread of COVID-19. **Proc Natl Acad Sci USA**, v. 117, n. 26, p. 14857-14863, 2020.

ZHAO, Y.; *et al.* Longitudinal COVID-19 profiling associates IL-1Ra and IL-10 with disease severity and RANTES with mild disease. **JCI Insight***,* 2020.

ANEXO A

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

“Investigação de fatores imunogenéticos relacionados à resposta imune regulatória na infecção por SARS-CoV-2: associação com o prognóstico, morbidade e mortalidade.”

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo para participar da pesquisa **“Investigação de fatores imunogenéticos relacionados à resposta imune regulatória na infecção por SARS-CoV-2: associação com o prognóstico, morbidade e mortalidade.”**, a ser realizada “**no Laboratório de genética molecular e Imunologia da UEL”.** O objetivo da pesquisa é avaliar se variações genéticas existentes na população podem influenciar na progressão da COVID-19. Sua participação é muito importante e ela se daria da seguinte forma: 1) você responderá a um questionário sobre dados como idade, escolaridade, renda familiar, hábito tabagista, e presença de comorbidades; 2) você doará 5mL de sangue periférico, que serão coletados por equipe capacitada; 4) permitirá a consulta de dados de seu prontuário médico para análise do quadro clínico e acompanhamento da doença;

Esclarecemos que sua participação é totalmente voluntária, podendo o (a) senhor (a): recusar-se a participar, ou mesmo desistir a qualquer momento, sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Esclarecemos, também, que **suas informações serão utilizadas somente para os fins desta pesquisa e para pesquisas futuras** e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade. Os materiais biológicos serão armazenados no laboratório de genética molecular e imunologia da UEL, e serão mantidos codificados, de modo a não permitir a sua identificação, e serão armazenados por 10 anos para a realização desta pesquisa e de pesquisas futuras, que serão submetidas ao comitê de ética em pesquisa antes de sua realização.
Esclarecemos ainda, que você não pagará e nem será remunerado por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação.

Os **benefícios** esperados são indiretos, uma vez que este estudo permitirá um maior entendimento da imunopatologia da COVID-19, fornecendo subsídios para a identificação de pacientes com pior prognóstico e desfecho clínico, possibilitando a implantação de tratamento personalizado com impacto direto na morbidade e mortalidade dos pacientes.

Quanto aos **riscos** desta pesquisa são mínimos, pois os únicos procedimentos extras aos quais você será submetida são: 1) a realização de questionário sociodemográfico, que será aplicado em local reservado, a fim de garantir a sua privacidade e você poderá se recusar a responder as perguntas caso não se sinta confortável; 2) a realização de coleta de sangue que poderá gerar algum desconforto, dor, hematoma no local, e raramente desmaios, contudo serão realizadas por profissionais capacitados a fim de minimizar estes possíveis riscos, e serão realizadas em ambiente hospitalar. Em relação à coleta de secreção nasal, não há riscos diretos da participação nesta pesquisa, tendo em vista que você já seria submetido aos procedimentos descritos, para seu diagnóstico

e tratamento, independentemente de sua participação neste estudo. Contudo caso ocorra algum tipo de desconforto você será prontamente atendido e amparado por mim (pesquisadora responsável) e / ou pela equipe de trabalho deste projeto.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos poderá nos contatar Karen Brajão de Oliveira (Telefone (43) 3371-5728/ (43) 3336-9948, karen.brajao@uel.br)**,** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, situado junto ao LABESC – Laboratório Escola, no Campus Universitário, telefone 3371-5455, e-mail: cep268@uel.br.

Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas devidamente preenchida, assinada e entregue a você.

Londrina, ___ de _____de 202_.

Pesquisador Responsável Karen Brajão de Oliveira

RG::_6.538.742-5_____

_____ (NOME POR EXTENSO DO SUJEITO DE PESQUISA), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____

*Termo de Consentimento Livre Esclarecido apresentado, atendendo, conforme normas da Resolução 466/2012 de 12 de dezembro de 2012.

ANEXO B

CÓPIA DO PARECER DO COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS DA UEL

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Investigação de fatores imunogenéticos relacionados à resposta imune regulatória na infecção por SARS-CoV-2: associação com o prognóstico, morbidade e mortalidade.

Pesquisador: Karen Brajão de Oliveira
Área Temática:
Versão: 1
CAAE: 36247920.1.0000.5231
Instituição Proponente: CCB - Departamento de Ciências Patológicas
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.204.004

Apresentação do Projeto:
De acordo com o documento PB_Informações Básicas de 06/08/2020
Desenho:
Trata-se de estudo caso-controle, seguido de estudo longitudinal de acompanhamento dos pacientes por 30 dias.
Este é um subprojeto de um projeto maior já aprovado pelo CEP, intitulado "Investigação de fatores genéticos e imunológicos na infecção por SARSCoV-2: associação com o prognóstico, morbidade e mortalidade", cuja CAAE é 31656420.0.0000.5231, parecer nº 4.053.033.
O envolvimento da resposta imunológica na fisiopatologia e prognóstico da infecção pelo SARS-CoV-2 ainda não está totalmente estabelecida. Têm sido demonstrado intenso desequilíbrio na produção de citocinas pró e anti-inflamatórias em pacientes com doença pulmonar grave ("tempestade de citocinas"), e parece ser diferenciada daquela apresentada por outras infecções respiratórias. Embora fatores como idade e comorbidades préexistentes estejam diretamente associadas ao pior prognóstico e desfecho clínico de pacientes com COVID-19, casos graves e alta mortalidade também tem ocorrido na ausência destes fatores. Variações nos genes de citocinas podem alterar a sua expressão proteica exercendo assim um papel importante tanto na susceptibilidade como na progressão da COVID-19. Assim o objetivo deste estudo é avaliar a associação de biomarcadores imunogenéticos da resposta anti-inflamatória com o prognóstico e

Endereço: LABESC - Sala 14
Bairro: Campus Universitário **CEP:** 86.057-970
UF: PR **Município:** LONDRINA
Telefone: (43)3371-5455 **E-mail:** cep268@uel.br

desfecho clínico em pacientes com COVID-19. Trata-se de estudo
caso-controle, prospectivo em que serão selecionados 300 participantes alocados em dois grupos: Controle (n=150 indivíduos sem comorbidades,
sem evidências clínicas e laboratoriais de doenças infecciosas agudas e/ou crônicas); COVID-19 (n=150, pacientes infectados pelo SARS-CoV-2).
Os participantes serão provenientes do Hospital Universitário de Londrina - PR. Todos os participantes serão avaliados no início do estudo para
obtenção dos dados clínicos, coleta de amostra biológica e determinação dos indicadores de prognóstico. Os pacientes do grupo COVID-19 serão
reavaliados após 7, 14 e 30 dias para obtenção de dados clínicos: duração de hospitalização, necessidade de UTI, dias em uso de ventilação
mecânica, infecções secundárias, sucesso terapêutico ao tratamento convencional instituído, desfecho clínico (óbito/sobrevivência). Será realizada a
genotipagem dos seguintes polimorfismos dos genes, TGFBR2 (rs308465), TGFB1 (rs1800468, rs1800469, rs1800470, rs1800471), IL10 (rs1800896, rs1800871, rs1800872) e FOXP3(rs2232365 e rs3761548). Os níveis plasmáticos de citocinas (IL-10 e TGF-) serão determinados por imunofluorimetria. Espera-se com este estudo evidenciar a influência destes polimorfismos no desenvolvimento da COVID-19, colaborando assim
para o entendimento da imunopatologia desta doença, fornecendo subsídios para a identificação de pacientes com pior prognóstico e desfecho clínico, possibilitando a implantação de tratamento personalizado com impacto direto na morbidade e mortalidade dos pacientes.

Critério de Inclusão:

Serão incluídos no estudo indivíduos com diagnóstico confirmado pela técnica de RT-PCR, de SARS-CoV-2 sintomáticos e assintomáticos atendidos no HU-UEL.

Grupo Controle: será constituído por doadores de sangue controlados quanto ao sexo, idade e raça (cor). Apresentarão sorologia não reagente para
todos os testes sorológicos realizados na triagem de doadores de sangue, assim como deverão apresentar sorologia negativa para Dengue.

Critério de Exclusão:

Serão excluídos das análises indivíduos com sorologia positiva para outros vírus, como vírus da imunodeficiência humana (HIV), vírus da hepatite C
(HCV), vírus da hepatite B (HBV), vírus linfotrópico de células T humanas tipos I e II (HTLV I/II), vírus da Dengue e Chikungunya, assim como amostras reagentes para o Trypanosoma cruzi e para

o teste não treponêmico para sífilis (VDRL).

Objetivo da Pesquisa:

De acordo com o documento PB_Informações Básicas de 06/08/2020

Objetivo Primário:

Avaliar biomarcadores genéticos e imunológicos da resposta imune anti-inflamatória associados ao prognóstico, morbidade e mortalidade da doença causada por SARS-CoV-2

Avaliação dos Riscos e Benefícios:

De acordo com o documento PB_Informações Básicas de 06/08/2020

Riscos:

Os riscos da pesquisa são relacionados a coleta de sangue periférico, uma vez que a coleta de secreção nasal para diagnóstico de COVID-19 será realizada independentemente do envolvimento do participante na pesquisa.

Assim, há um pequeno risco de hematoma na região da coleta de sangue, bem como de queda de pressão e desmaio. Para minimizar os riscos a coleta será realizada em ambiente hospitalar por profissionais capacitados, e quaisquer riscos que venham a ocorrer, os participantes serão prontamente amparados pela equipe do projeto.

Benefícios:

Os benefícios serão indiretos, uma vez que este estudo permitirá um maior entendimento da imunopatologia da COVID-19, fornecendo subsídios para a identificação de pacientes com pior prognóstico e desfecho clínico, possibilitando a implantação de tratamento personalizado com impacto direto na morbidade e mortalidade dos pacientes.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante para área e temática de COVID-19.

Considerações sobre os Termos de apresentação obrigatória:

Apresentou folha de rosto devidamente preenchida e assinada. Apresentou autorização do HU para coleta de dados. Apresentou TCLE em acordo com a resolução. Apresentou declaração de biorrepositório assinado. A coleta de dados está prevista para 08/09, o orçamento é de R$ 13.900,00 custeado pela própria pesquisadora que destaca que os reagentes já foram adquiridos com recursos de projetos anteriores.

Conclusões ou Pendências e Lista de Inadequações:

A pesquisadora faz parte da equipe do projeto "Investigação de fatores genéticos e imunológicos na infecção por SARSCoV-2: associação com o prognóstico, morbidade e mortalidade", cuja CAAE é 31656420.0.0000.5231 e que foi avaliado e aprovado por este CEP (parecer nº 4.053.033). A coleta de dados dos prontuários, questionário e de sangue será executado pelo projeto maior, sendo que neste projeto a pesquisadora receberá as amostras de sangue para fazer as avaliações dos biomarcadores. Portanto, recomenda-se aprovação.

Considerações Finais a critério do CEP:

Prezado(a) Pesquisador(a),

Este é seu parecer final de aprovação, vinculado ao Comitê de Ética em Pesquisas Envolvendo Seres Humanos da Universidade Estadual de Londrina. É sua responsabilidade apresenta-Lo aos órgãos e/ou instituições pertinentes.

Ressaltamos, para início da pesquisa, as seguintes atribuições do pesquisador, conforme Resolução CNS 466/2012 e 510/2016:

A responsabilidade do pesquisador é indelegável e indeclinável e compreende os aspectos éticos e legais, cabendo-lhe:

- conduzir o processo de Consentimento e de Assentimento Livre e Esclarecido;
- apresentar dados solicitados pelo sistema CEP/CONEP a qualquer momento;
- desenvolver o projeto conforme delineado, justificando, quando ocorridas, a sua mudança ou interrupção;
- elaborar e apresentar os relatórios parciais e final;
- manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período mínimo de 5 (cinco) anos após o término da pesquisa;
- encaminhar os resultados da pesquisa para publicação, com os devidos créditos aos pesquisadores e pessoal técnico integrante do projeto;
- justificar fundamentadamente, perante o sistema CEP/CONEP, interrupção do projeto ou a não publicação dos resultados.

Coordenação CEP/UEL.

Endereço: LABESC - Sala 14
Bairro: Campus Universitário **CEP:** 86.057-970
UF: PR **Município:** LONDRINA
Telefone: (43)3371-5455 **E-mail:** cep268@uel.br

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1608049.pdf	06/08/2020 14:57:28		Aceito
Folha de Rosto	FolhaDeRostoAssinada.pdf	06/08/2020 14:56:50	Karen Brajão de Oliveira	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	declaracao_Biorrepositorio_COVID_polimorfismos.pdf	06/08/2020 14:56:38	Karen Brajão de Oliveira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	05/08/2020 20:41:53	Karen Brajão de Oliveira	Aceito
Outros	AutorizacaoHU_projeto_maior.pdf	05/08/2020 20:30:49	Karen Brajão de Oliveira	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_4053033_projeto_maior.pdf	05/08/2020 20:30:22	Karen Brajão de Oliveira	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Pesquisa_COVID_produtividade_CEP.pdf	05/08/2020 20:29:08	Karen Brajão de Oliveira	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

LONDRINA, 10 de Agosto de 2020

Assinado por:
Adriana Lourenço Soares Russo
(Coordenador(a))