IL-10 EXPRESSION LEVELS AS POTENTIAL BIOMARKER IN WOMEN WITH SLE

Recebido em: 14/05/2024
Aceito em: 10/12/2024
DOI: 10.25110/arqsaude.v28i2.2024-11246

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ABSTRACT: Systemic Lupus Erythematosus (SLE) is a multi-systemic autoimmune disease. Its etiology is unclear; however, genetics, environmental, and immunological factors have already been related to the pathogenesis of the disease. There is evidence that cytokines can contribute to SLE, modulating innate and adaptive immune responses. Interleukin-10 (IL-10) is a pleiotropic cytokine previously related to SLE patients and disease activity status. Genetic polymorphisms at the promoter region of *IL-10* have been associated with differential protein production, but the findings are conflicting. To assess the potential involvement of polymorphisms -1082 A/G (rs1800896) and -819 A/C (rs1800871) on the susceptibility to disease and clinical features, we identified these variants by TaqMan® SNP assay in a sample of 135 SLE cases and 130 matched controls from an admixed Brazilian population. High *IL-10* expression genotypes (-1082 AG or GG) were associated with a protection for SLE (OR 0.317; 95% CI 0.189–0.525; p = 0.001), while no significant results were found for -819 A/C (OR 1.907; 95% CI 0.966–3.921; p = 0.063). Haplotypes of medium or high *IL-10* expression were associated with anti-RO positive autoantibodies (p = 0.038), early diagnosis (p = 0.022), and longer fertility time (p = 0.019), possibly predicting a premature but milder disease. These findings indicate that *IL-10* polymorphisms influence the susceptibility to SLE in this Brazilian South population and could predict a phenotype characterized by positivity to Anti-RO, premature diagnosis, and longer fertility time.
KEYWORDS: rs1800896; rs1800871; anti-RO; Brazil.

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NÍVEIS DE EXPRESSÃO DE IL-10 COMO POTENCIAL BIOMARCADOR EM MULHERES COM LES

RESUMO: Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune multissistêmica. Sua etiologia não é clara; entretanto, fatores genéticos, ambientais e imunológicos já foram relacionados à patogênese da doença. Há evidências de que as citocinas possam contribuir para o LES, modulando as respostas imunes inata e adaptativa. A interleucina-10 (IL-10) é uma citocina pleiotrópica previamente relacionada a pacientes com LES e ao status de atividade da doença. Polimorfismos genéticos na região promotora da *IL-10* têm sido associados à produção diferencial de proteína, mas os resultados são conflitantes. Para avaliar o envolvimento potencial dos polimorfismos -1082 A/G (rs1800896) e -819 A/C (rs1800871) na suscetibilidade à doença e características clínicas, identificamos essas variantes pelo ensaio TaqMan® SNP em uma amostra de 135 casos de LES e 130 controles pareados de uma população brasileira miscigenada. Genótipos predizendo níveis elevados de *IL-10* (-1082 AG ou GG) foram associados à proteção para LES (OR 0,317; IC 95% 0,189–0,525; p = 0,001), enquanto nenhum resultado significativo foi encontrado para -819 A/C (OR 1,907; IC 95% 0,966–3,921; p = 0,063). Haplótipos de média ou alta expressão de *IL-10* foram associados a autoanticorpos anti-RO positivos (p = 0,038), diagnóstico precoce (p = 0,022) e maior tempo de fertilidade (p = 0,019), possivelmente predizendo uma doença prematura, porém mais leve. Esses achados indicam que polimorfismos da *IL-10* influenciam a suscetibilidade ao LES nesta população do Sul do Brasil e podem predizer um fenótipo caracterizado por positividade a Anti-RO, diagnóstico prematuro e maior tempo de fertilidade.
PALAVRAS-CHAVE: rs1800896, rs1800871, anti-RO, Brasil.

NIVELES DE EXPRESIÓN DE IL-10 COMO BIOMARCADOR POTENCIAL EN MUJERES CON LES

RESUMEN: El lupus eritematoso sistémico (LES) es una enfermedad autoinmune multisistémica. Su etiología no está clara; sin embargo, factores genéticos, ambientales e inmunológicos ya se han relacionado con la patogénesis de la enfermedad. Existe evidencia de que las citoquinas pueden contribuir al LES, modulando las respuestas inmunes innatas y adaptativas. La interleucina-10 (IL-10) es una citocina pleiotrópica previamente relacionada con los pacientes con LES y el estado de actividad de la enfermedad. Los polimorfismos genéticos en la región promotora de IL-10 se han asociado con la producción diferencial de proteínas, pero los hallazgos son contradictorios. Para evaluar la posible implicación de los polimorfismos -1082 A/G (rs1800896) y -819 A/C (rs1800871) en la susceptibilidad a la enfermedad y las características clínicas, identificamos estas variantes mediante el ensayo TaqMan® SNP en una muestra de 135 casos de LES y 130 controles emparejados de una población brasileña mestiza. Los genotipos elevados de *IL-10* (-1082 AG o GG) se asociaron con una protección para LES (OR 0,317; IC 95% 0,189-0,525; p = 0,001), mientras que no se encontraron resultados significativos para -819 A/C (OR 1,907; IC 95% 0,966–3,921; p = 0,063). Los haplotipos de expresión media o alta de *IL-10* se asociaron con autoanticuerpos anti-RO positivos (p = 0,038), diagnóstico temprano (p = 0,022) y mayor tiempo de fertilidad (p = 0,019), lo que posiblemente predice una enfermedad prematura pero más leve. Estos hallazgos indican que los polimorfismos de IL-10 influyen en la susceptibilidad al LES en esta población del sur de Brasil y podrían predecir un fenotipo

caracterizado por positividad a Anti-RO, diagnóstico prematuro y mayor tiempo de fertilidad.
PALABRAS CLAVE: rs1800896; rs1800871; anti-RO; Brasil.

1. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a multisystemic disease predominantly affecting females (Kim *et al*., 2022). Despite the intermittent illness course, vital organs/tissues such as kidneys, brain, and blood are compromised in most patients. The condition shows wide clinical heterogeneity, justifying the search for personalized therapy (Tsokos, 2020). However, its pathophysiology is only partially understood, hampering patient stratification and the selection among available treatments (Caielli; Wan; Pascual, 2023).

SLE is characterized by breaking B cell tolerance and producing pathogenic autoantibodies that bind to self-antigens (particularly nuclear). These self-antigens are presented to autoreactive helper-T cells that can induce the differentiation of autoreactive B-cells in antibody-producing plasma cells. Large amounts of autoantibodies lead to immune complex formation, which, once deposited, destroys tissue (Geginat *et al*., 2019).

The disease results from an interplay between genetics, environmental, and hormonal factors. SLE genetic background is complex, with more than 150 loci yet identified by Genome-Wide-association-Studies (GWAS) as disease-associated (Deng and Tsao, 2017). Although its etiology remains elusive, altered immune signaling is recognized as a critical step in disease development, with dysregulation of several cytokines (Tsokos *et al*., 2016).

Cytokines play an important role in modulation of immune response, by regulating proliferation and differentiation of target cells and interfering with the production of other cytokines. In recent years, have been noticed that differences in cytokine levels are related with certain allelic variants, which could modify the susceptibility to diverse diseases (Trifunović *et al*., 2015).

Several pieces of evidence suggest a pathogenic role for IL-10 in SLE, with the cytokine being part of a mechanism that contributes to disease. High IL-10 levels are found in lupus patients and are associated with active disease status (Hagiwara *et al*., 1996). Moreover, anti-IL-10 treatment can decrease disease activity (Llorente *et al*., 2000). However, the role of IL-10 in lupus models is highly variable (Geginat *et al*., 2019).

It has been suggested that IL-10 is protective in autoimmune diseases since it inhibits pathogenic inflammation and induces self-tolerance to downregulate Th1 responses (Yin *et al*., 2002). Nevertheless, IL-10 acts as a growth factor for cytotoxic lymphocytes and B-cells, besides promoting the survival of autoreactive B-cells from germinal centers in vitro (Llorente *et al*., 1995). Additionally, IL-10 increases the threshold for T-cell activation, inducing an anergic state in T-cells, which may be necessary for autoimmunity mechanisms (Akdis and Blaser, 2001).

The IL-10 gene is located at chromosome 1, comprised of five exons, and codifies for a 178 amino acids long protein (Spits and Malefyt, 1992). Many polymorphisms have been identified in the promoter gene region (Sabat *et al*., 2010) and around 75% of interindividual differences in IL-10 production are attributed to genetic factors (Westendorp 1997), with the involvement of three main polymorphisms located at positions -1082 A>G (rs1800896), -819 C>T (rs1800871), and -592 C>A (rs1800872). These SNPs give rise to three main haplotypes, GCC, ACC, and ATA, characterized by differences in expression levels, with the alleles -1082A, -819T, and -592A associated with low IL-10 production (Rigo *et al*., 2017; Turner *et al*., 1997; Zhao *et al*., 2017).

Several studies have evaluated the role of these polymorphisms (Liu *et al*., 2013; Zhou *et al*., 2013) and haplotypes (Liu *et al*., 2013) in SLE pathogenesis, but with controversial results. Moreover, aiming the precision medicine development, these findings should be evaluated in the target population once that strategy requires prior knowledge about the genetic background. Therefore, this study aimed to analyze the association of *IL-10* haplotypes with SLE clinical and epidemiological manifestations in a sample of the Brazilian population.

2. METHODOLOGY

2.1 Sample

A total of 135 women SLE patients diagnosed according to the American College of Rheumatology (ACR) criterium were sampled at the Polydoro Ernani of São Thiago University Hospital (HU/UFSC), and 130 healthy women with no autoimmune family history comprising the control group were sampled at the same institution. The research was approved by the Ethics Committee of Universidade Federal de Santa Catarina (CEPSH/UFSC) number 423.535, and all participants gave consent. Epidemiologic data was retrieved from a structured questionnaire applied to participants, with the following

data collected: age, menarche age, menopause age, fertility time, and parity. Clinical data was extracted from medical records.

2.2 Laboratory analysis

DNA was extracted from whole blood using the salting-out method (Miller; Dykes; Polesky, 1987). The polymorphisms located at the promoter IL-10 region: rs1800896 (-1082 A/G) e rs1800871 (-819 A/C) were genotyped by Real-time PCR (rtPCR) using TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific Inc., Waltham, MA, USA), following the manufacturer's instructions.

2.3 Statistical analysis

Allelic, genotypic, and haplotype frequencies were computed by the direct counting method. Adherence of genotypic proportions to expectations under Hardy–Weinberg equilibrium was evaluated by the exact test of Guo and Thompson (Guo and Thompson, 1992), using ARLEQUIN v.3.5.1.2 (Excoffier and Lischer, 2010). The linkage disequilibrium (LD) pattern and the most likely haplotypic combinations were estimated in MLOCUS v.2.0.

The haplotypes obtained were classified according to information available in the literature on the level of IL-10 expression. Meanwhile, A*allele at position -1082 and T*allele at position -819 are related to lower IL-10 expression levels; G*allele at position -1082 and C*allele at position -819 are linked to higher IL-10 expression (Rigo *et al*., 2017; Turner *et al*., 1997; Zhao *et al*., 2017). The genotypes were sorted according to the presence of the allele coding for the highest expression of IL-10 versus homozygotes for the allele with the lowest expression.

All statistical analyses were performed at SPSS v.25.0, considering $p \leq 0.05$ as significant. Continuum variables were described in average and standard deviation and the Student t test was employed to compare variables between groups. Categoric variables were expressed by frequency and absolute number and compared using the Chi-square test. Logistic regression was used to test the association of genotypes and haplotypes to disease. The correlation between alleles and haplotypes with clinical symptoms were assessed using Poisson regression with robust variance.

3. RESULTS

Allele frequencies from cases and controls for the SNPs -1082 A/G (rs1800896) and -819 A/C (rs1800871) did not fit Hardy-Weinberg Equilibrium. Regarding epidemiologic data, no association was verified between case and control groups (Table 1). Otherwise, the presence of low expression haplotypes increased in 87% the risk for SLE (OR 1.87; IC 1.15 – 3.06; *p*= 0.011).

Table 1: Epidemiological characterization in case and control groups.

		Controls (*n*= 130)	Cases (*n*= 135)	*p*-value
Age	Average (SD)	40.0 (17.3)	37.8 (12.5)	0.252
Menarche age		12.8 (1.7)	13.2 (1.8)	0.088
Menopause age		45.9 (6.4)	45.0 (5.5)	0.597
Fertility time		32.7 (6.5)	30.9 (4.7)	0.288
Parity	no children	41 (40.2)	28 (29.2)	0.051
	1-2 children	27 (26.5)	41 (42.7)	
	≥ 3 children	34 (33.3)	27 (28.1)	
IL-10 haplotypes	Low expression	53 (40.8)	76 (56.3)	**0.016**
	Medium/high expression	77 (59.2)	59 (43.7)	

Notes: *n*= absolute frequency.

The only clinical variable showing a significant difference among haplotype groups was the antibody anti-RO (*p*= 0.038). Concerning evaluation of epidemiological features, once all parameters are related, only fertility was included in final model for Poisson regression calculation (Table 2).

Table 2: SLE clinical characterization according to haplotype expression levels.

		Low expression	Medium or high expression	*p*-value
Diagnosis time (months)	Average (SD)	79.0 (73.4)	117.3 (95.9)	**0.022**
Fertility time		28.2 (4.6)	32.8 (3.8)	**0.019**
Parity	no children	21 (38.9)	7 (16.7)	**0.034**
	1-2 children	22 (40.7)	19 (45.2)	
	≥ 3 children	11 (20.4)	16 (38.1)	
antiRO status	Negative	49 (73.1)	27 (52.9)	**0.038**
	Positive	18 (26.9)	24 (47.1)	

4. DISCUSSION

SLE is a multisystemic disease characterized by hyperactivity of B- and T-lymphocytes, production of autoantibodies, and formation of immune complexes that, once deposited, produce organ damage. Its manifestation is diverse and unpredictable, with a heterogeneous clinical presentation that the patient's genetic background may influence (Tsokos, 2020). Therefore, it is paramount to identify biomarkers that can predict the risk for disease and future organ involvement.

Cytokines play an essential role in the pathogenesis of SLE, and their balance determines disease activity (Talaat *et al*., 2015). IL-10 is a pleiotropic cytokine related to immunoregulation and inflammation, produced by most classes of leukocytes. IL-10 participates in B cells hyperactivity, inducing antibody production (Llorente *et al*., 1995; Sabat *et al*., 2010). Otherwise, it inhibits the function of T cells and antigen-presenting cells, potentially contributing to impaired immunity (Akdis and Blaser, 2001).

Several studies suggest a role for IL-10 in SLE. High IL-10 levels have been associated with SLE risk and IL-10 serum levels are related with disease activity status (Godsell *et al*., 2016). Finally, anti-IL-10 therapy could decrease disease activity (Llorente *et al*., 2000).

Polymorphisms in *IL-10* promoter region may alter the cytokine production (Turner *et al*., 1997). Among them the *IL-10* SNPs -1082G/A (rs1800896), - 819T/C (rs1800871), and -592A/C (rs1800872) comprise haplotypes related to IL-10 differential production. The rs1800896 has been the most well studied among the three polymorphisms comprising these haplotypes (Liu *et al*., 2013; Zhou *et al*., 2013). The -1082*G allele and its related haplotype were associated with SLE risk in Europeans, while in Asians it is associated with a protection for disease (Liu *et al*., 2013).

According to the meta-analysis conducted by Liu *et al*. (2013), neither rs1800871 nor rs1800872 were associated with SLE in the European population. Moreover, considering haplotype analysis, GCC haplotypes were associated with SLE risk and ACC haplotypes with SLE protection (Liu *et al*., 2013).

Functional studies do not provide enough support for these associations, although there is some evidence. The -1082*G allele was related to higher IL-10 production in lymphocytes from blood (Turner *et al*., 1997). The -819*C allele was associated with increased IL-10 production (Eskdale *et al*., 1998).

According to Talaat *et al*. (2015), SLE patients show higher plasma levels of IL-10 (Talaat *et al*., 2015). In turn, we have found that an increase of 87% for SLE risk in patients showing low expression haplotypes (OR 1.87; IC 1.15 – 3.06; $p = 0.011$).

Promoter SNPs affect gene expression regulation, which may occur due to differential transcription factors (TF) binding. Therefore, an SNP can change the transcriptional factor binding sites (TFBS), affecting the initiation of the transcriptional machinery, which may impact gene expression. Different outcomes may result from a nucleotide change in a TFBS. This genetic variant may not affect the TF binding once the protein recognizes different binding motifs with no impairment. Still, the modification may also decrease or increase the affinity for TF binding, yielding an allele-specific gene expression regulation. Nucleotide change may yet abrogate the binding motif for TF. According to data provided by the HaploReg v.4.1 database (http://www.broadinstitute.org/mammals/haploreg/haploreg.php), the allelic variation in site for SNP rs1800896 modify the binding for twelve TFs (COMP1, ELF1_known1, Ik-1_2, MAZR, MZF1::1-4_2, MZF1::1-4_3, Maf_known2, PRDM1_known1, PU.1_disc1, PU.1_known2, PU.1_known3, and RXRA_disc4). Also, from information derived from Roadmap Epigenomics Consortium 2015, it is possible to observe differences in chromatin state at this polymorphic site for effector T cells, T regulatory cells, T CD8+ memory cells, B cells, and natural killer cells from the blood. By making chromatin accessible, these epigenetic modifications activate promoters and enhancers, thus allowing the transcriptional machinery to assemble in the promoter region (Ward and Kellis, 2016).

Evidence indicates that B cell hyperactivation and T cell abnormalities contribute to the pathogenesis of SLE. Therefore, the overactivation of T cells may have a leading role in SLE once autoreactive T cells are precursors to B cell hyperactivation, producing immunoglobulins and immune complexes. After antigen stimulation, CD4+ T cells produce cytokines, T helper cells (Th1, Th2, and Th17), and regulatory T cells (Tregs). Different Th cells produce different cytokines. While Th1 cells produce interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α, Th2 cells produce IL-4, IL-5, IL-6, and IL-10, which stimulate antibody production and induce humoral immunity (Xiang *et al*., 2022).

SLE is a Th2-cytokine dependent disease (Xiang *et al*., 2022); however, it has also been reported to have a role for abnormality of Th1 cytokines in its pathogenesis

(Takahashi *et al*., 1996). Although the mechanism why IL-10 affects SLE development is not clear (Geginat *et al*., 2019), some studies argue that IL-10 stimulates B-cells and inhibits the antigen-presenting cells and T-cells activity (Liu *et al*., 2013). In contrast, a previous study suggests that deficiencies in IL-10 production induce activation of CD4+ T-cells by pre-B-cells, potentially triggering autoimmunity (Sim *et al*., 2015).

We found that haplotypes codifying for low concentrations of IL-10 are associated with SLE risk, premature diagnosis time, and shorter fertility time (Table 2). These results are also consistent with our finding that a positive anti-RO status is more frequent in individuals with haplotypes for medium/high IL-10 expression.

IL-10 has been shown to suppress Th1 profile responses in several models of autoimmune diseases, suggesting this cytokine is an essential modulator of SLE, mainly during earlier stages. Th1 cytokines, including IFN-y, are found in patients with a more aggressive disease. Corroborating that, animals showing deficiency in IL-10 production develop more intense symptoms with earlier skin lesions, increased lymphadenopathy, more severe glomerulonephritis, and earlier and enhanced mortality (Yin *et al*., 2002). These previous findings agree with our results that low IL-10 levels are a risk factor for SLE.

Notwithstanding, for these authors, IL-10 may have multiple effects depending on the stage of disease. At the first stages of development, IL-10 deficiency increases IFN-y production by T CD4+ and T CD8+ cells. IFN-y upregulates the expression of MCH class II antigens, leading to antigen presentation to CD4+ T cells and immune activation. However, IFN-y is not required for the later stages of the disease, which could explain the dual role of IL-10 in lupus patients. Excessive cytokine amounts at these stages may lead to enhanced autoantibody production and pathogenic autoantibody complexes deposition (Yin *et al*., 2002).

We found that individuals with high *IL-10* expression haplotypes have earlier diagnoses than those with low *IL-10* expression haplotypes. While a low IL-10 expression profile has been diagnosed for approximately 6.5 months, a medium/high IL-10 expression profile has been diagnosed for at least 9.5 months, on average (Table 2). These agree with the findings that the anti-IL-10 treatment substantially delayed disease onset (Ishida *et al*., 1994).

The most substantial risk factor for SLE development appears to be female sex. The female-to-male ratio ranges from 8:1 to 15:1 and occurs mainly during the peak reproductive years, with a gradual decline after menopause (Kim *et al*., 2022).

Steroid hormones can modulate the T-helper 1 (Th1)/Th2 cytokine balance. These hormones, which include estrogen, progesterone, testosterone, dihydrotestosterone, and dehydroepiandrosterone, are related to the modulation of innate and adaptive immune responses. While progesterone and testosterone mainly show immunosuppressive and anti-inflammatory effects, estrogen is usually associated with immune-stimulatory effects. Nonetheless, the concentrations of oestrogens produce distinct effects. At higher concentrations, oestrogens inhibit Th1 cells and are anti-inflammatory, while they are pro-inflammatory in physiological or low concentrations. Additionally, earlier menopause is associated with an increased risk of developing SLE (Cutolo and Straub, 2020).

We have found that individuals carrying haplotypes for elevated IL-10 levels show a longer fertility time than ones showing reduced IL-10 levels (Table 2). Then, it is suggested that higher exposure to oestrogens in high IL-10 producers could protect against SLE development, supporting the hypothesis that these hormones regulate the intensity and severity of SLE.

During pregnancy, steroid hormones undergo profound changes. For SLE, pregnancy has been considered a high-risk event. Fluctuations in sex hormones during this period can also alter the disease activity, with frequent disease flares. Given that a steroid induced Th2 cytokine polarization is an immunological effect of pregnancy and that SLE is Th2 cytokine driven, these flares in pregnant SLE patients would be expected; however, studies have been showing a lower percentage of disease flares in the third trimester of pregnancy (Doria *et al*., 2002). Considering the following: that imbalances in Th1 cytokines are also related to disease pathogenesis (Takahashi *et al*., 1996), that prolonged high estradiol and progesterone levels during pregnancy inhibit Th1 immune responses, that higher estrogen levels promote IL-10 production (Doria *et al*., 2002), and our results showing that high IL-10 producers women have more children than low IL-10 producers, we could hypothesize that high producers of IL-10 are more propensity to achieve a full-term pregnancy.

SLE is characterized by anti-nuclear autoantibodies (ANA), including anti-double stranded DNA (anti-dsDNA), anti-histone, anti-nucleosome, and antibodies to extractable

nuclear antigens (ENA) [anti-Sm, anti-SSA/Ro, anti-SSB/La, anti-small nuclear ribonucleoproteins (anti-snRNP)]. Autoantibody levels were associated with disease activity. Then, the antibody level measures have been suggested as biomarkers for SLE (Chun *et al*., 2007).

Anti-Ro antibodies are part of a class of autoantibodies against protein components of small cytoplasmic ribonucleoproteins (scRNPs) (Meilof *et al*., 1997). According to our results, carries of -1082*A and -819*T alleles, which codify for low IL-10 expression, show a lower frequency of anti-Ro positive status. Anti-Ro autoantibodies were detected in 35% of patients in this study, like 32% presented by Novak *et al*. (2017) (Novak *et al*., 2017). These molecules were already associated with mild disease manifestations, such as cutaneous and musculoskeletal involvements (Xiang *et al*., 2022).

Although the serum levels of IL-10 have been correlated with anti-dsDNA antibody titers, no association has been found between anti-RO titles and anti-dsDNA responses. Consequently, anti-dsDNA does not follow the pattern of anti-Ro antibody levels in time (Meilof *et al*., 1997).

These findings support the hypothesis that higher IL-10 producers are anti-Ro autoantibodies positive and develop milder disease symptoms. However, it is necessary to interpret these results carefully due to the HWE deviation, which may have occurred due to selection bias or population stratification.

5. CONCLUSIONS

Low expression haplotypes were associated with SLE susceptibility and negative status for Anti-Ro autoantibody. Therefore, *IL-10* genotypes could predict different SLE phenotypes, with higher cytokine levels indicating a premature but milder disease. However, our study shows some limitations, such as HWE deviation and limited sample size.

6. STATEMENTS AND DECLARATIONS

Competing Interests and Funding: The authors declared no potential conflict of interest concerning this article's research, authorship, and publication.

Ethics Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later

amendments or comparable ethical standards. This work is part of a project approved by the Human Research Ethics Committee of the Universidade Federal de Santa Catarina (CEPSH-UFSC), protocol no 922.167.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent to publish: We confirm that all named authors have read and approved the manuscript. The participants have consented to the submission of the obtained data to the journal.

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