LORENA FLOR DA ROSA FRANCHI SANTOS

COMPARAÇÃO DO PERFIL DE MOLÉCULAS DE ADESÃO CELULAR E DO INIBIDOR DO ATIVADOR DO PLASMINOGÊNIO 1 EM PACIENTES COM LÚPUS ERITEMATOSO SISTÊMICO E ARTRITE REUMATOIDE:

INFLUÊNCIA DO TRATAMENTO MEDICAMENTOSO E DA SÍNDROME METABÓLICA

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Orientador: Profa. Dra. Andréa Name Colado Simão.

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SANTOS, Lorena Flor da Rosa Franchi. **Comparação do perfil de moléculas de adesão celular e do inibidor do ativador do plasminogênio 1 em pacientes com lúpus eritematoso sistêmico e artrite reumatoide:** influência do tratamento medicamentoso e da síndrome metabólica. 2019. 145 f. Tese (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina. 2019.

RESUMO

INTRODUÇÃO Lúpus eritematoso sistêmico (LES) e artrite reumatoide (AR) são doenças autoimunes com característica inflamatória crônica e risco cardiovascular (CV) aumentado que apresentam alteração do perfil de moléculas de adesão celular (MACs). O aumento do risco CV também têm sido avaliado frente a influência de alterações nos níveis do inibidor do ativador do plasminogênio tipo 1 (PAI-1), o principal inibidor da fibrinólise.
OBJETIVOS Os principais objetivos dessa tese foram: 1) revisar a influência dos principais medicamentos utilizados no tratamento do LES e da AR nos níveis plasmáticos das MACs; 2) comparar o perfil de MACs e PAI-1 no LES e na AR e examinar o impacto da síndrome metabólica (SM) nestas moléculas. **SUJEITOS E MÉTODOS** Esta tese apresenta dois artigos científicos; o primeiro é um artigo de revisão que inclui o objetivo 1 e o segundo, um artigo original, que inclui o objetivo 2. Artigo 1: Foi realizada pesquisa bibliográfica nas bases de dados eletrônicas PUBMED, Lilacs, Biblioteca Eletrônica Científica Online (SCIELO) e *Science Direct*. A pesquisa incluiu estudos em humanos, *in vivo* ou *in vitro,* com delineamento experimental ou observacional, e sem limite de data de publicação ou número de sujeitos. Foram excluídos estudos em modelos animais ou com tratamentos não padronizados. A lista de referências dos artigos selecionados também foi revisada.
Artigo 2: Foram selecionados 104 pacientes com LES e 124 pacientes com AR recrutados pelo ambulatório de Reumatologia do Hospital Universitário de Londrina, de ambos os sexos, com idade entre 18 e 69 anos. Foram determinados os níveis plasmáticos por imunofluorimetria utilizando a plataforma Luminex® das seguintes MACs: molécula de adesão celular endotelial plaquetária 1 (PECAM-1), molécula de adesão intercelular 1 (ICAM-1), molécula de adesão celular vascular 1 (VCAM-1), E-selectina, P-selectina e do PAI-1. A SM foi diagnosticada de acordo com os critérios propostos pelo Painel de tratamento de adultos III (ATP III). **RESULTADOS** Artigo 1: Foram incluídos 21 estudos, três no LES e 18 na AR, relatados em monoterapia ou terapia combinada. Os medicamentos mais avaliados nos artigos analisados foram ciclofosfamida (CY, n=2) e pulso de metilprednisolona (pMP, n=2) no LES; metotrexato (MTX, n=9) e infliximabe (IFX, n=4) na AR. Além disso, as MACs mais utilizadas para avaliar a resposta ao tratamento foram VCAM-1 (n=2) no LES e ICAM-1 (n=12), VCAM-1 (n=12) e E-selectina (n=14) na AR. Pacientes com LES ou AR submetidos ao tratamento usualmente demonstraram diminuição significativa nos níveis de MACs. Artigo 2: O diagnóstico (LES *versus* AR) apresentou alta associação com MACs e PAI-1, explicando 53,9% de sua variação, com efeito particularmente forte em PECAM-1 (45,7%) e VCAM-1 (38,3%). Todas as 5 MACs e PAI-1 foram significativamente maiores no LES do que na AR após o ajuste para possíveis efeitos da idade, SM e circunferência abdominal. A presença de SM pode interferir em 8,2% da variação dos níveis plasmáticos das 6 moléculas, e os níveis de VCAM-1, E-selectina e PAI-1 foram significativamente maiores nos indivíduos com SM. Os resultados da análise de regressão logística mostraram que níveis aumentados de PECAM-1, VCAM-1 e P-selectina foram melhores preditores de LES (x^2 = 143,99, df = 3, p <0,001, Nagelkerke = 0,700) com 87,1% de todos os casos corretamente classificados com sensibilidade de 87,8%,

especificidade de 86,5% e a área sob a curva de operação do receptor (AUC ROC) de 0,956 (± 0,020). Nossas análises de *machine learning* (incluindo SIMCA) provaram que ambos os grupos são qualitativamente diferentes com relação aos valores de MACs e PAI-1 e que, de fato, todos os valores das 6 moléculas avaliadas contribuem para a discriminação entre ambos os grupos. Os resultados de redes neurais confirmaram que PECAM-1 e VCAM-1 foram as MACs mais significativas que diferenciaram o LES da AR e puderam predizer o LES com sensibilidade de 96,8%, especificidade de 85,4% e AUC ROC de 0,956. Nós também verificamos que as MACs (ICAM-1, VCAM-1, E- e P-selectina) e PAI-1 foram associadas aos componentes da SM, tais como pressão arterial e parâmetros metabólicos. **CONCLUSÕES** Artigo 1: O principal resultado na presente revisão foi que os pacientes com LES ou AR, principalmente com doença ativa submetidos a tratamento específico, demonstraram diminuição significativa nos níveis de MACs. ICAM-1, VCAM-1 e E-selectina foram as moléculas frequentemente determinadas para avaliação da resposta ao tratamento. Desta forma, as MACs podem refletir a atividade da doença e a resposta ao tratamento em pacientes com LES e AR. Artigo 2: No artigo original, nossos dados demonstraram perfil diferencial de MACs e PAI-1 no LES e AR, e PECAM-1 e VCAM-1 podem ser usados para discriminar pacientes com LES de AR como um critério de validação externo. Além disso, a SM foi mais frequente em pacientes com LES, mas poderia influenciar os níveis plasmáticos de MACs e PAI-1 em ambas as doenças, e deve ser avaliada concomitantemente com eles. Os componentes da SM foram associados à MACs e PAI-1 e poderiam explicar o risco CV diferencial no LES e na AR.

Palavras-chave: Lúpus eritematoso sistêmico. Artrite reumatoide. Moléculas de adesão celular. Inibidor do ativador do plaminogênio 1. Síndrome metabólica.

SANTOS, Lorena Flor da Rosa Franchi. **Comparison of cell adhesion molecule profile and the plasminogen inhibitor 1 in patients with systemic lupus erythematosus and rheumatoid arthritis:** influence of drug treatment and metabolic syndrome. 2019. 145 p. Thesis (Doctoral Degree in Experimental Pathology) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

INTRODUCTION Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are chronic autoimmune diseases that present increased cardiovascular (CV) risk. These diseases are characterized by changes in cell adhesion molecules (CAM). Increased CV risk has also been evaluated in view of the influence of changes in the plasminogen activator inhibitor type 1 (PAI-1) levels, the main inhibitor of fibrinolysis. **OBJETIVOS** The main objectives of this thesis were: 1) to review the influence of the main drugs used in the treatment of SLE and RA on the plasma levels of CAM; 2) to compare the profile of CAM and PAI-1 in SLE and RA and to examine the impact of metabolic syndrome (MetS) on these molecules. **SUBJECTS AND METHODS** This thesis presents 2 scientific articles, the first one is a review article that includes objective 1 and the second, an original article, which includes objective 2. Article 1: A bibliographic search was performed in the electronic databases PUBMED, Lilacs, Scientific Electronic Library Online (SCIELO), and Science Direct. The research included human studies, *in vivo* or *in vitro*, with experimental or observational design, and with no limit of publication date or number of subjects. It was excluded studies in animal models or with non-standard treatments. The list of references of the selected articles was also revised. Article 2: We selected 104 patients with SLE and 124 patients with RA enrolled from the ambulatory of Rheumatology of the University Hospital of Londrina, Paraná, Brazil, of both sexes aged 18 to 69 years. Plasma levels of the following CAM were determined by immunofluorimeria using the Luminex® platform: platelet endothelial cell adhesion molecule 1 (PECAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, P-selectin, and PAI-1. MetS was diagnosed according to the criteria proposed by the Adult Treatment Panel III criteria (ATP III). **RESULTS** Article 1: There were included 21 studies, three on SLE and 18 on RA. Monotherapy or combined trials were reported. The most evaluated drugs in the articles analyzed were cyclophosphamide (CY, n=2) and methylprednisolone pulse (pMP, n=2) in SLE; and methotrexate (MTX, n=9) and infliximab (IFX, n=4) in RA. In addition, the most CAM used to assess response to treatment was VCAM-1 (n=2) in SLE; and ICAM-1 (n=12), VCAM-1 (n=12) and E-selectin (n=14) in RA. SLE or RA patients submitted to treatment usually demonstrated a significant decrease in CAM levels. Article 2: The diagnosis (SLE *versus* RA) had a high association with CAM and PAI-1, explaining 53.9% of their variation, with a particularly strong effect in PECAM-1 (45.7%) and VCAM-1 (38.3%). All 5 CAM and PAI-1 were significantly higher in SLE than in RA after adjusting for possible effects of age, MetS, and waist circumference. MetS presence could interfere in 8.2% of variation of 6 molecules plasma levels, while VCAM-1, E-selectin and PAI-1 were significantly higher in individuals with the MetS. The results of logistic regression analysis showed that SLE was best predicted by increased levels of PECAM-1, VCAM-1 and P-selectin (x^2=143.99, df=3, p<0.001, Nagelkerke=0.700) with 87.1% of all cases correctly classified with a sensitivity of 87.8%, specificity of 86.5% and an the area under the Receiver Operating Curves (AUC ROC) of 0.956 (±0.020). Our machine learning techniques (including SIMCA) proved that both groups are qualitatively different with respect to the CAM and PAI-1 values and that in fact all six evaluated molecules

contribute to the discrimination between both groups. The results of neural networks analysis agreed that PECAM-1 and VCAM-1 were the most significant CAM differentiating SLE from RA and could predict SLE with sensitivity of 96.8%, specificity of 85.4% and AUC ROC of 0.956). We also verified that CAM (ICAM-1, VCAM-1, E- and P-selectin) and PAI-1 were associated with MetS components, such as blood pressure and metabolic parameters. **CONCLUSION** Article 1: The most remarkable result in the present review was that SLE or RA patients, mainly with active disease, submitted to treatment demonstrated a significant decrease in CAM levels. ICAM-1, VCAM-1 and E-selectin were the molecules often determined for evaluation of treatment response. In this way, CAM markers may reflect the disease activity and the response to treatment in SLE and RA patients. Article 2: In conclusion, our data demonstrated the differential CAM profile and PAI-1 levels in SLE and RA, and PECAM-1 and VCAM-1 may be used to differentiate SLE *versus* RA patients as an external validating criterion. In addition, MetS were more frenquent in SLE patients but it could influence CAM and PAI-1 plasma levels in both disease, and it should be evaluated concomitantly with them. The MetS components were associated with CAM and PA-1 and could be explain the differential CV risk in SLE and RA.

Keywords: Systemic lupus erythematosus. Rheumatoid arthritis. Cell adhesion molecules. Plasminogen activator inhibitor type-1. Metabolic syndrome.

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Artigo 1

Artigo 2

LISTA DE ABREVIATURAS E SIGLAS

ACR	Colégio Americano de Reumatologia
AINEs	Anti-inflamatórios não esteroidais
ANA	Anticorpos antinucleares
ANOVA	Análises de variância
Anti-C1q	Anticorpo anti-componente do complemento 1q Anti-CCP Anticorpo anti-peptídeo cíclico citrulinado
Anti-RNP	Anticorpo anti-ribonucleoproteína Anti-Sm Anti-Smith
Anti-SSA	Anticorpo contra o antígeno A relacionado a Síndrome de Sjögren (anti-Ro) Anti-SSB Anticorpo contra o antígeno B relacionado a Síndrome de Sjögren (anti-La)
APC	Célula apresentadora de antígenos
AR	Artrite reumatoide
ATPIII	Painel de tratamento de adultos III
AUC ROC	Área sob as curvas características de operação do receptor
BICLA	Avaliação do Lúpus Combinado baseado no BILAG
BILAG	Índice de Atividade da Doença avaliado pelo Grupo de Lúpus das Ilhas Britânicas
BLys	Estimulador de Linfócitos B
CA	Circunferência abdominal
CD	Cluster de diferenciação
CP	Componente Principal
CV	Cardiovascular
DAS28	Escore de atividade da doença em 28 articulações ds
DNA	DNA dupla fita
EBV	Vírus Epstein-Barr
ECLAM	Medida de Atividade de Lúpus do Consenso Europeu
EDTA	Ácido etilenodiamino tetraacético
EMEA	Agência Europeia de Avaliação de Produtos Médicos
EPZ	Epratuzumabe
EROs	Espécies reativas de oxigênio
EULAR	Liga Europeia Contra o Reumatismo

FDA	*Food and Drug Administration*
FLSs	Sinoviócitos tipo fibroblastos
FR	Fator reumatoide
GLM	Modelo linear geral multivariado
HCAM	Molécula de adesão celular *homing*
HDL	Lipoproteína de alta densidade
ICAM-1	Molécula de adesão celular intercelular 1
IFN	Interferon
IgSF	Membros da superfamília das imunoglobulinas
IL	Interleucina
IMC	Índice de massa corporal
LDL	Lipoproteína de baixa densidade
LES	Lúpus eritematoso sistêmico
MACs	Moléculas de adesão celular
MeSH	*Medical Subject Headings*
MLP	Perceptron multicamadas
NCAM-1	Molécula de adesão celular neural 1
NFkB	Fator nuclear kappa B
NL	Nefrite lúpica
NN	Rede neural
oxLDL	Lipoproteína de baixa densidade oxidada
PAD	Pressão arterial diastólica
PAI-1	Inibidor do Ativador do Plasminogênio 1
PAS	Pressão arterial sistólica
PCR	Proteína C-reativa
PECAM-1	Molécula de adesão celular endotelial plaquetária 1
RAI	Índice articular de Ritchie
SAAF	Síndrome do anticorpo antifosfolípide
SCIELO	Biblioteca Eletrônica Científica Online
SE	Médias marginais estimadas
SIMCA	Modelagem suave independente por analogia de classe
SLEDAI	Índice de atividade da doença Lúpus Eritematoso Sistêmico
sLe^X	Tetrassacárideo sialil Lewis X
SM	Síndrome metabólica

SOD	Superóxido dismutase
TCLE	Termo de consentimento livre e esclarecido
TNF-α	Fator de necrose tumoral alfa
t-SNE	Incorporação de vizinhança estocástica distribuída em t
UV	Ultravioleta
VCAM-1	Molécula de adesão celular vascular 1
VHS	Velocidade de hemossedimentação

SUMÁRIO

1. INTRODUÇÃO

1.1 Lúpus Eritematoso Sistêmico

O LES é uma doença autoimune sistêmica e multifatorial com envolvimento de fatores genéticos, epigenéticos, imunológicos e hormonais. Os fatores de risco predominantes na fisiopatologia do LES envolvem interação de componentes genéticos com fatores ambientais, particularmente exposição à luz ultravioleta (UV), infecção pelo vírus Epstein-Barr (EBV) e fatores hormonais (KAUL *et al.*, 2016). Na maioria dos pacientes, a doença é herdada de maneira poligênica por meio de várias mutações de baixa penetrância; entretanto, também podem ocorrer mutações raras de alta penetrância, que incluem aquelas que causam deficiências na via do complemento (JAMES, 2015). Quanto aos fatores ambientais, a radiação UV pode induzir quebras de DNA que alteram a expressão gênica, geram fragmentos de ácidos nucléicos e/ou levam a morte celular por necrose ou apoptose. Já a infecção pelo EBV contribui para ativação do sistema imune inato por meio da expressão de interferon (IFN) tipo I, e para diferenciação de células B que estimulam a produção de anticorpos específicos para sequências de aminoácidos compartilhadas por autoproteínas e proteínas codificadas por EBV. Além disso, anticorpos específicos contra EBV podem reagir de forma cruzada com DNA de dupla fita (dsDNA), sugerindo que a infecção por EBV poderia induzir resposta autoimune (YADAV *et al.*, 2011).

Mulheres geneticamente predispostas expostas cronicamente a estrógenos, endógenos e exógenos, podem apresentar perda de tolerância aos antígenos nucleares com surgimento de autoanticorpos e subsequente lesão imunomediada (HUGHES; CHOUBEY, 2014). As taxas de incidência global de LES variam de aproximadamente 0,3 a 23,7 por 100.000 pessoas/ano, enquanto as taxas de prevalência variam de 6,5 a 178,0 por 100.000 pessoas (PONS-ESTEL *et al.*, 2017). Quanto à influência do sexo na epidemiologia da doença, a razão de prevalência entre homens e mulheres varia em torno de 9:1 e é ainda mais elevada durante as idades de pico da doença, ou seja, entre a menarca e a menopausa (fase reprodutiva) (HUGHES; CHOUBEY, 2014).

O LES apresenta evidências de distúrbios nas imunidades inata e adaptativa manifestadas por depuração defeituosa de células apoptóticas, produção aumentada de moléculas proinflamatórias e resposta inadequada de células B e T (FARIDI *et al.*, 2017; LISNEVSKAIA; MURPHY; ISENBERG, 2014). Essas anormalidades da resposta imune resultam no aumento da produção de autoanticorpos, formação de imunocomplexos com ativação do sistema complemento e produção de citocinas, como o estimulador de linfócitos B (BLys), interleucina (IL) -6, IL-17, IL-18, IFN tipo I e fator de necrose tumoral alfa (TNF-α) (JACOB; STOHL, 2011). Os complexos imunes depositam-se nos tecidos favorecendo a resposta inflamatória com danos teciduais e orgânicos (AHMADPOOR; DALILI; ROSTAMI, 2014).

Os mecanismos multissistêmicos complexos no LES culminam em amplo espectro de manifestações clínicas, que vão desde o envolvimento da pele e articulações até os sistemas renal e nervoso (ZHANG *et al.*, 2016). Alguns fenótipos estão fortemente correlacionados com o aparecimento de autoanticorpos específicos. A presença de autoanticorpos específicos no LES pode estar correlacionada com (a) diagnóstico da doença [anti-dsDNA, anti-Smith (anti-Sm)]; (b) atividade da doença (anti-dsDNA), (c) alterações dos sistemas renal e/ou nervoso (anti-dsDNA e anti-nucleossomo); (d) ocorrência de lúpus eritematoso neonatal e fotossensibilidade [anticorpo contra o antígeno A relacionado a Síndrome de Sjögren (Anti-SSA ou anti-Ro) e anticorpo contra o antígeno B relacionado a Síndrome de Sjögren (AntiSSB ou anti-La)]; (e) síndrome do anticorpo antifosfolípide (SAAF) e lúpus neuropsiquiátrico (anti-fosfolipídio e anti-ribossomal P); (f) lúpus cutâneo e nefrite [(anticorpos anti-componente do complemento 1q (Anti-C1q)]; (g) lúpus induzido por drogas (anti-histona); e (h) doença mista do tecido conjuntivo [Anti-ribonucleoproteína (anti-RNP)] (COZZANI *et al.*, 2014).

O tratamento do paciente com LES inicia-se com agentes antimaláricos (hidroxicloroquina) e secundariamente com glicocorticoides, entretanto outros fármacos podem ser empregados. Entre eles estão os imunossupressores (azatioprina, metotrexato ou micofenolato mofetil) frequentemente utilizados em associação com outros medicamentos ou quando não for possível a redução ou descontinuação da dose de glicocorticoides dentro de um prazo razoável, mesmo para pacientes sem envolvimento orgânico. Finalmente, o uso da ciclofosfamida e/ou anticorpos monoclonais (belimumabe e rituximabe) pode ser recomendado em casos de manifestações clínicas graves, como alterações renais e do sistema nervoso central, e refratárias à terapia convencional. Os objetivos do tratamento desses pacientes são atingir remissão ou controlar a atividade da doença, além de prevenir dano orgânico com a dose mínima possível de glicocorticoides (KUHN *et al.*, 2015).

1.2 Artrite Reumatoide

A AR é uma doença inflamatória crônica sistêmica de acometimento articular e natureza autoimune caracterizada pela produção de autoanticorpos contra proteínas citrulinadas [anticorpo anti-peptídeo cíclico citrulinado (anti-CCP)] e contra a fração Fc da imunoglobulina G [fator reumatoide (FR)], apesar de alguns indivíduos serem soronegativos para esses autoanticorpos (MALMSTRÖM; CATRINA; KLARESKOG, 2017). A fisiopatologia da AR também envolve fatores genéticos e ambientais. Entre os fatores genéticos, vale ressaltar a forte associação dos indivíduos afetados com a presença do epítopo compartilhado *HLA-DRB1*04* e a forte associação desse epítopo com o anticorpo anti-CCP (SMOLEN *et al.*, 2018). Quanto aos fatores ambientais, o tabagismo e processos infecciosos têm sido relacionados com a patogênese da doença. O mecanismo pelo qual o tabagismo favorece o aparecimento da AR não está completamente elucidado; entretanto, parece envolver a indução da citrulinização de peptídeos e formação de radicais livres que favorecem ativações genéticas e formação de autoanticorpos (GOELDNER *et al.*, 2011). Similarmente, infecções pelo EBV, citomegalovírus e bactérias como *Proteus mirabilis* e *Escherichia coli* parecem induzir, por mimetismo celular, processo inflamatório com formação de imunocomplexos e autoanticorpos contra a porção Fc das imunoglobulinas (MCINNES; SCHETT, 2011).

Uma revisão publicada em 2016 demonstrou que a incidência de AR era de 0,5 a 1,0% em populações do Hemisfério Norte (SMOLEN; ALETAHA; MCINNES, 2016). Posteriormente, Smolen e colaboradores (2018) concluíram que a maioria dos estudos

epidemiológicos em AR têm sido realizados em países ocidentais, com valores de prevalência na faixa de 0,5 a 1,0% em indivíduos caucasianos. Quanto a influência do sexo na epidemiologia da doença, a razão de prevalência entre mulheres e homens é 3:1 com evidências de que a AR aparece com maior frequência em mulheres após a menopausa com pico de incidência na faixa etária entre 45 a 75 anos (HUGHES; CHOUBEY, 2014).

A AR caracteriza-se por uma resposta inflamatória inicialmente na membrana sinovial (sinovite) que favorece a formação de novos vasos, e o influxo de células inflamatórias (células T, células B, plasmócitos, células dendríticas, macrófagos e mastócitos) por ação de citocinas proinflamatórias (como TNF-α, IL-1 e IL-17), moléculas de adesão celular (MACs) e quimiocinas (MCINNES; SCHETT, 2011). Na articulação, neutrófilos juntamente com sinoviócitos e condrócitos liberam enzimas (proteases) e espécies reativas de oxigênio (EROs) que levam à degradação da cartilagem. A camada de revestimento torna-se hiperplásica (maior que 20 camadas de células) e a membrana sinovial se expande para formar vilosidades. Os espaços articulares se tornam estreitos ou desaparecem como um sinal de degradação da cartilagem e destruição do osso adjacente em um processo denominado erosão (ALAM *et al.*, 2017). A porção destrutiva da membrana sinovial é denominada *pannus* e os elementos celulares destrutivos são os osteoclastos (SMOLEN *et al.*, 2007). A produção de autoanticorpos, resposta inflamatória, destruição da cartilagem e do osso subcondral perpetuam o dano articular e a inflamação na AR (MCINNES; SCHETT, 2011).

A principal característica clínica da AR é a formação do *pannus* com consequente rigidez e dor nas articulações (CHOY, 2012). Entretanto, sintomas extra articulares podem aparecer, entre eles, os nódulos reumáticos que caracterizam a manifestação cutânea mais comum na AR (LORA; CERRONI; COTA, 2018). Manifestações cardiovasculares (CV) e pulmonares também podem ocorrer (BERMAN *et al.*, 2018).

Diversas estratégias podem ser utilizadas no tratamento do paciente com AR. As drogas antirreumáticas modificadoras de doenças (DMARDs) constituem uma classe terapêutica importante no tratamento desses pacientes. DMARDs podem ser de três tipos: biológicos (bDMARDs), sintéticos tradicionais ou convencionais (csDMARDs) e sintéticos direcionados (tsDMARDs). Os csDMARDs incluem metotrexato, leflunomida, hidroxicloroquina e sulfasalazina. bDMARDs incluem abatacept, tocilizumab e inibidores do TNF-α. Os tsDMARDs são representados pelo tofacitinib, um inibidor da Janus quinase (JAK). A primeira escolha recomendada é o metotrexato e glicocorticoides podem ser usados como terapia adjuvante. Se a remissão não for atingida faz-se a combinação ou troca dentro da classe dos csDMARDs. Se ainda assim a remissão não for alcançada a combinação ou

troca é realizada com os bDMARDs ou tsDMARDS. Na AR, os objetivos do tratamento incluem a remissão ou controle da atividade da doença, prevenção de danos nas articulações e incapacidades, redução da mortalidade e eventos cardiovasculares (BURMESTER; POPE, 2017).

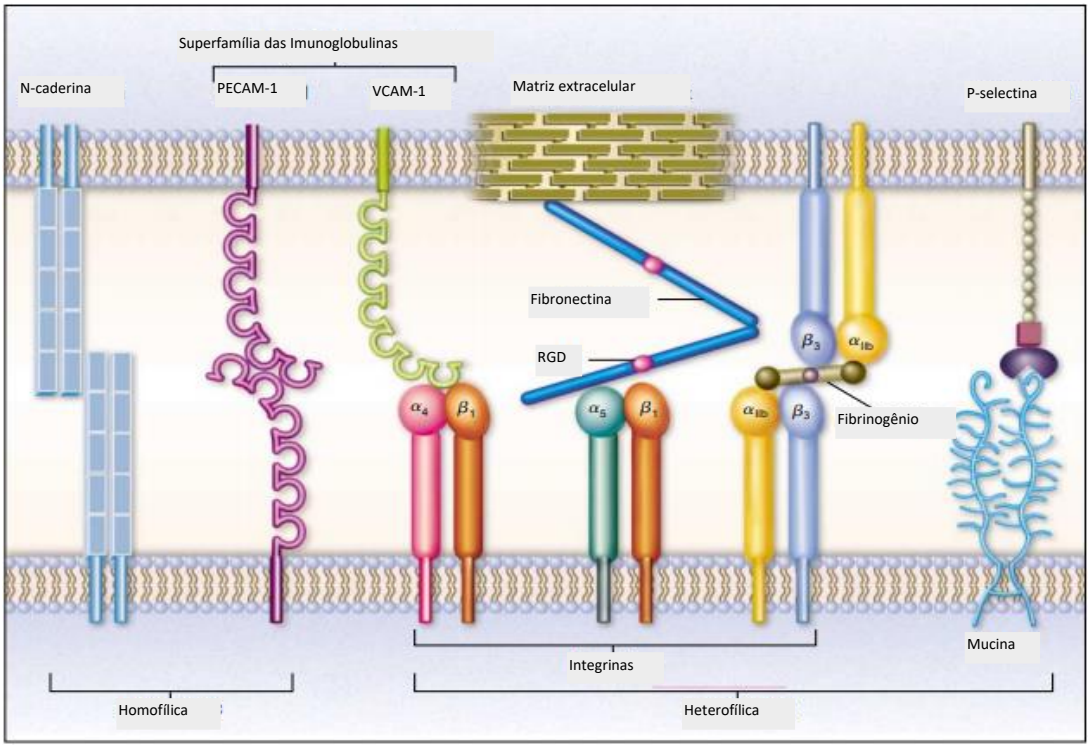
1.3 Moléculas de Adesão Celular e Autoimunidade

As MACs são proteínas integrais de membrana que compreendem domínios extracelular, transmembrana e citoplasmático. Elas podem ser classificadas em quatro tipos principais, denominados integrinas, membros da superfamília das imunoglobulinas (IgSF), selectinas e caderinas (MCMURRAY, 1996). As integrinas são glicoproteínas transmembrana heterodiméricas, compostas por cadeias α e β, das quais os membros da IgSF são importantes ligantes (PAN *et al.*, 2016). Os membros da IgSF são amplamente expressos em células endoteliais, têm sua estrutura caracterizada por repetidos domínios similares aqueles encontrados nas imunoglobulinas e são representadas pelas icônicas molécula de adesão celular intercelular 1 (ICAM-1), molécula de adesão celular vascular 1 (VCAM-1) e molécula de adesão celular endotelial plaquetária 1 (PECAM-1) (GOLIAS *et al.*, 2011). As selectinas são glicoproteínas transmembrana que reagem com carboidratos, compartilham propriedades similares às lectinas e são expressas em células endoteliais (P- e E-selectina), plaquetas (P-selectina) ou leucócitos (L-selectina). Além disso, as selectinas estão envolvidas principalmente na captura e rolamento de leucócitos sobre o endotélio (BARTHEL *et al.*, 2007). Por fim, os dímeros de caderina se reúnem em junções aderentes para manter células adjacentes em contato próximo em uma estrutura semelhante a um zíper. Elas estão ligadas ao citoesqueleto através das cateninas e são dependentes de cálcio. Um representante desse grupo é a E-caderina, expressa em diferentes epitélios e importante componente dos desmossomos que se concentram em junções intercelulares conhecidas como zona de aderência (FRENETTE; WAGNER, 1996) **(Figura 1)**.

As MACs podem se ligar a elas mesmas (interação homofílica) ou a superfície de outras moléculas (interação heterofílica) ou ambas. Podem, ainda, mediar a adesão entre duas células do mesmo tipo (adesão homotípica) ou de diferentes tipos (adesão heterotípica) (MCGARY; LEV; BAR-ELI, 2002). São liberadas na circulação e atuam como marcadores de ativação e disfunção endotelial, sendo reguladas positivamente por citocinas proinflamatórias, como IL-1, IFN-γ e TNF-α. Elas são expressas na superfície de células endoteliais e/ou leucócitos e têm papel fundamental na organogênese, homeostasia,

manutenção da integridade da arquitetura tecidual, sinalização celular, regulação imune, adesão celular, recrutamento e migração seletiva de células inflamatórias dos vasos sanguíneos até o local da inflamação (SKEOCH *et al.*, 2014).

Figura 1 – As quatro principais classes de moléculas de adesão celular mostrados incorporados em uma membrana plasmática.

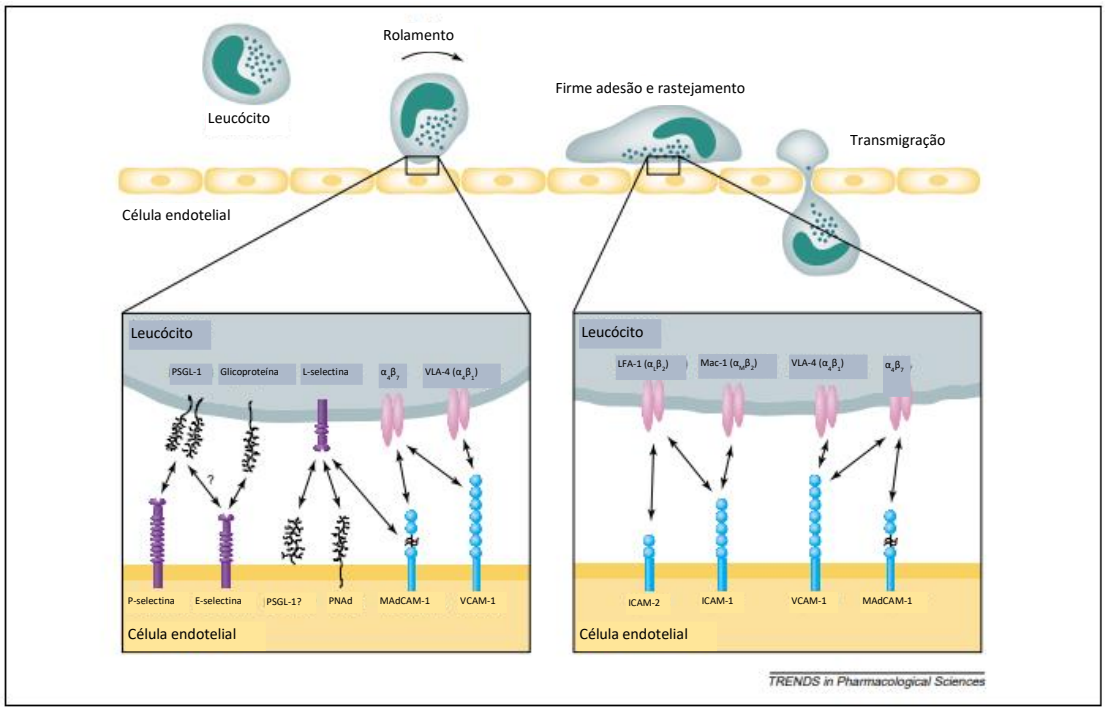


Fonte: FRENETTE; WAGNER (1996, p. 1527, tradução nossa). PECAM-1: Molécula de adesão celular endotelial plaquetária 1; VCAM-1: Molécula de adesão celular vascular 1; ICAM-1: Molécula de adesão celular intercelular 1; RGD: tripeptídeo ácido arginina-glicina-aspártico.

No processo inflamatório, as MACs participam das etapas da cascata de adesão e migração leucocitária que consistem em (a) captura; (b) rolamento; (c) ativação das integrinas por meio de quimiocinas; (d) fortalecimento da aderência; e, finalmente, (e) rastejamento intravascular e (f) transmigração paracelular e/ou transcelular mediados por membros de IgSF (LEY *et al.*, 2007). Esse contato intercelular precisa ser transitório e cíclico para o leucócito ser capaz de ser reutilizado e de se deslocar para outras áreas onde a inflamação está ocorrendo (MONTEFORT; HOLGATE, 1991). A captura inicial e rolamento de leucócitos é mediada predominantemente pela interação transitória de moléculas de selectina presentes em leucócitos (L-selectina) e células endoteliais (P-selectina e E-selectina) com baixa e alta afinidade. O rolamento de leucócitos também pode ser mediado por interações de baixa

afinidade entre integrinas de leucócitos e membros da IgSF no endotélio. A firme adesão leucocitária é mediada pela ligação entre integrinas e membros da IgSF (ULBRICH; ERIKSSON; LINDBOM, 2003) **(Figura 2)**. Uma das etapas da cascata de adesão e migração leucocitária é a fase de ativação das integrinas que envolve as denominadas sinalizações *outside-in* e *inside-out*. Essas duas sinalizações são responsáveis pelo controle da polaridade celular, estrutura do citoesqueleto, expressão genética, sobrevivência e proliferação celular; bem como pela adesão/migração celular e remodelação/montagem da matriz extracelular, respectivamente (SHATTIL; KIM; GINSBERG, 2010).

Figura 2 – Moléculas de adesão celular (MACs) envolvidas na adesão de leucócitos a células endoteliais.

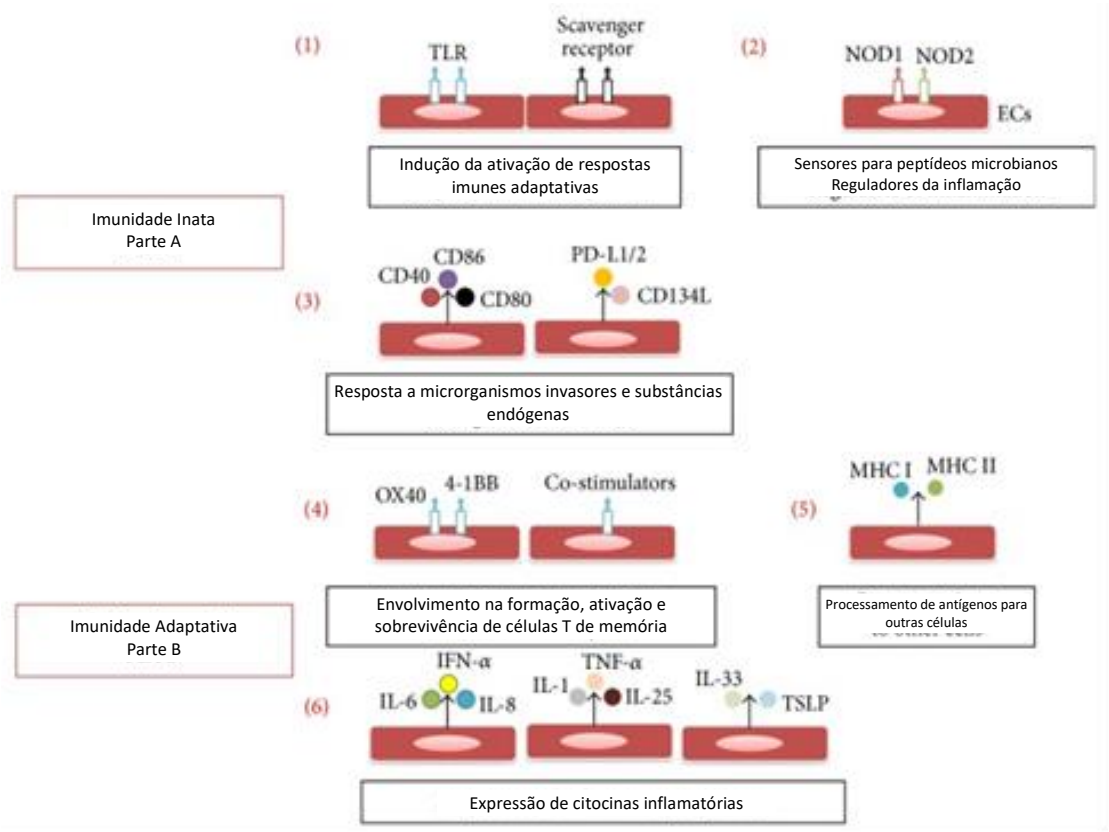


Fonte: ULBRICH; ERIKSSON; LINDBOM (2003, p. 641, tradução nossa). PSGL-1: Glicoproteína ligante da P-selectina tipo 1; VLA-4: antígeno *very late* tipo 4; PNAd: endotelina de nó periférico; MAdCAM-1: molécula de adesão celular endotelina mucosa 1; VCAM-1: Molécula de adesão celular vascular 1; LFA-1: Molécula associada a função leucocitária tipo 1; Mac-1: Antígeno macrofágico tipo 1; ICAM-1/2: Molécula de adesão celular intercelular 1 ou 2.

As células endoteliais também exercem papel no sistema imune tanto como primeira linha de defesa, quanto como mediadoras via diversos receptores. Entretanto, quando a imunidade inata não elimina os estímulos inflamatórios, a resposta imune adaptativa será desencadeada e as células endoteliais estarão envolvidas no estabelecimento da inflamação

crônica atuando como célula apresentadora de antígenos (APC) e via interações com células efetoras especializadas. Assim, células endoteliais secretam substâncias ativas, entre elas as MACs, que promoverão a regulação e modulação das respostas imunes inatas e adaptativas a fim de controlar o recrutamento e o influxo de células inflamatórias para locais de ação específicos (YANG; CHANG; WEI, 2016) **(Figura 3)**.

Figura 3 – Células endoteliais e imunidade inata e adaptativa.



Fonte: YANG; CHANG; WEI (2016, p. 3, tradução nossa). TLR: Toll-like receptor; NOD1/2: receptores de tipo domínio de oligomerização de ligação a nucleotídeos 1 ou 2; CD: Cluster de diferenciação; PD-L1/2: ligante de morte programada 1 ou 2; MHC: complexo principal de histocompatibilidade, IFN: Interferon; IL: Interleucina; TNF-α: Fator de necrose tumoral alfa; TSLP: Linfopoietina estromal tímica.

Portanto, MACs podem afetar as interações entre células T e APCs, e entregar sinais necessários para a efetiva função de células T auxiliar, T citotóxica e células B (MOJCIK; SHEVACH, 1997). Aberrações dessas moléculas, por sua vez, têm sido implicadas na patogênese de doenças autoimunes que apresentam caráter inflamatório crônico, como LES e AR (FROSTEGÄRD, 2014; TURKCAPAR *et al.*, 2005), apresentando grande importância tanto como um componente fisiopatológico (ATEHORTÚA *et al.*, 2017), quanto como um

biomarcador (KLIMEK *et al.*, 2014; LEWIS *et al.*, 2016). Além disso, elas têm sido consideradas alvos terapêuticos para doenças inflamatórias e autoimunes (GIRALDO *et al.*, 2012).

1.3.1 Evidências do Envolvimento das Moléculas de Adesão Celular na Fisiopatologia do Lúpus Eritematoso Sistêmico

Pacientes com LES têm demonstrado níveis elevados de MACs quando comparados a indivíduos saudáveis (EGERER *et al.*, 2000; HAJIALILO *et al.*, 2018; MOK *et al.*, 2010; SANTOS *et al.*, 2018; SKEOCH *et al.*, 2014; WU *et al.*, 2007); e esses altos níveis têm sido associados com a atividade da doença (CRISPÍN *et al.*, 2010; LEWIS *et al.*, 2016) e suas manifestações clínicas (LU *et al.*, 2010; MOK *et al.*, 2018; NYBERG; ACEVEDO; STEPHANSSON, 1997; SOLIMAN *et al.*, 2017; WANG *et al.*, 2018), especialmente com os eventos CV (BLANKENBERG; BARBAUX; TIRET, 2003; EL-MESALLAMY; HAMDY; IBRAHIM, 2011; GUSTAFSSON *et al.*, 2012; PAMUK *et al.*, 2014; SKEOCH *et al.*, 2014). Além disso, estudos também relacionaram os níveis de MACs com marcadores inflamatórios, como por exemplo velocidade de hemossedimentação (VHS) e E-caderina (JIN *et al.*, 2013; SEDIE *et al.*, 2018), e presença de autoanticorpos como anti-dsDNA (MAHAYIDIN *et al.*, 2014).

Crispín e colaboradores (2010) avaliaram pacientes com LES e demonstraram correlação positiva entre os níveis da molécula de adesão celular *homing* (HCAM) e o Índice de Atividade da Doença Lúpus Eritematoso Sistêmico (SLEDAI) (Anexo A) (CRISPÍN *et al.*, 2010). Posteriormente, Lewis e colaboradores (2016) mostraram correlação positiva dos níveis elevados de VCAM-1 com a Medida de Atividade de Lúpus do Consenso Europeu (ECLAM) (Anexo B) (LEWIS *et al.*, 2016).

Nyberg, Acevedo e Stephansson (1997) encontraram níveis elevados de ICAM-1, VCAM-1 e E-selectina em pacientes com lúpus eritematoso cutâneo e a radiação UV, um conhecido fator de risco do LES, pode induzir a produção de ICAM-1 (NYBERG; ACEVEDO; STEPHANSSON, 1997). Lu e colaboradores (2010) demonstraram que os níveis de VCAM-1 e P-selectina no líquido cefalorraquidiano foram parâmetros adequados para o monitoramento da atividade do LES neuropsiquiátrico e da resposta ao tratamento, já que essas moléculas estavam inicialmente aumentadas, e após o tratamento, ocorreu redução significativa (LU *et al.*, 2010). Soliman e colaboradores (2017) e Mok e colaboradores (2018) descreveram VCAM-1 como um bom biomarcador urinário de nefrite lúpica (NL) (MOK *et*

al., 2018; SOLIMAN *et al.*, 2017). Wang e colaboradores (2018) demonstraram que os níveis de ICAM-1 e molécula de adesão celular neural 1 (NCAM-1) foram significativamente maiores na urina de pacientes com NL ativa (WANG *et al.*, 2018).

Têm sido demonstrado que a VCAM-1 (GUSTAFSSON *et al.*, 2012), E-selectina (EL-MESALLAMY; HAMDY; IBRAHIM, 2011; SKEOCH *et al.*, 2014), ICAM-1 (BLANKENBERG; BARBAUX; TIRET, 2003) e PECAM-1 (PAMUK *et al.*, 2014) estão associadas, em maior ou menor grau, com o aparecimento da aterosclerose e/ou doenças CV em pacientes com LES. Esse dado é de especial importância uma vez que o risco de aparecimento dessas comorbidades no LES é elevado quando comparado a população geral (MCMAHON; HAHN; SKAGGS, 2011), possivelmente devido à inflamação crônica e disfunção endotelial desses pacientes que acarreta em recrutamento celular intenso e potencialização da resposta imune (SANTOS *et al.*, 2012). Além disso, pacientes com LES e alterações CV parecem estar mais propensos ao aparecimento de autoanticorpos, como anticorpos anti-lipoproteína de baixa densidade oxidada (oxLDL) e anticorpos antifosfolípide que, por sua vez, favorecem a produção de MACs (MCMAHON; HAHN; SKAGGS, 2011).

Outros autores ainda evidenciaram correlação positiva entre os níveis de ICAM-1 e os marcadores inflamatórios VHS (SEDIE *et al.*, 2018) ou E-caderina (JIN *et al.*, 2013). Mahayidin e colaboradores (2014) demonstraram significativa correlação de VCAM-1 com anti-dsDNA, o que corrobora a teoria de que a formação de complexos imunes e a regulação positiva de MACs estão envolvidas na patogênese do LES (MAHAYIDIN *et al.*, 2014).

Nós demonstramos previamente que pacientes com LES apresentaram níveis aumentados de PECAM-1, VCAM-1, E-selectina, P-selectina e inibidor do ativador do plasminogênio tipo-1 (PAI-1) quando comparados ao grupo controle. Também propusemos um modelo utilizando PECAM-1 e PAI-1 que foi capaz de predizer o diagnóstico de LES com 86,5% de sensibilidade e 81,3% de especificidade; e outro modelo utilizando PECAM-1 e presença de síndrome metabólica (SM) que foi capaz de predizer 14,8% de variações no SLEDAI. Além disso, observamos que a presença de SM e os níveis de anticorpos antinucleares (ANA) e cortisol podem modular os níveis de MACs (SANTOS *et al.*, 2018).

Nesse sentido, o envolvimento das MACs na fisiopatologia do LES também tem sido relatado na literatura, embora seus exatos mecanismos ainda sejam desconhecidos. A relação das MACs com a patogênese do LES parece envolver a ativação do inflamassoma (KAHLENBERG; KAPLAN, 2014), a presença de autoanticorpos (MOK *et al.*, 2005; NOJIMA *et al.*, 2008) e o aumento do estresse oxidativo e nitrosativo (MCGARRY *et al.*,

2018), que, por sua vez, promovem aumento na produção de citocinas proinflamatórias e MACs.

1.3.2 Evidências do Envolvimento das Moléculas de Adesão Celular na Fisiopatologia da Artrite Reumatoide

Na AR, a sinovite parece ser o ponto-chave para compreender o envolvimento das MACs na fisiopatologia da doença. Com o aparecimento da sinovite, há migração celular que é ocasionada pela ativação endotelial da microvasculatura sinovial, promovendo aumento da expressão de MACs e quimiocinas (HWANG; KIM, 2017; MCINNES; SCHETT, 2011). Além disso, em uma articulação com AR, sinoviócitos tipo fibroblastos (FLSs) tornam-se invasivos e aptos para interagir com linfócitos T e resultar na indução de uma variedade de mediadores inflamatórios, incluindo IL-6, TNF-α, IFN-γ, VCAM-1 e ICAM-1 (BARTOK; FIRESTEIN, 2010). Por fim, FLSs alterados promovem um microambiente permissivo e contribuem diretamente para a destruição da cartilagem/articulação local e a cronicidade da inflamação sinovial (KOMATSU; TAKAYANAGI, 2012).

Enquanto no LES o envolvimento das MACs está melhor definido, na AR os resultados têm sido controversos. Em geral, pacientes com AR apresentam altos níveis de MACs, principalmente ICAM-1 (SÖDERGREN *et al.*, 2010; UGUR *et al.*, 2004) e VCAM-1 (KLIMEK *et al.*, 2014; WANG *et al.*, 2015). Klimiuk e colaboradores (2007) encontraram níveis elevados de VCAM-1, ICAM-1 e E-selectina em pacientes com AR não tratada quando comparados a indivíduos com osteoartrite (grupo controle) (KLIMIUK *et al.*, 2007). Além disso, Wang e colaboradores (2014) observaram correlação positiva entre VCAM-1 e os níveis de FR (WANG *et al.*, 2015). Entretanto, diversos autores têm encontrado nenhuma ou inversa correlação de parâmetros relacionados à AR e os níveis de MACs. Ugur e colaboradores (2004) revelaram correlação positiva entre os níveis de ICAM-1, e o índice articular de Ritchie (RAI) e os níveis de proteína C-reativa (PCR), mas negativa correlação entre os níveis de ICAM-1 e a atividade da superóxido dismutase extracelular (SOD) (UGUR *et al.*, 2004). Gonzales-Gay e colaboradores (2006) não encontraram correlação entre a atividade da doença, avaliada pelo Escore de atividade da doença em 28 articulações (DAS28) (Anexo C) e os níveis plasmáticos de ICAM-1, VCAM-1, E- ou P-selectina (GONZALES-GAY *et al.*, 2006). Södergren e colaboradores (2010) demonstraram que o DAS28 foi inversamente correlacionado aos níveis de VCAM-1 e L-selectina (SÖDERGREN *et al.*, 2010).

Recentemente, nosso grupo de pesquisa demonstrou que pacientes com AR apresentavam níveis diminuídos de VCAM-1 e aumentados de PAI-1, enquanto os níveis de ICAM-1, E-selectina e P-selectina permaneceram inalterados quando comparados ao grupo controle. Foi proposto um modelo estatístico, utilizando VCAM-1, PCR e TNF-α, que foi capaz de predizer o diagnóstico de AR com 98,9% de sensibilidade e 89,5% de especificidade. Foi demonstrado também que as variáveis PAI-1, TNF-α, índice de massa corporal (IMC) e PECAM-1, conjuntamente, são responsáveis por 42,9% da variação da atividade da doença, avaliada pelo DAS28-VHS. Além disso, nossos dados mostraram que os níveis diminuídos de VCAM-1 foram associados à AR independentemente da SM, os níveis aumentados de PAI-1 foram associados a AR e SM, e os níveis aumentados de selectinas (E-selectina e P-selectina) foram associadas exclusivamente a SM e não a AR (DE SÁ *et al.*, 2018).

1.4 Moléculas de Adesão Celular, Inibidor do Ativador do Plasminogênio Tipo-1 e Síndrome Metabólica

A SM é um transtorno complexo definido como um conjunto de fatores interconectados que aumentam o risco de doenças CV ateroscleróticas e diabetes mellitus tipo 2. Atualmente, existem várias definições diferentes de SM que utilizam fatores de classificação de risco CV clássicos ou não (KASSI *et al.*, 2011). Entre as condições epidemiologicamente já confirmadas como fatores de risco clássicos ou tradicionais, e que são utilizadas para classificar SM estão à dislipidemia [lipoproteína de baixa densidade (HDL) reduzida e hipertrigliceridemia], hipertensão, obesidade central e resistência à insulina. Outros fatores envolvidos são estresse crônico causado por hipersecreção de hormônios do estresse, desequilíbrio de citocinas pró e anti-inflamatórias, além do estresse oxidativo, fatores maternos e alterações do microbioma intestinal (MENDRICK *et al.*, 2018).

A prevalência da SM afeta 20,0 a 30,0% da população geral e aumenta com a idade (PUCCI *et al.*, 2017). Adicionalmente, pacientes com doenças de caráter inflamatório e autoimune, como LES e AR, apresentam aumento da frequência ou prevalência de SM e alta incidência de doenças CV, apesar dos avanços no tratamento de doenças reumáticas (CASTAÑEDA *et al.*, 2015). A prevalência de SM em pacientes com LES em diferentes estudos variaram de 16,3% a 45,2% (MOBINI *et al.*, 2018), e o risco relativo de distúrbios CV é de 7 a 17 vezes maior do que a população em geral (SUN *et al.*, 2017). Semelhantemente, a prevalência de SM também é elevada na AR, variando de 14,3 a 37,8%

(HALLAJZADEH *et al.*, 2017). Os fatores de risco CV tradicionais não explicam totalmente o risco aumentado nessa população, o que sugere a existência de outros fatores envolvidos nestas condições (CASTAÑEDA *et al.*, 2015).

Nas doenças autoimunes, a ativação de vias sinalizadoras proinflamatórias resulta na indução de vários marcadores biológicos de inflamação crônica que contribuem para a doença CV e o desenvolvimento de SM (MEDINA *et al.*, 2018). Na SM, baixos níveis de adiponectina e altos níveis de citocinas proinflamatórias, como TNF-α e IL-6, contribuem para altos níveis de lipoproteína de baixa densidade (LDL) e baixos níveis de HDL. Esse ambiente proinflamatório também favorece o aumento de PCR e de EROs que, por sua vez, desencadeiam disfunção endotelial, uma resposta bem estabelecida aos fatores de risco CV. Estas alterações aumentam os níveis de ICAM-1 e VCAM-1 que ligam as moléculas de LDL às paredes dos vasos sanguíneos e levam ao aumento da quimioatração de monócitos e do risco de doenças CV. Portanto, o aumento de citocinas proinflamatórias, o acúmulo de EROs e a regulação positiva de MACs parece ser denominador comum na patogênese de distúrbios CV associados a doenças autoimunes (FAVERO *et al.*, 2014; MURDACA *et al.*, 2012; STEYERS; JR. MILLER, 2014).

Gustafsson e colaboradores (2012) relataram altos níveis circulantes de VCAM-1 associados com a mortalidade CV em estudo de coorte com pacientes lúpicos (GUSTAFSSON *et al.*, 2012). E-selectina já foi apresentada como um potencial marcador de risco CV em pacientes com LES (SKEOCH *et al.*, 2014), além de ser o elo entre obesidade e comorbidades relacionadas (EL-MESALLAMY; HAMDY; IBRAHIM, 2011). Na AR, Dessein, Joffe e Singh (2005) relataram associação entre os níveis de VCAM-1 e a espessura da íntima-média da artéria carótida e presença de placa aterosclerótica (DESSEIN; JOFFE; SINGH, 2005).

O PAI-1 é o principal inibidor da fibrinólise e contribui para o aumento do risco CV em indivíduos obesos e/ou em indivíduos com doença autoimune de caráter inflamatório (KASSI *et al.*, 2011). O tecido adiposo abdominal é a maior fonte de PAI-1 (BARNARD; PIETERS; DE LANGE, 2016) e seu nível plasmático elevado é considerado marcador bioquímico da obesidade (PHELAN; KERINS, 2014), além de ser um componente envolvido na SM (ALESSI; JUHAN-VAGUE, 2008). No LES, níveis elevados de PAI-1 têm sido associados ao estado de hipercoagulabilidade na SAAF primária e secundária (SINGH *et al.*, 2013), enquanto na AR, PAI-1 foi descrito em níveis elevados (WÅLLBERG-JONSSON *et al.*, 2002) e apresentou correlação com atividade da doença avaliada pelo DAS28-VHS

(SÖDERGREN *et al.*, 2010). Nesse contexto, a avaliação do PAI-1 proporciona informações complementares se avaliado juntamente com as MACs.

Como descrito anteriormente, as MACs tem sido amplamente avaliadas no LES, AR e eventos CV associados, entretanto ainda são escassos os trabalhos que compararam o perfil dessas moléculas no LES e AR. Até o presente momento, apenas 3 artigos foram publicados comparando os níveis de MAC entre pacientes com AR e LES, não havendo ainda nenhum que tenha comparado o PAI-1 nestas doenças. Machold e colaboradores (1993) não encontraram diferenças significativas nos níveis de ICAM-1 entre os grupos avaliados (LES, AR e controle). No entanto, Santos e colaboradores (2012) encontraram níveis significativamente maiores de ICAM-1 em pacientes com LES quando comparados aos pacientes com AR e ao grupo controle. Eles também avaliaram os níveis plasmáticos de VCAM-1 mas não encontraram diferenças significativas entre os grupos. Apenas os níveis de ICAM-1 permaneceram significativamente maiores em pacientes com LES quando comparados aos pacientes com AR (e não ao grupo controle) após o ajuste para possíveis efeitos de covariáveis (idade, duração da doença, colesterol total, HDL, LDL, triglicerídeos, uso de aspirina, hidroxicloroquina, metotrexato e dose de prednisolona). Os autores também encontraram níveis significativamente maiores de ICAM-1 e VCAM-1 em pacientes com LES ativo quando comparado aqueles com LES em remissão. Semelhantemente, pacientes com AR ativa também apresentaram níveis significativamente maiores apenas de ICAM-1 quando comparado aos pacientes com AR em remissão (SANTOS *et al.*, 2012). Finalmente, Pamuk e colaboradores (2014) encontraram níveis significativamente maiores de PECAM-1 em pacientes com AR quando comparados aos pacientes com LES e ao grupo controle. Entretanto, os níveis séricos de PECAM-1 não foram associados à atividade da doença, marcadores inflamatórios e envolvimento extra-articulares ou de órgãos principais nos grupos AR e LES (PAMUK *et al.*, 2014).

1.5 Moléculas de Adesão Celular: influência dos medicamentos utilizados em doenças autoimunes e potenciais alvos terapêuticos

Atualmente vários medicamentos utilizados para o tratamento de pacientes com LES e AR, embora não tenham como alvo a inibição das MACs, atuam sobre essas, controlando a atividade e alterando o curso da doença, respectivamente. Esses medicamentos geralmente atuam na redução do processo inflamatório e, consequentemente, inibem a produção das MACs (AMANO *et al.*, 2000; BONELLI *et al.*, 2013; DARIDON *et al.*, 2010; DOMINICAL

et al., 2011; GONZALES-GAY *et al.*, 2006; HJELTNES *et al.*, 2013; KLIMIUK *et al.*, 2007; KLIMIUK *et al.*, 2009; KRAGSTRUP *et al.*, 2016; PARKER *et al.*, 2014; RUIZ-LIMÓN *et al.*, 2017).

Diferentes grupos de medicamentos em uso clínico interferem com a função de MACs direta ou indiretamente. Por exemplo, a inibição de IL-1β ou TNF-α por meio de anticorpos ou receptores solúveis tem efeitos potentes na expressão de MACs em células endoteliais. Além disso, corticosteroides, anti-inflamatórios não esteroidais (AINEs) e antioxidantes também diminuem a expressão de MACs e quimiocinas inflamatórias por meio do bloqueio de fator nuclear kappa B (NF-kB) (GONZÁLEZ-AMARO; SÁNCHEZ-MADRID, 2001). As estatinas demonstraram potente efeito sobre a ativação da integrina, o que mostra que interferências com MACs podem ter potencial terapêutico (WEITZ-SCHMIDT, 2002).

Por outro lado, diversos medicamentos têm sido delineados para atuar diretamente sobre as MACs e modificar o curso do LES e/ou AR. Vários mecanismos podem modular os níveis de MACs, entre eles o bloqueio competitivo, a expressão alterada na superfície celular e, para integrinas, a interferência com a ativação do receptor. O objetivo terapêutico final de cada mecanismo é quebrar o caminho de múltiplas etapas da cascata de recrutamento celular (ULBRICH; ERIKSSON; LINDBOM, 2003). Ulbrich, Eriksson e Lindbom (2003) descreveram, em seu artigo de revisão, diversos medicamentos, em fase de estudo, que têm como alvo a inibição direta da função das MACs. Entre eles o efazulimabe, um anticorpo monoclonal contra α_L integrina [aguardava avaliação da Agência Europeia de Avaliação de Produtos Médicos (EMEA) e do Administração de alimentos e medicamentos (FDA)] que promoveu diminuição da área da psoríase e do seu índice de gravidade após duas semanas; além do natalizumabe, um anticorpo monoclonal contra α_4 integrina (fase III) que demonstrou diminuição de lesões cerebrais e recidivas em pacientes com esclerose múltipla recidivante (ULBRICH; ERIKSSON; LINDBOM, 2003). O efalizumabe foi aprovado para uso clínico em 2003 (RØNHOLT; IVERSEN, 2017) e é um dos imunobiológicos disponíveis na Europa mais utilizados para o tratamento da psoríase (LAMAZZA *et al.*, 2009), enquanto o natalizumabe tem sido opção terapêutica para esclerose múltipla refratária, apesar de ter uso restrito, pois foi observada alta ocorrência de reações adversas graves (POLMAN *et al.*, 2006). No LES, o epratuzumabe (EPZ), um anticorpo monoclonal humanizado que tem como alvo a molécula de superfície de células B CD22 (também chamada de molécula de adesão de linfócitos B) (JACOBI *et al.*, 2008) tem sido amplamente estudado. Um exemplo, é o artigo publicado por Daridon e colaboradores (2010) que demonstrou que o EPZ reduziu significativamente a expressão das MACs CD62L (também chamada L-selectina), β7

integrina e β1 integrina após a incubação, por 90 minutos, de subgrupos de células B com EPZ. Em artigo de revisão, Geh e Gordon (2018) descreveram que já foram realizados 7 ensaios clínicos (1 ensaio clínico aberto de fase IIa, 1 fase I/II, 3 ensaios clínicos randomizados controlados de fase IIb e 2 de fase III), além de 3 estudos abertos para determinar a eficácia, segurança e farmacocinética do EPZ no LES. No total, 2167 doentes foram recrutados para estes ensaios com um total de 1370 doentes recebendo EPZ. O resultado primário em todos os ensaios envolveu a redução da atividade da doença lúpica definida pelo Índice de Atividade da Doença avaliado pelo Grupo de Lúpus das Ilhas Britânicas (BILAG) (Anexo D) ou Avaliação do Lúpus Combinado baseado em BILAG (BICLA) (GEH; GORDON, 2018).

Assim, estudos que avaliaram MACs como novos alvos terapêuticos têm demonstrado várias possibilidades de intervenção, como: (a) inibição de selectinas por meio de anticorpos, análogos de carboidratos como o tetrassacárideo sialil Lewis X (sLe^X) e/ou inibidores de baixo peso molecular; (b) bloqueio de integrinas por meio de anticorpos; (c) bloqueio com anticorpos da interação entre membros da IgSF e integrinas (DEDRICK; BODARY; GAROVOY, 2003; JOIS; JINING; NAGARAJARAO, 2006; ULBRICH; ERIKSSON; LINDBOM, 2003). Entretanto, apesar de resultados promissores em estudos pré-clínicos e/ou em modelos animais, resultados de ensaios clínicos ainda são inconsistentes. Uma grande limitação encontrada nesses estudos envolve o fato de que o bloqueio a longo prazo da função de MACs poderia comprometer indevidamente mecanismos de defesa importantes para o organismo humano, embora o bloqueio transitório possa ser útil em diversas desordens inflamatórias.

De acordo com o exposto, a inibição das MACs tem sido considerada potencial terapêutico em muitos distúrbios autoimunes, tais como LES e AR. Entretanto, mais estudos são necessários para determinar a eficácia e segurança a longo prazo dessas estratégias, além de esclarecer quais são as moléculas mais promissoras e desenvolver inibidores seletivos que proporcionem alcance adequado do objetivo terapêutico.

2. JUSTIFICATIVA

Não há até o presente momento, estudos que tenham revisado os dados de literatura disponíveis a respeito do efeito dos principais fármacos utilizados no tratamento do LES e da AR nos níveis plasmáticos de MACs. Assim, a pesquisa bibliográfica para avaliar o efeito dessas drogas em diferentes MACs poderá acrescentar informações importantes para compreender o envolvimento dessas moléculas na fisiopatologia destas doenças autoimunes e alterações metabólicas associadas, além de sugerir biomarcadores e alvos terapêuticos mais promissores que os atualmente utilizados.

Estudos anteriores, publicados por nosso grupo de pesquisa, avaliaram o perfil das MACs e os níveis plasmáticos de PAI-1 em pacientes com LES (SANTOS *et al.*, 2018) e naqueles com AR (DE SÁ *et al.*, 2018), e a influência da SM nas concentrações plasmáticas destas moléculas. Além disso, temos demonstrado a associação das MACs com a fisiopatologia destas doenças, sendo propostos modelos preditores para seu diagnóstico e determinação da atividade da doença utilizando-as como biomarcadores. No entanto, até presente momento, poucos estudos compararam os níveis plasmáticos das MACs nestas duas doenças (MACHOLD *et al.*, 1993; PAMUK *et al.*, 2014; SANTOS *et al.*, 2012), que embora apresentem etiologia autoimune e muitas vezes se manifestem concomitantemente e com manifestações clínicas em comum, possuem características imunológicas e perfis de citocinas bastante distintos. Além disso, esses estudos que compararam as MACs nestas doenças, avaliaram moléculas individualmente e não estabeleceram um perfil para diferenciação destas duas doenças.

Embora o LES e AR sejam doenças autoimunes de caráter inflamatório crônico e compartilhem manifestações clínicas, elas possuem diferenças importantes nos mecanismos fisiopatológicos envolvidos e apresentam diferentes graus de risco CV (ABELLA *et al.*, 2014; MEDINA *et al.*, 2018). Além do mais, os fatores de risco tradicionais não conseguem explicar, por si só, a aterosclerose acelerada e o aumento do risco de doenças CV. Desta forma, este estudo teve como hipótese principal que o estabelecimento do perfil das MACs e do PAI-1 nestas doenças, poderia contribuir, pelo menos em parte, para o melhor entendimento das diferenças encontradas na fisiopatologia destas doenças autoimunes, bem como suas associações com a SM.

3. OBJETIVOS

3.1 Objetivo Geral

Comparar o perfil dos níveis séricos de MACs e PAI-1 em pacientes com LES e AR, bem como examinar o efeito da SM e seus componentes nessas moléculas.

3.2 Objetivos Específicos

- Revisar os artigos disponíveis na literatura a fim de verificar o efeito dos principais fármacos utilizados no tratamento do LES e AR nos níveis plasmáticos de MACs;
- Identificar os medicamentos frequentemente utilizados na avaliação de seu efeito sobre os níveis de MACs;
- Descrever as MACs que são mais utilizadas para avaliar resposta ao tratamento em pacientes com LES e AR;
- Comparar os níveis plasmáticos de MACs e PAI-1 entre pacientes com LES e AR;
- Determinar o tamanho do efeito do diagnóstico e da presença da SM nos níveis plasmáticos de MACs e do PAI-1 em pacientes com LES e AR;
- Propor modelos preditores para auxiliar na diferenciação entre LES e AR, utilizando as MACs e o PAI-1
- Verificar associação entre as MACs e PAI-1 com os componentes da SM.

4. CASUÍSTICA E MÉTODOS

4.1 Delineamento e Amostragem

Esta tese apresentará dois delineamentos. O primeiro, para confecção do artigo de revisão, envolverá revisão de literatura; enquanto, o segundo compreenderá estudo transversal para realização do artigo original.

4.1.1 Delineamento para artigo 1: Revisão bibliográfica

Para o artigo de revisão, foi realizada busca bibliográfica nas bases de dados eletrônicas PUBMED, Lilacs, Biblioteca Eletrônica Científica Online (SCIELO) e *Science Direct*, em inglês, espanhol ou português. A lista de referências dos artigos selecionados também foi investigada.

As seguintes combinações de termos do *Medical Subject Headings* (MeSH) foram usadas para o LES: 1-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Antimalarial medications";* 2-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "hydroxychloroquine";* 3-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "chloroquine";* 4-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Glucocorticoids";* 5-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Prednisone";* 6-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Immunosuppressive";* 7-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Mycophenolate";* 8-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Cyclophosphamide";* 9-*"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Antibodies, Monoclonal";* 10- *"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Cyclosporine";* 11- *"Lupus Erythematosus, Systemic" AND "Adhesion Molecule" OR "Selectin" AND "Azathioprine".* As seguintes combinações de termos MeSH foram usadas para AR: 1- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Methotrexate";* 2-*"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Abatacept";* 3-*"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Tocilizumab";* 4-*"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "hydroxychloroquine";* 5- *"Arthritis,*

Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Sulfasalazine"; 6- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Leflunomide";* 7- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Glucocorticoids";* 8- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Prednisone";* 9- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Antibodies, Monoclonal, Humanized";* 10- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Infliximab";* 11- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Adalimumab";* 12- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Rituximab";* 13- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Etanercept";* 14- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Golimumab";* 15- *"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Certolizumab".*

Os critérios de inclusão foram artigos originais, estudos realizados em humanos sem limite de data de publicação ou número de sujeitos, estudos com delineamento experimental (ensaios clínicos, randomizados ou não) ou observacionais (caso-controle e estudos de coorte). Os critérios de exclusão foram estudos realizados em modelos animais com lúpus ou artrite induzidos, ou tratamentos não padronizados.

Foram encontrados 25 estudos, entretanto, 4 estudos foram excluídos pois envolviam modelos animais (n=1) ou tratamentos não padronizados (n=3). Assim, 21 estudos, incluindo quatro ensaios clínicos randomizados e controlados e 17 ensaios clínicos não randomizados. Apenas três estudos foram desenvolvidos em pacientes com LES, enquanto 18 estudos foram desenvolvidos em pacientes com AR. Artigos que avaliaram medicamentos por meio de ensaios *in vitro* também foram avaliados.

4.1.2 Delineamento para artigo 2: Estudo transversal

Trata-se de estudo transversal em que foram selecionados 228 indivíduos, sendo 104 pacientes com LES e 124 pacientes com AR, de ambos os sexos, com idade entre 18 e 69 anos e selecionados do Ambulatório de Reumatologia do Hospital Universitário de Londrina, Paraná, Brasil. Os critérios de exclusão foram doença renal crônica, doenças cardíacas, tireoidianas, hepáticas, gastrointestinais, oncológicas ou outras doenças autoimunes de acordo com dados clínicos e laboratoriais.

Os pacientes com LES não apresentavam NL em atividade e nenhum dos pacientes apresentou histórico de uso de álcool. Informações referentes a estilo de vida e histórico médico foram obtidos a partir de avaliação clínica de cada paciente (Apêndices A e B).

O diagnóstico do LES foi realizado a partir dos critérios revisados do Colégio Americano de Reumatologia (ACR) de 1997 (HOCHBERG, 1997). O diagnóstico da AR foi realizado a partir dos critérios revisados do Colégio Americano de Reumatologia e da Liga Europeia Contra o Reumatismo (EULAR) (ALETAHA *et al.*, 2010).

4.2 Artigo 2: Estudo Comparativo das Moléculas de Adesão Celular e do Inibidor do Ativador do Plasminogênio Tipo-1 em pacientes com Lúpus Eritematoso Sistêmico e Artrite Reumatoide

4.2.1 Aspectos Éticos

O estudo foi aprovado pelo Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina (UEL) (CAAE 01865212.0.0000.5231; CAAE 06405812.1.0000.5231, Anexo E). Os indivíduos foram convidados a participar voluntariamente da pesquisa e um termo de consentimento livre e esclarecido (TCLE) foi obtido dos indivíduos envolvidos na pesquisa ou de seus responsáveis (Apêndices C e D).

4.2.2 Medidas Antropométricas e Determinação da Pressão Arterial

O peso corporal foi avaliado com aproximação de 0,1 kg utilizando uma balança eletrônica e com os indivíduos vestindo roupas leves, sem sapatos e no período da manhã; a altura foi medida com precisão de 0,1 cm, utilizando-se um estadiômetro. IMC foi calculado como peso (kg) dividido pela altura (m) ao quadrado. A circunferência abdominal (CA) foi medida com uma fita suave na região entre a última costela e a crista ilíaca, sempre com os indivíduos na posição em pé. Três medidas da pressão arterial foram registradas com intervalo de um minuto entre elas depois do indivíduo se sentar. A média destas medições foi utilizada na análise. A hipertensão foi considerada diagnosticada quando a pressão arterial sistólica (PAS) e diastólica (PAD) foi ≥140/90 mmHg ou quando os pacientes estavam em uso de medicação anti-hipertensiva (PICKERING *et al.*, 2005).

4.2.3 Biomarcadores Bioquímicos, Imunológicos e Hematológicos

Após jejum de 12h, foram coletadas amostras em tubo de coleta a vácuo sem anticoagulante e com anticoagulante ácido etilenodiamino tetraacético (EDTA). O material foi encaminhado imediatamente ao laboratório para registro, processamento e armazenamento das amostras. Plasma e soro foram obtidos após centrifugação (10 min à 2500 rpm) e armazenados a -80ºC até o momento das análises, quando estas não eram analisadas no mesmo dia. Todos os pacientes e suas respectivas amostras foram identificados por número e letra para garantir o anonimato e confidencialidade dos indivíduos e dos resultados obtidos.

Os níveis de glicose, colesterol total, colesterol HDL, colesterol LDL e triglicerídeos foram determinados por autoanalisador bioquímico (Dimension Dade AR Dade Behring Deerfield, IL, EUA), utilizando kits Dade Behring®. SM foi definida de acordo com os critérios propostos pelo Painel de tratamento de adultos III (ATP III) quando o paciente apresentava 3 dos 5 componentes a seguir: CA ≥ 94 cm para homens, e ≥ 80 cm para mulheres; níveis de triglicerídeos ≥ 150 mg/dL; colesterol HDL menor que 40 mg/dL para homens e menor que 50 mg/dL para mulheres; glicose em jejum ≥ 100 mg/dL ou uso de hipoglicemiantes; PAS ≥130 mmHg e PAD ≥85 mmHg ou uso de anti-hipertensivos, (GRUNDY *et al.*, 2005). Os níveis de insulina foram avaliados por imunoensaio de quimiluminescência em micropartícula (Architect, Abbott Laboratory, Abbott Park, IL, USA). VHS foi obtida pelo método cinético-fotométrico automatizado (Ves-MaticCUBE 30, DIESSE, Siena, Itália).

4.2.4 Dosagem das Moléculas de Adesão Celular e do Inibidor do Ativador do Plasminogênio Tipo 1

Os níveis de PECAM-1, ICAM-1, VCAM-1, E-Selectina, P-Selectina e PAI-1 foram determinados por imunofluorimetria utilizando o kit *Human Magnetic Adhesion 6-Plex Panel* (Novex Life Technologies, Frederick, United States of America) para plataforma Luminex®.

4.2.5 Análise Estatística

Para avaliar as diferenças entre os grupos em variáveis contínuas, nós utilizamos análises de variância (ANOVA). Para investigar diferenças entre grupos em variáveis categóricas, empregamos análises de tabelas de contingência (x^2-teste). Os resultados das

comparações múltiplas foram corrigidos com p para taxa de descoberta falsa. Análises univariadas e do modelo linear geral multivariado (GLM) foram usadas para determinar os efeitos das variáveis explanatórias (diagnóstico e SM) sobre os níveis de MACs (variáveis dependentes), ajustando para fatores confundidores (como idade, SM e CA). Consequentemente, utilizamos testes para efeitos entre sujeitos para delinear os efeitos das variáveis explanatórias sobre MACs. Diferenças nos níveis de MACs em ambos os grupos de diagnóstico (e indivíduos com e sem SM) foram avaliadas usando médias marginais estimadas (SE) obtidas por análises GLM. A análise de regressão múltipla foi utilizada para definir as MACs significativamente associadas às variáveis metabólicas, ajustando para variáveis confundidoras, que incluíram idade, SM, CA, tratamento com drogas anti-hipertensivas e hipoglicemiantes.

A análise de regressão logística binária foi empregada para delinear MACs significativamente associadas ao LES *versus* AR. Os valores de Nagelkerke foram usados como estimativas do tamanho do efeito. Calculamos também a porcentagem de casos corretamente classificados com valores de sensibilidade, especificidade, e área sob as curvas características de operação do receptor (AUC ROC). As análises estatísticas foram realizadas usando o IBM SPSS Windows versão 22. Os testes foram bicaudais e níveis alfa de 0,05 indicaram resultados estatisticamente significativos.

Calculamos escores compostos pela unidade z ponderada, a saber: a) soma dos escores z de todos os valores de MACs (índice de níveis aumentados de MACs, zCAM); b) valor z do colesterol total (zCHOL) - zHDL-colesterol (zCHOL-HDL) como índice que reflete o índice de risco Castelli I, e z triglicerídeos (zTG) - zHDL (zTG_HDL) como índice validado que reflete o índice aterogênico do plasma; c) escore composto zInsulina + zGlicose (zGlicose + Insulina), índice de resistência à insulina, e zInsulina-zGlicose (zInsulina_Glicose), índice de função das células β.

Foram utilizadas as técnicas de Modelagem suave independente por analogia de classe (SIMCA), Rede Neural (NN) e Incorporação de vizinhança estocástica distribuída em t (t-SNE). SIMCA foi utilizado para modelar cada classe (LES e AR) por um modelo de Componente Principal (CP), que melhor representa a classe. Consequentemente, os casos são projetados em cada modelo de classe e sua distância da classe é calculada; os casos são atribuídos a uma classe por comparação das distâncias de cada modelo. Como tal, o SIMCA é uma técnica de modelagem de classes que descreve as semelhanças entre os casos nas classes. O poder de modelagem dos recursos descreve o impacto para o modelo de cada variação de classe. Recursos com uma potência de modelagem inferior a 0,300, que indica baixa potência

de modelagem, podem ser omitidos do modelo (seleção dos recursos). O SIMCA também calcula o poder discriminatório de todas as variáveis, indicando o impacto dos recursos para classificar objetos nas diferentes classes. O SIMCA calcula distâncias modelo a modelo com uma distância maior que 3, indicando diferenças qualitativas entre as classes modeladas e uma distância próxima de 0, mostrando que não há diferenças significativas entre as classes. O SIMCA foi realizado usando o programa de software Unscrambler X10.5.

Utilizamos também Perceptron Multicamadas (MLP), para examinar as complexas relações não-lineares entre variáveis de entrada e saída, empregando uma arquitetura *feedforward* automatizada com valores MACs (combinados com idade e variáveis metabólicas) como variáveis de entrada e diagnóstico (LES e AR) como variáveis de saída. Uma ou duas camadas ocultas foram consideradas com um número variável de nós (máximo 6). Erro, taxa de previsões incorretas baseadas em treinamento, testes e amostras de validação, bem como a matriz de confusão particionada para variáveis de diagnóstico de saída. Também calculamos a AUC ROC e a importância (relativa) das variáveis de entrada nas análises de sensibilidade. Finalmente, utilizamos o t-SNE para visualizar os pontos de dados de todos os casos em uma plotagem bidimensional.

5. RESULTADOS

Os resultados obtidos neste trabalho foram apresentados e discutidos em um artigo de revisão e um artigo original intitulados:

Artigo 1 – "Influence of drugs treatment on cell adhesion molecules in patients with systemic lupus erythematosus and rheumatoid arthritis: a review", que foi submetido ao periódico científico Inflammopharmacology (qualis A2 e fator de impacto 3,304),

Artigo 2 - "Differential Profile Of Cellular Adhesion Molecules And Plasminogen Activator Inhibitor Type-1 In Systemic Lupus Erythematosus versus Rheumatoid Arthritis Are Associated With Metabolic Syndrome: A Machine Learning Study", que foi submetido ao periódico científico e Immunologic Research (qualis A2 e fator de impacto 2,610).

5.1 Artigo 1

Influence of drugs treatment on cell adhesion molecules in patients with systemic lupus erythematosus and rheumatoid arthritis: a review

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Abstract

Background Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases characterized by changes in cell adhesion molecules (CAM) that may be correlated with disease activity and clinical manifestations.

Objective To review the influence of the main drugs used in the treatment of SLE and RA in the plasma levels of CAM.

Methods A bibliographic search was performed in the electronic databases PUBMED, Lilacs, Scientific Electronic Library Online (SCIELO), and Science Direct. The research included human studies, *in vivo* or *in vitro* with experimental or observational design, and with no limit of publication date or number of subjects. It was excluded studies in animal models or with non-standard treatments. The list of references of the selected articles was also investigated.

Results There were included 21 studies, three on SLE and 18 on RA. Monotherapy or combined trials were reported. The most evaluated medicaments in the articles analyzed were cyclophosphamide (CY, n=2) and methylprednisolone pulse (pMP, n=2) in SLE; and methotrexate (MTX, n=9) and infliximab (IFX, n=4) in RA. In addition, the most CAM used to assess response to treatment was vascular cell adhesion molecule 1 (VCAM-1, n=2) in SLE; and intercellular adhesion molecule-1 (ICAM-1, n=12), VCAM-1 (n=12) and E-selectin (n=14) in RA. SLE or RA patients submitted to treatment usually demonstrated a significant decrease in CAM levels.

Conclusions The most remarkable result in the present review was that SLE or RA patients, mainly with active disease, submitted to treatment demonstrated a significant decrease in CAM levels. ICAM-1, VCAM-1 and E-selectin were the molecules often determined for evaluation of treatment response. In this way, CAM markers may reflect the disease activity and the response to treatment in SLE and RA patients.

Keywords: Antirheumatic Drugs. Systemic Lupus Erythematosus. Rheumatoid Arthritis. Cell Adhesion Molecules.

Introduction

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases characterized by chronic inflammation, autoantibodies production and endothelial dysfunction (McInnes and Schett 2011; Tsokos 2011). The pathophysiology of these autoimmune diseases has been extensively studied and their mechanisms have not yet been fully elucidated. However, cell adhesion molecules (CAM) have shown great importance both as a pathophysiological component (Atehortúa et al. 2017), and also as a biomarker (Klimek et al. 2014; Lewis et al. 2016) of these immunoinflammatory conditions.

CAM are integral membrane proteins (Lodish H, Berk A, Zipursky SL 2000) upregulated by proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), and responsible for capture, adhesion, rolling and migration of leukocytes along endothelial cells surfaces into inflamed tissues (Skeoch et al. 2014). These molecules are mostly grouped into three different types called immunoglobulin supergene family (IGSF) members, selectins and integrins. Among the first group are the platelet endothelial cell adhesion molecule 1 (PECAM-1), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Selectins are mainly represented by endothelial and platelet selectin (E- and P-selectin) (McMurray 1996). Integrins are heterodimers consisting α and β subunits. There are 24 αβ heterodimers linked by non-covalent bonds; one of its representatives is the lymphocyte function-associated antigen 1 (LFA-1) (Pan et al. 2016).

Patients with SLE (Mok et al. 2018; Santos et al. 2018) and RA (de Sá et al. 2018; Oleszowsky and Seidel 2018) have mostly demonstrated increased CAM levels. Abnormalities on CAM seem to favor the onset of autoimmune diseases (Dedrick et al. 2003). On the other hand, their typical organic dysfunctions also may contribute to increase CAM (Steyers and Jr. Miller 2014). Thus, more studies are needed to determine the exact role of CAM and thereafter to propose new therapeutic targets.

In SLE and RA, CAM demonstrated important clinical value: (a) to differentiate patients from healthy controls (Sabry, A.; Sheashaa, H.; El-Husseini, A.; El-Dahshan, K.; Abdel-Rahim, M.; Elbasyouni 2007); (b) as a biomarker of disease activity (Klimek et al. 2014; Lewis et al. 2016); (c) as an indicator of associated comorbidities, mainly cardiovascular (CV) events (Santos et al. 2012); (d) as a marker of distinct clinical manifestations (Klimiuk et al. 2002; Singh et al. 2012); (e) and as a marker of clinical response to treatment, mainly in RA (Smith et al. 2001).

In this way, most articles focus on changes in CAM and their relationship with pathophysiology, disease activity and clinical manifestations in SLE, RA, and associated CV

diseases. However, few studies have studied the effect of the main drugs used in the SLE or RA treatment on CAM levels. The search for the effect of these drugs on CAM could add important information for a better comprehension of these diseases. This article reviews the available data on the effect of the main drugs used in the treatment of SLE and RA on CAM.

Therapy for systemic lupus erythematosus and rheumatoid arthritis

The SLE management is still a clinical challenge. Traditionally, glucocorticoids, antimalarial agents (hydroxychloroquine), immunosuppressive drugs (mycophenolate mofetil and cyclophosphamide), and B-cell blockers [such as belimumab, a fully humanized monoclonal antibody that inhibits B lymphocyte stimulator (BLys)] have been employed as drugs of choice in SLE and are well established. New therapies have been proposed such as atacicept that blocks BLys and APRIL (a proliferation-inducing ligand) through its binding to transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) receptors; blisibimod, a selective antagonist of BLys (Adinolfi et al. 2016); epratuzumab (EPZ), a humanized anti-CD22 monoclonal antibody (Daridon et al. 2010); and sifalimumab, a fully human immunoglobulin G1 κ monoclonal antibody that binds to and neutralises the majority of IFN-α subtypes (Khamashta et al. 2016).

On the other hand, the current therapeutic approach of RA includes glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs). New drugs have expanded the therapeutic options for rheumatologists and patients, improved the safety of therapies and provided tools for the optimization of long-term management of RA (Bortoluzzi et al. 2018).

Thus, CAM have also been considered as possible therapeutic targets in SLE and RA treatment. More studies are needed for effective application of CAM blockers. However, the understanding of CAM role in the pathophysiology of these diseases may be the key to achieve this goal.

The role of cellular adhesion molecules in the systemic lupus erythematosus pathophysiology

SLE patients with an activated type I interferon (IFN) system have impaired endothelial function, observed by high CAM levels, connecting central pathogenic processes in SLE with endothelial dysfunction and CV disease (Tydén et al. 2017). In this way, the comprehension of the type I IFN effects on endothelium seems to be critical to understand how CAM are involved in SLE pathophysiology.

Type I IFN suppresses expression of the pro-angiogenic cytokine interleukin (IL)-1β and upregulates NOD-like receptor containing protein 3 (NLRP3) and caspase-1 in human endothelial progenitor cells (EPC) cultures. The inflammasome activation enhances the synthesis of IL-18 that has inhibitory effects on EPC differentiation to mature endothelial cells (Kahlenberg et al. 2011) and leads to upregulation of IL-6, IL-8, and CAM (Gracie et al. 2003), resulting in endothelial dysfunction independently of disease activity (Kahlenberg et al. 2011). Thus, the balance of production of IL-1β and IL-18 via inflammasome activation, as influenced by type I IFN exposure, may have important consequences for vascular health in SLE promoting inflammation and atherosclerosis (Kahlenberg and Kaplan 2014). It is well known that atherogenesis is closely related to endothelial dysfunction and increased levels of CAM (Schram and Stehouwer 2005). In addition, plasmacytoid dendritic cells also produce type I IFN in response to nuclear antigens, immune complexes and ultraviolet light in cutaneous lupus erythematosus. This cytokine increases leukocyte recruitment to the skin via proinflammatory cytokines, chemokines and CAM, thereby inducing a cycle of cutaneous inflammation (Robinson and Werth 2015).

The role of CAM in SLE also seems to involve the presence of autoantibodies. Lupus anticoagulant factor and anti-cardiolipin antibodies cross-react with multiple plasma and tissue antigens (beta-2-glycoprotein-I/oxLDL, HDL, apolipoprotein-A1) and have a prominent role in initiation, progression and acceleration of atherosclerosis (Nojima et al. 2008). As described above, SLE and CV diseases are connected by endothelial dysfunction. Investigators also showed the central role of endothelial cells in induction of thrombosis and fetal death in antiphospholipid syndrome, a major adverse condition in the prognosis of SLE patients. About 40% of SLE patients have antiphospholipid antibodies, although not all of them present thrombotic events (Mok et al. 2005). In the presence of these autoantibodies, endothelial cells become activated, express CAM and enhance the production of tissue factor. Upregulated CAM and tissue factor induce a procoagulant state that together with other factors promote thrombosis (Ruiz-Irastorza et al. 2010).

CD44, also known as homing cell adhesion molecule (HCAM), an CA that facilitates homing of T cells to inflamed tissues (e.g., skin and kidney) (Tsokos 2011), is abnormally increased in T cells from patients with SLE (Li et al. 2007). This high amount of HCAM increases T cells infiltration in SLE and contribute to organ damage (Crispín et al. 2010).

Additionally, oxidative and nitrosative stress can also positively affect the CAM levels since reactive oxygen and nitrogen species (ROS and RNS, respectively) may amplify the inflammation process, particularly via activation of the nuclear transcription factor NF-kB

(McGarry et al. 2018). Studies have already demonstrated increased levels of ROS and RNS in SLE (Lozovoy et al. 2014; Shah et al. 2014). Finally, we previously reported that CAM, especially PECAM-1, are significantly associated with SLE pathophysiology and disease activity (Santos et al. 2018). We also proposed a model to predict SLE diagnosis based on lipid and protein oxidation, PECAM-1, and body mass index (BMI) (Scavuzzi et al. 2018). The role of CAM in SLE pathophysiology is shown in Fig. 1A.

The role of cellular adhesion molecules in the rheumatoid arthritis pathophysiology

Classical reviews have already emphasized the role of CAM in the pathogenesis of RA (Postigo et al. 1993; Cronstein 1994). In this context, the main role of CAM in RA pathophysiology appears in the onset of synovitis when leukocytes infiltrate the synovial compartment by endothelial activation in synovial microvessels, which increases the expression of CAM and chemokines (Szekanecz et al. 2009). Koch and colleagues (1991) reported for the first time the abundant expression of CAM including ICAM-1, VCAM-1 and E-selectin in RA synovial tissues (Koch et al. 1991). The presence of PECAM-1 and P-selectin were also described in RA synovium previously (Johnson et al. 1993).

In RA synovium, fibroblast-like synoviocytes (FLSs), which are normally resident in the synovium, proliferate and change their phenotype (Bradfield et al. 2003). The cell contact between FLSs and T cells results in the induction of a variety of inflammatory mediators and CAM, including IL-6, TNF-α, IFN-γ, VCAM-1 and ICAM-1 (Bombara et al. 1993). In turn, the interaction of T cell integrins with their ligands, besides an additional antigenic stimulus, could trigger a mitogenic response on these cells, a phenomenon that can contribute to increased cellularity observed into the rheumatoid synovial membrane (Postigo et al. 1993). Thus, altered FLSs promote a permissive microenvironment that sustains T- and B- cells survival (Filer et al. 2006) and directly contribute to local cartilage/joint destruction and the chronicity of synovial inflammation by the production of a variety of proteases (Muller-ladner et al. 1996). In addition, TNF-α receptor signaling induces the transcription factor NF-κB leading to enhanced expression of CAM (Steyers and Jr. Miller 2014).

Kong and colleagues (2018) reported in their review that the interaction between VCAM-1 and α4β1 integrin seems to be critical for RA (Kong et al. 2018). They found in previous studies that: (a) mice with collagen-induced arthritis treated with a neutralizing monoclonal antibody to VCAM-1 presented significantly reduction of the overall disease clinical severity with fewer arthritic joints in their paws (Carter et al. 2002); (b) the treatment with an anti-VCAM-1 blocking antibody reduced the survival of synovial fluid B cells by

inhibiting their interaction with FLS (Reparon-Schuijt et al. 2000); (c) the adhesion of T cells to synovial cells was inhibited by a murine anti-VCAM-1 monoclonal antibody or anti-α4β1 integrin (Shimada et al. 1994); and (d) the interaction between VCAM-1 and α4β1 integrin mediates the recruitment of endothelial progenitor cells and promote neovascularization in RA synovial cells (Silverman et al. 2007). All these findings support the close relationship between α4β1 integrin and VCAM-1 in RA pathogenesis.

In addition, similar effects of oxidative stress on SLE can be observed in RA since elevated levels of ROS and RNS have also been reported in this disease (Costa et al. 2016; Quinonez-Flores et al. 2016). Lastly, we previously reported that lowered VCAM-1 and increased plasminogen activator inhibitor type-1 (PAI-1) levels were associated with RA, whilst ICAM-1, E-selectin and P-selectin levels were not altered (de Sá et al. 2018). The role of CAM in RA pathophysiology is shown in Fig. 1B.

Methods

A diagram for the selection of the studies is shown in Fig. 2. A bibliographic search was carried out in the electronic databases PUBMED, Lilacs, Scientific Electronic Library Online (SCIELO), and Science Direct, in English, Spanish or Portuguese. The list of references of the selected articles was also investigated.

The following combinations of Medical Subject Headings (MeSH) terms were used to SLE: 1-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Antimalarial medications”; 2-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “hydroxychloroquine”; 3-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “chloroquine”; 4-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Glucocorticoids”; 5-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Prednisone”; 6-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Immunosuppressive”; 7-”Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Mycophenolate”; 8-“Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Cyclophosphamide”; 9-“Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Antibodies, Monoclonal”; 10- “Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Cyclosporine”; 11- “Lupus Erythematosus, Systemic” AND “Adhesion Molecule” OR “Selectin” AND “Azathioprine”. The following combinations of MeSH terms were used to RA: 1-”Arthritis, Rheumatoid” AND “Adhesion Molecule” OR “Selectin” AND “Methotrexate”; 2-”Arthritis,

Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Abatacept"; 3-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Tocilizumab"; 4-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "hydroxychloroquine"; 5-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Sulfasalazine"; 6-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Leflunomide"; 7-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Glucocorticoids"; 8-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Prednisone"; 9-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Antibodies, Monoclonal, Humanized"; 10-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Infliximab"; 11-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Adalimumab"; 12-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Rituximab"; 13-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Etanercept"; 14-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Golimumab"; 15-"Arthritis, Rheumatoid" AND "Adhesion Molecule" OR "Selectin" AND "Certolizumab".

Inclusion criteria were original articles, human studies with no limit of publication date or number of subjects, studies with experimental design (clinical assays, controlled or not, randomized or not) or observational (case–control and cohort studies). Exclusion criteria were studies in animal models with induced lupus or arthritis, or non-standard treatments, or with a score which included CAM, but was also composed by several other biomarkers

There were found 25 studies, although 4 studies in animal models (1) or with non-standard treatments (3) were excluded. Thus, 21 studies, including 17 non-randomized clinical trials and 4 controlled randomized clinical trials, were selected. Only three and 18 studies on SLE and RA were found, respectively. Articles with drugs that were carried out *in vitro* were also evaluated.

Effects of the main SLE drugs on cellular adhesion molecules

Studies on the effects of SLE drugs are shown in Table 1.

Immunosuppressive therapies and Rituximab

Patients with active disease who need to excessively increase the dose of steroids to achieve adequate disease control may associate other immunosuppressive drugs in a combined therapeutic strategy to reduce inflammation and organ damage. These agents may be azathioprine (AZA), mycophenolate mofetil (MM), cyclosporine, tacrolimus and

methotrexate (MTX). Cyclophosphamide (CY) or rituximab (RTX) are used less frequently and in cases of severe clinical manifestations, such as changes in the kidneys and central nervous system, and refractory to conventional therapy. In general, these drugs suppress lymphocyte proliferation, and production of antibodies and CAM, essential for the migration of leukocytes to sites of inflammation. Rituximab is a chimeric mouse-human monoclonal antibody that binds to the CD20 antigen expressed on B cells and causes depletion of these lymphocytes via antibody-dependent cell-mediated cytotoxicity and induction of apoptosis. It is effective for the treatment of refractory lupus (Amissah-Arthur and Gordon 2010; Kuhn et al. 2015). We described one study with immunosuppressive therapies (Parker et al. 2014).

Parker et al. (2014) submitted twelve SLE patients to standard immunosuppressive therapies and ten to rituximab. These patients had active SLE and returned to follow-up after a median interval of 20 weeks. They observed an improvement in disease activity associated with a significant reduction in endothelial microparticles (EMPs composed by PECAM-1 or CD31) after both treatments. These levels were comparable to those seen in healthy controls when disease is controlled. The VCAM-1 levels were also evaluated; however, no significant results were obtained post treatment. PECAM-1 was higher in SLE patients than healthy controls. The results indicated that the suppression of inflammation possibly was the key factor to decrease EMPs and to improve the disease control (Parker et al. 2014).

Prednisolone and Cyclophosphamide

Glucocorticoids are often used to keep the patient in remission. CY has immunosuppressive activity in B cell and consequently formation of antibodies with dose-dependent action. These agents act by different mechanisms; however, all of them promote disruption of DNA function and cell death (Boumpas et al. 1992). We included in this review two study with CY (Amano et al. 2000).

Lu et al. (2010) evaluated 54 neuropsychiatric (NP) SLE patients with an A score in the nervous system domain according to the British Isles Lupus Assessment Group (BILAG) activity index. Each patient received intravenous pulse methylprednisolone (pMP) 500–1000mg daily for 3–5 days as induction treatment, followed by intravenous CY 0.75 g/m^2 monthly as the combined treatment. Therapy was maintained until clinical remission (from a nervous system BILAG A score to C or D score). They found that P-selectin and VCAM-1 levels were significantly upregulated in patients with NPSLE compared with SLE controls. A significant decline in the levels of P-selectin and VCAM-1 could be observed after sufficient induction therapy resulting in clinical remission. The authors concluded that the intrathecal

cytokine/chemokine profile is different among patients with NPSLE and SLE without NP manifestations, and that VCAM-1 and P-selectin are potential biomarkers to monitor NPSLE disease activity and response to treatment (Lu et al. 2010).

Amano et al. (2000) enrolled 34 SLE patients with SLE disease activity index (SLEDAI) greater than 8 points to receive CY. All patients had moderate to severe disease activity and those with resistance to steroid pulse therapy received CY intravenously at an initial dose of 500 mg per square meter of body-surface area every 4 weeks for six doses. In these patients, oral prednisolone (oPRED) was adjusted to a daily dose of 30 ± 60 mg at the beginning of the study, and tapered during the study period when the improvement of disease was ensured. Patients who were given steroid pulse therapy received 500 mg per day of pMP intravenously for three days and received more than 1mg/kg/day of oPRED after the pulse therapy. Soluble CAM were investigated on days 0, 7 and 14 of the first month, and at weeks 4, 8, 12, 16 and 20 after the first administration of IVCY or steroid pulse therapy, when patients were considered responsive to treatment (or achieved incomplete or complete remission). The authors found increased CD11a (integrin also known as LFA-1) on T cells, and decreased CD54 (also known as ICAM-1) on B cells with CY treatment, while decreased CD11a/LFA-1 and increased CD54/ICAM-1 with pMP. Therefore, combined therapy with CY and pMP seems to be more effective to treat SLE because these drugs presented different effects (Amano et al. 2000).

Effect of the main RA drugs (in monotherapy) on cellular adhesion molecules

Studies on the effects of DMARDs, and glucocorticoids in monotherapy are shown in Table 2. Studies on the effects of TNF-α inhibitors in monotherapy are shown in Table 3.

Disease-modifying antirheumatic drugs

DMARDs are a class of drugs indicated for RA treatment. The goals of therapy with these drugs are to achieve remission. In general, DMARDs interfere in combinations of critical pathways in the inflammatory cascade and can be of three types: biologics (bDMARDs), traditional or conventional synthetic (csDMARDs) and targeted synthetic (tsDMARDs). csDMARDs include MTX, leflunomide (LEF), hydroxychloroquine (HCQ), and sulfasalazine (SSZ). On the other hand, bDMARDs include abatacept (ABA), tocilizumab (TCZ) and TNF-α inhibitors. tsDMARDs are represented by tofacitinib, a Janus kinase (JAK) inhibitor. The choice of RA treatment involves many factors ranging from severity of disease and disability to patient preferences and the presence of adverse events (Burmester and Pope 2017;

Benjamin and Lappin 2018). Ultimately, treatment will include either monotherapy or a combination of therapies. In this review, we included two studies evaluating CAM markers comparing different DMARDs in monotherapy (Smith et al. 2001; Dominical et al. 2011).

Dominical et al. (2011) compared the effect of different treatments performed by *in vitro* analyses in neutrophils from blood samples of RA patients and healthy controls. Patients were divided into those with active [Disease Activity Score in 28 joints (DAS28)>3.2] or inactive (DAS28<2.6) RA; and subdivided into three groups according to their treatment: DMARDs group [most patients with MTX (7.5-25 mg/week)], infliximab (IFX) group (3mg/kg intravenously every 8 weeks), and non-treated group. They reported lower expressions of L-selectin and CD11a/LFA-1 on the surface of neutrophils from inactive RA subjects on DMARDs therapy when compared to the neutrophils of healthy controls, which could reflect priming of neutrophils to activation. Patients on anti-TNF-α therapy and in remission also showed lower expressions of L-selectin but in contrast they did not demonstrate any significant alterations in neutrophil CD11a/LFA-1, an interesting data since integrins modulate adhesive interactions by changes in ligand affinity and not in surface protein expression. No significant alterations in the adhesive and chemotactic properties of neutrophils from active RA were observed when compared to control neutrophils, independently of treatment regimen. Conversely, no significant alterations in CD11b/Mac-1 expression were found on the neutrophils of patients, in remission, that were on either DMARDs or anti-TNF-a therapy. Therefore, significant decreases in CAM were more apparent on neutrophils of patients on DMARDs and in remission (Dominical et al. 2011).

Smith et al. (2001) selected thirteen RA patients with mean C-reactive protein (CRP) levels of 70.7 mg/L who received treatment with MTX, SSZ or intramuscular gold. Serum CRP was used as a laboratory assessment of inflammation. The authors demonstrated that the expression of ICAM-1, VCAM-1 and E-selectin, but not PECAM-1 and P-selectin, was decreased in the synovial membrane of RA patients who clinically respond to treatment with DMARDs, specifically MTX and intramuscular gold. They also verified a good clinical and laboratorial response to DMARDs treatment in 11 of 13 patients with 7 achieving disease remission (three with intramuscular gold, two with MTX, one with MTX, and one with SSZ) (Smith et al. 2001).

Methotrexate

MTX shows good efficacy in RA patients with 40% of treated patients achieving an American College of Rheumatology improvement score (ACR) 50 response. Its mechanism of action is

not fully defined; however, several mechanisms have been proposed to explain its clinical effects. MTX is a structural analog of folic acid and acts by antagonism of folate-dependent processes, stimulation of adenosine signaling, inhibition of methyl-donor production, generation of reactive oxygen species, downregulation of adhesion-molecule expression, modification of cytokine profiles and downregulation of eicosanoids and matrix metalloproteinases (Brown et al. 2016). Two studies evaluated CAM markers with MTX alone (Dolhain et al. 1998; Klimiuk et al. 2007).

Klimiuk et al. (2007) evaluated 32 RA patients that received MTX at a mean dose of 9.1 mg/week during 6 months. In comparison with osteoarthritis patients (control group), higher serum concentrations of ICAM-1, VCAM-1, and E-selectin were observed in untreated patients with early RA. They also found serum concentrations of ICAM-1, VCAM-1 and E-selectin diminished during treatment with MTX; however, the CAM levels were still higher than in controls, suggesting that higher doses of MTX are needed to satisfactorily suppress CAM production in early stages of RA. Additionally, they showed that MTX decrease clinical markers of RA activity, such as the number of painful and swollen joints, rheumatoid arthritis index (RAI), DAS, erythrocyte sedimentation rate (ESR), and CRP levels, demonstrating correlation between CAM levels and the mentioned clinical markers of disease activity. They concluded that serum concentrations of ICAM-1, VCAM-1 and E-selectin seem to be useful markers of disease activity in early stages of RA (Klimiuk et al. 2007).

Dolhain et al. (1998) included eleven patients with seropositive erosive RA in their study. The patients were treated with MTX in a mean dose of 9 mg/week. Before and after 16 weeks of treatment, levels of VCAM-1, ICAM-1 and E-selectin were analyzed in the synovial tissue. They verified that treatment of RA patients with MTX diminished significantly the mean scores for E-selectin and VCAM-1 in the synovial membrane (Dolhain et al. 1998).

Sulfasalazine

SSZ is an antirheumatic agent used in RA treatment for its anti-inflammatory effects; however, it was initially used because of its bactericidal properties. SSZ and their metabolites, 5-aminosalicylic acid and sulphapyridine, act by inhibiting important enzymes (cyclooxygenase, lipoxygenase and prostaglandin dehydrogenase) in the inflammatory cascade, and also inhibiting both folate transport across the intestine and various folate-metabolizing enzymes. The activity of SSZ seems to occur through inhibition of various inflammatory cell functions, such as chemotaxis and migration; reduction of activity of the immune system; and inhibition of oxidative stress. Because its effects, SSZ reduces pain,

swelling, damage to the joints and prevent disability in the long term (Smedegård and Björk 1995).

We found one article with SSZ monotherapy (Veale et al. 1998), where the researchers recruited thirteen patients with active RA who received SSZ therapy at an initial dose of 500 mg/day, increasing to 1-gram bid at four weeks, or the maximum tolerated dose. After three months, the clinical parameters and levels of serum CAM were evaluated. The authors found significant higher levels of ICAM-1 and P-selectin in active RA patients at baseline compared with healthy controls; and a significant reduction in these levels in patients with active RA following 3 months therapy with SSZ. Despite significant reductions in several parameters (CRP, Health Assessment Questionnaire, RAI, morning stiffness and platelet count) suggesting a clinical improvement in disease activity in response to SSZ therapy, only reduction of ICAM-1 correlated with improvement in CRP levels, whereas P-selectin did not. Therefore, they concluded that the decreased ICAM-1 and P-selectin levels could be a direct result of reduced CAM shedding, as a consequent reduced expression by the endothelium in response to therapy, or an indirect marker of decreased disease activity (Veale et al. 1998).

Prednisolone

Prednisolone belongs to glucocorticoids class. Because of its effects on physiological and pathological inflammation, it is widely used in different inflammatory diseases, and frequently is accompanied by clinically significant side effects. Their mechanisms include direct effects on gene expression by the binding of glucocorticoid receptors to glucocorticoid-responsive elements, indirect effects on gene expression through the interactions of glucocorticoid receptors with other transcription factors or second-messenger cascades. The glucocorticoid resistance may commonly appear in RA patients, so prolonged therapy is contraindicated, while associated therapy is often required (Rhen and Cidlowski 2005). One article assessed pMP alone in RA patients (Youssef et al. 1996).

Youssef et al. (1996) studied ten RA patients who had active synovitis in multiple joints and at least one inflamed knee joint. All patients received 1,000 mg of pMP intravenously. Before and 24 hours after pMP administration, concentration of CAM were analyzed in the synovial membrane samples. In this study, pMP treatment markedly decreased the expression of E-selectin and ICAM-1, indicating that one of the possible mechanisms by which pMP inhibited neutrophil trafficking in vivo is by reducing expression of these CAM on the synovial vascular endothelium. They also investigated P-selectin and PECAM-1 expression; however, these CAM did not show similar effects from those verified with E-

selectin and ICAM-1 on synovial membrane. They concluded that pMP decreased E-selectin and ICAM-1 expression in the RA synovium *in vivo* within 24 hours of therapy, which may lead to decreased neutrophil trafficking into inflamed RA joints and improved joint symptoms (Youssef et al. 1996).

Tocilizumab

TCZ is a recombinant humanized antihuman IL-6 receptor monoclonal antibody that acts by binding both soluble and membrane IL-6 receptor with consequent block of the pro-inflammatory signaling of IL-6 (Nishimoto and Kishimoto 2008).

We found one clinical trial that evaluated levels of E-selectin and VCAM-1 in plasma from twelve RA patients before and after TCZ therapy for 6 months. The authors found that both CAM levels were reduced by treatment with TCZ. Similar results were found in assays *in vitro* with monocytes from RA patients incubated with TCZ. Incubation of these cells with IL-6 increased VCAM-1 expression and thereafter decreased by the addition of TCZ (data not shown). Thus, these data evidenced that TCZ restored endothelial function, inhibited inflammation and CAM, and possibly can prevent CV events (Ruiz-Limón et al. 2017).

Abatacept

ABA is a soluble recombinant human fusion protein comprising a Fc domain of human IgG1 and an extracellular domain of human cytotoxic T lymphocyte antigen-4 that has been widely used in RA patients with early disease or non-responsive to methotrexate (MTX) or with inadequate responses to anti-TNF drugs (Tanaka et al. 2014). One study with ABA was included in this review (Bonelli et al. 2013).

Bonelli et al. (2013) assessed twelve RA patients who received 10 mg/kg of ABA at baseline, week 2, and week 4. After, peripheral blood mononuclear cell culture (PBMCs) were isolated from heparinized blood. They observed a significant reduction in the expression of certain CAM (CD106/VCAM-1, CD54/ICAM-1, CD50/ICAM-3, CD58/LFA-3, CD62E/E-selectin) involved in adhesion and transmigration of monocytes through endothelial barriers. The authors also performed spreading assays that revealed a substantial influence of ABA on actin reorganization as well as formation of focal contacts, mechanisms that are operatives in the migratory capacity of cells. Thus, the down-regulation of CAM by ABA appears to be mediated by reducing actin dynamics (Bonelli et al. 2013).

Etanercept

Etanercept (ETA) is a fusion protein of the extracellular portion of the TNF receptor type II (p75) and the Fc portion of the human IgG1. It binds with high affinity to the soluble and transmembrane forms of TNF, promoting its effects by competitive inhibition (Marotte and Cimaz 2014). In this review, we describe one study with ETA in monotherapy (Klimiuk et al. 2009).

Klimiuk et al. (2009) studied eighteen consecutive patients who had active RA and were administered with 50mg ETA subcutaneously and weekly for 12 months. The analyses were performed before treatment with ETA and 3, 6, 9, and 12 months after the prior injection. The authors demonstrated a sustained inhibition of ICAM-1, VCAM-1 and E-selectin along all different duration of treatments with ETA accompanied by a decrease in DAS28. TNF-α blocking with ETA seemed to downregulate the cytokine cascade responsible for endothelium activation and consequently to diminish interactions between CAM and leucocytes, chemotaxis and migration of inflammatory cell into rheumatoid synovium. Decreased CAM levels also correlated with markers of disease activity such as ESR and DAS28 (Klimiuk et al. 2009).

Adalimumab

Adalimumab (ADA) is a recombinant human IgG1 monoclonal antibody approved for RA treatment that has been associated with reductions from baseline in CRP, ESR and IL-6. It binds specifically to TNF-α and blocks its interaction with surface TNF-α receptors, p55 and p75. ADA inhibits the CAM responsible for leukocyte migration (E-selectin, ICAM-1 and VCAM-1) and induces lysis of cells expressing surface TNF in the presence of complement (Hoy 2017).

We found one clinical trial with ADA monotherapy (den Broeder et al. 2002) that evaluated 47 patients with active RA defined by DAS greater than 3.2 at baseline. The patients were divided into two groups: placebo group (from six to eight weeks placebo controlled, randomized, dose ranging 0.5 - 10 mg/kg) and ADA group (dosages of 3 mg/kg intravenously biweekly or 1 mg/kg subcutaneously weekly). All patients received ADA treatment, including those initially randomized to receive placebo; and were followed up for a period of two years. The study of CAM levels before and after ADA treatment was not the main goal of this article, but as the CAM results were clearly described, this study was used in the present review. After two weeks from baseline, 46% of RA patients treated with ADA but none in the placebo group had achieved proper clinical responses. E-selectin and ICAM-1

decreased significantly within two weeks after starting anti-TNFα treatment, while only ICAM-1 maintaining thereafter a modest reduction in patients who were still receiving ADA. Thus, ICAM-1 and E-selectin seem to be differentially regulated and do not have the same properties as disease markers, since variations of ICAM-1 was even associated with radiological progression course and may have prognostic value of the radiological evolution (den Broeder et al. 2002).

Infliximab

IFX is a chimeric IgG1 mAb that consists of human constant regions and murine variable regions (Cohen et al. 2013). Initially called chimeric anti-tumor necrosis factor α monoclonal antibody (cA2), it was first evaluated in 1993 by Elliott et al. in 20 refractory RA patients and improved clinical and laboratory parameters of disease activity. The effect of these drugs seems to occur by reduced production of proinflammatory cytokines and down-regulation of cytokine-inducible CAM (Elliott et al. 1993). We evaluated, in this review, three articles with IFX monotherapy in patients with RA (Tak et al. 1996; Klimiuk et al. 2004; Gonzales-Gay et al. 2006).

Gonzalez-Gay et al. (2006) included, in this cohort, thirty-four RA patients with severe and active disease and DAS28 greater than 5.1. IFX was administered intravenously (over 120 minutes) in an initial dosage of 3 mg/kg at 0, 2, 6 weeks and then every 8 weeks. The levels of soluble CAM were determined immediately prior to the onset of IFX infusion (time 0) and at the end of IFX infusion (time 120). The results confirmed the rapid and beneficial effect of IFX infusion in most RA patients treated periodically with a significant decrease of ICAM-3 and P-selectin levels probably due to a direct effect of TNF-α blockade. No significant differences for ICAM-1, VCAM-1 and E-selectin were observed. (Gonzales-Gay et al. 2006).

Klimiuk et al. (2004) studied eleven patients with active RA as defined by 6 or more tender joints, 6 or more swollen joints, and two of the following: morning stiffness for more than 45 min, CRP level of more than 20 mg/l, or erythrocyte sedimentation rate (ESR) of more than 28 mm/h. The patients received 9 IFX infusions always in the same dose of 3 mg/kg at 0, 2, 6 weeks and every 8 weeks. They evaluated CAM from blood samples obtained at weeks 0, 2, 6, 14, and 38 prior to infusion, and at the week 62 (8 weeks after the last drug administration). In general, the authors found that levels of CAM (ICAM-1, VCAM-1 and E-selectin) significantly decreased after the initial IFX administration, and this reduction persisted after repeated IFX infusions but with less effect. This was also observed in the

correlation between CAM and markers of disease activity. Only for E-selectin, its concentration was especially suppressed after the second drug infusion, as evaluated on week 6 prior to the third IFX administration. Before the initial IFX infusion, ICAM-1, VCAM-1 and E-selectin correlated with ESR levels, the number of tender and swollen joints. After further drug administrations, these correlations were also found, but were less significant. A rapid reduction in disease activity was also observed. The authors concluded that their results may reflect a suppressed activation of the endothelium, resulting in a decreased migration of leukocytes into the rheumatoid synovium, and, therefore, CAM might be useful as markers of disease activity in patients undergoing anti-TNF-α monoclonal antibody therapy (Klimiuk et al. 2004).

Tak et al. (1996) studied fourteen patients with active RA, seven in an open-label trial, and seven in a randomized double-blind placebo-controlled study, who received cA2 or placebo. In the open-label study, seven patients were administered with 2 infusions of 10 mg/kg of cA2, at study entry and 14 days later; or 4 infusions of 5 mg/kg of cA2, at entry and on days 4, 8, and 12. In the placebo-controlled study, two patients and five were administered with a single infusion of placebo or 10 mg/kg of cA2 at study entry, respectively. Before infusion and 4 weeks after, concentrations of CAM were analyzed in the synovial tissue samples. This study demonstrated significant reduction in the mean scores for VCAM-1 and E-selectin after therapy with 10 mg/kg or 20 mg/kg of the chimeric anti-TNF-α Mab in patients with RA with beneficial effects in clinical parameters. The group treated with placebo did not exhibit any response. The authors concluded that the effects observed in this study could be explained by down-regulation of cytokine-inducible CAM in synovial tissue after cA2 therapy, with a consequent reduction of cell traffic into joints (Tak et al. 1996).

Effect of the main RA drugs (in associated therapy) on cellular adhesion molecules

We found 5 studies that evaluated the effect of different associated therapies. Three of them evaluated the association of MTX with TNF-α inhibitors (Visvanathan et al. 2009; Hjeltnes et al. 2013; Kragstrup et al. 2016), one studied MTX with SSZ and HCQ (Çobankara et al. 2004), and, finally, one verified the effects of treatment of MP with others DMARDs (Dessein and Joffe 2006). Table 4 shows the effects of RA drugs in combined therapy on CAM.

Methotrexate and Adalimumab

Kragstrup et al. (2016) randomly selected 152 early RA patients to a MTX protocol in combination with either ADA or placebo. They collected plasma samples from the initiation

of treatment (0 months) and after 3, 6, and 12 months of treatment. Plasma from patients with chronic RA formed a subgroup for *in vitro* analysis with use of PBMC and synovial fluid mononuclear cells (SFMC) culture in stimulation and inhibition experiments. The treatment strategy showed a biphasic pattern of response. Initially, CD18 (also known as β2 integrin) levels decreased after 3 months of treatment what could reflect autoimmunity in transition from early to chronic disease. After 12 months of treatment, there was a gradual increase in CD18 levels to healthy controls levels, and this effect was particularly pronounced in patients who quickly achieved an ACR response. This normalization in response to treatment could reflect autoimmunity in remission. The *in vitro* assay with ADA reproduced the initial phase of treatment with similar results and therefore decreased concentration of CD18 in both SFMC and PBMC cultures. Shedding of CD18 from RA SFMC and PBMC was increased by adding recombinant human TNFα and decreased by neutralizing TNFα with ADA. There were no significant differences between MTX+ADA or MTX+placebo groups (Kragstrup et al. 2016).

Methotrexate and TNF-α inhibitors (adalimumab, infliximab or etanercept)

Hjeltnes et al. (2013) studied 64 consecutive RA patients treated with MTX (15-25mg orally once a week) monotherapy or in combination with ADA (40mg subcutaneously every other week), IFX (3-5mg/kg intravenously at baseline) or ETA (50 mg subcutaneously once a week). The analyses were performed at baseline, and after 6 weeks and 6 months of treatment. The authors found decreased levels of E-selectin and a strong relationship between E-selectin and serum lipoprotein(a) [s-Lp(a)] during treatment with MTX monotherapy or MTX in combination with a TNF-α-inhibitor in RA patients. These results may suggest direct or indirect relationship between the levels of these two molecules, or may indicate that they have a common cause such as inflammation. Therefore, both may be useful in assessing cardiovascular risk in RA patients. ICAM-1 levels were significantly decreased in RA patients only after 6 weeks in both treatment groups. VCAM-1 levels were also evaluated, but no significant results were found (Hjeltnes et al. 2013).

Methotrexate and Golimumab

Golimumab (GOL) is a fully human IgG1 anti-TNF-α antibody with structure very similar to IFX, except the portion of mouse protein. It was generated and affinity matured in an *in vivo* system (Cohen et al. 2013). We found one clinical trial with GOL (Visvanathan et al. 2009).

Visvanathan et al. (2009) assessed the GOL plus MTX treatment compared with placebo plus MTX in patients with active RA. The patients were randomly placed in one of 5 treatment groups: placebo or GOL in 4 different doses (50 mg every 4 weeks; 50 mg every 2 weeks; 100 mg every 4 weeks, or 100 mg every 2 weeks) subcutaneously administered from Week 0 to Week 18. All patients received stable doses of MTX (at least 10 mg/week). Serum samples were collected and analyses were performed at Weeks 0, 4, and 16. Treatment with GOL+MTX resulted in greater and more rapid reductions in serum levels of E-selectin as early as week 4 and continuously through week 16. Reductions of E-selectin in the week 4 significantly correlated with improvement in swollen joint count and DAS28-CRP score at week 16 in GOL+MTX treated patients. These findings suggest that E-selectin levels at week 4 after GOL+MTX treatment could be useful as a marker of improved clinical response at week 16 (Visvanathan et al. 2009).

Methotrexate and Sulfasalazine and Hydroxychloroquine

The combination of triple conventional synthetic DMARDs (MTX, SSZ and HCQ) is indicated to RA patients whose disease is not well controlled. This triple therapy (tDMARD) promotes downregulation of genes included those involved in T cell activation and signaling and plasmablast/plasma cell differentiation and function, showing remarkable efficacy in early RA (Walsh et al. 2017). One study assessed CAM levels with tDMARD in RA patients (Çobankara et al. 2004).

Çobankara et al. (2004) evaluated consecutively eleven RA patients that were followed up at 0, 1, 3 and 6 months of triple treatment (SSZ 1–3 g/day, MTX 7.5–15 mg/week, and HCQ 200 mg/day) for clinical and laboratory analyses. All patients had active disease defined by ESR ≥ 20 mm/h for males, ≥ 30 mm/h for females, the presence of morning stiffness lasting more than 60 min, and the presence of at least one joint with active arthritis (defined as the presence of swelling or limitation of motion, with either pain on movement or tenderness). All clinical and laboratory parameters improved after successful triple treatment. E-selectin was significantly higher in patients than in healthy controls before treatment; but these levels decreased returning to normal (similar to healthy controls group) levels after triple treatment. E-selectin levels correlated with morning stiffness, tender joint count, swollen joint count, patients' global assessment, physician global assessment, ESR and CRP before the treatment. This support that E-selectin could participate in the pathogenic mechanisms in the rheumatoid joint. Thus, E-selectin levels probably reflect disease activity

and can be helpful in monitoring disease status and response to therapy (Çobankara et al. 2004).

Metilprednisolone and Disease-modifying Antirheumatic Drugs

Dessein and Joffe (2006) enrolled 21 patients with active RA. On the same day, all patients were treated with intraarticular (ia) MP in all or most swollen joints using a mean dose of 417 mg, and with DMARDs (MTX or minocyclin or LEF or SSZ). After two weeks, the authors evaluated disease activity parameters and circulating CAM levels. In the study, patients with active disease submitted to combined treatment of iaMP with DMARDs had a significant reduction in levels of VCAM-1, ICAM-1 and ELAM-1 (Dessein and Joffe 2006).

Conclusions

The present review evaluated 21 articles that assessed CAM levels in SLE and RA patients. In SLE, 3 studies were evaluated in which reduced CAM levels after treatment were found five occasions (62.5%); while increased results were reported twice (25.0%) and non-significant changes only once (12.5%). On other hand, in the 18 papers that evaluated CAM levels in RA patients, these molecules were significantly reduced after treatment on 73 occasions (73.7%); while non-significant changes were described in 25 occasions (25.3%), and increased results only once (1.0%). Thus, this improvement in CAM levels may be directly related to the success of therapy or indirectly to control of disease activity. The most evaluated medicaments in the articles analyzed were CY (n=2) and pMP (n=2) in SLE; and MTX (n=9) and IFX (n=4) in RA. In addition, the most CAM used to assess response to treatment was VCAM-1 (n=2) in SLE; and VCAM-1 (n=12), ICAM-1 (n=12) and E-selectin (n=14) in RA. Thus, our results suggest that patients submitted to specific treatments usually showed significant decrease in markers of CAM. In accordance with the results of this review, ICAM-1, VCAM-1 and E-selectin were the molecules often determined for evaluation of treatment response.

This review has some limitations. Some characteristics of the studies make it difficult to compare them, such as heterogeneity of used tests, disease duration and activity, presence of autoantibodies and comorbidities. However, most of the studies evaluated groups with active disease, revealing that CAM may have great relevance in this context

Endothelial dysfunction has often been related to the high prevalence of CV diseases associated with rheumatic diseases. Although the articles analyzed in this review seem to demonstrate a similar behavior of the levels of CAM in SLE and RA, with high initial levels

and with a significant reduction after treatment, the low amount of articles found in the literature on CAM in SLE is a limiting factor to determine whether a different profile occur between these two diseases. However, some studies, including data obtained by our research group (Santos et al. 2012; Santos et al. 2019), have shown that CAM levels are higher in SLE patients when compared to RA regardless of used drugs. Thus, CAM may be useful as markers of response to treatment and disease condition, favoring strategies to achieve remission and avoid typical long-term outcomes and comorbidities. Further studies are needed to better understand the role of CAM in the pathophysiology of SLE and RA, allowing its application in the clinical context and in the elaboration of new therapeutic targets.

Author contributions LFRFS, ID and ANCS designed the experimental plan; LFRFS, ANCS and ID wrote the manuscript; LFRFS performed the tables, figures and diagram; ID revised the language grammar and style. Note: the figures presented in this manuscript are original. CorelDRAW 2017 was used to create the artwork.

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Table 1. Effects of the main drugs used in the treatment of patients with systemic lupus erythematosus (SLE) on the cell adhesion molecules (CAM).

Design	Drug	n	Age (years±SD)	Dur. (years±SD)	Disease activity Score	CAM markers	Conclusions	Refs.
Non-randomized clinical trial	S-IST/RTX	22 SLE 22 HC	41.5±14.1	7.0±3.5	Active SLE SLEDAI-2K T0: 6.0 T_{w20}: 4.0	VCAM-1 and PECAM-1 ↑ PECAM-1 T_{W20}∨ VCAM-1 T_{W20}*	The use of S-IST or RTX decreased the endothelial damage and dysfunction in patients with active SLE. In addition, disease activity improved significantly over time.	Parker et al. (2014)
Non-randomized clinical trial	pMP + CY	54	28.1±10.7	NR	Active SLE (nervous system BILAG A score)	P-selectin VCAM-1 ↑ T_{final} ∨	VCAM-1 and P-selectin in CSF are effective parameters to monitor NPSLE disease activity and response to treatment.	Lu et al. (2010)
Non-randomized clinical trial	CY or pMP	34 21	28.8±NR	NR	Active SLE SLEDAI >8	CY: CD11a/LFA-1 T_{w8}∧ CD54/ICAM-1 T_{w8}∨ MP pulse: CD11a/LFA-1 T_{w4}∨ CD54/ICAM-1 T_{w4} ∧	CY and pMP affects CD11a/LFA-1 and CD54/ICAM-1 levels differently. Thus, these markers may contribute to predict the responsiveness of CY and combined therapy with CY and pMP seems to be more effective.	Amano et al. (2000)

SD: standard deviation; Dur: duration of illness; CAM: cell adhesion molecules; Refs.: references; S-IST: Standard immunosuppression therapies; HC: healthy controls; RTX: Rituximab; SLEDAI(-2K): SLE Disease Activity Index (2000); T_W: weeks after intervention; EMPs: Endothelial microparticles; PECAM-1: platelet endothelial cell adhesion molecule 1; VCAM-1: vascular cell adhesion molecule-1; NR: not reported in the article; CY: cyclophosphamide; pMP: methylprednisolone pulse; BILAG: British Isles Lupus Assessment Group; T_{final}: after treatment (until clinical remission); CSF: cerebrospinal fluid; NPSLE: Neuropsychiatric systemic lupus erythematosus; oPRED: prednisolone oral; LFA1: lymphocyte function associated antigen-1; ICAM-1: intercellular adhesion molecule-1.

* Without significant difference

∧Significantly elevated levels in comparison with T0

∨Significantly diminished levels in comparison with T0

↑Significantly elevated levels in comparison with control group in T0

Table 2. Effects of biological (TCZ and ABA) and conventional synthetic (MTX and SSZ) disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids (MP) in monotherapy on cell adhesion molecules (CAM) in patients with rheumatoid arthritis (RA).

Design	Drug	n	Age (years±SD)	Dur. (years±SD)	Disease activity Score	CAM markers	Conclusions	Refs.
Non-randomized clinical trial	TCZ	20	47.8±2.3	7.6±1.8	DAS28 T0: 4.2±0.2 T_{M6}: 2.9±0.4$^{\vee}$	VCAM-1 and E-selectin $T_{M6}^{\vee}$	Evidence of TCZ for 6 months improved endothelial function, inhibiting inflammation and CAM.	Ruiz-Limón et al. (2017)
Non-randomized clinical trial	ABA	12	56.0±NR	NR	DAS28-CRP T0: 3.7±0.6 T_{W2}: 3.7±0.6 T_{W4}: 3.8±0.6	VCAM-1, ICAM-1, and ICAM-3$^{\#}$ $T_{W2}^{\vee}$ LFA-3 and E-selectin$^{\#}$ $T_{W2}^{\vee}$ $T_{W4}^{\vee}$	The expression of several CAM was significantly diminished with ABA treatment.	Bonelli et al. (2013)
Non-randomized clinical trial	DMARD IFX	Active RA DMARD: 55 IFX: 21 Inactive RA DMARD: 15 IFX: 9	Active RA DMARD:48.3±1.4 IFX: 49.7±1.9 Inactive RA DMARD: 55.7±nr IFX: 53.0±nr	Active RA DMARD:9.5±0.8 IFX: 12.2±1.5 Inactive RA DMARD:8.0±1.7 IFX: 17.1±3.7	Active RA: DAS28>3.2 Inactive RA: DAS28<2.6 DAS28: Active RA DMARD:4.9±0.1 IFX:5.1±0.2 Inactive RA: NR	Active RA DMARD: L-selectin, LFA-1 and Mac-1 T_{M3}^{*} IFX: L-selectin, LFA-1 and Mac-1 T_{M3}^{*} Inactive RA DMARD: L-selectin and LFA-1$^{\#}$ $T_{M3}^{\vee}$ Mac-1* IFX: L-selectin$^{\#}$ $T_{M3}^{\vee}$ Mac-1 and LFA-1*	Significant decreases in CAM expression were more apparent on neutrophils of patients on DMARDs and in remission.	Dominical et al. (2011)
Non-randomized	MTX	32 RA 20 OA	51.0±14.2 53.5±12.0	16.4±9.8 24.6±17.5	DAS T0: 3.7±0.8	ICAM-1, VCAM-1 and E-selectin ↑	MTX resulted in clinical improvement and reduced	Klimiuk et al. (2007)

clinical trial				(in months)	PJ, RAI, ESR, CRP and DAS $T_{M6}^{\vee}$	$T_{M6}^{\vee}$ ICAM-1, VCAM-1 and E-selectin correlated with DAS, CRP, ESR and SJC	CAM concentrations in RA patients. Moreover, ICAM-1, VCAM-1, and E-selectin correlate with laboratory and clinical markers of the disease activity.	
Non-randomized clinical trial	DMARD (MTX or IM gold or SSZ)	13	72.3±NR	10.8±NR	CRP (normal<6mg/L) T0: 70.7 $T_{M12}^{\vee}$, $T_{M18}^{\vee}$	Responders: E-selectin# $T_{M6}^{\vee}$, $T_{M12}^{\vee}$, $T_{M18}^{\vee}$ ICAM-1# $T_{M6}^{\vee}$, $T_{M12}^{\vee}$, $T_{M18}^{\vee}$ VCAM-1# $T_{M18}^{\vee}$ PECAM-1 and P-selectin#*	E-selectin and ICAM-1 expression were decreased at the synovial membrane in patients who respond clinically to drug treatment. There was a significant reduction in VCAM-1 expression by the synovial lining in some, but not all patients.	Smith et al. (2001)
Non-randomized clinical trial	MTX	11	NR	> 5 years	DAS T0: 5.6±1.1 $T_{W16}^{\vee}$: 4.3±1.2	E-selectin and VCAM-1# $T_{W16}^{\vee}$ ICAM-1#*	MTX is associated with downregulation of E-selectin and VCAM-1.	Dolhain et al. (1998)
Non-randomized clinical trial	SSZ	13 13 HC	63.0±nr	≤ 3 months	Active RA CRP T0: 3.9±0.9mg/L $T_{W12}^{\vee}$: 2.0±0.5mg/L	ICAM-1 and P-selectin ↑ ICAM-1 and P-selectin $T_{W12}^{\vee}$ VCAM-1 and P-selectin* Decreased ICAM-1 correlated with improved CRP	SSZ therapy was effective by a reduction of P-selectin and ICAM-1 levels in active RA. In addition, ICAM-1 correlates with disease activity as measured by CRP.	Veale et al. (1998)

Non-randomized clinical trial	pMP	10	69.5±11.2	4.5±7.2	Active synovitis	E-selectin and ICAM-1[#] $T_{24}^{\vee}$ PECAM-1 and P-selectin[#*]	pMP significantly reduces E-selectin and ICAM-1 expression in the RA synovium within 24 hours of therapy.	Youssef et al. (1996)

SD: standard deviation; Dur: duration of illness; CAM: cell adhesion molecules; Refs: references; TCZ: tocilizumab; T_M: months after intervention; DAS28: Disease Activity Score in 28 joints; VCAM-1: vascular cell adhesion molecule-1; ABA: abatacept; NR: not reported in the article; CRP: C-reactive protein; T_W: weeks after intervention; ICAM-1 or -3: intercellular adhesion molecule-1 or -3; LFA-1 or -3: lymphocyte function associated antigen-1 or -3; DMARD: disease-modifying antirheumatic drugs; IFX: infliximab; Mac-1 MTX: methotrexate; OA: osteoarthritis; DAS: three-parametrical disease activity score (Ritchie articular index, number of swollen joints, and erythrocyte sedimentation rate); PJ: painful joints; RAI: Ritchie articular index; ESR: erythrocyte sedimentation rate; SJC: swollen joint count; IM gold: intramuscular sodium aurothiomalate; SSZ: sulphasalazine; HC: healthy controls; pMP: methylprednisolone pulse; $T_{24:}$ 24 hours after intervention.

* Without significant difference

$^{\wedge}$Significantly elevated levels in comparison with T0

$^{\vee}$Significantly diminished levels in comparison with T0

↑Significantly elevated levels in comparison with control group in T0

[#] Evaluation of CAM expression

Table 3. Effects of biological (TNF-α inhibitors) disease-modifying antirheumatic drugs in monotherapy on cell adhesion molecules (CAM) in patients with rheumatoid arthritis (RA).

Design	Drug	n	Age (years±SD)	Dur. (years±SD)	Disease activity Score	CAM markers	Conclusions	Refs.
Non-randomized clinical trial	ETA	18	45.3±NR	6.1±4.7	DAS28 T0: 6.2±0.4 $T_{M3}^{\vee}$: 4.6±0.5 $T_{M6}^{\vee}$: 4.4±0.5 $T_{M9}^{\vee}$: 4.1±0.6 $T_{M12}^{\vee}$: 3.7±0.8	ICAM-1, VCAM-1 and E-selectin $T_{M3}^{\vee}$, $T_{M6}^{\vee}$, $T_{M9}^{\vee}$, $T_{M12}^{\vee}$ ICAM-1, VCAM-1, and E-selectin correlated with ESR or DAS28	There were a significant fall in ICAM-1, VCAM-1 and E-selectin concentrations in RA patients during treatment with ETA. CAM levels correlated with markers of disease activity.	Klimiuk et al. (2009)
Non-randomized clinical trial	IFX	34	55.4±12.6	NR	Active RA DAS28>5.1 T0 DAS28: 4.3±1.2 T120' DAS28$^{\vee}$	ICAM-3 and P-selectin: T120'$^{\vee}$ ICAM-1, VCAM-1 and E-selectin*	Decrease of P-selectin and ICAM-3 levels after 120 minutes after the infusion with IFX in RA patients.	Gonzalez-Gay et al. (2006)
Non-randomized clinical trial	IFX	11	45.2±NR	10.6±6.6	Active RA: TJC or SJC≥6; and MS>45 min or CRP≥20 mg/l or ESR≥28 mm/h.	ICAM-1, VCAM-1 and E-selectin: $T_{W2}^{\vee}$, $T_{W6}^{\vee}$, $T_{W14}^{\vee}$, $T_{W38}^{\vee}$, $T_{W62}^{\vee}$ ICAM-1 and E-selectin correlated with improved ESR and SJC; VCAM-1 correlated with improved ESR, TJC and SJC before and after (with less impact) IFX infusions.	Significant decrease in CAM levels in RA patients after first IFX infusion. For E-selectin, it concentration was especially suppressed after the second IFX administration. Further IFX infusions also significantly reduced CAM levels, although these were less effective.	Klimiuk et al. (2004)
Controlled randomized clinical trial	ADA	ADA: 35 Placebo: 12	ADA+Placebo: 56.0±15.0	ADA+Placebo: 13.0±8.0	Active RA DAS>3.2	E-selectin $T_{W2}^{\vee}$	ICAM-1 levels were prognostic of the radiological course.	den Broeder et al. (2002)

					T0: DAS28: 5.2±1.0 DAS28 $T_{W2}^{\vee}$ $T_{Y2}^{\vee}$	ICAM-1 $T_{W2}^{\vee}$ $T_{Y2}^{\vee}$	E-selectin and ICAM-1 decreased significantly within two weeks after use of ADA. ICAM-1 values still showed a modest decrease in patients who were still receiving ADA after two years.	
Controlled randomized clinical trial	cA2	cA2: 12 Placebo: 2	NR	NR	Active RA TJC, SJC, ESR: $T_{W4}^{\vee}$	E-selectin and VCAM-1[#] $T_{W4}^{\vee}$	There were significant reductions in concentrations of E-selectin and VCAM-1, and in parameters of disease activity measured 4 weeks after cA2 treatment.	Tak et al. (1996)

SD: standard deviation; Dur: duration of illness; CAM: cell adhesion molecules; Refs: References; ETA: etanercept; NR: not reported in the article; DAS28: Disease Activity Score in 28 joints; T_M: months after intervention; ICAM-1 or -3: intercellular adhesion molecule-1 or -3; VCAM-1: vascular cell adhesion molecule-1; ESR: erythrocyte sedimentation rate; IFX: infliximab; T120': 120 minutes after infusion; TJC: tender joint count; SJC: swollen joint count; MS: morning stiffness; CRP: C-reactive protein; T_W: weeks after intervention; ADA: adalimumab; DAS: disease activity score; T_{Y2}: 2 years after intervention; cA2: chimeric anti-tumor necrosis factor α monoclonal antibody.

* Without significant difference

$^{\wedge}$Significantly elevated levels in comparison with T0

$^{\vee}$Significantly diminished levels in comparison with T0

[#] Evaluation of CAM expression

Table 4. Effects of combined therapy with TNF-α inhibitors and/or disease-modifying antirheumatic drugs (DMARDs) on cell adhesion molecules (CAM) in in patients with rheumatoid arthritis (RA).

Design	Drug	n	Age (years±SD)	Dur. (years±SD)	Disease activity Score	CAM markers	Conclusions	Refs.
Controlled randomized clinical trial	MTX+ADA MTX+placebo	*In vivo:* 152 *In vitro:* 6	*In vivo*: Early RA MTX+ADA/ MTX+placebo: 56.0 *In vitro*: Chronic RA ADA: 58.5	84±NR NR	MTX+ADA/ MTX+placebo: DAS28-CRP T0: 5.6 $T_{M3}^{\vee}$: 2.2 $T_{M6}^{\vee}$: 2.4 $T_{M9}^{\vee}$: 2.0 $T_{M12}^{\vee}$: 2.0	*In vivo*: β2 integrin $T_{M3}^{\vee}$, $T_{M6}^{\vee}$, $T_{M9}^{\vee}$, $T_{M12}^{\wedge}$ MTX+ADA vs. MTX+placebo *In vivo:* β2 integrin* ADA *In vitro*: β2 integrin ↓	CD18 levels exhibited a biphasic course during treatment with an initial decline followed by a gradual increase to HC levels. Changes in CD18 levels did not differ between MTX+ADA or MTX+placebo groups. The concentration of CD18 from RA SFMC and PBMC was decreased by ADA compared with UT cultures.	Kragstrup et al. (2016)
Non-randomized clinical trial	MTX MTX+ TNF-α inhibitors (ADA or IFX or ETA)	MTX: 34 MTX+ TNF-α inhibitors: 30	56.0±11.0 58.0±8.0	3.0±6.0 8.0±8.0	DAS28-ESR MTX T0: 5.1 $T_{W6}^{\vee}$: 4.0 $T_{M6}^{\vee}$: 2.8 MTX+ TNF-α inhibitor T0: 5.0 $T_{W6}^{\vee}$: 3.1 $T_{M6}^{\vee}$: 2.7	E-selectin (for both groups) $T_{W6}^{\vee}$ $T_{M6}^{\vee}$ ICAM-1 (for both groups) $T_{W6}^{\vee}$ VCAM-1* E-selectin correlated with Lp(a)	Significant reduction of E-selectin (at T_{W6} and T_{M6}) and ICAM-1 (at T_{W6} only) during treatment with MTX in monotherapy or in combination with a TNF-α inhibitor in RA patients. There were a strong association between E-selectin and Lp(a) over time.	Hjeltnes et al. (2013)
Controlled randomized clinical trial	MTX+GOL MTX+placebo	GOL+MTX 50mg/4wks: 37 50mg/2wks: 32	57.0 48.0	NR	Active RA CRP $T_{W4}^{\vee}$ $T_{W16}^{\vee}$	E-selectin: $T_{W4}^{\vee}$ (for all GOL+MTX dose groups) $T_{W16}^{\vee}$ (except	GOL+MTX significantly decreased E-selectin levels vs. Placebo+MTX group at T_{W4}. This decrease was sustained through T_{W16}. Decreases in E-	Visvanathan et al. (2009)

		100mg/4wks: 33 100mg/2wks: 35 MTX+placebo: 34	57.5 53.5 52.0			for the 50mg/4wks group) GOL+MTX: E-selectin: $T_{W4}^{\vee}$ and $T_{W16}^{\vee}$ correlated with improved DAS28-CRP, SJC and TJC at $T_{W16}^{\vee}$	selectin levels at T_{W4} may be useful in predicting clinical response at T_{W16}.	
Non-randomized clinical trial	SSZ+MTX+HCQ	11 12 HC	35.0	32.0 (in months)	Active RA: ESR≥20 mm/h (males); or ESR≥30 mm/h (females); or the presence of MS>60min; or joint with active arthritis ≥1.	E-selectin ↑ E-selectin $T_{M6}^{\vee}$ T0: E-selectin correlated with improvement of all clinical (MS, TJC, SJC, VAS-PDA and VAS-DDA) and laboratorial (ESR and CRP) parameters.	The combination treatment successfully reduced E-selectin levels in RA patients. E-selectin levels probably reflect disease activity and can be helpful in monitoring disease status and response to therapy.	Çobankara et al. (2004)
Non-randomized clinical trial	iaMP+ DMARD (MTX or LEF or SSZ)	21	59.0±9.0	6.0±4.0	Active RA HAQ-DI, VAS-P, VAS-PDA, VAS-DDA, ESR and CRP $T_{W2}^{\vee}$	VCAM-1, ICAM-1 and ELAM-1 $T_{W2}^{\vee}$	After 2 weeks, the use of iaMP+DMARD in active RA patients resulted in reduced endotelial activation.	Dessein; Joffe (2006)

SD: standard deviation; Dur: duration of illness; CAM: cell adhesion molecules; Refs: References; MTX: methotrexate; ADA: adalimumab; NR: not reported in the article; NA: not applicable; DAS28: Disease Activity Score in 28 joints; CRP: C-reactive protein; ACR: American College of Rheumatology improvement score; SFMC: synovial fluid mononuclear cell culture; PBMC: peripheral blood mononuclear cell culture; UT: untreated; T_M: months after intervention; TNF-α: tumor necrosis factor alpha; IFX: infliximab; ETA: etanercept; T_W: weeks after intervention;ESR: erythrocyte sedimentation rate; ICAM-1: intercellular adhesion molecule-1; Lp(a): lipoprotein a; GOL: golimumab; wks: weeks; SJC: swollen joint count; TJC: tender joint count; SSZ: sulphasalazine; HCQ: hydroxychloroquine; HC: healthy controls; MS: morning stiffness; VAS-P or -PDA or -DDA: visual analog scale (range 0-10 for both pain, patient and doctor disease activity); iaMP: intraarticular methylprednisolone; DMARD: disease-modifying antirheumatic drugs; MCY: minocycline; LEF: leflunomide; HAQ-DI: Health Assessment Questionnaire-Disability Index (range 0-3); VCAM-1: vascular cell adhesion molecule-1; ELAM-1: endothelial leukocyte adhesion molecule-1.

* Without significant difference

∧Significantly elevated levels in comparison with T0

∨Significantly diminished levels in comparison with T0

↑Significantly elevated levels in comparison with control group in T0

↓Significantly diminished levels in comparison with untreated cultures

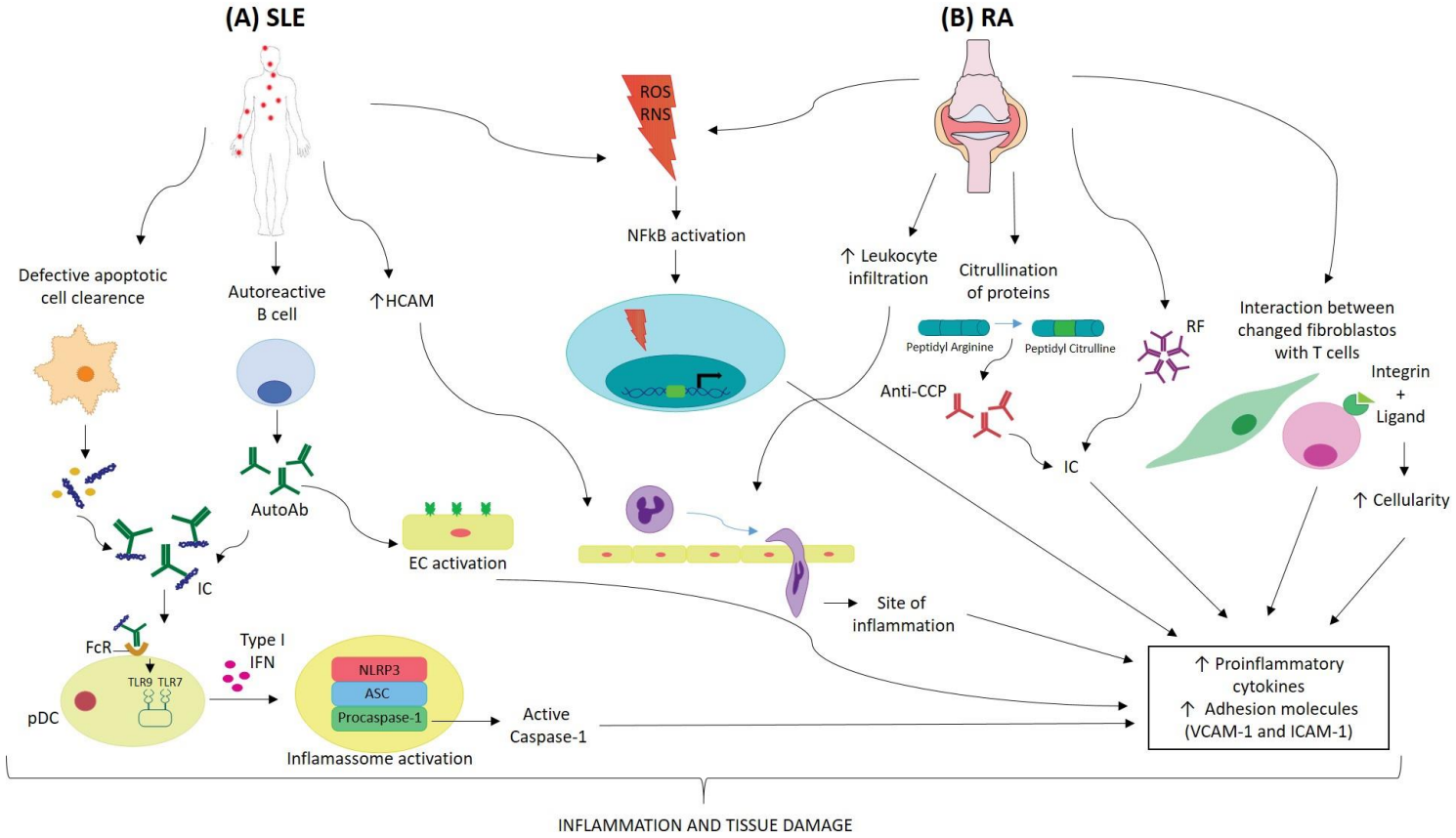

Fig. 1 The role of cellular adhesion molecules in the (A) Systemic Lupus Erythematosus (SLE) and (B) Rheumatoid Arthritis (RA) pathophysiology. IC: immune complexes; FcR: Fc-gamma receptors; pDC: plasmacytoid dendritic cells; TLR: Toll like receptor; IFN: interferon; NLRP3: NOD-like receptor containing protein 3; ASC: apoptosis-associated speck-like protein containing a CARD; AutoAb: autoantibodies; EC: endothelial cells; HCAM: homing cell adhesion molecule; ROS: reactive oxygen species; RNS: reactive nitrogen species; NFkB: nuclear transcription factor NF-kB; anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1.

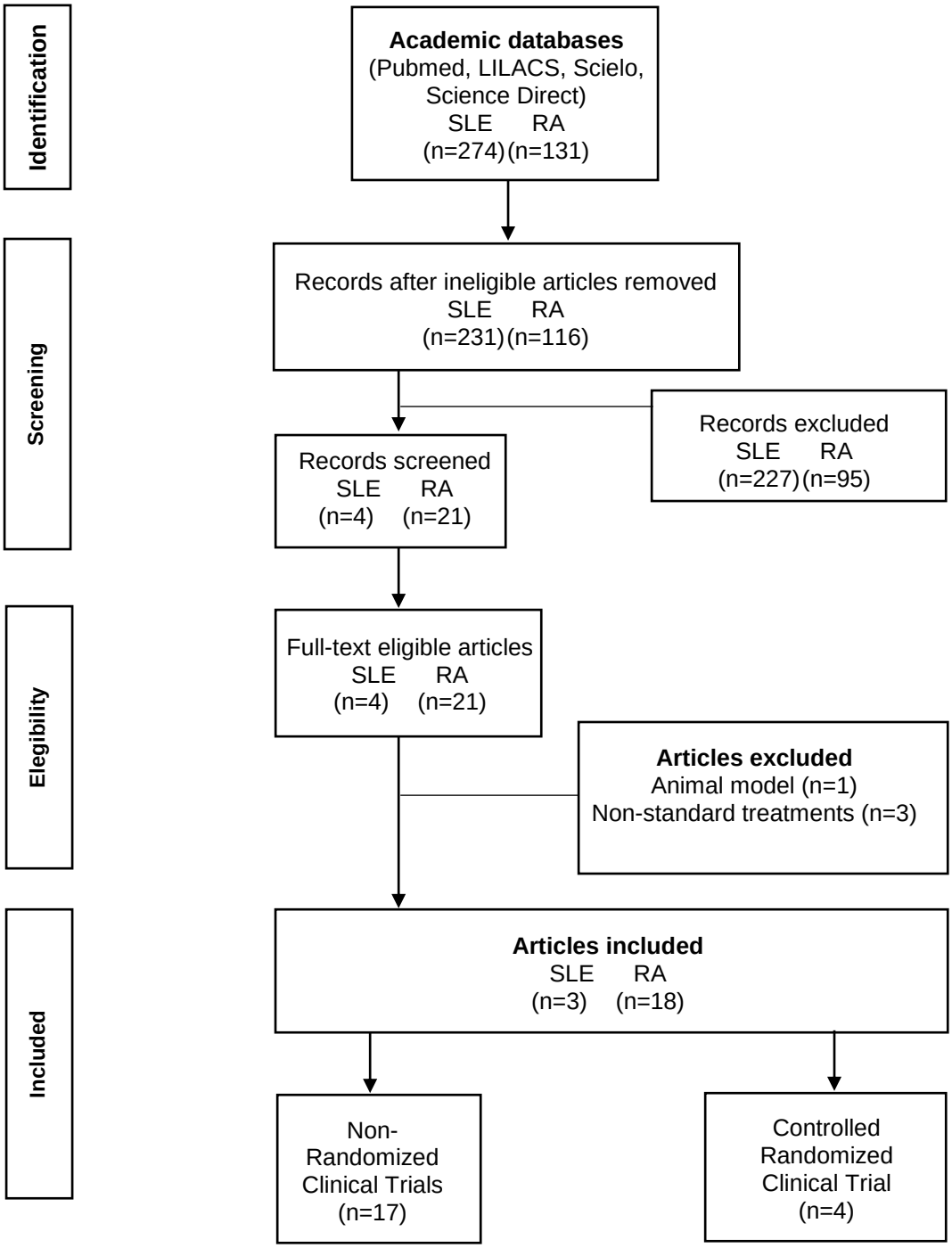

Fig. 2 Scheme for selection of the studies to be included in this review. SLE: Systemic Lupus Erythematosus; RA: Rheumatoid Arthritis; CAM: cell adhesion molecules.

List of abbreviations

ABA	Abatacept
ACR50	American College of Rheumatology improvement score
ADA	Adalimumab
APRIL	Proliferation-inducing ligand
AZA	Azathioprine
BILAG	British Isles Lupus Assessment Group
BLyS	B lymphocyte stimulator
BMI	Body mass index
cA2	Chimeric anti-tumor necrosis factor α monoclonal antibody
CAM	Cell adhesion molecules
CRP	C-reactive protein
CV	Cardiovascular
CY	Cyclophosphamide
DAS28	Disease Activity Score in 28 joints
DMARDs	Disease-modifying antirheumatic drugs
EMPs	Endothelial microparticles
EPC	Endothelial progenitor cells
EPZ	Epratuzumab
E-selectin	Endothelial selectin
ESR	Erythrocyte sedimentation rate
ETA	Etanercept
FLS	Fibroblast-like synoviocytes
GOL	Golimumab
HCAM	Homing cell adhesion molecule
HCQ	Hydroxychloroquine
HDL	High density lipoprotein
ICAM-1	Intercellular adhesion molecule-1
IFN	Interferon

IFX	Infliximab
Ig	Immunoglobulin
IGSF	Immunoglobulin supergene family
IL	Interleukin
LEF	Leflunomide
LFA-1	Lymphocyte function-associated antigen 1
Lp(a)	Lipoprotein(a)
MM	Mycophenolate mofetil
MTX	Methotrexate
NLRP3	NOD-like receptor containing protein 3
NP	Neuropsychiatric
oPRED	Oral prednisolone
oxLDL	Oxidized low density lipoprotein
PAI-1	Plasminogen activator inhibitor type-1
PBMCs	Peripheral blood mononuclear cells
PECAM-1	Platelet endothelial cell adhesion molecule 1
pMP	Pulse methylprednisolone
P-selectin	Platelet selectin
RA	Rheumatoid arthritis
RAI	Rheumatoid arthritis index
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RTX	Rituximab
SFMC	Synovial fluid mononuclear cells
SLE	Systemic lupus erythematosus
SLEDAI	SLE disease activity index
SSZ	Sulfasalazine
TACI	Transmembrane activator and calcium-modulator and cyclophilin ligand interactor
TCZ	Tocilizumab
tDMARD	Triple DMARD therapy

TNF-α	Tumor necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule-1

5.2 Artigo 2

Differential Profile Of Cellular Adhesion Molecules And Plasminogen Activator Inhibitor Type-1 In Systemic Lupus Erythematosus versus Rheumatoid Arthritis Are Associated With Metabolic Syndrome: A Machine Learning Study

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Abstract

The aims of the present study were to compare the cellular adhesion molecules (CAM) profile and plasminogen activator inhibitor type-1 (PAI-1) levels in Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA), and to examine the impact of the metabolic syndrome (MetS) on CAM. 104 SLE patients and 124 RA patients were enrolled. CAM and PAI-1 were determined by immunofluorimetry. MetS was determined by Adult Treatment Panel III. The diagnosis (SLE *versus* RA) had a high association on the CAM and PAI-1, explaining 53.9% of their variation, with impact in platelet endothelial cell adhesion molecule 1 (PECAM-1) 45.7% and vascular cell adhesion molecule-1 (VCAM-1) 38.3%. All 5 CAM and PAI-1 were significantly higher in SLE than in RA. MetS could interfere in 8.2% of variation of 6 molecules, while VCAM-1, E-selectin and PAI-1 were significantly higher in individuals with MetS. The results of the different machine learning techniques agreed that PECAM-1 and VCAM-1 were the most significant CAM differentiating SLE from RA and could predict SLE (sensitivity 96.8%, specificity 85.4%, AUC ROC of 0.956). In conclusion, our data demonstrated the differential CAM profile and PAI-1 levels in SLE and RA, and that PECAM-1 and VCAM-1 may be used to differentiate SLE *versus* RA patients as an external validating criterion. In addition, MetS were more frequent in SLE patients but it could influence CAM and PAI-1 plasma levels in both disease. The MetS components were associated with CAM and PAI-1 and could explain the differential cardiovascular risk in SLE and RA.

Keywords: Systemic Lupus Erythematosus. Rheumatoid Arthritis. Cellular adhesion molecules. Plasminogen activator inhibitor type 1. Metabolic Syndrome.

Introduction

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are chronic autoimmune diseases and the development of both diseases requires the combination of genetic susceptibility factors and environmental influences [1]. Despite many inflammatory factors involved in the pathophysiology of SLE and RA, it is known that cellular adhesion molecules (CAM) play an important role [2,3].

Among these CAM are platelet endothelial cell adhesion molecule 1 (PECAM-1) [4], vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endothelial and platelet selectin (E- and P-selectin) [5]. Changes in CAM levels have been demonstrated in SLE [2,4,6] and RA [7,8] and have been considered as potential biomarkers [3,4,8,9] of diagnosis and prognostic. CAM are strongly related to inflammation [6], endothelial dysfunction [10], onset of cardiovascular (CV) diseases [11], and metabolic syndrome (MetS) [12]. The pathogenetic factors of increased CV events in patients with autoimmune diseases is not yet fully understood. However, SLE, RA and MetS may develop interdependently because they have a shared immunopathogenesis, such as inflammatory process. So, the presence of MetS may provide a substantial link between accelerated atherosclerosis, inflammation and autoimmunity [13].

Therefore, endothelial dysfunction is characterized by increased of CAM and a shift towards prothrombotic activity of the endothelium with altered molecules, such as plasminogen activator inhibitor type-1 (PAI-1) [14]. In addition, SLE and RA patients have showed high frequency of MetS [15,16] that is characterized by endothelial dysfunction, central obesity, and insulin resistance.

A recent meta-analysis demonstrated a prevalence of MetS in SLE patients of 26.0% throughout the world with differences in the geographical areas: 21.0% in Europe, 25.0% in North America, 35.0% in South America, 26.0% in Asia, and 38.0% in Africa [15]. Another meta-analysis found an overall prevalence of MetS of 30.6% in RA [17]. Studies performed in the Brazilian population revealed a MetS prevalence of 45.2% and 51.3% in SLE and RA patients, respectively [18]. This variability can be explained by multiple factors, such as differences in the definition of MetS, ethnicity, geographic area, and study design [15,16]. We previously reported that MetS directly contributes to increased inflammatory potential and imbalance of CAM in SLE [4] and RA [8]. In addition, CAM and MetS has been associated with activity disease in SLE [9] and RA [7].

Although SLE and RA share clinical manifestation, those diseases are different in immunologic characteristics, cytokines profile and showed differential CV risk [13,19].

However, scarce studies compared some CAM and PAI-1 plasma levels in both diseases [11,20,21]. In addition, there are not studies that evaluated those molecules as biomarkers to differentiate those diseases. Thus, we hypothesized that CAM and PAI-1 could be involved in those differences between SLE and RA. This way, the main aims of the present study were to compare the CAM profile and PAI-1 plasma levels in SLE and RA, and to examine the impact of the MetS and their components on those molecules.

Subjects and Methods

Subjects

This study included 228 subjects, consisting of 104 SLE patients and 124 RA patients who were selected from ambulatory of Rheumatology of the University Hospital of Londrina, Paraná, Brazil. SLE and RA were diagnosed using the American College of Rheumatology (ACR) criteria (1997) [22] and the 2010 RA classification criteria [23], respectively. SLE patients did not have lupus nephritis in activity. None of the participants presented chronic renal disease, heart, thyroid, hepatic, gastrointestinal, oncological or other autoimmune disease and none of the patients were alcoholic according clinical and laboratory data.

MetS was defined following the Adult Treatment Panel III (ATP III) criteria. MetS is present if three or more of the following five criteria are met: 1) waist circumference (WC) over 94 cm in men and 80 cm in women, 2) fasting triglyceride (TG) levels greater than or equal to 150 mg/dL, 3) high density lipoprotein (HDL) lower than 40 mg/dL in men or 50 mg/dL in women; 4) blood pressure (BP) over 130/85 mmHg (or antihypertensive medication use), and 5) fasting glucose levels greater than or equal to 100 mg/dL or the use of antidiabetic medication [24].

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the Ethical committee of the University of Londrina, Paraná, Brazil approved all procedures involving SLE (CAAE 01865212.0.0000.5231) and RA patients (CAAE 06405812.1.0000.5231). Written informed consent was obtained from all subjects.

Anthropometric and Blood Pressure Measurements

Body weight was measured to the nearest 0,1 kg by using an electronic scale, with individuals wearing light clothing, but no shoes, in the morning. Height was measured to the nearest 0,1 cm by using a stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. WC was measured with a soft tape in the region between the last rib and the iliac crest, always in the standing position. Three BP measurements using a calibrated

sphygmomanometer were taken with a 1-min interval after the participant had been seated were recorded on the left arm. The mean of these measurements was used in the analysis. BP≥140/90 mmHg or use of antihypertensive medications were the considered criteria to define hypertension [25].

Biochemical and Hematological biomarkers

After fasting for 12 hours, the patients underwent the following laboratory blood analysis: glucose and lipid profile [TG, total cholesterol (CHOL), HDL, and low density lipoprotein (LDL)] were evaluated by a biochemical autoanalyzer (Dimension Dade AR, Dade Behring, Deerfield, IL, USA), using Dade Behring kits. Insulin was evaluated by chemiluminescence microparticle immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). Erythrocyte sedimentation rate (ESR) was obtained by automated kinetic-photometric method (Ves-MaticCUBE 30, DIESSE, Siena, Italy).

CAM and PAI-1 measurement

Plasma levels of PECAM-1, VCAM-1, ICAM-1, E-selectin, P-selectin and PAI-1 were determined by Human Magnetic Adhesion 6-Plex Panel according to the manufacturer (Novex Life Technologies, Frederick, United States of America), for Luminex® platform. The assay sensitivity for PECAM-1 and PAI-1 were <0.05 ng/mL, while for ICAM-1 was <5.00 ng/mL, for VCAM-1 was <0.50 ng/mL, for E-selectin was <1.00 ng/mL, and for P-selectin was <0.10 ng/mL. The inter-assay precision were 2.40% for PECAM-1, 3.70% for ICAM-1, 2.20% for VCAM-1, 1.80% for E-selectin, 1.90% for P-selectin, and 1.70% for PAI-1.

Statistics

To assess differences among groups in scale variables we used analyses of variance (ANOVA). In order to investigate differences among groups in nominal variables we employed analyses of contingency tables (X^2-test). Results of multiple comparisons were p-corrected for false discovery rate [26]. Univariate and multivariate general linear model (GLM) analyses were used to determine the effects of explanatory variables (diagnosis and MetS) on CAM and PAI-1 (6 molecules) levels as dependent variables, while adjusting for confounders (e.g. age, MetS, WC). Consequently, we used tests for between-subjects effects to delineate the effects of the explanatory variables on the 6 molecules. Differences in 6 molecules in both diagnostic groups (and subjects with and without MetS) were assessed

using model-generated estimated marginal mean (SE) values obtained by multivariate GLM analyses.

Multiple regression analysis was used to define the most significant 6 molecules that were associated with metabolic variables while adjusting for confounding variables, including age, MetS, WC, treatment with antihypertensive and hypoglycemic drugs. Binary logistic regression analysis was employed to delineate the most significant molecules that are associated with SLE *versus* RA. Nagelkerke values were used as estimates of effect size. We also computed the percentage of correctly classified cases with sensitivity and specificity and additionally the area under the Receiver Operating Curves (AUC ROC). Statistical analyses were performed using IBM SPSS Windows version 22. Tests were 2-tailed, and an alpha level of 0.05 indicated a statistically significant effect.

We computed z unit weighted composite scores, namely a) sum of the z scores of all CAM values (index of increased CAM levels; zCAM); b) z value of total cholesterol (zCHOL) – zHDL-cholesterol (zCHOL-HDL) as an index which reflects Castelli risk index 1, and z triglycerides (zTG) – zHDL (zTG_HDL), which is a validated index reflecting atherogenic index of plasma [27,28]; and c) zInsulin + zGlucose composite score (zGlucose + Insulin), which is an index of insulin resistance, and zInsulin - zGlucose (zInsulin_Glucose), an index of β cell function.

As machine learning techniques, we used Soft Independent Modeling of Class Analogy (SIMCA), Neural Networks (NN) and t-distributed stochastic neighbor embedding (t-SNE). SIMCA was used to model each class (SLE and RA) by a Principal Component (PCs) model, which best represents the class [29,30]. The number of PCs, which model each class, is defined by cross-validation. Consequently, the cases are projected in each class model and their distance from the class is computed and cases assigned to a class by comparing the distances from each model. As such, SIMCA is a class-modeling technique which describes the similarities between the cases in the classes. The modeling power of the features describes the impact to model each class variation. Features with a modeling power lower than 0.300, which indicates a low modeling power, may be omitted from the model (feature selection). SIMCA also computes the discriminatory power of all variables indicating the impact of features to classify objects in the different classes. SIMCA computes the model-to-model distances with a distance greater than 3 indicating qualitative differences between the modeled classes and a distance closer to 0 showing that there are no significant differences between the classes. SIMCA was performed using the Unscrambler X10.5 software program.

We also used a NN procedure, namely multilayer perceptron (MLP), to examine the complex non-linear relationships among input and output variables employing an automated feedforward architecture with CAM values (combined with age and metabolic variables) as input variables and diagnosis (SLE and RA) as output variables. One or two hidden layers were considered with a variable number of nodes (max 6). Error, rate of incorrect predictions based on training, testing and holdout samples as well as the partitioned confusion matrix for output diagnostic variables. We also computed the AUC ROC and the (relative) importance of the input variables in sensitivity analyses. Finally, we used t-SNE to visualize the data points of all cases in a two-dimensional plot.

Results

Descriptive statistics

Table 1 shows the socio-demographic and biomarkers data in patients with SLE *versus* RA. All significant differences among both study groups remained significant after p-correction for false discovery rate. Thus, patients with RA were somewhat older than SLE patients although there were no significant differences in the ratio caucasians *versus* non-Caucasians. While there were no significant differences in BMI and WC between both groups, the prevalence of MetS was significantly greater in SLE than in RA. There were highly significant differences in all 5 CAM and PAI-1 (6 molecules) between both groups with higher levels in SLE as compared with RA. There were no significant differences in ESR between both groups, while systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in RA than SLE. zTG_HDL and zInsulin_Glucose composite scores were significantly higher in SLE than in RA. There were no significant differences in zCHOL_HDL and zGlucose+Insulin composite scores between both groups.

Effects of diagnosis and MetS on the 6 molecules (CAM + PA-1)

In order to investigate the possible cumulative effects of diagnosis and MetS on the 6 molecules, while adjusting for age, WC and BMI, we performed multivariate GLM analysis with the 5 CAM and PAI-1 levels as dependent variables and diagnosis and MetS as primary explanatory variables and age, BMI and WC as additional explanatory variables. **Table 2** shows that diagnosis (SLE *versus* RA) had a much stronger impact (partial-eta squared=0.539) than MetS (8.2%), while age and WC also exerted moderate effect on 6 molecules (16.5% and 15.0%, respectively). Tests for between-subject effects showed that

diagnosis was associated with all 6 molecules, while MetS was significantly associated with VCAM-1, E-selectin, and PAI-1 but not with PECAM-1, ICAM-1, and P-selectin.

Table 3 shows the model-generated estimated marginal means of the z-transformed CAM scores in SLE *versus* RA and MetS, adjusted for age and WC. All 5 CAM and PAI-1 were significantly higher in SLE than in RA. VCAM-1, E-selectin and PAI-1 were significantly higher in individuals with the MetS as compared with those without MetS.

Effects of extraneous variables

Age was significantly associated with PECAM-1 and VCAM-1 (both positively) and E-selectin (negatively). WC was significantly associated with PECAM-1 and E-selectin (both positively). BMI was not significant in this regression analysis (F=1.51, df=6/181, p=0.176). Univariate GLM analysis showed that diagnosis explained an important part of the variance in z-unit CAM-score, namely 38.3%, whereas MetS and WC explained only 2.7% of the variance, and age was not significant.

In addition, we examined possible effects of drugs, namely in SLE patients: antimalarial medications (n=72), immunosuppressive (n=42), mycophenolate (n=23) and prednisolne (n=89). Multivariate GLM analysis with the 5 CAM and PAI-1 values as dependent variables showed that there were no significant effects of antimalarial medications, immunosuppressive drugs and prednisolone, while mycophenolate had a significant effect (F=3.25, df=6/86, p=0.006). Tests for between-subject effects showed that there was (without p=correction) a significant effect of mycophenolate on E-selectin only (higher in mycophenolate treated patients). However these differences disappeared after p-correction for false discovery rate (p=0.210). In RA patients 72 were treated with methotrexate, TNF inhibitor (n=28), leflunomide (n=55), prednisone (n=74), and antimalarial medications (n=43). There were no significant effects of these drugs on the 5 CAM and PAI-1 (as dependent variables in multivariate GLM analysis). We also examined the possible impact of treatments with glucocorticoids across SLE and RA patients combined. Adding the latter as an additional explanatory variable to the multivariate GLM analysis showed that this drug had no significant effect (F=1.34, df=6/181, p=0.240, partial-eta squared=0.043). In both RA and SLE combined, 90 participants were treated with antihypertensive and 20 with hypoglycemic drugs. Multivariate GLM analysis did not show and significant effects of antihypertensives and hypoglycemic drugs on the 5 CAM and PAI-1 values (data not shown).

Discrimination of SLE versus RA using logistic regression

Table 4 (BLRA#1) shows the results of logistic regression analysis with SLE and RA as dependent variables and the 6 molecules as explanatory variables. SLE was best predicted by increased levels of PECAM-1, VCAM-1 and P-selectin (X^2=143.99, df=3, p<0.001, Nagelkerke=0.700). 87.1% of all cases were correctly classified with a sensitivity of 87.8%, specificity of 86.5% and an AUC ROC curve of 0.956 (±0.020). In order to examine whether these results remained significant after considering the effects of MetS, WC and age, we have ran another logistic regression analysis with forced entry of these 3 confounding variables. BLRA #2 shows that PECAM-1 and VCAM-1 were highly significant predictors of SLE *versus* RA, while the effects of P-selectin were no longer significant after considering the effects of the three confounding variables, of which only age was significant.

Discrimination of SLE versus RA using Machine Learning techniques

Table 5 shows the results of SIMCA analysis. All 6 CAM yielded a significant modeling power for both SLE and RA (all values > 0.300). Also, the discriminatory power of all 6 CAM was significant, while VCAM-1, PECAM-1 and E-selectin were the top-3 features in SIMCA. The model-to-model SIMCA distance was significant, namely 5.8496.

The same table also shows the results of NN analysis. Automatic architecture training of the network delineated 2 hidden layers with 5 units in hidden layer 1 and 5 units in hidden layer 2. The hidden layers used hyperbolic tangent as activation function, while the output layer used identity as activation function. The sum of squares error was lower in the testing than in the training sample showing that the NN model learnt to generalize from the trend. The percentage of incorrect predictions was fairly constant in the training (9.0%), testing (6.3%) and holdout (9.7%) samples showing that the model is not overstrained (or overfitted). Table 5 shows the partitioned confusion matrices with a sensitivity of 96.8% and specificity of 85.4% in the holdout sample, whilst the AUC ROC curve was 0.956. **Figure 1** shows the impact of the input variables in the neural model as (relative) importance. P-selectin, PECAM-1 and VCAM-1 were the most important determinants of the predictive power of the NN model, followed at a distance by PAI-1.

t-SNE was used to visualize the underlying data structure of the 5 CAM and PAI-1 in a two-dimensional plot. **Figure 2** displays the distribution of the subjects' data points and also shows SLE and RA membership. While both SLE and RA subjects segregate at two different parts of the plot, there is an overlap among the two classes.

Associations between CAM, PAI-1, and components of MetS

In order to investigate the associations between the metabolic variables listed in Table 1 and CAM we have performed regression analyses with the metabolic variables as dependent variables and 6 molecules as explanatory variables, while adjusting for effects of MetS, WC, BMI, age and use of hypoglycemic or antihypertensive drugs, all entered as additional explanatory variables. **Table 6** shows the results of these regression analyses. We found that 23.0% of the variance in SBP (MRA #1) was explained by the regression on ICAM-1, RA, MetS and use of antihypertensive. 17.2% of the variance in DBP (MRA #2) was explained by the regression on ICAM-1, RA and MetS. 33.3% of the variance in zTG_HDL composite score (MRA #3) was explained by the regression on E-selectin and P-selectin and MetS. Up to 35.5% of the variance in zInsulin+Glucose score (MRA #4) was explained by MetS, WC, RA and use of hypoglycemic drugs. 25.8% of the variance in zInsulin_Glucose score (MRA #5) was explained by the regression on PAI-1, WC, RA, age and use of hypoglycemic drugs.

Discussion

The first major finding of this study is that diagnosis (SLE *versus* RA) had a high association on CAM and PAI-1, explaining 53.9% of their variation, with a particularly strong impact in PECAM-1 45.7% and VCAM-1 38.3%. All 5 CAM and PAI-1 were significantly higher in SLE than in RA after adjusting for possible effects of age, MetS, WC and treatment. MetS presence could interfere in 8.2% of variation of 6 molecules plasma levels, while VCAM-1, E-selectin and PAI-1 were significantly higher in individuals with the MetS as compared with those without MetS. The machine learning techniques (including SIMCA) proved that both groups are qualitatively different with respect to CAM and PAI-1 values and in fact all 6 molecules values contribute to the discrimination of both groups. Nevertheless, the results of the different machine learning techniques, such as SIMCA, NN and t-SNE, agreed that PECAM-1 and VCAM-1 were the most significant CAM differentiating SLE from RA. Using PECAM-1 and VCAM-1 levels (with or without the inclusion of the P-selectin, MetS, WC and age) we were able to establish a Neural Network model predicting increased risk towards SLE. These markers correctly classified SLE (sensitivity 96.8% and specificity 85.4%) with a discriminating power AUC ROC curve of 0.956. tSNE showed a good separation of both classes with some degree of overlap.

Due to the similarity of symptoms and concomitantly disease, the differential diagnosis and treatment of these two diseases is sometimes challenging. Laboratory tests may be performed using traditional serum markers but they are not always sensitive or specific

enough to detect ongoing disease activity and related clinical manifestations [31]. In this way, increased levels of CAM have demonstrated important prognostic value in rheumatic diseases [11]. In SLE, they have been able to discriminate between active and inactive disease [9]. On other hand, CAM seems to play an essential role in the generation of chronic synovitis [32] and to correlate with disease activity in RA [7]. Different serum profile of CAM could be associated with distinct histological appearances of rheumatoid synovitis [3] and could modulate in RA patients who respond clinically to drug treatment [33]. In the present study, we demonstrated that SLE had higher CAM and PAI-1 plasma levels than RA. In literature, patients with SLE have often demonstrated elevations on CAM values. Several authors found increased levels of PECAM-1 [4], ICAM-1 [34], VCAM-1 [35], E-selectin [2,34], P-selectin [36], and PAI-1 [37] significantly associated with SLE pathophysiology. VCAM-1 was previously validated to be a good serum marker of active lupus nephritis [35]. We previously reported that PECAM-1 was significantly associated with SLE and disease activity, suggesting that CAM play a role in SLE pathophysiology [4]. In addition, we also proposed a model to predict SLE diagnosis with PECAM-1 with high sensitivity and specificity [6].

In contrast, results on CAM in RA patients have been conflicting. Several studies have shown increased [7], unchanged [20] or decreased [8] CAM levels in RA patients. We previously reported that lowered VCAM-1 levels and increased PAI-1 levels were associated with RA, whilst ICAM-1, E-selectin and P-selectin levels were not altered [8]. Although several studies have evaluated CAM levels in SLE and RA, few studies have compared CAM differences in SLE *versus* RA. Santos and colleagues (2012) reported early endothelial changes in SLE and RA female patients and found higher ICAM-1 levels, but not VCAM-1 in SLE when compared with RA patients [11]. The authors suggested that differences in CAM in these two patient groups could explain the different CV risk. On the other hand, Pamuk and colleagues (2014) reported higher PECAM-1 levels in RA than in SLE [21]. However, those studies evaluated some CAM or a single one and not a profile such as in our study what could have restricted the phenomenon interpretation. Thus, in the present study, we demonstrated a differential CAM profile between SLE and RA.

There are some possible mechanisms explaining that different CAM profile: (a) Firstly, systemic inflammation is detected in SLE patients, while there is a more predominant local inflammation in synovial joints of RA patients [32]. (b) Secondly, the presence of lupus anticoagulant factor and anti-cardiolipin antibodies in SLE. These autoantibodies cross-react with multiple plasma and tissue antigens (anti beta-2-glycoprotein-I/oxLDL, anti-HDL, anti-apolipoprotein-A1) and have a prominent role in initiation, progression and acceleration of

atherosclerosis [38]. It is well known that atherogenesis is closely related to endothelial dysfunction and increased levels of CAM [10]. (c) Thirdly, SLE patients with an activated IFN type I system have impaired endothelial function, connecting central pathogenic processes in SLE with endothelial dysfunction and CV diseases. These patients, by IFN type I action, have repression of IL-1β expression and upregulation of NOD-like receptor 3 (NLRP3), caspase-1 and IL-18 in human endothelial progenitor cells (EPC) cultures, an important type of cell for vascular repair. This effect results in increased production of IL-18 that has inhibitory effects on EPC differentiation to mature endothelial cells. Therefore, the balance between IL-1β and IL-18 production via inflammasome activation may have important consequences for vascular health in SLE promoting atherosclerosis [39].

Our analyses of logistic regression and machine learning agreed that PECAM-1 and VCAM-1 were the most significant CAM differentiating SLE from RA. The proinflammatory feature of PECAM-1 mainly involves the final stage of leukocyte recruitment (transmigration), because this molecule favors the opening of endothelial junctions and promote chemokine-mediated leukocyte migration that directs leukocytes through the activation of integrins to the site of inflammation. This mechanism could explain the involvement of PECAM-1 with the diagnosis and prognosis of inflammatory diseases, such as SLE and RA [21]. Additionally, VCAM-1 mediates the contact and binding between cells or between the cells and stroma, and so, is also involved in trafficking of inflammatory cells and lymphocytes. Besides, it can interact with and influence several inflammatory cytokines, such as IL-4, IL-8 and TNF-α [2]. Therefore, VCAM-1 could affect some inflammatory reactions, which may be associated with occurrence and development of SLE and RA.

Our data are also in agreement with previous studies, which demonstrated that SLE is associated with the presence of MetS and a more atherogenic lipid profile, as measured with a higher atherogenic index of plasma (AIP) composite score [40]. The pathogenesis of MetS in SLE seems to involve chronic systemic inflammation, production of adipokines, and endothelial dysfunction [19]. It was previously reported that SLE [4,13] or RA [8,13]-linked inflammatory processes associated with MetS may affect CAM and PAI-1 levels. So, MetS presence could also increase CAM and PAI-1 plasma levels and worse inflammatory process. In the present study, we demonstrated that MetS could influence VCAM-1, E-selectin, and PAI-1 plasma levels in SLE and RA patients. The findings of the present study are in agreement with previous reports that have demonstrated associations between E-selectin and metabolic changes [41], and between VCAM-1 and presence of MetS [12]. Associations between CAM and MetS are expected because these molecules are regulated positively by

proinflammatory cytokines, such as IL-1β and TNF-α; and negatively by adiponectin. Circulating levels of proinflammatory cytokines are known to be increased in MetS, while adiponectin tend to be low in obese patients and in patients with MetS [13]. This phenomenon favors the elevation of CAM expression and the mean BMI of our patients showed that they were overweight and additionally 55.7% and 34.3% of SLE and RA patients, respectively, had MetS. Furthermore, we showed that WC, a measure of adiposity evaluated together with BMI, in SLE or RA patients had also a moderate effect on CAM levels. On other hand, high PAI-1 level is not surprising in patients with autoimmune diseases and MetS since this molecule was already associated with impaired endothelial function [37], and abdominal fat is a great source of PAI-1 [42]. Additionally, we previously reported that the evaluation of PECAM-1 and MetS together was associated with SLE pathophysiology [4], confirming the importance of PECAM-1 in the context of autoimmune diseases associated with metabolic disorders. A moderate effect of age was also demonstrated on CAM levels, possibly due to the endothelial dysfunction observed in aging and caused by increased inflammation and oxidative stress [43].

In addition, in the present study, we did not find differences in insulin resistance between both SLE and RA, but observed that an index of β cell function (zInsulin_Glucose composite score) was higher in SLE than in RA. Chung and colleagues (2008) reported that RA and SLE are both associated with IR as evaluated with homeostasis model assessment (HOMA) index, but the pathogenesis mechanisms and relationship with inflammation and coronary atherosclerosis may differ. They found that the major contributing factors to IR were BMI in SLE, and IL-6 and TNF-α in RA [44]. Thus, these different factors may be modulating β cell function in these two diseases. Additionally, drug intake, particularly prednisone and hydroxychloroquine, may have influenced these metabolic variables. In SLE, corticosteroids have been shown to decrease glucose tolerance by increasing hepatic glucose production and decreasing peripheral glucose uptake via a decrease in skeletal muscle and adipose tissue insulin sensitivity [45]. Moreover, RA patients showed higher SBP and DBP than SLE patients. In accordance with our finding, a study demonstrated high SBP in adjuvant-induced arthritis (AIA) rats treated with etanercept, a TNF-α inhibitor [46]. The underlying mechanisms of etanercept on endothelial function are still unknown, but hypertension is a little-known side effect of anti-TNF-α therapy in RA [47]. In addition, in the present study 28 patients was taking etanercept.

Although several studies have reported the influence of drugs traditionally used in the treatment of SLE and RA patients on levels of CAM and PAI-1 [33,48], our findings did not

show this data. A possible explanation for this could be the fact that these articles consist of follow-up studies in patients usually with active disease, and often with maintenance of treatment until achieving the remission. Meanwhile, our design consisted of a cross-sectional study with most patients presenting low disease activity index and undergoing to specific treatment for at least 6 months.

Finally, we evaluated which components of MetS could be associated with CAM and PAI-1 levels and we observed that SBP and DPB were associated with RA and ICAM-1 levels. Similarly, Ibrahim and colleagues (2013) investigated the effect of leptin administration in pregnant rats and found higher SBP and ICAM-1 levels. Thus, obesity seems to be correlated with endothelial activation and altered metabolic markers [49]. For atherogenic index, we found that changes in zTG_HDL score were associated with E- and P-selectin levels. Our data are in agreement with Adamska and colleagues (2014) study that demonstrated higher E-selectin levels in obese patients and higher E-selectin levels correlated positively with BMI, WC, percentage of body fat and plasma TG levels [41]. For the indexes related to glucose metabolism, we showed only PAI-1 as a marker for zInsulin_Glucose score, since adipokines, such as PAI-1, are elevated in patients with obesity, insulin resistance, and type 2 diabetes [42].

In the present study, the cross-sectional design does not allow for inference causality and this is a limitation that may be considered. Some strengths also deserves to be mentioned including the robust statistical analysis and the simultaneous evaluation of two autoimmune diseases SLE and RA. Furthermore, our study was designed to control for the cumulative effects of diagnosis, age, MetS, WC and treatment for SLE and RA in the included subjects.

To the best of our knowledge, this is the first human study to demonstrate associations between CAM levels and components of MetS: (a) SBP was explained by RA, MetS, ICAM-1 and use of antihypertensive drugs; (b) DBP was explained by RA, MetS and ICAM-1; (c) zTG_HDL composite score was explained by MetS, E-selectin and P-selectin; (d) zInsulin+Glucose score was explained by WC, MetS, RA and use of hypoglycemic drugs; and (e) zInsulin_Glucose score was explained by age, RA, WC, PAI-1 and use of hypoglycemic drugs. Together, our data suggested differential CAM profile and PAI-1 plasma levels could explain, in part, the different CV risk in SLE and RA.

Conclusions

Our data demonstrate that CAM were strongly associated with SLE pathophysiology and exhibit different behaviors in SLE and RA. Besides to the classical forms of biomarkers

already reported in the literature and often used in clinical management, which allows to distinguish between SLE and RA, we also propose a model to contribute in this complex differentiation. This model was based on PECAM-1 and VCAM-1 with high sensibility and specificity. These results show that this CAM profile and PAI-1 may be used to differentiate SLE *versus* RA patients as an external validating criterion. In addition, MetS were more frequent in SLE patients but it could influence CAM and PAI-1 plasma levels in both disease, and it should be evaluated concomitantly with them. The MetS components were associated with CAM and PAI-1 and could explain the differential cardiovascular risk in SLE and RA.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests.

Ethical 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 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent

All the participants included in this study provided written informed consent.

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Table 1. Socio-demographic and biomarker data in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Variables	SLE (n= 104)	RA (n=124)	F/X^2	df	p value
Age (years)	43.9 (±11.9)	50.7 (±9.7)	15.65	1/203	**<0.001**
Caucasian/Not-caucasian	62/42 (59.6/40.4)	78/46 (62.9/37.1)	0.14	1	0.708
BMI kg/m^2	28.3 (±5.7)	28.5 (±5.9)	0.08	1/199	0.771
Waist circumference (cm)	93.7 (±13.8)	93.1 (±13.7)	0.11	1/196	0.740
MetS (No/Yes)	45/59 (43.3/56.7)	84/40 (6.7/32.3)	9.33	1	**0.002**
PECAM-1 (pg/mL)	39,973 (±10,619)	24,089 (±7,997)	162.46	1/204	**<0.001**
ICAM-1 (pg/mL)	994,202 (±1,787,781)	451,800 (±892,397)	9.91	1/204	**0.002**
VCAM-1 (pg/mL)	967,217 (±422,488)	549,115 (±221,044)	111.92	1/204	**<0.001**
E-selectin (pg/mL)	201,136 (±110,098)	109,878 (±74,179)	69.38	1/204	**<0.001**
P-selectin (pg/mL)	167,033 (±74,735)	106,056 (±57,728)	51.25	1/204	**<0.001**
PAI-1 (pg/mL)	115,101 (±101,040)	80,681 (±47,499)	5.27	1/204	**<0.001**
ESR (mm/h)	28.4 (±26.2)	24.9 (±22.6)	0.58	1/135	0.446
SBP (mmHg)	122.5 (±16.7)	135.1 (±18.8)	25.18	1/198	**<0.001**
DBP (mmHg)	79.2 (±12.6)	88.3 (±13.7)	24.23	1/198	**<0.001**
zTG_HDL	+0.29 (1.65)	-0.27 (1.72)	5.52	1/201	**0.020**
zCHOL_HDL	-0.03 (1.21)	+0.12 (1.37)	0.08	1/201	0.772
zGlucose + Insulin	-0.13 (1.45)	+0.09 (1.78)	0.91	1/198	0.342
zInsulin_Glucose	+0.33 (1.13)	-0.36 (1.06)	19.76	1/198	**<0.001**

Data are shown as mean (±SD). F/X^2: values of ANOVA (F), analysis of contingency tables (X^2). SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; BMI: body mass index; MetS: metabolic syndrome; PECAM-1: platelet endothelial cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1; ESR: erythrocyte

sedimentation rate; SBP: systolic blood pression; DBP: diastolic blood pression; TG: triglycerides; HDL: high density lipoprotein; CHOL: cholesterol.

zTG_HDL: computed as z-score of trygliceride (zTG) – zHDL (atherogenic index of plasma).

zCHOL_HDL: computed as z-score of cholesterol (zCHOL) – zHDL (index for CHOL/HDL ratio).

zGlucose + Insulin: computed as z score of glucose (zGlucose) + zInsulin (index of insulin resistance).

zInsulin_Glucose: computed as zInsulin – zGlucose (index of β cell function).

Table 2. Results of multivariate GLM analysis with 6 adhesion molecules as dependent variables.

Tests	Dependent variables	Explanatory variables	F	df	p value	Partial Eta squared
Multivariate	6 molecules	SLE – RA	35.66	6/183	<0.001	**0.539**
	(5 CAM + PAI-1)	MetS	2.72	6/183	0.015	0.082
		Age	6.04	6/183	<0.001	0.165
		Waist Circumference	5.39	6/183	<0.001	0.150
Between-subject effects	PECAM-1	SLE – RA	158.51	1/188	<0.001	**0.457**
		Age	4.87	1/188	0.029	0.025
		Waist Circumference	7.09	1/188	0.008	0.036
	ICAM-1	SLE – RA	5.32	1/188	0.022	0.028
	VCAM-1	SLE – RA	116.86	1/188	<0.001	**0.383**
		MetS	4.02	1/188	0.046	0.021
		Age	11.78	1/188	0.001	0.059
	E-selectin	SLE – RA	53.29	1/188	<0.001	**0.221**
		MetS	9.33	1/188	0.003	0.047
		Age	4.16	1/188	0.043	0.022
		Waist Circumference	20.20	1/188	<0.001	0.097
	P-selectin	SLE – RA	58.10	1/188	<0.001	**0.236**
	PAI-1	SLE – RA	5.38	1/188	0.021	0.028
		MetS	4.57	1/188	0.034	0.024
Univariate	z-unit CAM score	SLE – RA	116.66	1/188	<0.001	**0.383**
		MetS	5.31	1/188	0.022	0.027
		Age	0.06	1/188	0.810	0.000
		Waist Circumference	5.20	1/188	0.024	0.027

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MetS: metabolic syndrome; PECAM-1: platelet endothelial cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1; z-unit CAM score: computed as the sum of all z scores of the 6 adhesion molecules (index of concentrations of all 6 adhesion molecules together).

Table 3. Model-generated estimated marginal means (SE) of z scores of serum adhesion molecules in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) divided into those with (MetS) and without (No MetS) metabolic syndrome.

Variables	SLE	RA
PECAM-1	+ 0.752 (0.078)	- 0.668 (0.075)
ICAM-1	+ 0.164 (0.105)	- 0.185 (0.100)
VCAM-1	+ 0.705 (0.084)	- 0.602 (0.080)
E-selectin	+ 0.504 (0.085)	- 0.389 (0.081)
P-selectin	+ 0.554 (0.090)	- 0.436 (0.086)
PAI-1	+ 0.235 (0.104)	- 0.113 (0.099)
Z-unit CAM score	+ 2.919 (0.342)	- 2.392 (0.326)
Variables	**No MetS**	**MetS**
VCAM-1	- 0.078 (0.083)	+ 0.181 (0.088)
E-selectin	- 0.142 (0.084)	+ 0.257 (0.089)
PAI-1	- 0.110 (0.103)	+ 0.232 (0.108)
z unit CAM score	- 0.344 (0.337)	+ 0.865 (0.356)

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MetS: metabolic syndrome; PECAM-1: platelet endothelial cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1.

z-unit CAM score: computed as the sum of all z scores of the 6 adhesion molecules (index of concentrations of all 6 adhesion molecules together).

Table 4. Results of binary logistic regression analyses (BLRA) with Systemic Lupus Erythematosus (SLE) as dependent variable (Rheumatoid Arthritis as reference group).

Tests	Explanatory Variables	Wald	df	p value	OR	CI 95%
BLRA #1	PECAM-1	19.14	1	<0.001	5.17	2.48 – 10.78
	VCAM-1	13.57	1	<0.001	3.79	1.86 – 7.70
	P-selectin	6.19	1	0.013	2.06	1.17 – 3.63
BLRA #2	PECAM-1	14.84	1	<0.001	5.75	2.36 – 13.99
	VCAM-1	13.29	1	<0.001	5.45	2.19 – 13.57
	P-selectin	2.07	1	0.150	1.60	0.84 – 3.05
	Waist Circumference	0.55	1	0.458	0.98	0.95 – 1.03
	MetS	1.99	1	0.158	2.22	0.73 – 6.70
	Age	15.36	1	<0.001	0.89	0.84 – 0.94

OR: odds ratio; CI: confidence interval; PECAM-1: platelet endothelial cell adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; MetS: metabolic syndrome.

BLRA#1: X^2=143.99, df=3, p<0.001, Nagelkerke=0.700. 87.1% of all cases were correctly classified with a sensitivity of 87.8%, specificity of 86.5% and an AUC ROC curve of 0.956 (±0.020).

BLRA#2: forced entry of these 3 confounding variables (MetS, age, and waist circumference).

Table 5. Results of Soft Independent Modeling of Class Analogy (SIMCA) analysis performed with the 6 cell adhesion molecules (CAM) as modelling/discriminatory features and results of Neural Network (NN) analysis separating systemic lupus erythematosus (SLE) from rheumatoid arthritis (RA).

SIMCA	Model to Model distance	PECAM-1	ICAM-1	VCAM-1	E-selectin	P-selectin	PAI-1
Modelling power RA		0.5991	0.9956	0.8438	0.7601	0.9360	0.9646
Modelling power SLE		0.4882	0.9459	0.9385	0.6161	0.5180	0.7723
Discriminatory power	5.8496	2.6778	1.8776	2.7979	2.6110	1.9459	1.8831

NN Output: SLE versus RA	NN samples	Sum of squares error	% incorrect predictions	Observed	Predicted	AUC ROC
Input: 6 CAM	**Training**	7.582	9.0%	RA	87.2%	
				SLE	95.2% (overall: 91.0%)	
	Testing	1.847	6.3%	RA	100.0%	0.956
				SLE	88.2% (overall: 93.8%)	
	Holdout	-	9.7%	RA	85.4%	
				SLE	96.8% (overall: 90.3%)	

CAM: cellular adhesion molecules; PECAM-1: platelet endothelial cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1.

Table 6. Results of automatic multiple regression analysis (MRA) with metabolic variables as dependent variables and adhesion molecules as explanatory variables.

Tests	Dependent variables	Explanatory variables	t	p value	F	df	p value	R^2
MRA #1	SBP	RA	+ 6.52	<0.001	13.79	4/185	<0.001	0.230
		MetS	+ 2.64	0.009				
		AH drugs	+ 2.43	0.016				
		ICAM-1	+ 1.98	0.049				
MRA #2	DBP	RA	+ 5.77	<0.001	12.87	3/186	<0.001	0.172
		MetS	+ 2.95	0.004				
		ICAM-1	+ 2.66	0.009				
MRA #3	zTG_HDL	MetS	+ 6.50	<0.001	30.99	3/186	<0.001	0.333
		E-selectin	+ 4.33	<0.001				
		P-selectin	- 2.58	0.011				
MRA #4	zInsulin + Glucose	Waist circumference	+ 4.15	<0.001	25.34	4/184	<0.001	0.355
		Hypoglycemic drugs	+ 3.91	<0.001				
		MetS	+ 4.42	<0.001				
		RA	+ 2.02	0.044				
MRA #5	zInsulin_Glucose	Age	- 3.92	<0.001	12.74	5/183	<0.001	0.258
		SLE	+ 3.59	<0.001				
		Waist circumference	+ 4.02	<0.001				
		Hypoglycemic	- 3.58	<0.001				

	drugs		
	PAI-1	- 2.21	0.028

RA: rheumatoid arthritis; MetS: metabolic syndrome; SBP: systolic blood pression; DBP: diastolic blood pression; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1; TG: triglycerides; HDL: high density lipoprotein; AH drugs: antihypertensive drugs.

zTG_HDL: computed as z-score of trygliceride (zTG) – zHDL (atherogenic index of plasma).

zGlucose + Insulin: computed as z score of glucose (zGlucose) + zInsulin (index of insulin resistance).

zInsulin_Glucose: computed as zInsulin – zGlucose (index of β cell function).

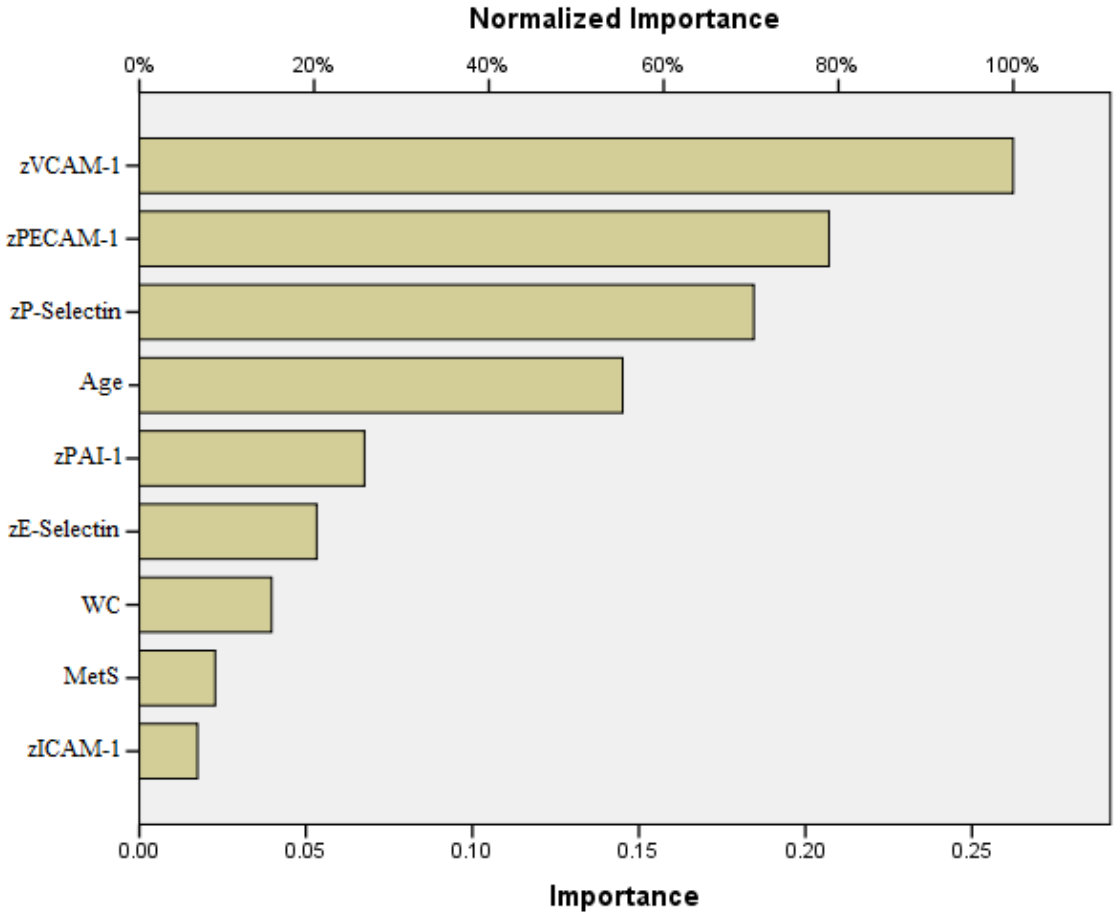

Fig. 1 Impact of the input variables in the neural network (NN) model as (relative) importance. VCAM-1: vascular cell adhesion molecule 1; PECAM-1: platelet endothelial cell adhesion molecule 1; PAI-1: plasminogen activator inhibitor-1; WC: Waist Circumference; MetS: Metabolic Syndrome; ICAM-1: intercellular adhesion molecule 1.

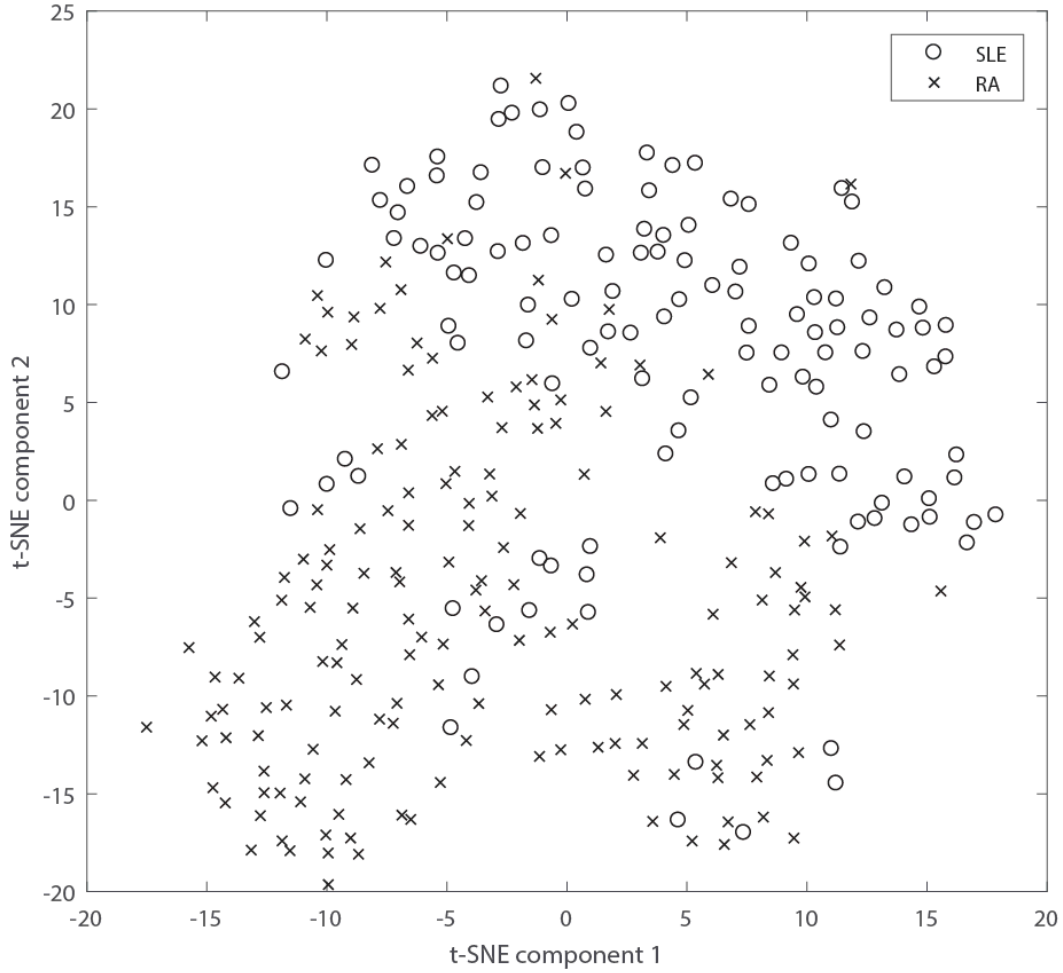

Fig. 2 Distribution of the subjects' data points based on t-distributed stochastic neighbor embedding (t-SNE) model to visualize the underlying data structure of the 5 CAM and PAI-1 in a two-dimensional plot. SLE: systemic lupus erythematosus; RA: rheumatoid arthritis.

6. CONCLUSÃO

A presente tese permite as seguintes conclusões de acordo com os objetivos propostos e os resultados obtidos nos dois artigos:

Artigo 1:

- Os medicamentos mais avaliados nos artigos analisados foram ciclofosfamida e pulso de metilprednisolona no LES; e metotrexato e infliximabe na AR;
- As MACs mais utilizadas para avaliar a resposta ao tratamento foram VCAM-1 no LES; e ICAM-1, VCAM-1 e E-selectina na AR;
- ICAM-1, VCAM-1 e E-selectina foram as moléculas frequentemente determinadas para avaliação da resposta ao tratamento.
- Os pacientes com LES ou AR submetidos a tratamento específico demonstraram diminuição significativa nos níveis de MACs, aparentemente relacionados ao sucesso da terapia, indicando que esses poderiam ser bons biomarcadores para o monitoramento do tratamento.

Artigo 2:

- Pacientes com LES apresentaram níveis plasmáticos das MACs (PECAM-1, ICAM-1, VCAM-1, E-Selectina, P-Selectina) e do PAI-1 significativamente maiores quando comparados aos pacientes com AR, independentemente das variáveis confundidoras idade, SM, CA e tratamento;
- O diagnóstico de LES/AR pode explicar 53,9% da variação das MACs e PAI-1 nestas doenças, sendo o maior efeito na PECAM-1 (45,7%) e VCAM (38,3%);
- A presença de SM pode explicar 8,2% da variação das MACs e PAI-1, e pacientes com SM possuem níveis plasmáticos elevados de VCAM-1, E-selectina e PAI-1;
- O modelo de regressão logística binária proposto utilizou PECAM-1, VCAM-1 e P-selectina, e foi capaz de diferenciar casos de LES daqueles de AR, classificando corretamente 87,1% de todos os casos, com sensibilidade de 87,8%, especificidade de 86,5% e AUC ROC de 0,956;
- Nossas análises mostraram que ambos os grupos são qualitativamente diferentes em relação aos valores de MACs e que esses valores contribuem para a discriminação de ambos os grupos. Os resultados das análises de *machine learning* confirmaram que PECAM-1 e

VCAM-1 foram as MACs mais significativas que diferenciaram o LES da AR e puderam predizer o LES com sensibilidade de 96,8%, especificidade de 85,4% e curva AUC ROC de 0,956);

- As análises de regressão demonstraram associação entre MACs e PAI-1, e os componentes da SM. A PAS/PAD, zTG_HDL (índice aterogênico do plasma) e zInsulina_Glicose (índice da função da célula β) estão associadas aos níveis plasmáticos de ICAM-1, E-selectina e P-selectina, e PAI-1, respectivamente.

7. CONSIDERAÇÕES FINAIS

Algumas limitações no presente estudo devem ser consideradas. Primeiro, o estudo de revisão e o desenho transversal do artigo original não permitem a inferência de causalidade. Entretanto, alguns pontos fortes também merecem ser mencionados, incluindo a análise estatística robusta e a avaliação simultânea de duas doenças autoimunes (LES e AR). Além disso, nosso estudo foi desenhado para controlar os efeitos cumulativos do diagnóstico, SM, CA e idade.

Nossos dados demonstraram que as MACs estão fortemente associadas à fisiopatologia do LES e exibem comportamentos diferentes no LES e na AR. Além disso, a presença de SM pode influenciar os níveis de MACs em ambas as doenças autoimunes. Além biomarcadores tradicionais já relatados na literatura e frequentemente utilizados no manejo clínico do paciente com LES ou AR, também mostramos que as MACs podem ser utilizadas para diferenciação do diagnóstico de LES e AR a partir de um modelo preditor baseado nos níveis séricos de PECAM-1, VCAM-1 e E-selectina. Portanto, a associação de um perfil ed MACs com os atuais critérios do LES ou AR poderiam ser úteis para o diagnóstico e monitoramento dessas doenças. Além disso, sugerimos, de acordo com a revisão de literatura realizada, que as MACs também podem ser utilizadas como marcadores de resposta ao tratamento e condição das doenças, favorecendo estratégias para alcançar a remissão e evitar desfechos e comorbidades típicos em longo prazo. Novos estudos são necessários para melhor compreender o papel das MACs na fisiopatologia do LES e AR, permitindo sua aplicação no contexto clínico e no desenvolvimento de novos alvos terapêuticos.

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APÊNDICES

APÊNDICE A

Ficha de avaliação dos pacientes.

FICHA DE AVALIAÇÃO - PROJETO LES

NOME:	PRONTUÁRIO:
DATA NASC:	CAUCASIANO ( ) NAO CAUC ( )
END:	TEL:
MEDICAMENTOS PREDNISONA: HIDROXICLOROQUINA/CLOROQUINA: METOTREXATE: AZATIOPRINA: MICOFENOLATO MOFETIL: OUTROS IMUNOSSUPRESSORES: OUTROS:	
OUTRAS DOENÇAS: HAS SIM ( ) NÃO ( ) DIABETES SIM ( ) NÃO ( ) AVC/IAM SIM ( ) NÃO ( ) OUTROS:	
NL SIM ( ) NÃO ( ) OBS:	
TEMPO DE DOENÇA:	
ESCORE SLEDAI:	
TABAGISMO: SIM ( ) NÃO ( )	
ATIVIDADE FÍSICA: SIM ( ) NÃO ( )	

PESO	ALTURA	IMC	CIRC. ABDOMINAL	PRESSÃO ARTERIAL

APÊNDICE B

FICHA DE AVALIAÇÃO DOS PACIENTES COM ARTRITE REUMATOIDE

NOME:_____
RG: _____
IDADE ou DN: _____
SEXO: feminino ( ) masculino ( ) Etnia_____

TEMPO DE DIAGNÓSTICO: _____ DAS 28: _____

COMPROMETIMENTO SISTÊMICO EXTRA-ARTICULAR:
Pulmonar ( ) / Vasculite ( ) / Ocular ( ) / Nódulos reumatoides ( ) / Cardíaco ( ) / SNC ( )

OUTRAS DOENÇAS:
HAS ( ) / DM ( ) / dislipidemia ( ) / IAM ( ) / AVE ( ) / depressão ( )
Outros:_____
Outra colagenoses: _____

MEDICAÇÕES:
( ) Prednisona – dose: _____
( ) Metotrexate – dose: _____
( ) Hidroxicloroquina/Cloroquina – dose: _____
( ) Sulfassalazina – dose: _____
( ) Leflunomide – dose: _____
( ) Etanercepte – dose: _____
( ) Adalimumabe – dose: _____
( ) Infliximabe – dose: _____
( ) Golimumabe – dose: _____
( ) Certolizumabe pegol – dose: _____
( ) Tocilizumabe – dose: _____
( ) Abatacepte – dose: _____
( ) Rituximabe – dose: _____
( ) Ciclofosfamida – dose: _____
( ) Tofacitinibe – dose: _____
( ) Outros: _____

TABAGISMO: sim ( ) não ( )

ATIVIDADE FÍSICA: sim ( ) não ( )
Tipo: _____ frequência: _____ há quanto tempo: _____

DADOS ANTROPOMÉTRICOS

Altura (cm)	**Peso (Kg)**	**IMC (Kg/m2)**	**Circunferência abdominal (cm)**	**Pressão arterial (mmHg)**

APÊNDICE C

Termo de Consentimento Livre e Esclarecido

Título da pesquisa:
"Associação entre polimorfismos genéticos e a susceptibilidade ao Lúpus Eritematoso Sistêmico em pacientes atendidos no Ambulatório do Hospital de Clínicas da Universidade Estadual de Londrina, Londrina, Paraná"

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo (a) a participar da pesquisa **"Associação entre polimorfismos genéticos e a susceptibilidade ao Lúpus Eritematoso Sistêmico (LES) em pacientes atendidos no Ambulatório do Hospital de Clínicas da Universidade Estadual de Londrina, Londrina, Paraná,"** realizada no "Hospital Universitário da Universidade Estadual de Londrina (HU/UEL), Londrina, Paraná". O objetivo da pesquisa é "determinar se existe associação entre fatores genéticos do indivíduo e a chance de desenvolver LES e se existe associação com o quadro clínico da doença". A sua participação é muito importante e ela se daria da seguinte forma: no momento da entrada no projeto de pesquisa, será realizada uma avaliação clínica e coleta de 20 mL de sangue periférico para realização de exames laboratoriais relacionados ao LES, e uma entrevista para você fornecer informações sobre estilos de vida como dieta e exercícios físicos. Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos, ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

As amostras de sangue coletadas serão identificadas por códigos com letra e número garantindo o absoluto sigilo e confidencialidade dos resultados. Após sua utilização, as amostras serão armazenadas em *freezer* sob a responsabilidade do pesquisador responsável para outros estudos genéticos relacionados ao LES.

A participação no projeto não apresenta riscos ao (a) senhor (a) e a população poderá ser beneficiada com os resultados obtidos, caso a equipe de pesquisa determine fatores genéticos que possam estimar a chance de um indivíduo desenvolver a doença ou a chance de um

indivíduo previamente com a doença em desenvolver quadros clínicos mais graves como a NL.

Informamos que o(a) senhor(a) não pagará 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 na pesquisa.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contatar: **Professora Dra. Andrea Name Colado Simão, no Setor de Imunologia Clínica do Laboratório de Análises Clínicas do HU/UEL, fone 43-3371-2321, e-mail:** deianame@yahoo.com.br**,** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490.

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

Londrina, _____ de _____ de 2018.

Pesquisador Responsável: Profa. Dra. Andrea Name Colado Simão

1. RG: 6226736-4

_____, tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____

Data:_____

Obs: Caso o participante da pesquisa seja menor de idade, deve ser incluído o campo para assinatura do menor e do responsável.

APÊNDICE D

Termo de Consentimento Livre e Esclarecido

Título da Pesquisa:
"Avaliação do estresse oxidativo, fatores de risco cardiovasculares e frequência de síndrome metabólica em pacientes com artrite reumatoide"

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo(a) a participar da pesquisa **"AVALIAÇÃO DO ESTRESSE OXIDATIVO, FATORES DE RISCO CARDIOVASCULARES E FREQUÊNCIA DE SÍNDROME METABÓLICA EM PACIENTES COM ARTRITE REUMATOIDE"**, realizada no "Hospital Universitário de Londrina (HU), da Universidade Estadual de Londrina". O objetivo da pesquisa é os fatores de risco cardiovasculares em pacientes com artrite reumatoide. A sua participação é muito importante e ela se daria da seguinte forma: avaliação clínica pelo médico reumatologista e coleta de sangue. Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas para os fins dessa pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar sua identidade.

Serão realizados testes laboratoriais para a confirmação do diagnóstico e prognóstico da artrite reumatoide, determinação da atividade da doença, perfil metabólico, resposta imunológica, estresse oxidativo e outras análises que se façam necessárias.

Os benefícios esperados são: 1) o conhecimento da prevalência de síndrome metabólica em pacientes com artrite reumatoide, de uma amostra da população brasileira, permite estratificação de risco cardiovascular, o que implica em manejo mais adequado de acordo com as características da nossa população; 2) o envolvimento da fisiopatologia que envolve as alterações do estresse oxidativo e inflamação na artrite reumatoide possibilita melhor monitoramento da doença e desenvolvimento de novas intervenções medicamentosas.

Informações que o(a) senhor(a) não pagará 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 na pesquisa.

Caso você tenha dúvida ou necessite de maiores esclarecimentos, pode nos **contactar (médica reumatologista – Tatiana Mayumi Veiga Iriyoda 3371-2218)**, ou procurar o

Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Koch nº 60, ou no telefone 3371-2490. Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas devidamente preenchida e assinada entregue a você.

Londrina, _____ de _____ de 2018.

- Pesquisadores Responsáveis:

Tatiana Mayumi Veiga Iriyoda (telefone: 9615-5476)
RG: 197.669-5
Andréa Name Colado Simão (telefone: 9627-8181)
RG: 6.226.736-4

_____ (nome por extenso do sujeito da pesquisa), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar voluntariamente da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica): _____

Data: _____

Obs.: Caso o participante da pesquisa seja menor de idade, deve ser incluído o campo para assinatura do menor e do responsável.

ANEXOS

ANEXO A

Escore SLEDAI - Índice de atividade da doença Lúpus Eritematoso Sistêmico

A descrição deve estar presente na visita ou nos ùltimos 10 dias.

SLE Daily Activity Index: Data Collection Sheet

SLEDAI Score	Descriptor	Definition
8	Seizures	Recent onset. Exclude metabolic, infectious or drug causes.
8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized or catatonic behavior. Exclude uremia and drug causes.
8	Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness or increased or decreased psychomotor activity. Exclude metabolic, infection or drug causes.
8	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid or optic neuritis. Exclude hypertension, infection or drug causes.
8	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	Lupus headache	Severe, persistent headache: may be migrainous, but must be nonresponsive to narcotic analgesia.
8	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infraction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	Arthritis	More than 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4	Urinary casts	Heme-granular or red blood cell casts.
4	Hematuria	>5 red blood cells high power field. Exclude stone, infection or other cause.
4	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	New rash	New onset or recurrence of inflammatory type rash.
2	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	Mucosal ulcers	New onset or recurrence of oral or nasal ulcerations.
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion or electrocardiogram or echocardiogram confirmation.
2	Low complement	Decrease in CH50, C3 or C4 below the lower limit of normal for testing laboratory.
2	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory.
1	Fever	>38°C. Exclude infectious cause.
1	Thrombocytopenia	<100.000 platelets/mm^3.
1	Leukopenia	<3,000 white blood cells/mm^3. Exclude drug causes.

TOTAL SLEDAI SCORE: _____

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ANEXO B

Escore ECLAM - Medida de Atividade de Lúpus do Consenso Europeu

EUROPEAN CONSENSUS LUPUS ACTIVITY MEASUREMENT INDEX (ECLAM)		
Generalized manifestations	Fever, fatigue	0.5
Articular manifestations	Arthritis, evolving arthralgia	1
Active mucocutaneous manifestations	Malar rash, generalized rash, discoid rash, skin vasculitis, oral ulcers	0.5
Evolving mucocutaneous¥		1
Myositis*		2
Pericarditis		1
Intestinal manifestations	Intestinal vasculitis, sterile peritonitis	2
Pulmonary manifestations	Pleurisy, pneumonitis, ingravescent dyspnea	1
Evolving neuropsychiatric manifestations*	Headache/migraine, seizures, stroke, organic brain disease, psychosis	2
Renal manifestations*+	Proteinuria, urinary casts, hematuria, raised serum creatinine or reduced creatinine clearance	0.5
Evolving renal manifestations		2
Hematologic features	Nonhemolytic anemia, hemolytic anemia*, leukopenia (or lymphopenia), thrombocytopenia	1
Erythrocyte sedimentation rate	Raised ESR	1
Hypocomplementemia	C3, CH50	1
Evolving hypocomplementemia		1

Final score #

¥ If any of the above mucocutaneous manifestations are new or have worsened since the last 1 manifestations observation, add **1 point.**
* If this system (or manifestation) is the only involvement present from among items 1–10, add 2 more points.
+ Excluding patients with end-stage chronic renal disease.
# If the final total score is not an integer number, round off to the lower integer for values <6 and to the higher integer for values >6. If the final score is >10, round off to 10.

Details about the items of ECLAM

1. Generalized manifestations
Fever = Documented basal morning temperatures of 37.5°C not due to an infective process.
Fatigue = A subjective feeling of extraordinary tiredness.

2. Articular manifestations
Arthritis = Non-erosive arthritis involving at least 2 peripheral joints (wrist, metacarpophalangeal or proximal, interphalangeal joints).
Evolving arthralgia = New onset or worsening of specific localized pain without objective symptoms in at least two peripheral joints.

3a. Active mucocutaneous manifestations
Malar rash = Fixed erythema, flat or raised over the malar eminences, and tending to spare the naso-labial folds.
Generalized rash = A maculo-papular rash not induced by drugs, that may be located anywhere on the body, and that is not strictly dependent on sun exposure.
Discoid rash = Erythematosus, raised patches with adherent keratotic scaling and follicular plugging.
Skin vasculitis = Including digital ulcers, purpura, urticaria, bullous lesions.
Oral ulcers = Oral or naso-pharyngeal ulcers, usually painless, observed by a physician.

3b. Evolving mucocutaneous
If any of the above mucocutaneous manifestations are new or have worsened since the last 1 manifestations observation, add **1 point.**

4. Myositis*
Confirmed by raised muscle enzymes and/or EMG examination and/or histology.

5. Pericarditis
Documented by ECG or rub or evidence of pericardial effusion on ultrasound.

6. Intestinal manifestations
Intestinal vasculitis = Evidence of acute intestinal vasculitis.
Sterile peritonitis = Evidence of abdominal effusion in the absence of infective processes.

7. Pulmonary manifestations
Pleurisy = Clinical or radiological evidence of pleural effusion in the absence of infective processes.
Pneumonitis = Single or multiple lung opacities on chest X-ray thought to reflect active disease not due to an infective process.
Ingravescent dyspnoea = Due to an evolving interstitial involvement.

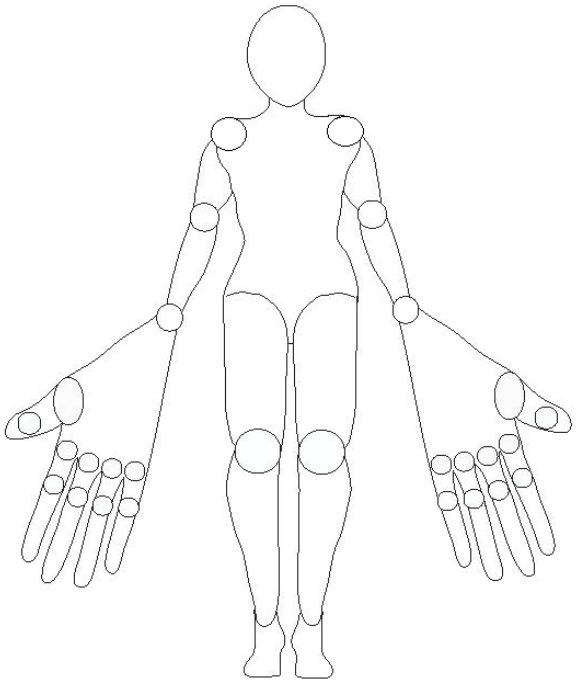
ANEXO C

Escore DAS28 - Escore de atividade da doença Artrite Reumatoide em 28 articulações

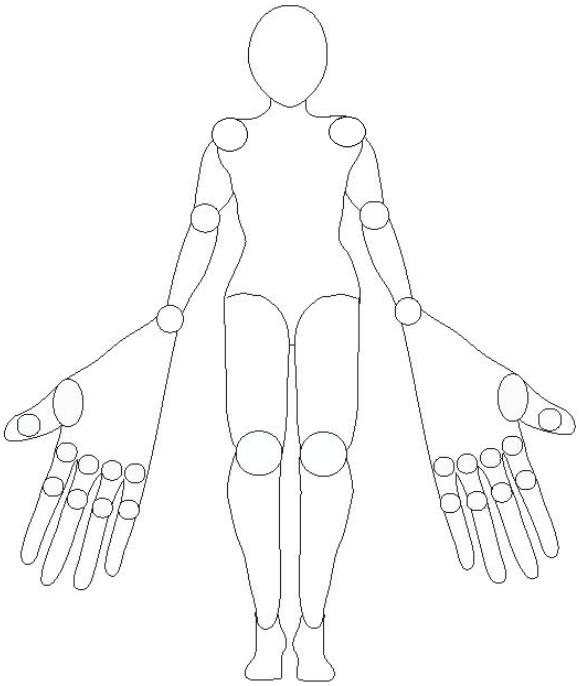
NOME:_____

DATA DA VISITA: _____

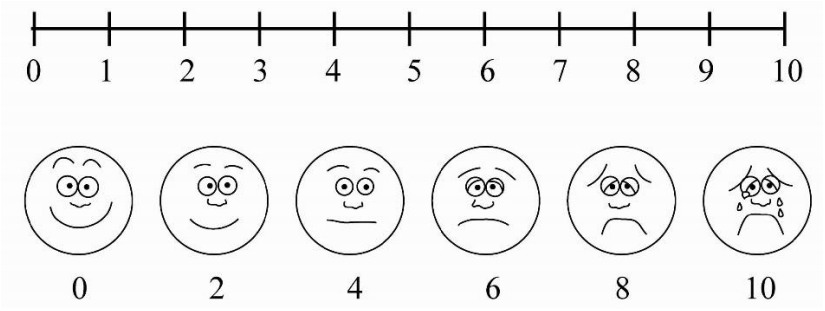
ARTICULAÇÕES DOLOROSAS
Total: _____



ARTICULAÇÕES EDEMACIADAS
Total: _____



EVA Global: _____



RESULTADOS LABORATORIAIS
Data: _____
VHS: _____ mm/h e/ou
PCR: _____ mg/L

AVALIAÇÃO DO DAS
Cálculo do DAS 28 (VHS): _____ e/ou
Cálculo do DAS 28 (PCR): _____

ANEXO D

Escore BILAG - Índice de Atividade da Doença avaliado pelo Grupo de Lúpus das Ilhas Britânicas

SCORING OF DISEASE ACTIVITY OF THE BILAG-2004 BASED ON THE PRINCIPLE OF PHYSICIAN'S INTENTION TO TREAT			
Scoring by grade	**Disease severity**	**Numerical scores**	**Assumption about the treatment for each grade**
A = Active	Severe	12	Severe disease activity requiring any of the following treatment: 1. systemic high-dose oral glucocorticoids (equivalent to prednisolone >20 mg/day) 2. intravenous pulse glucocorticoids (equivalent to pulse methylprednisolone ≥500 mg) 3. systemic immunomodulators (include biologicals, immunoglobulins and plasmapheresis) 4. therapeutic high-dose anticoagulation in the presence of high-dose steroids or immunomodulators; e.g., warfarin with target INR 3–4
B = Beware	Moderate	8	Moderate disease activity requiring any of the following treatment: 1. systemic low dose oral glucocorticoids (equivalent to prednisolone ≤20 mg/day) 2. intramuscular or intra-articular or soft tissue glucocorticoids injection (equivalent to methylprednisolone <500 mg) 3. topical glucocorticoids 4. topical immunomodulators 5. antimalarials or thalidomide or prasterone or acitretin 6. symptomatic therapy; e.g., NSAIDs for inflammatory arthritis
C = Contentment	Mild	1	Patient requires symptomatic treatment (e.g., analgesics or NSAIDs)
D = Discount	Inactive but previously affected	0	Not applicable
E = No Evidence	Inactive with no previous involvement	0	Not applicable

ANEXO E

Parecer do Comitê de Ética em Pesquisa em seres humanos da Universidade Estadual de Londrina

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Associação entre polimorfismos genéticos e a susceptibilidade ao Lúpus Eritematoso Sistêmico em pacientes atendidos no Ambulatório do Hospital de Clínicas da Universidade Estadual de Londrina, Londrina, Paraná

Pesquisador: Andréa Name Colado Simão

Área Temática:

Versão: 4

CAAE: 01865212.0.0000.5231

Instituição Proponente: Universidade Estadual de Londrina - UEL

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 210.328

Data da Relatoria: 19/12/2012

Apresentação do Projeto:

Estudos com famílias e gêmeos sugerem que os fatores genéticos desempenham um papel significativo na predisposição ao Lupus Eritematoso Sistêmico (LES). Assim, a hipótese levantada neste projeto é de que indivíduos que apresentam polimorfismo genético nos genes que codificam a Proteína C Reativa, o HLA e o TNF apresentam maior susceptibilidade ao desenvolvimento de LES e apresentam maior estresse oxidativo. Para isso, o sangue dos indivíduos selecionados será colhido para realização de investigação gênica e dosagem de Proteína C Reativa e TNF.

Objetivo da Pesquisa:

Este projeto objetiva determinar a associação de polimorfismos genéticos e a susceptibilidade ao LES e ao aumento do estresse oxidativo em pacientes atendidos no Ambulatório do Hospital de Clínicas (AHC) da Universidade Estadual de Londrina (UEL), Londrina, Paraná.

Avaliação dos Riscos e Benefícios:

O projeto não apresenta riscos ao paciente e a população poderá ser beneficiada com os resultados obtidos, caso a equipe de pesquisa determine fatores genéticos que possam estimar a chance de um indivíduo desenvolver a doença ou a chance de um indivíduo previamente com a

Endereço: AVENIDA ROBERT KOCH, 60
Bairro: VILA OPERÁRIA **CEP:** 86.038-440
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doença em desenvolver quadros clínicos mais graves como a nefrite lúpica. Além disso, os resultados obtidos neste estudo poderão, também, indicar uma possível relevância da inclusão na rotina laboratorial de testes de genotipagem dos
genes indicados para indivíduos atendidos no AHC e no Hospital Universitário da UEL. Indivíduos que apresentarem um genótipo ou um conjunto de haplótipos associado ao LES poderão ser beneficiados com estratégias terapêuticas diferentes ou serem submetidos a um monitoramento clínico e laboratorial em intervalos menores de tempo, ou ambos procedimentos, o que poderá contribuir para uma melhor avaliação e monitorização clínica
destes indivíduos.

Comentários e Considerações sobre a Pesquisa:
O Projeto está bem estruturado e é relevante para o avanço das investigações sobre LES.

Considerações sobre os Termos de apresentação obrigatória:
Todas as pendências foram respondidas adequadamente.

Recomendações:
Encaminhar relatório ao final do estudo.

Conclusões ou Pendências e Lista de Inadequações:
Não há.

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:
Prezada Pesquisadora,

Favor retirar seu parecer de aprovaçaõ junto ao CEP/UEL.

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

LONDRINA, 04 de Março de 2013

Assinador por:
Alexandrina Aparecida Maciel Cardelli
(Coordenador)

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