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CHRISTIANE AGUIAR NOBRE

AVALIAÇÃO DE RISCO CARDIOVASCULAR EM PACIENTES COM ARTRITE REUMATOIDE: POSSÍVEL ASSOCIAÇÃO COM ATIVIDADE DA DOENÇA, CITOCINAS E A VIA NRF2/HO-1

FORTALEZA/CE

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Tese apresentada ao Programa de Pós-Graduação em Medicina Translacional da Universidade Federal do Ceará, como requisito parcial à obtenção do título de doutor em Medicina Translacional.

Orientadora: Profa. Dra. Mirna Marques Bezerra
Coorientador: Prof. Dr. Carlos Ewerton Maia Rodrigues

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Aprovada em: ___/___/_____.

BANCA EXAMINADORA

_____
Profa. Dra. Mirna Marques Bezerra (Orientadora)
Universidade Federal do Ceará (UFC)

_____
Prof. Dr. Carlos Ewerton Maia Rodrigues (Coorientador)
Universidade de Fortaleza (UNIFOR)

_____
Profa. Dra. Adriana Rolim Campos
Universidade de Fortaleza (UNIFOR)

_____
Profa. Dra. Andrea Rocha de Saboia MontAlverne
Universidade de Fortaleza (UNIFOR)

_____
Profa. Dra. Profa. Dra. Caroline de Fátima Aquino Moreira Nunes
Universidade Federal do Ceará (UFC)

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“A dor passa, mas a beleza permanece.”

Pierre-Auguste Renoir

RESUMO

Avaliação de risco cardiovascular em pacientes com artrite reumatoide: possível associação com atividade da doença, citocinas e a via NrF2/HO-1. Christiane Aguiar Nobre. Mirna Marques Bezerra. Tese de Doutorado. Programa de Pós-Graduação em Medicina Translacional. Núcleo de Pesquisa e Desenvolvimento de Medicamentos, Faculdade de Medicina, UFC. Fortaleza, 2026.

Introdução: A inflamação participa do risco cardiovascular (RCV) na artrite reumatoide (AR) e, de forma complementar, o estresse oxidativo mediado pelo fator eritroide 2 (Nrf2)/ hemeoxigenase 1 (HO-1) pode contribuir nesse processo. Objetivo: Avaliar o RCV, a atividade da doença, os níveis séricos de citocinas e a expressão da via Nrf2/HO-1 na AR. Métodos: Estudo transversal com 120 participantes, distribuídos entre grupos AR e controle. Foram coletados dados clínicos, laboratoriais e ultrassonográficos (US) de coração e carótidas; dosadas citocinas por ELISA (TNF-α, IL-1β, IL-6, IL-4, IL-10) e a expressão gênica (RNAm) de Nrf2 e HO-1 por qRT-PCR. A atividade da doença foi mensurada pelo DAS28, SDAI e CDAI, enquanto o RCV através do pró-BNP sérico, US e escores clínicos (Framingham e SCORE). As análises utilizaram SPSS v26.0 ($p<0,05$). Resultados: A amostra foi predominantemente feminina (90%), com idade média de 52 anos. O grupo controle apresentou maiores valores de dislipidemia ($p=0,027$), circunferência abdominal ($p=0,017$), IMC ($p=0,010$) e glicemia ($p<0,001$). No grupo AR, a duração média da doença foi de 10,4 ± 7,5 anos, com 63,3% positivos para fator reumatoide e/ou anti-CCP. Em relação ao tratamento, 91,7% usavam DMARDs, 21,7% biológicos e 11,7% agentes alvo-específicos. Não foram observadas diferenças entre os grupos nos escores clínicos de RCV e achados de US. Contudo, os níveis de pró-BNP foram mais elevados na AR ($p=0,009$). Observamos maiores níveis de IL-6 ($p=0,002$) e IL-10 ($p=0,004$), com redução de IL-1β ($p=0,001$). Encontramos aumento de RNAm-Nrf2 ($p<0,001$) e redução de RNAm-HO-1 ($p=0,030$). Doença com mais de 10 anos associou-se à hipertensão arterial ($p=0,023$), maior DAS28-VHS ($p=0,017$) e SDAI ($p=0,025$), além do uso mais frequente de anti-hipertensivos ($p<0,001$), estatinas ($p=0,011$), hipoglicemiantes ($p=0,038$) e maiores níveis de glicemia ($p=0,029$). O CDAI correlacionou-se com maiores níveis de IL-6 ($p=0,033$) e o DAS28-PCR à menor escolaridade ($p=0,038$), maior tempo de doença ($p=0,012$), disfunção ventricular ($p=0,044$) e triglicerídeos elevados ($p=0,030$). Conclusão: Pacientes com AR apresentaram desregulação da via Nrf2/HO-1 e elevação de pró-BNP. A maior atividade e o maior tempo de doença relacionaram-se a maior prevalência de comorbidades e complicações cardiovasculares na AR.

Palavras-chave: Artrite reumatoide; Doença cardiovascular; Citocinas; Nrf2; HO-1.

ABSTRACT

Cardiovascular risk assessment in patients with rheumatoid arthritis: possible association with disease activity, cytokines, and the NrF2/HO-1 pathway. Christiane Aguiar Nobre. Mirna Marques Bezerra. Doctoral thesis. Graduate Program in Translational Medicine. Center for Research and Development of Medicines, School of Medicine, UFC. Fortaleza, 2026.

Introduction: Inflammation contributes to cardiovascular risk (CVR) in rheumatoid arthritis (RA), and oxidative stress mediated by erythroid factor 2 (Nrf2)/heme oxygenase 1 (HO-1) may also contribute to this process. Objective: To evaluate CVR, disease activity, serum cytokine levels, and Nrf2/HO-1 pathway expression in RA. Methods: Cross-sectional study with 120 participants, divided into RA and control groups. Clinical, laboratory, and ultrasound (US) data were collected from the heart and carotid arteries; cytokines were measured by ELISA (TNF-α, IL-1β, IL-6, IL-4, IL-10) and gene expression (mRNA) of Nrf2 and HO-1 by qRT-PCR. Disease activity was measured by DAS28, SDAI, and CDAI, while CVR was measured by serum pro-BNP, US, and clinical scores (Framingham and SCORE). Analyses were performed using SPSS v26.0 ($p<0.05$). Results: The sample was predominantly female (90%), with a mean age of 52 years. The control group had higher values for dyslipidemia ($p=0.027$), abdominal circumference ($p=0.017$), BMI ($p=0.010$), and blood glucose ($p<0.001$). In the RA group, the mean duration of the disease was 10.4 ± 7.5 years, with 63.3% positive for rheumatoid factor and/or anti-CCP. Regarding treatment, 91.7% used DMARDs, 21.7% used biologics, and 11.7% used target-specific agents. No differences were observed between the groups in clinical CVR scores and US findings. However, pro-BNP levels were higher in RA ($p=0.009$). We observed higher levels of IL-6 ($p=0.002$) and IL-10 ($p=0.004$), with a reduction in IL-1β ($p=0.001$). We found an increase in mRNA-Nrf2 ($p<0.001$) and a reduction in mRNA-HO-1 ($p=0.030$). Disease lasting more than 10 years was associated with hypertension ($p=0.023$), higher DAS28-ESR ($p=0.017$) and SDAI ($p=0.025$), as well as more frequent use of antihypertensive drugs ($p<0.001$), statins ($p=0.011$), hypoglycemic agents ($p=0.038$), and higher blood glucose levels ($p=0.029$). CDAI correlated with higher IL-6 levels ($p=0.033$) and DAS28-PCR with lower education ($p=0.038$), longer disease duration ($p=0.012$), ventricular dysfunction ($p=0.044$), and elevated triglycerides ($p=0.030$). Conclusion: Patients with RA presented with Nrf2/HO-1 pathway dysregulation and elevated pro-BNP. Higher disease activity and longer disease duration were associated with a higher prevalence of comorbidities and cardiovascular complications in RA.

Keywords: Rheumatoid arthritis; Cardiovascular disease; Cytokines; Nrf2; HO-1;

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LISTA DE ABREVIATURAS E SIGLAS EM INGLÊS

Anti-CCP	Anti-cyclic citrullinated peptide
ACR	American College of Rheumatology
BMI	Body mass index
BNP	Brain natriuretic peptide
CDAI	Clinical Disease Activity Index
CEP/UFC/PROPESQ	Research ethics committee of the Federal University of Ceara
CRP	C-reactive protein
CVD	Cardiovascular disease
CVR	Cardiovascular risk
DAS28	Disease Activity Score-28
DMARD	Disease-modifying anti-rheumatic drugs
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HDL	High-density lipoprotein
HO-1	Heme oxygenase-1
LDL	Low-density lipoprotein
mSCORE	Modified Systematic Coronary Risk Evaluation
Nrf2	Nuclear factor-erythroid 2-related factor 2
ProBNP	N-terminal prohormone of brain natriuretic peptide
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SCORE	Systematic Coronary Risk Evaluation
SDAI	Simplified Disease Activity Index
SPSS	Statistical Package for the Social Sciences
TC	Total cholesterol
TG	Triglyceride
WC	Waist circumference
RR	Relative risk
CI	Confidence interval

LISTA DE ABREVIATURAS E SIGLAS EM PORTUGUÊS

AR	Artrite Reumatoide
DCV	Doença Cardiovascular
RCV	Risco Cardiovascular
HLA-DRB1	Antígeno Leucocitário Humano – subtipo DRB1
CD4+	Linfócitos T auxiliares com marcador CD4
TNF-α	Fator de Necrose Tumoral Alfa
IL-4, IL-10	Interleucinas (citocinas anti-inflamatórias)
IL-1β, IL-2, IL-6, IL-7, IL-17, IL-18, IL-21, IL-23, IL-33	Interleucinas (citocinas pró-inflamatórias)
GM-CSF	Fator Estimulador de Colônias de Granulócitos e Macrófagos
ROS	Espécies Reativas de Oxigênio
RNS	Espécies Reativas de Nitrogênio
Nrf2	Fator nuclear relacionado ao fator eritroide 2
HO-1	Heme Oxigenase-1
FR	Fator Reumatoide
anti-CCP	Anticorpo Anti-Peptídeo Citrulinado Cíclico
VHS	Velocidade de Hemossedimentação
PCR	Proteína C Reativa
ANP	Peptídeo Natriurético Atrial
BNP	Peptídeo Natriurético do Tipo B
CNP	Peptídeo Natriurético Tipo C

LISTA DE SÍMBOLOS

%	Porcentagem
α	Alfa
β	Beta
=	Igual
>	Maior que (comparações estatísticas)
±	Média ± desvio padrão
<	Menor que (comparações estatísticas)
nº	Número
/	Usado em unidades (ex: kg/m²), relações ou separações
p	Valor-p (significância estatística)

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1 INTRODUÇÃO - REVISÃO BIBLIOGRÁFICA, RELEVÂNCIA E JUSTIFICATIVA

1.1 Epidemiologia e manifestações clínicas da artrite reumatoide

A artrite reumatoide (AR) é uma doença inflamatória crônica e autoimune caracterizada primariamente por poliartrite crônica, simétrica e aditiva de grandes e pequenas articulações que pode levar à destruição óssea e cartilaginosa. Estima-se uma prevalência de 0,5 a 1% da população, sendo de duas a três vezes mais comum no sexo feminino, com pico de incidência entre a quarta e quinta décadas de vida (HOCHBERG, 2011). No Brasil, a prevalência em adultos varia de 0,2% a 1%, o que corresponderia a uma estimativa de até 2 milhões de pessoas acometidas (MARQUES et al., 1993). Além das manifestações articulares, a AR tem comorbidades variadas, sendo a doença cardiovascular (DCV) a principal causa de mortalidade nesses pacientes (GUALTIEROTTI R. et al., 2017).

1.2 Critérios de Classificação da Artrite Reumatoide

Os primeiros critérios de classificação da AR foram publicados em 1987 pelo American College of Rheumatology (ACR), com sensibilidade de 77% a 98% e especificidade de 85% a 98%, incluindo critérios como rigidez matinal de pelo menos uma hora, artrite em três ou mais regiões por no mínimo seis semanas, artrite das mãos, artrite simétrica, nódulos reumatoides, fator reumatoide e alterações radiográficas. Para classificar a doença, era necessário cumprir pelo menos quatro desses critérios (ARNETT et al., 1988).

Com o objetivo de obter diagnóstico precoce e prevenir o dano estrutural articular, foram estabelecidos em 2010 os critérios ACR/EULAR (American College of Rheumatology/European League Against Rheumatism), que contemplam contagem articular, fator reumatoide (FR), anticorpo anti-peptídeo citrulinado cíclico (anti-CCP), velocidade de hemossedimentação (VHS), proteína C reativa (PCR) e sintomas por pelo menos seis semanas (ALETAHA et al., 2010). Estudos demonstram que esses critérios apresentam sensibilidade entre aproximadamente 80% e 82% e especificidade entre 61% e 71%, superando a sensibilidade dos critérios de 1987 às custas de uma redução da especificidade.

1.3 Índices de atividade de doença na artrite reumatoide

Os índices mais utilizados para avaliar atividade de doença na AR são o CDAI (*Clinical Disease Activity Index)*, SDAI (*Simplified Disease Activity Index*) e DAS28 (*Disease Activity Score*). Estes índices além de medir a atividade da doença numa escala contínua, permitem categorizar o paciente em estratos de atividade, com o uso de diferentes pontos de corte: remissão, atividade leve, moderada e alta, orientando desta forma o tratamento. São 28 articulações avaliadas nos três índices: ombros, cotovelos, punhos, metacarpofalangeanas, interfalangeanas proximais, incluindo polegar e joelhos (PREVOO et al., 1995).

O CDAI é uma soma direta do número de articulações doloridas (0 a 28), edemaciadas (0 a 28), avaliação global da doença pelo paciente (PGA), variando de 0 a 10, 10 significando atividade máxima, e a avaliação global da doença pelo examinador (EGA), variando de 0 a 10, 10 significando atividade máxima. O valor < 2,8 significa remissão clínica; se > 2,8 até 10, baixa atividade; > 10 até 22, moderada atividade; e > 22, alta atividade de doença (ALETAHA et al; 2005). O SDAI também é uma soma direta que inclui os parâmetros do CDAI mais o valor do PCR (mg/dL). O valor < 3,3 significa remissão clínica; se >3,3 até 11, baixa atividade; > 11 e até 26, moderada atividade; e > 26 alta atividade de doença (SMOLEN et al., 2003). O DAS28 é um índice combinado de atividade da AR que inclui número de articulações doloridas (0 a 28), edemaciadas (0 a 28), avaliação global da doença pelo paciente (0 a 10, 10 significando atividade máxima) e VHS (velocidade de hemossedimentação) ou PCR (proteína C reativa). O valor < 2,6 significa remissão clínica; se >2,6 até 3,1, baixa atividade; >3,2 até 5,0, moderada atividade; e > 5,1, alta atividade de doença (PREVOO et al., 1995). Para a obtenção de tal resultado, utiliza-se uma calculadora específica no aplicativo RheumaHelper para Smartphone.

1.4 Fisiopatologia da artrite reumatoide

A fisiopatologia da AR envolve uma complexa interação entre fatores genéticos, ambientais e imunológicos. (ALETAHA; SMOLEN, 2018; WEYAND; GORONZY, 2021). Geneticamente, alelos do HLA-DRB1, aumentam o risco de AR, facilitando a apresentação de autoantígenos modificados, como proteínas citrulinadas, para linfócitos T CD4+ (WEYAND; GORONZY, 2021). Fatores ambientais, como tabagismo, periodontite e alterações no microbioma, contribuem para a quebra da tolerância imunológica (MUENCH *et al.*, 2022; WEYAND; GORONZY, 2025). A AR resulta da perda de tolerância imunológica, ativação sustentada de células T e B, produção de autoanticorpos, formação de complexos imunes, ativação de macrófagos e fibroblastos sinoviais, e liberação de citocinas pró-inflamatórias, culminando em destruição articular progressiva (KONDO; KURODA; KOBAYASHI, 2021).

Entre as moléculas inflamatórias, o fator de necrose tumoral alfa (TNF-α) e a interleucina-6 (IL-6) desempenham um papel central com investigações mais recentes tendo demonstrado que outras citocinas, como a IL-7, IL-17, IL-21, IL-23, GM-CSF, IL-1β, IL-18, IL-33 e IL-2, também participam na progressão da doença (GORONZY; WEYAND, 2024).

1.5 Estresse oxidativo na artrite reumatoide

Além dos níveis aumentados de citocinas pró-inflamatórias, a patogênese da AR também está relacionada ao estresse oxidativo (FONSECA et al., 2019) que contribui de maneira significativa para progressão da doença. O estresse oxidativo resulta do desequilíbrio entre a produção de espécies reativas de oxigênio (ROS) e nitrogênio (RNS) e a capacidade dos sistemas antioxidantes de neutralizá-las (MATEEN *et al.*, 2016; KHOJAH *et al.*, 2016). Para manter essa homeostase, as células desenvolveram mecanismos de proteção adaptativa, como o fator nuclear eritróide 2 relacionado fator 2 (Nrf2), que regula a transcrição de muitos genes envolvidos no equilíbrio redox, desintoxicação e inflamação (PALL AND LEVINE, 2015). A ativação de Nrf2 resulta na síntese da heme oxigenase-1 (HO-1), um dos mecanismos citoprotetores mais críticos ativados durante o estresse celular (LOBODA et al., 2016).

A indução desta resposta protetora necessita da ativação da via Keap1-Nrf2-ARE. A ativação do Nrf2 ocorre quando Keap1 é exposta aos indutores de estresse oxidativo. Após essa exposição Keap1 é modificado, levando à liberação do Nrf2 e subsequente translocação para o núcleo. Ao chegar ao núcleo, Nrf2 se liga a um elemento de resposta antioxidante (ARE) para codificação de enzimas antioxidantes, como por exemplo, heme-oxigenase 1 (HO-1) (LOBODA et al., 2016).

A HO-1 é a enzima limitante da taxa de degradação do heme para gerar monóxido de carbono (CO), ferro e biliverdina (BR), funcionando como um dos fatores mais importantes de adaptação celular ao estresse oxidativo, bem como um regulador de programas de sinalização inflamatória. A atividade da HO é modulada por 3 isoenzimas, uma isoforma induzível (HO-1) e duas isoformas expressas constitutivamente (HO-2 e HO-3). A HO-1 é indetectável em condições basais, mas altamente induzível em condições de estresse ou inflamação (WAZA et al., 2018).

A indução de HO-1 em células endoteliais pode ser um componente importante dos efeitos vasculoprotetores das estatinas e a prevenção da aterosclerose em pacientes com doenças inflamatórias sistêmicas (ALI et al., 2009). Logo, HO-1 pode desempenhar um papel importante, conferindo proteção às células renais contra danos oxidativos e reduzir a reatividade vascular a estímulos constritores e hipertensão. Como resultado, o controle da inflamação por HO-1 pode levar a uma redução do risco de eventos CV em pacientes com AR (BLUM;

ADAWI, 2019).

Portanto, considerando que a ativação de Nrf2 resulta na síntese de HO-1 que pode modular a ativação/metabolismo de condrócitos, sinoviócitos e células do sistema imune, evidências sugerem que a via Nrf2-HO-1 pode conferir proteção contra o estresse oxidativo e a resposta inflamatória na AR, oferecendo assim novas oportunidades para terapias alvo direcionadas nas doenças articulares (ALCARAZ; FERRÁNDIZ, 2019).

1.6 Risco cardiovascular na artrite reumatoide

Pacientes com AR vivenciam eventos cardiovasculares precoces e têm menor expectativa de vida comparados à população geral (PETERS et al., 2010), De fato, o risco de mortalidade por causas cardiovasculares é 50% superior em pacientes com AR, quando comparados com a população em geral (GUALTIEROTTI et al., 2017).

Estudos epidemiológicos mostraram que pacientes com AR tem maior probabilidade de desenvolver cardiopatia isquêmica silenciosa, insuficiência cardíaca e sofrer morte súbita em comparação com os controles (SOLOMON et al., 2015). O aumento da morbimortalidade CV em pacientes com AR não pode ser inteiramente explicado pelos fatores de risco CV tradicionais (hipertensão, diabetes mellitus, dislipidemia, obesidade e estilo de vida sedentário). Foi demonstrado que pacientes com AR apresentam aterosclerose e calcificação coronária mais precoce e mais extensa em relação a indivíduos controles sem AR (ADAWI et al., 2019). A resposta imune/inflamatória, que forma a base da fisiopatologia da AR, parece atuar como um fator de risco CV independente, de modo que o maior risco CV em pacientes com AR poderia ser creditado em parte, ao aumento de citocinas pró-inflamatórias e autoanticorpos circulantes, o que promoveria a formação de placa aterosclerótica e o remodelamento cardíaco (SOLOMON et al., 2015). Por isso, a AR é considerada um fator de risco independente para DCV (DEL RINCON et al., 2001), com risco comparável ao do diabetes mellitus (SOLOMON et al., 2006).

1.7 Fisiopatologia da doença cardiovascular na artrite reumatoide

Embora se reconheça o papel da inflamação na aterogênese, os mecanismos celulares e moleculares da aterosclerose acelerada na AR ainda são pouco claros. As citocinas têm papel central: o desequilíbrio entre citocinas pró-inflamatórias (TNF-α, IL-1β, IL-6) e anti-inflamatórias (IL-4, IL-10) perpetua a resposta autoimune e a lesão articular (ALAM et al., 2017), sendo seus níveis séricos indicadores da gravidade da doença (MCINNES et al., 2016).

O estresse oxidativo também contribui para a patogênese da AR. A via de sinalização Nrf2 regula a expressão de genes antioxidantes como a heme-oxigenase 1 (HO-1), essencial na resposta ao estresse oxidativo (LU et al., 2016; SAHA, 2024), com HO-1 degradando o heme

e gerando substâncias protetoras, exercendo funções anti-inflamatórias e vasculoprotetoras (LOBODA et al., 2016; WAZA et al., 2018).

Apesar da informação limitada sobre o papel da via Nrf2/HO-1 no sistema cardiovascular, o fator de transcrição Nrf2 tem sido estudado como um potencial mediador do risco cardiovascular em doenças metabólicas (DA COSTA et al., 2019), o que pode ter implicações relevantes nos mecanismos fisiopatológicos da DCV associada à AR.

1.8 Avaliação do Risco cardiovascular na artrite reumatoide

Ainda não se dispõe de um método padrão ouro para avaliação do RCV em pacientes com AR. Uma das estratégias utilizadas é a aplicação de escores clínicos de predição de risco cardiovascular a 10 anos, como o Systematic Coronary Risk Evaluation (SCORE), o SCORE modificado (mSCORE) e o escore de Framingham, os quais permitem a estratificação individual do risco de eventos cardiovasculares maiores a partir de variáveis clínicas e laboratoriais padronizadas (CONROY et al., 2003; D'AGOSTINO et al., 2008; WILSON et al., 1998).

O SCORE é utilizado para estimar o risco de mortalidade cardiovascular em 10 anos, considerando idade, sexo, tabagismo, níveis de pressão arterial sistólica e colesterol total, conforme as tabelas propostas pela European Society of Cardiology (ESC, 2021). A classificação de risco seguiu as categorias convencionais: baixo risco (<1%), risco moderado (1–4%), alto risco (5–9%) e risco muito alto (≥10%) de mortalidade cardiovascular em 10 anos (CONROY et al., 2003; ESC, 2021). Reconhecendo que determinadas condições inflamatórias crônicas, como a AR, estão associadas a risco cardiovascular adicional não totalmente capturado pelos escores tradicionais, é aplicado o SCORE modificado (mSCORE), obtido pela multiplicação do valor do SCORE por um fator de correção de 1,5, conforme recomendado pela EULAR (PETERS et al., 2010; AGCA et al., 2017). A estratificação de risco do mSCORE segui os mesmos pontos de corte do SCORE original. Adicionalmente, o escore de Framingham , que estima o risco de ocorrência de eventos cardiovasculares fatais e não fatais em 10 anos, incorporando variáveis como idade, sexo, colesterol total, HDL-colesterol, pressão arterial sistólica (tratada ou não), tabagismo e diabetes mellitus. Os indivíduos são classificados em baixo risco (<10%), risco intermediário (10–20%) ou alto risco (>20%) de eventos cardiovasculares em 10 anos (WILSON et al., 1998; D'AGOSTINO et al., 2008). Diante da importância da DCV na AR e da limitação dos escores de risco que subestimam a exposição inflamatória crônica, a EULAR recomendou o uso de um escore adaptado (mSCORE) com fator multiplicador de 1,5 para pacientes com AR (AGCA et al., 2017). Mesmo assim, muitos pacientes com AR em alto risco podem não ser identificados (CHOY E. et al., 2014).

Com o objetivo de aprimorar a avaliação do RCV, a ultrassonografia cardíaca e das artérias carótidas tem se mostrado útil na detecção de lesões subclínicas e de alterações funcionais, contribuindo para uma estratificação mais acurada do risco cardiovascular em pacientes com artrite reumatoide. Trata-se de um método não invasivo, de baixo custo, reprodutível e sensível, capaz de identificar precocemente alterações estruturais, como o aumento da espessura médio-intimal carotídea e a presença de placas ateroscleróticas (CORRALES et al., 2013). Ademais, a disfunção diastólica avaliada por ecocardiografia vem sendo reconhecida como um marcador cada vez mais relevante de dano miocárdico nessa população (VIZZARDI et al., 2012; ABHAYARATNA et al., 2006).

Adicionalmente, os métodos de laboratório representam uma outra estratégia para obter uma compreensão da patogênese da doença em níveis celulares ou moleculares, através do monitoramento das concentrações de biomarcadores de disfunção cardíaca e de inflamação secretados no sangue, que representam preditores de risco de DCV e mortalidade na AR (PROVAN et al., 2010). Dentre os biomarcadores de disfunção cardíaca, os peptídeos natriuréticos (NPs), NPs atriais (ANP), fração cerebral (BNP) e NP endoteliais (CNP) são liberados pelo coração e vasos para a circulação sistêmica em resposta à sobrecarga de volume ou pressão (SANTAGUIDA et al.,2014). O processamento intracelular do pró-peptídeo proBNP resulta na formação do BNP biologicamente ativo e do fragmento N-terminal inerte (NT-proBNP), ambos liberados na circulação sistêmica (HILL et al., 2014). A dosagem plasmática do BNP e do NT-proBNP apresenta reconhecido valor diagnóstico e prognóstico na insuficiência cardíaca, com níveis que se correlacionam diretamente com a gravidade da disfunção miocárdica (MAISEL et al., 2002; JANUZZI et al., 2005). Embora apresentem desempenho clínico semelhante para a detecção e o acompanhamento da disfunção cardíaca, o NT-proBNP possui meia-vida plasmática mais prolongada em comparação ao BNP, o que confere maior estabilidade e otimização analítica à sua dosagem (HUNT et al., 2005; JANUZZI et al., 2005). Além disso, evidências indicam que o NT-proBNP atua como preditor de doença cardiovascular e mortalidade tanto em populações gerais quanto em pacientes com artrite reumatoide (PROVAN et al., 2010)

1.8 Relevância e justificativa

Diante da associação consistente entre a artrite reumatoide e a presença de aterosclerose subclínica, com consequente aumento do risco cardiovascular, bem como da possibilidade de que esses pacientes se beneficiem de estratégias de triagem mais refinadas para fatores de risco cardiovascular incluindo a identificação de biomarcadores capazes de detectar aterosclerose precoce e acelerada e considerando a escassez de estudos que avaliem o papel modulador da via Nrf2/HO-1 no risco cardiovascular nessa população, o presente estudo investigou a associação entre o risco cardiovascular na artrite reumatoide e parâmetros clínicos, inflamatórios e relacionados ao estresse oxidativo. Foram exploradas as relações entre o risco cardiovascular e a atividade da doença, alterações estruturais cardíacas e carotídeas, escores clínicos de predição de risco cardiovascular, perfis de citocinas e a modulação desses eventos pela via Nrf2–HO-1. A compreensão integrada desses fatores pode contribuir para a identificação de marcadores de risco e de potenciais alvos terapêuticos, favorecendo o desenvolvimento de estratégias custo-efetivas de prevenção primária e secundária das doenças cardiovasculares na artrite reumatoide, com potencial impacto na redução da morbimortalidade, das internações e dos custos associados ao Sistema Único de Saúde (SUS).

2 OBJETIVOS

2.1 Objetivo Geral

Avaliar o risco cardiovascular em pacientes com AR e a possível associação entre atividade da doença, citocinas e a via Nrf2/HO-1.

2.2 Objetivos Específicos

- Caracterizar o perfil clínico e epidemiológico dos pacientes com artrite reumatoide e sua associação com o risco cardiovascular.
- Investigar a associação entre o risco cardiovascular e os níveis séricos de citocinas inflamatórias.
- Avaliar a relação entre alterações cardiovasculares à ultrassonografia e a via Nrf2/HO-1.
- Estabelecer a associação entre alterações ultrassonográficas cardiovasculares, escores de risco cardiovascular e índices de atividade da doença.
- Analisar variáveis independentes associadas ao risco cardiovascular nos pacientes com artrite reumatoide.
- Identificar potenciais biomarcadores relacionados ao risco cardiovascular na artrite reumatoide.

Ressalta-se que a defesa desta tese será baseada no artigo já publicado, o qual contempla os principais achados do estudo; contudo, os objetivos propostos foram integralmente alcançados por meio do conjunto dos três artigos produzidos ao longo da pesquisa, que, de forma complementar, abordam os diferentes eixos investigados.

3 ARTIGO PUBLICADO EM REVISTA INDEXADA

3.1 Revista, fator de impacto e Qualis

A presente tese foi publicada na revista Clinical Immunology (ISSN 1521-6616; Elsevier) – ANEXO C, classificada como A3 no Qualis/CAPES para a área de Medicina (quadriênio 2017–2000). Conforme o Journal Citation Reports (Web of Science), o fator de impacto da revista no ano-base 2023 é de 3,8**,** com índice de impacto em cinco anos de 4,3.

3.2 Artigo

3.2.1 Article title

ProBNP, cytokines, and the Nrf2/HO-1 signaling pathway: A cross-sectional study on cardiovascular risk in rheumatoid arthritis

3.2.2 Abstract

We evaluated associations between clinical/laboratory findings and serum cytokines, Nrf2/HO-1 pathway expression and cardiovascular risk in both RA patients and controls. Sixty RA patients and 60 controls were included in the study. Serum cytokine and proBNP levels were assessed by ELISA, while serum Nrf2 and HO-1 mRNA levels were quantified by qRT-PCR. The RA group (91.7% women) and the control group (90% women) were aged 52 ± 12 and 52 ± 13 years, respectively. ProBNP levels were higher in the RA group than in controls ($p=0.009$). Nrf2 mRNA levels were higher ($p<0.001$) and HO-1 mRNA levels were lower ($p=0.030$) in the RA group than in controls. CDAI scores were significantly associated with serum IL-6 levels ($p=0.033$). This study found a significant dysregulation in Nrf2/HO-1 pathway activity in RA patients, although without association with cardiovascular risk, RA-related clinical and laboratory variables. Moderate/high disease activity was positively associated with IL-6 levels.

Key-words: rheumatoid arthritis, Nrf2/HO-1 signaling pathway, cytokines, ProBNP, oxidative stress, cardiovascular risk factors

3.2.3 Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by symmetrical polyarthritis compromising the bones, with an overall prevalence of 0.5-1% [1]. RA not only affects the joints, but is associated with several comorbidities of which cardiovascular disease (CVD) is the main cause of mortality [2]. RA patients tend to experience cardiovascular events earlier and have lower life expectancy than the general population [3]. Evidence suggests that inflammatory response to RA is an independent cardiovascular risk (CVR) factor accelerating rapid atherosclerosis [4,5].

In addition to inflammatory activity, the pathogenesis of RA also involves oxidative stress [3]. In order to maintain homeostasis, cells employ adaptive protective mechanisms, such as the nuclear factor-erythroid 2-related factor 2 (Nrf2) signaling pathway, which regulates the transcription of several genes involved in redox balance, detoxification and inflammation [6]. Nrf2 activation results in the production of heme oxygenase-1 (HO-1), one of the most important cytoprotective mechanisms activated during cellular stress [7]. Recent studies have

shown that the Nrf2/HO-1 pathway allows for the formation of bioactive metabolites protective against oxidative stress and inflammatory response in RA [8,9]. Moreover, reduced Nrf2 expression is known to contribute to the pathophysiology of cardiovascular disease in conditions such as obesity, diabetes mellitus, hypertension and atherosclerosis [10]. However, little is known about the Nrf2/HO-1 pathway and how it relates to cardiovascular protection in RA patients.

To better understand the possible modulatory role of the Nrf2/HO-1 signaling pathway in RA, in this study we evaluated associations between clinical/laboratory findings and serum cytokines, Nrf2/HO-1 pathway expression and CVR in both RA patients and controls.

3.3.4 Methods

3.3.4.1 Approval of study protocol

This cross-sectional study was conducted at a secondary-level outpatient service in Northeastern Brazil between October 2021 and October 2022. The protocol complied with the tenets of the Helsinki Declaration [11], all procedures being performed according to well-established standards and guidelines, and was approved under file #110729/2020 by the research ethics committee of the Federal University of Ceará (CEP/UFC/PROPESQ). All participants gave their informed written consent prior to study entry.

3.3.4.2 Patients and controls

Initially, 123 participants were recruited and assigned to one of two groups: A control group of healthy volunteers and an RA group. RA was diagnosed according to the 2010 American College of Rheumatology criteria [12]. One RA patient was excluded due to mitral valve disease caused by rheumatic fever, and two other participants were excluded from the control group (one did not complete the study protocol, the other was diagnosed with hypertensive heart disease and non-dialysis-dependent chronic kidney disease), leaving a final sample of 120 participants (60 in each group). The non-RA participants were sex and age-paired residents from the local community. The exclusion criteria were: age under 18 years, pregnancy, history of congestive heart failure, coronary artery disease, cerebrovascular disease, atrial fibrillation, cancer, chronic kidney disease and/or other collagen diseases such as systemic lupus erythematosus, Sjögren syndrome, scleroderma and inflammatory myopathy.

3.3.4.3 Clinical and anthropometric evaluation

The physical examination included tender and swollen joint count (RA group), and arterial blood pressure, waist circumference (WC) and body mass index (BMI) (both groups). WC was measured with a tape held horizontally between the last rib and the iliac crest. BMI was obtained by dividing weight (kg) by height squared (m^2). Individuals with BMI in the range

25-29.9 were considered overweight, while BMI >30 was interpreted as obesity [13]. The 10-year CVR was estimated based on the Systematic Coronary Risk Evaluation (SCORE), the modified SCORE [14], and the Framingham Risk Score [15]. The modified SCORE (mSCORE) was obtained by multiplying SCORE by 1.5 in RA patients meeting at least two of the following criteria: i) disease duration >10 years, ii) positivity for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP), and iii) presence of extra-articular manifestations [16]. Disease activity was quantified with the Clinical Disease Activity Index (CDAI), the Simple Disease Activity Index (SDAI), and the Disease Activity Score-28 (DAS28). CDAI scores were interpreted as follows: <2.8 clinical remission, >2.8-10 low activity, >10-22 moderate activity, and >22 high activity. SDAI scores were interpreted as follows: <3.3 clinical remission, >3.3-11 low activity, >11-26 moderate activity, and >26 high activity. Based on C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR), DAS28 scores were interpreted as follows: <2.6 clinical remission, >2.6-3.1 low activity, >3.2-5.0 moderate activity, and >5.1 high activity [17].

3.3.4.4 Laboratory evaluation

Laboratory data, including complete blood count, rheumatoid factor (RF), anti-CCP, glycemia, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and uric acid, were obtained from patient records.

For the quantification of proBNP and cytokines (TNF-α, IL-1β, IL-6, IL-10) by ELISA, as well as the assessment of Nrf2 and HO-1 mRNA expression by qRT-PCR, a 25 mL peripheral blood sample was collected from each participant.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Following collection, blood samples were anticoagulated, diluted in phosphate-buffered saline (PBS), and carefully layered over a Ficoll-Paque solution, then centrifuged at 4000 g for 15 minutes at 4°C, without brake. The PBMC layer was collected, washed twice with PBS (300 g, 10 min, 4°C), and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) for subsequent experiments.

An aliquot of 1–2 × 10^6 PBMCs was used for cytokine assays or RNA extraction, while the remaining cells were stored at -80°C for future analyses.

ProBNP levels were estimated with a clinically validated automated immunoassay (Cobas® e 801, Roche Diagnostics, Basel, Switzerland). The controls used the manufacturer's acceptability limits, with measurement intervals of 5-35,000 pg/mL. No cross-reactivity with atrial natriuretic peptide, N-terminal atrial natriuretic peptide or BNP was detectable.

The levels of TNF-α, IL-1β, IL-6 and IL-10 were measured with the commercial kit

DuoSet ELISA (R&D Systems Inc., MN, USA), along with standard curves. All kits were used following the manufacturer's directions, and the results were expressed in pg/mL.

The qRT-PCR analysis of the peripheral RNA samples was performed as follows: the total RNA was extracted from peripheral blood collected in EDTA tubes using the reagent Trizol (Invitrogen, São Paulo, Brazil). The reverse transcription was performed with SuperScript IV (Invitrogen, São Paulo, Brazil), according to the manufacturer's directions. A StepOne Real-Time PCR thermocycler (Applied Biosystems, Warrington, UK) with SYBR Green Master Mix (Applied Biosystems, Warrington, UK) was employed for the qRT-PCR reaction, following the manufacturer's directions. The relative gene expression was determmined with the 2-ΔΔCt method [18]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as endogenous control to normalize the mRNA expression. The specificity of each pair of primers was confirmed by dissociation curve analysis of the PCR products. The primer sequences for real-time PCR were: Nrf2 (Forward 5′-3′: GTCCCAGCAGGACATGGAT; Reverse 5′-3′: GCTCATACTCTTTCCGTCGC; GenBank serial number: HM446346.1), HO-1 (Forward 5′-3′: GGCCTAAACTTCAGAGGGGG; Reverse 5′-3′: CAGCTGCCACATTAGGGTGT; GenBank serial number: NM_002133.3) and GAPDH, the reference gene (Forward 5′-3′: GTGGCTGGCTCAGAAAAAGG; Reverse 5′-3′: GGTGGTCCAGGGGTCTTACT; GenBank serial number: AY340484.1).

3.3.4.5 Statistical analysis

Clinical and demographic variables were expressed as mean ± standard deviation (normally distributed continuous variables) or as absolute numbers (n) and percentages (%) (categorical variables) and submitted to Pearson's chi-squared test (χ^2). Medians (minimum-maximum) were calculated for continuos variables not normally distributed.The Shapiro-Wilk test was used to confirm the normal distribution of each quantitative data set. Non-parametric data were analyzed with the Mann-Whitney test or the Kruskal-Wallis test, as required, followed by Dunn's post-test. Spearman's correlation test was employed to calculate the correlation coefficient (r) and the determination coefficient (r^2) of the quantitative variables. All analyses were performed with the software SPSS for Windows (v. 20.0), with the level of significance set at $p<0.05$.

3.3.5 Results

3.3.5.1 Epidemiological, clinical and laboratory profile

The participants (n=120) were predominantly female (90%). In the RA group 91.7% were female. The mean age was 52 ± 13 years (controls) and 52 ± 12 years (RA group).

Table 1 shows the traditional CVR factors and the clinical and laboratory findings.

Higher frequencies of the following cardiovascular risk factors were observed in controls compared to RA patients: dyslipidemia (55% vs 33%, p=0.027), WC (91.67 ± 10.63 vs. 87.65 ± 10.99, p=0.017), BMI (28.58 ± 4.17 vs. 26.83 ± 5.22, p=0.010) , glycemia (109.95 ± 30.86 vs. 93.92 ± 26.35, p<0.001), role and triglycerides (154.27 ± 93.00 vs. 114.37 ± 54.96, p=0.004).

The mean disease duration was 10.43 ± 7.55 years, and 63.3% were positive for RF and/or anti-CCP. Disease activity was quantified and each RA patient was classified as either 'in remission/low activity' or 'moderate/high activity'. The latter category was assigned to 38.3% (DAS-28 PCR), 50% (DAS28 ESR), 53.3% (SDAI) and 45% (CDAI).

Almost all RA patients (98.3%) were in treatment, receiving corticosteroids (26.7%) and/or synthetic disease-modifying anti-rheumatic drugs DMARDs (91.7%), the most common of which was methotrexate (72.9%). DMARDs were not associated with other classes of antirheumatic drugs in 38.3%. Biologic DMARDs were used in 21.7%, targeted DMARDs in 11.7%.

Table 1: Traditional cardiovascular risk factors and clinical and laboratory findings of subjects with and without rheumatoid arthritis (RA).

Variable	Controls (n=60) (n/%)	RA (n=60) (n/%)	*p*-value
Family history of cardiovascular disease			
Yes	6 (10.0)	9 (15.0)	0.582 [a]
No	54 (90.0)	51 (85.0)	
Smoking (current)			
Yes	6 (10.0)	1 (1.7)	0.114 [a]
No	54 (90.0)	59 (98.3)	
Smoking (previous)			
Yes	10 (16.7)	14 (23.3)	0.360 [a]
No	50 (83.3)	46 (76.7)	
Systemic arterial hypertension			
Yes	21 (35.0)	21 (35.0)	1.000 [a]
No	39 (65.0)	39 (65.0)	
Diabetes mellitus			
Yes	11 (18.3)	5 (8.3)	0.178 [a]
No	49 (81.7)	55 (91.7)	
Dyslipidemia			
Yes	33 (55.0)	20 (33.3)	**0.027** [a]
No	27 (45.0)	40 (66.7)	
Sedentary lifestyle			
Yes	39 (65)	47 (78.3)	0.152 [a]

Variable	Controls (n=60) (n/%)	RA (n=60) (n/%)	*p*-value
Family history of cardiovascular disease			
Yes	6 (10.0)	9 (15.0)	0.582 [a]
No	54 (90.0)	51 (85.0)	
Smoking (current)			
Yes	6 (10.0)	1 (1.7)	0.114 [a]
No	54 (90.0)	59 (98.3)	
No	21 (35)	13 (21.7)	
Physical examination			
Systolic blood pressure	123.33 ± 18.66	120.50 ± 9.46	0.905 [b]
Diastolic blood pressure	81.50 ± 11.02	80.67 ± 5.48	0.602 [b]
Waist circumference (cm)	91.67 ± 10.63	87.65 ± 10.99	**0.017** [b]
Body mass index	28.58 ± 4.17	26.83 ± 5.22	**0.010** [b]
Body mass index			
Low weight + ideal weight	13 (21.7)	20 (33.3)	0.234 [a]
Overweight	27 (45)	28 (46.7)	
Obesity	20(33,3)	12 (20.0)	
Laboratory tests			
Blood glucose	109.95 ± 30.86	93.92 ± 26.35	**<0.001**[b]
Uric acid	3.29 ± 0.97	3.20 ± 1.29	0.371[b]
Total cholesterol	197.60 ± 43.63	192.05 ± 29.60	0.785[b]
HDL	56.60 ± 12.04	59.77 ± 14.17	0.301[b]
LDL	110.15 ± 40.83	108.43 ± 28.96	0.795[b]
TGs	154.27 ± 93.00	114.37 ± 54.96	**0.004**[b]

Data expressed as absolute numbers (n) and percentages (%) or mean ± standard deviation of the mean or absolute number (%). [a]Pearson's chi-square test. [b]Mann-Whitney test. Bold type=significant at the level of 5% ($p<0.05$). HDL=high density lipoprotein); LDL=low density lipoprotein; TG=triglycerides.

3.3.5.2 Cardiovascular risk

The two groups did not differ significantly with regard to 10-year CVR according to the SCORE/mSCORE ($p=0.261$) and Framingham scores ($p=0.069$) (Table 2). On the other hand, proBNP levels were significantly higher in the RA group (86.77 ± 76.03) than in the control group (60.12 ± 52.28) ($p=0.009$) (Table 2).

Table 2. Prediction of cardiovascular risk in SCORE/mSCORE and Framingham scores, and proBNP serum levels in subjects with and without rheumatoid arthritis (RA)**.**

Variable	Controls (n=60) (n/%)	RA (n=60) (n/%)	*p*-value
SCORE/mSCORE			

Low (<1%)	31 (55.4)	25 (43.9)	0.261[a]
Moderate (1% to <5%)	25 (44.6)	32 (56.1)	
Framingham			
Low (≤10%)	41 (68.3)	51 (85)	0.069[a]
Moderate (>10-20%)	14 (23.3)	5 (8.3)	
High (≥20%)	5 (8.3)	4 (6.7)	
ProBNP serum levels	60.12 ± 52.28	86.77 ± 76.03	**0.009**[b]

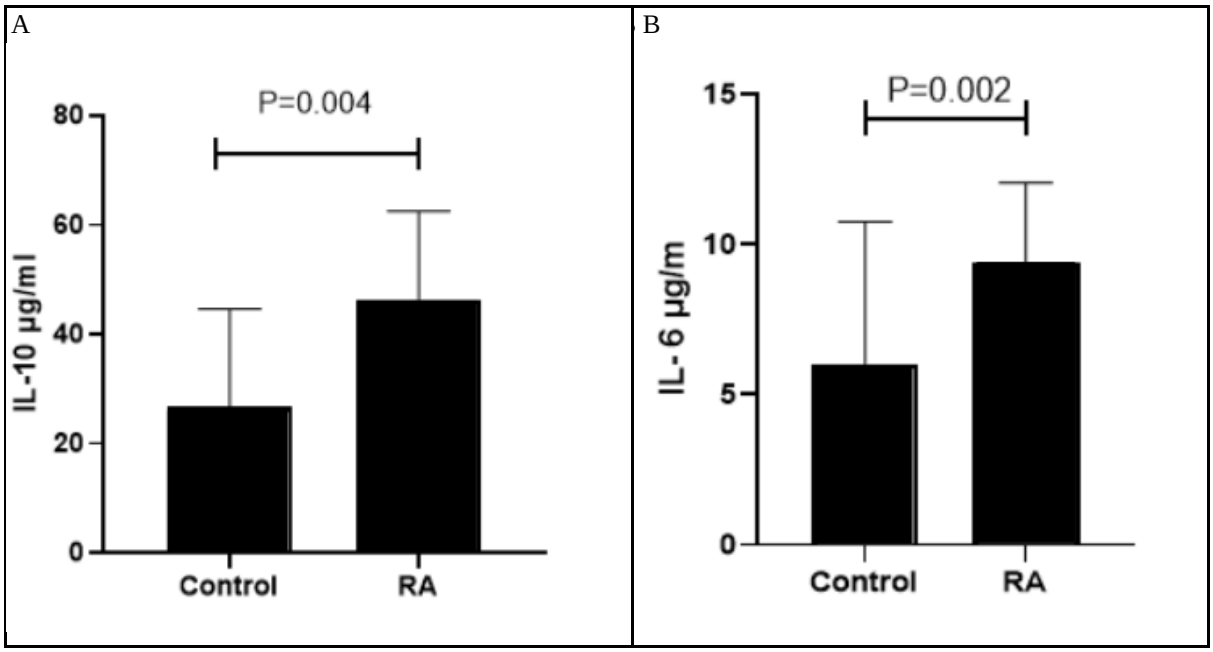
Data expressed as absolute numbers (n) and percentages (%) or mean ± standard deviation of the mean or absolute number (%). [a]Pearson's chi-square test. [b]Mann-Whitney test. Bold type=significant at the level of 5% (p<0.05). ProBNP=N-terminal prohormone brain natriuretic peptide.

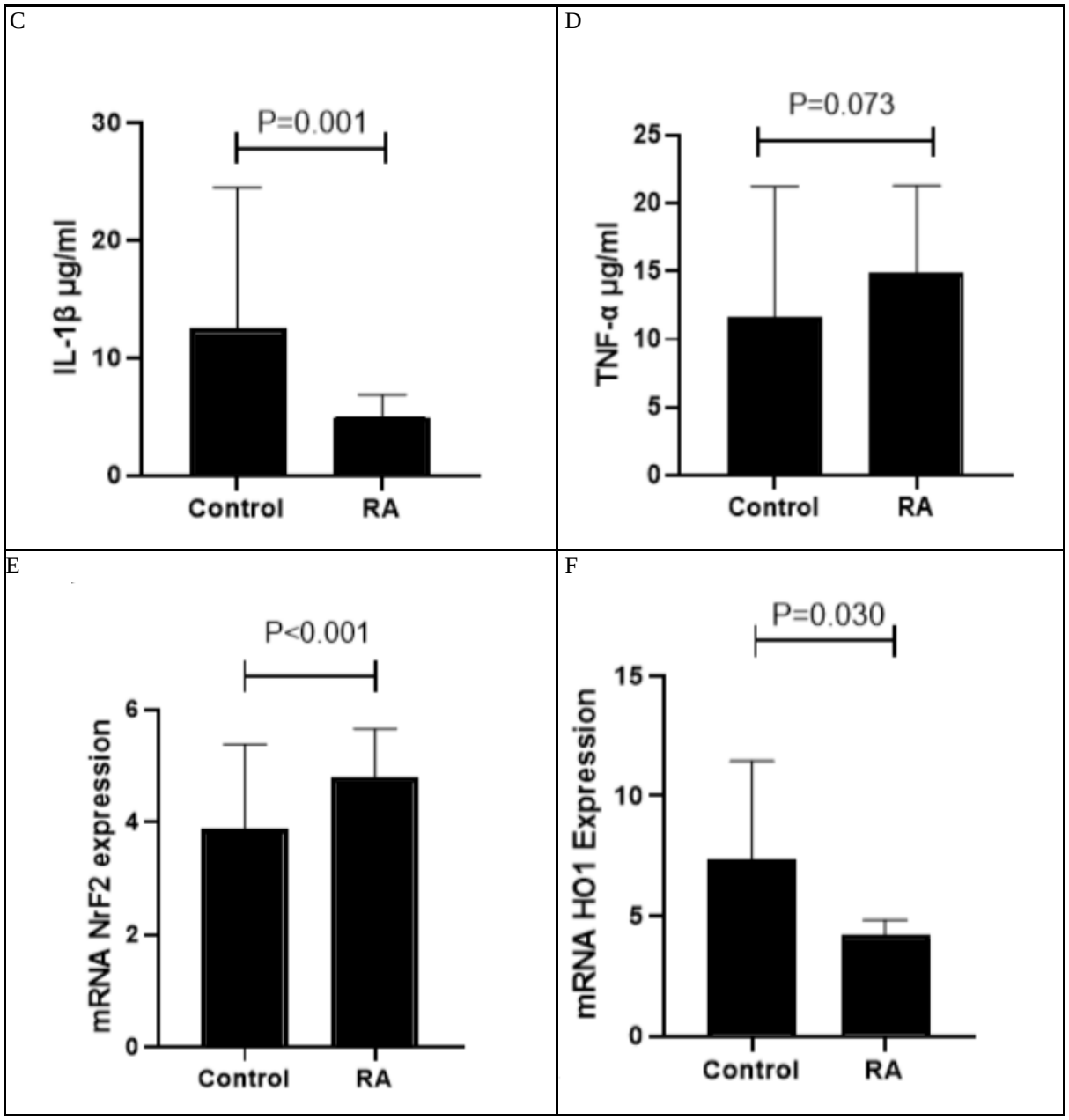
3.3.5.3 Serum and Nrf2/HO-1 pathway cytokines

The RA group displayed significantly higher levels of IL-10 (p=0.004) and IL-6 (p=0.002) and lower levels of IL-1β (p=0.001) than the control group (Figures 1A, 1B and 1C, respectively). No significant difference was observed between the groups for TNF-α (p=0.073) (Figure 1D).

Nrf2 mRNA levels were significantly higher in the RA group (4.79 ± 6.72) than in the control group (3.88 ± 11.46) (p<0.001) (Figure 1E). Conversely, HO-1 mRNA levels were significantly lower in the RA group (4.18 ± 5.15) than in the control group (7.33 ± 31.67) (p<0.030) (Figure 1F).

Figure 1: Serum levels of the cytokines IL-10 (A), IL-6 (B), IL-1 beta (C) and TNF-alpha (D), and serum levels of mRNA-Nrf2 (E) and mRNA-HO-1 (F) in subjects with and without rheumatoid arthritis.





RA= rheumatoid arthritis. Data expressed as mean ± standard deviation.

3.3.5.4 Analysis of association

In this study we evaluated the association between different disease activity indices and CVR. A significant association (p=0.018) was found between remission on DAS28 ESR and Framingham CVR. No other disease activity index (SDAI, CDAI, DAS 28 PCR) was significant (Table 3).

Table 3: Disease activity scores vs. Framingham scores in patients (n=60) with rheumatoid arthritis (RA).

Variable	**Framingham score**			***p*-value**
Disease activity scores	Low **(n/%)**	Moderate **(n/%)**	High **(n/%)**	
DAS 28 CRP				
Remission	26 (51.0)	4 (80.0)	1 (25.0)	0.163
Low	5 (9.8)	0 (0.0)	1 (25.0)	
Moderate	19 (37.3)	1 (20.0)	1 (25.0)	
High	1 (2.0)	0 (0.0)	1 (25.0)	
DAS 28 ESR				
Remission	21 (41.2)	1 (20.0)	0 (0.0)	**0.018**
Low	4 (7.8)	3 (60.0)	1 (25.0)	
Moderate	16 (31.4)	1 (20.0)	1 (25.0)	
High	10 (19.6)	0 (0.0)	2 (50.0)	
SDAI				
Remission	15 (29.4)	1 (20.0)	1 (25.0)	0.261
Low	8 (15.7)	3 (60.0)	0 (0.0)	
Moderate	17 (33.3)	1 (20.0)	2 (50.0)	
High	11 (21.6)	0 (0.0)	1 (25.0)	
CDAI				
Remission	20 (39.2)	1 (20.0)	1 (25.0)	0.258
Low	7 (13.7)	3 (60.0)	1 (25.0)	
Moderate	18 (35.3)	1 (20.0)	1 (25.0)	
High	6 (11.8)	0 (0.0)	1 (25.0)	

Data expressed as absolute numbers (n) and percentages (%). Pearson's chi-square test. Bold type=significant at the level of 5% ($p<0.05$). DAS28=Disease Activity Score-28; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; SDAI=Simplified Disease Activity Index; CDAI=Clinical Disease Activity Index.

When evaluating the association between disease activity and serum cytokines levels, we found a significant positive association between moderate/high activity on CDAI and IL-6 ($p=0.03$). Spearman correlation between CDAI and IL-6 was 0.17, p=0.197. No other cytokine (IL-10, IL-1β, TNF-α) was significantly associated with any of the disease activity indices (CDAI, SDAI, DAS 28 ESR, DAS 28 PCR) ($p>0.05$) (Table 4).

Table 4: Disease activity indices vs. serum cytokines and Nrf2/HO-1 gene expression in patients (n=60) with rheumatoid arthritis (RA).

Variable	SDAI		
	Remission / Low	**Moderate / High**	***p*-value**
Interleukins Median (Minimum-Maximum)			
IL-10	0.00 (0.00-693.14)	0.00 (0.00-580.10)	0.754[b]
IL-6	1.00 (0.00-80.96)	2.21 (0.00-85.69)	0.258[b]
TNF-alpha	0.00 (0.00-114.02)	0.00 (0.00-343.25)	0.771[b]
IL-1 beta	0.00 (0.00-87.35)	0.00 (0.00-59.80)	0.339[b]
Gene expression Median (Minimum-Maximum)			
Nrf2	3.81 (0.02-26.77)	2.37 (0.24-59.8)	0.477[b]
HO-1	1.65 (0.30-21.86)	1.08 (0.31-20.08)	0.900[b]
Variable	**CDAI**		
	Remission / Low	**Moderate/ High**	**p-value**
Interleukins Median (Minimum-Maximum)			
IL-10	0.00 (0.00-693.14)	1.95 (0.00-580.10)	0.405[b]
IL-6	0.00 (0.00-80.96)	3.96 (0.00-85.69)	**0.03**
TNF-alpha	0.00 (0.00-343.28)	0.00 (0.00-88.88)	0.986
IL-1 beta	0.00 (0.00-87.35)	0.00 (0.00-59.80)	0.819
Gene expression Median (Minimum-Maximum)			
Nrf2	3.59 (0.02-26.77)	2.72 (0.24-35.11)	0.567
HO1	1.14 (0.30-21.86)	2.00 (0.31-20.08)	0.789
Variable	**DAS 28 CRP**		
	Remission / Low	**Moderate/ High**	**p-value**
Interleukins Median (Minimum-Maximum)			
IL-10	0.00 (0.00-693.14)	1.95 (0.00-580.10)	0.638
IL-6	0.50 (0.00-80.96)	3.96 (0.00-85.69)	0.063
TNF-alpha	0.00 (0.00-343.28)	0.00 (0.00-88.88)	0.986
IL-1 beta	0.00 (0.00-87.35)	0.00 (0.00-59.80)	0.839
Gene expression Median (Minimum-Maximum)			
Nrf2	3.99 (0.02-35.11)	2.02 (0.24-23.00)	0.251
HO-1	1.31 (0.30-21.86)	0.83 (0.37-16.31)	0.988

Data expressed as absolute numbers (n) and percentages (%). Mann-Whitney test. Bold type=significant at the level of 5% (p<0.05). DAS28=Disease Activity Score-28; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; SDAI=Simplified Disease Activity Index; CDAI=Clinical Disease Activity Index; Nrf2=Nuclear factor erythroid 2-related factor 2; HO-1=heme oxygenase 1.

Finally, the cytokines (IL-6, IL-10, IL-1β, TNF-α) and the Nrf2/HO-1 signaling pathway were not significantly associated with RF/anti-CCP positivity or disease duration (p>0.05) (Table 5).

Table 5: Serum cytokines and Nrf2/HO-1 gene expression vs. FR/Anti-CCP positivity and disease duration in patients (n=60) with rheumatoid arthritis (RA).

Variable	**RF/Anti-CCP+**	**RF or Anti-CCP+**	**RF/Anti-CCP-**	***p*-value**
Interleukins Median (Minimum-Maximum)				
IL-10	0.00 (0.00–115.48)	0.00 (0.00–693.14)	0.00 (0.00–580.10)	0.978
IL-6	2.13 (0.00–81.77)	0.54 (0.00–80.96)	1.00 (0.00–85.69)	0.791
TNF-alpha	0.00 (0.00–88.88)	0.03 (0.00–114.02)	0.00 (0.00–343.28)	0.609
IL-1 beta	0.00 (0.00–28.07)	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.779
Gene expression. Median (Minimum-Maximum)				
Nrf2	2.49 (0.04–23.00)	1.92 (0.00–9.88)	3.74 (0.02–35.12)	0.584
HO-1	0.85 (0.31–16.32)	3.49 (0.37–21.86)	2.01 (0.30–20.08)	0.997
Variable	**Disease duration**			
	1-5 years	**5-10 years**	**>10 years**	***p*-value**
Interleukins Median (Minimum-Maximum)				
IL-10	1.39 (0.00–115.48)	3.06 (0.00–693.14)	0.00 (0.00–580.10)	0.671
IL-6	1.09 (0.00–37.38)	3.54 (0.00–81,77)	0.94 (0.00–85.69)	0.633
TNF-alpha	0.00 (0.00–49.94)	0.03 (0.00–114.02)	0.00 (0.00–343.28)	0.819
IL-1 beta	0.00 (0.00–28.07)	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.249
Gene expression. Median (Minimum-Maximum)				
Nrf2	2.49 (0.04–23.00)	1.92 (0.00–9.88)	3.74 (0.02–35.12)	0.504
HO-1	0.85 (0.31–16.32)	3.49 (0.37–21.86)	2.01 (0.30–20.08)	0.359

Data expressed as absolute numbers (n) and percentages (%). Mann-Whitney test. Bold type=significant at the level of 5% (p<0.05). RF=rheumatoid factor; Anti-CCP=anti-cyclic citrullinated peptide; Nrf2=Nuclear factor erythroid 2-related factor 2; HO-1=heme oxygenase 1.

3.3.6 Discussion

To our knowledge, this is the first study to investigate to what extent serum cytokines

and the Nrf2/HO-1 signaling pathway are associated with clinical and laboratory variables and CVR in RA patients. We found the Nrf2 gene expression to be stronger and the HO-1 expression weaker in RA patients than in healthy controls, although the tested serum cytokines and the Nrf2/HO-1 pathway could not be shown to be associated with RF/anti-CCP positivity, disease duration or disease activity. We also observed increased serum proBNP levels in RA patients and higher disease activity in patients with high serum levels of IL-6.

CVR scores have been used to make predictions for the general population, subsidize preventive measures and avoid unfavorable outcomes [16]. In this study, however, SCORE/mSCORE and Framingham scores were statistically similar for RA and non-RA subjects, while the traditional CVR factors (WC, BMI, glucose, triglycerides) were more prevalent among controls. This finding raises the hypothesis that CVR was increased in RA due to the pathogenesis of the condition, equating the deleterious effects of the more prevalent CVR factors observed in the control group [19].

Serum proBNP levels were increased in RA patients. A diagnostic biomarker of heart failure and dysfunction in clinical practice, high proBNP levels have been independently associated with CVD and mortality [20,21]. Matching our findings, a study involving patients with early RA and no history of DMARD or glucocorticoid use reported high levels of proBNP [22]. Likewise, proBNP was a predictor of mortality in the EURIDISS cohort study, which included RA patients with up to 4 years of disease duration followed up for 10 years, suggesting that proBNP is a simple, robust and non-invasive CVR indicator in RA patients which can help identify and stratify risk in this population [23].

In the present study, RA patients had significantly higher serum IL-10 and IL-6 levels and significantly lower IL-1β levels than healthy controls, but no difference was found for TNF-α levels. Other authors have found raised serum IL-10 levels in RA patients [24], possibly indicating a continuous effort of the organism to restore local homeostasis. However, increased levels of other inflammatory mediators in RA patients are known to counteract this effort, even when the clinical management is adequate [25,26].

Interestingly, in our RA patients the increase in IL-6 levels was associated with higher CDAI scores. Likewise, some authors [27] have reported serum IL-6 levels almost three times higher in RA patients than in controls and a positive correlation between IL-6 and DAS28 ESR, suggesting IL-6 may be a biomarker of disease activity. An earlier study [28] also identified significantly higher serum IL-6 levels in RA patients vs. non-RA controls, in addition to significant associations between IL-6, CRP and duration of morning stiffness. Others found serum IL-6 levels to be significantly correlated with RA activity and severity, suggesting IL-6

may be an important treatment target in RA [29].

Our RA patients displayed significantly lower levels of serum IL-1β than our healthy controls. Increased serum IL-1β levels are generally expected as a result of the presence of erosions in the early stages of RA. In fact, high serum IL-1β levels in RA patients correlate well with disease activity [30], but the predictive value of this marker may be compromised by the use of DMARDs [31,32]. The lower IL-1β levels observed in our study are therefore likely due to disease control achieved by long-term treatment of RA. Importantly, one study showed that RA patients with high disease activity appear to have the highest risk of developing CVD. These findings support the implementation of tight control (treat-to-target) strategies in daily clinical practice to achieve low disease activity or remission, also aiming to reduce CVD risk in this population [33]. Moreover, a systematic review and meta-analysis of observational and controlled trials-including 28 studies and 236,525 RA patients- found that DMARDs (particulary methotrexate) were associated with a 28% reduction in cardiovascular events (relative risk (RR) 0.72, 95% confidence interval (CI) 0.57 to 0.91) and TNF inhibitors with a 30% reduction(RR 0.70, CI 0.54 to 0.90), with specifically protective effects observed for myocardial infarction (RR 0.59,CI 0.36 to 0.97) and stroke (RR 0.57, CI 0.35 to 0.92)[34].

When analyzing the association between CVR and disease activity, we observed that patients with RA in remission on DAS 28 ESR had lower Framingham scores, matching the literature which shows that inflammation in RA increases CVR [33]. These findings reflect the multifactorial nature of CVR in RA, considering ~30% of the risk to be due to disease-related chronic inflammatory activity and ~70% to be explained by traditional CVR factors, with emphasis on arterial hypertension and smoking [33].

Notably, the gene expression of Nrf2 and HO-1 was significantly higher and lower, respectively, in the RA group than in the control group, indicating compromised antioxidant protection in RA. This finding aligns with previous studies reporting disturbances in the Nrf2 signaling pathway in this condition [35]. It has been demonstrated that RA synovial tissues exhibit abnormally low levels of Nrf2 targets heme oxygenase 1 (HO-1) mRNA and protein [36]. A possible explanation is that chronic inflammation and oxidative stress in RA may induce to compensatory upregulation of Nrf2 expression as a cellular response to oxidative damage [37]. However, despite increased Nrf2 mRNA levels, its downstream transcriptional activity may be impaired due to several factors, such as epigenetic modifications, post-translational alterations (e.g., excessive Keap1-mediated degradation), or disrupted nuclear translocation of the Nrf2 protein [38]. These mechanisms may prevent adequate upregulation HO-1 expression, even in the presence of elevated Nrf2 levels. Moreover, pro-

inflammatory cytokines associated with RA have been shown to suppress HO-1 transcription through epigenetic mechanisms, including promoter hypermethylation, histone deacetylation, and microRNA regulation [39].

The discrepancy between elevated Nrf2 mRNA levels and reduced HO-1 expression observed in RA patients suggests that additional regulatory mechanisms may disrupt the canonical Nrf2-HO-1 axis. Previous studies have reported increased Nrf2 expression in experimental models of RA, likely as a compensatory response to persistent inflammation and oxidative stress [35,36,39]. However, this transcription upregulation does not necessarily translate into activation of downstream target genes. Supporting this notion, Ray et al [40] demonstrated that HO-1 expression requires histone H3 phosphorylation at serine 10, indicating that permissive chromatin remodeling is a prerequisite for transcriptional activation. In addition, Reichard et al. showed that the transcriptional repressor BACH1 must be inactivated for Nrf2 to effectively induce HO-1 expression [41]. More recently, Yoo et al. reported that TonEBP (NFAT5) suppresses HO-1 transcription by preventing Nrf2 recruitment to its promoter, even in the context of elevated Nrf2 expression [42]. Collectively, these findings underscore the importance of transcriptional co-regulators and epigenetic modifications in enabling HO-1 induction, suggesting that Nrf2 abundance alone may be insufficient in the absence of a permissive epigenetic environment.

Little information on the role of the Nrf2/HO-1 pathway in the cardiovascular system is available, but one study suggests that a compromised Nrf2/HO-1 pathway can negatively affect the redox balance and mediate cardiovascular changes such as those observed in patients with obesity, diabetes mellitus and atherosclerosis [10]. Also, long-term hyperlipidemia reduces Nrf2/HO-1 pathway activation, inducing oxidative stress in endothelial cells [43], and Nrf2 reduces oxidative damage to endothelial cells and prevents endothelial dysfunction in cardiovascular diseases [44].

Overexpression of HO-1 has been shown to reduce the formation of atherosclerotic lesions by inhibiting lipid peroxidation, indicating HO-1 may have an intrinsic antioxidant function [45]. Likewise, under acute oxidative stress and inflammation (as in sepsis), induction of the Nrf2/HO-1 pathway reportedly improves cardiovascular function in rats [46].

The complexity of the pathogenesis of RA, which involves immune response, genetics, inflammation and oxidative stress, makes it difficult to identify the proinflammatory molecules causing vascular endothelial damage, but it should be considered that dysfunction of the Nrf2/HO-1 pathway likely contributes to vascular damage and disease-related cardiovascular risk by inducing oxidative stress and inflammation.

Our study was to some extent limited by i) the cross-sectional study design which did not allow us to longitudinally observe cardiovascular outcomes and their possible relationship with oxidative stress, ii) the higher prevalence of traditional CDR factors in the control group, iii) the potential bias associated with the recruitment of patients from a secondary-level outpatient service, with an arguably smaller proportion of severe cases and lower CVR, and iv) we cannot determine whether reduced HO-1 expression might also result from impaired Nrf2 nuclear translocation or post-translational modifications, as the current study focused solely on mRNA expression in peripheral blood samples and did not include protein quantification or subcellular localization assays. Nevertheless, our study provide a rationale for future investigations into the nuclear-cytoplasmic dynamics of Nrf2, which may further clarify the mechanisms underlying Nrf2-HO-1 uncoupling in RA.

3.3.7 Conclusion

In conclusion, we identified higher Nrf2 expression and lower HO-1 expression in the RA group, though not associated with CVR, RA-related clinical and laboratory variables, and serum cytokine levels. Moderate/ High disease activity was positively associated with IL-6 and high levels of proBNP were found in patients with RA compared to controls. This study found a significant dysregulation in Nrf2/HO-1 pathway activity in RA patients, providing clinical evidence to support future research on the role of this pathway in RA pathogenesis.

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4 CONSIDERAÇÕES FINAIS

Este estudo, pioneiro e inédito, integra um projeto de investigação voltado à avaliação do risco cardiovascular na artrite reumatoide por diferentes abordagens metodológicas, não se restringindo ao artigo aqui incluído. Nesse contexto, os resultados provenientes dessas análises fundamentaram a elaboração de outros dois artigos científicos, atualmente em fase de publicação que incluem : *"Subclinical diastolic dysfunction and its association with disease activity in patients with rheumatoid arthritis"* (Anexo D) e *"Lower educational level is associated with higher disease activity and increased cardiovascular risk in rheumatoid arthritis from northeastern Brazil: a cross-sectional study"* (Anexo E), os quais aprofundam, sob perspectivas complementares, a compreensão das interações entre atividade inflamatória, fatores socioeducacionais e comprometimento cardiovascular na artrite reumatoide.

5 CONCLUSÃO

Em conclusão, este estudo demonstrou uma desregulação significativa da via Nrf2/HO-1 em pacientes com artrite reumatoide, caracterizada por maior expressão de Nrf2 e menor expressão de HO-1. Destaca-se a avaliação integrada dessa via com parâmetros clínicos, laboratoriais e cardiovasculares. Adicionalmente, a identificação de níveis elevados de pró-BNP, mesmo na ausência de manifestações clínicas de doença cardiovascular, sugere a presença de possível envolvimento cardiovascular subclínico nesses pacientes. Em conjunto,nossos achados contribuem de para o maior entendimento e futuras investigações sobre o papel da via Nrf2/HO-1 na patogênese da artrite reumatoide e no risco cardiovascular associado.

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APÊNDICES

APÊNDICE A
TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto: AVALIAÇÃO DE RISCO CARDIOVASCULAR EM PACIENTES COM ARTRITE REUMATOIDE: POSSÍVEL ASSOCIAÇÃO COM ATIVIDADE DA DOENÇA, CITOCINAS E A VIA NRF2/HO-1

Pesquisador responsável: Christiane Aguiar Nobre

Instituição responsável: Universidade Federal do Ceará

Você está sendo convidado (a) a participar de uma pesquisa porque foi atendido (a) ou está sendo atendido (a) nesta instituição e teve diagnóstico de **ARTRITE REUMATOIDE**. Para que você possa decidir se quer participar ou não, precisa conhecer os benefícios, os riscos e as consequências pela sua participação. Este documento é chamado de Termo de Consentimento Livre e Esclarecido e tem esse nome porque você só deve aceitar participar desta pesquisa depois de ter lido e entendido este documento. Leia as informações com atenção e converse com o pesquisador responsável e com a equipe da pesquisa sobre quaisquer dúvidas que você tenha. Caso haja alguma palavra ou frase que você não entenda, converse com a pessoa responsável por obter este consentimento, para maiores esclarecimentos. Converse com os seus familiares, amigos e com a equipe médica antes de tomar uma decisão. Se você tiver dúvidas depois de ler estas informações, entre em contato com o pesquisador responsável. Após receber todas as informações, e todas as dúvidas forem esclarecidas, você poderá fornecer seu consentimento por escrito, caso queira participar. A sua participação neste estudo é voluntária e não é obrigatória. Você pode aceitar participar do estudo e depois desistir a qualquer momento. Isto não tirará nenhum direito do seu tratamento e assistência. Você também poderá pedir a qualquer momento que as suas informações sejam excluídas completamente deste estudo e que elas não sejam usadas para outros fins. A pesquisa estará sob a responsabilidade da pesquisadora Christiane Aguiar Nobre e tem como objetivo avaliar o risco cardiovascular (exemplo: risco de infarto, risco de AVC) em pacientes com artrite reumatoide. Para isso realizaremos uma pesquisa em seu prontuário e precisamos que você responda as perguntas contidas no questionário. Além disso, será realizado um exame físico (exame no seu corpo), exame de sangue e de ultrassonografia (exame de ultrassom) do coração e carótidas (vasos do pescoço). O seu nome assim como os resultados dos seus exames não será divulgado em nenhuma fase do estudo. Os dados coletados, os resultados dos exames ultrassonográficos e os de sangue realizados serão utilizados apenas nesta pesquisa e os resultados divulgados em eventos e/ou revistas científicas. Esta pesquisa poderá trazer maior conhecimento sobre o fato de que pacientes com artrite reumatoide tem mais doença no coração, o que poderá orientar novos

tratamentos. É possível que este estudo não traga benefícios diretos a você, mas ao final deste estudo, as informações que ele gerar, poderão trazer benefícios a outros pacientes. Se o preenchimento deste questionário trouxer algum constrangimento, comunique ao pesquisador. O contato do responsável pela pesquisa é **(88) 9-9914-2670**. O Comitê de Ética em Pesquisa (CEP) é formado de um grupo de profissionais de diversas áreas, cuja função é avaliar as pesquisas realizadas com seres humanos. O CEP a qual está submetida essa pesquisa é o CEP/UFC/PROPESQ. O CEP/UFC/PROPESQ é a instância da Universidade Federal do Ceará responsável pela avaliação e acompanhamento dos aspectos éticos de todas as pesquisas envolvendo seres humanos. Qualquer dúvida o Sr. (Sra.) poderá entrar em contato com o CEP/UFC/PROPESQ:

Endereço: Rua Coronel Nunes de Melo, 1000 - Rodolfo Teófilo - Fortaleza -CE. Telefone: +55 (85) 3366-8346 (segunda à sexta-feira) de 08h às 12h.

E-mail: comepe@ufc.br

O Termo de Consentimento Livre e Esclarecido é feito em duas vias, sendo que uma ficará com o Sr. (Sra.) e a outra com a pesquisadora. A sua participação nesse estudo não terá nenhum custo ou quaisquer compensações financeiras.

Sobral – CE, ____ de _____ de 20 _____

Participante da pesquisa _____

(digitais caso não assine)

Pesquisador Responsável _____

APÊNDICE B
FORMULÁRIO DE COLETA DE DADOS CLÍNICOS, LABORATORIAIS E ULTRASSONOGRÁFICOS

FORMULÁRIO DE COLETA DE DADOS CLINICOS, LABORATORIAIS E ULTRASSONOGRÁFICOS

Médico Assistente:_____ Data: ___/___/___

Informações Pessoais:

Nome:_____Prontuário:_____

DN:_____ Idade: ____ Sexo: ____ Raça: _____

Anos de estudo:_____ Telefone:_____

Endereço:_____

AR:

Tempo de doença:_____

Tempo de tratamento:_____

Exame Físico:

Peso: _____ Altura: ____ IMC: _____ CA: _____PA:_____

Dor:___ Edema:___

PGA: ___ EGA: ____ CDAI:____ SDAI:____ DAS28 (VHS):____ DAS (PCR):____

Exames laboratoriais:

HC:_____

PCR:____ VHS:____ FR:_____ anti-CCP:_____ Glic:____ Cr:____ Ur:_____ CT:_____ HDL:_____ LDL:_____ VLDL:____ TG:____ AU:_____TSH:___T4L:__ ProBNP_____

RNAm de NFr2 (qRT-PCR)_____

RNAm de HO-1 (qRT-PCR)_____

Citocinas

TNF-α	IL-1ß,	IL-6	IL-4	IL-10

Outros: _____

_____

Escores de predição de RCV

SCORE:_____ mSCORE_____ Framingham_____

Exames ultrassonográficos

US de carótidas_____

_____

Ecocardiograma_____

Medicações:

Uso atual: _____

Uso prévio:_____

( ) AAS ( ) clopidogrel ( ) anticoagulante ( ) estatinas

Especificar tempo de uso:

( ) corticoide ( ) cloroquina

( ) MTX ( ) LFN

( ) SSZ ( ) biológico

(qual):_____

Anti-hipertensivos:_____

Hipoglicemiantes:_____

Outros: _____

Antecedentes e Comorbidades:

( ) Diabetes ( ) pré-DM ( ) HAS ( ) Dislipidemia ( )Hipotireoidismo

( ) Osteoporose ( ) Osteopenia

( ) Etilismo atual ( ) Antecedente de Etilismo

( )Tabagismo atual ( ) Ex-tabagista (duração? há quanto tempo parou?)

( ) Exercício (qual?_____, frequência___x/sem, duração___min)

( ) HVE, IAM, ANGINA, AIT, AVE, DAOP, FA, ICC (circular os positivos)

( ) História familiar de IAM, AVE ou morte súbita em < 55 anos em homens e < 65 em mulheres (parentes de primeiro grau)

Outras doenças? Quais? _____

ANEXOS

ANEXO A
PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DE RISCO CARDIOVASCULAR EM PACIENTES COM ARTRITE REUMATOIDE: POSSÍVEL ASSOCIAÇÃO COM ATIVIDADE DA DOENÇA, CITOCINAS E A VIA NRF2/HO-1

Pesquisador: Christiane Aguiar Nobre

Área Temática:

Versão: 2

CAAE: 38586820.4.0000.5054

Instituição Proponente: UNIVERSIDADE FEDERAL DO CEARÁ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.491.484

Apresentação do Projeto:

O estudo pretende investigar a possível associação entre maior risco cardiovascular em pacientes com artrite reumatoide e alterações estruturais em coração e carótidas, níveis de citocinas circulantes e a modulação de respostas inflamatórias e imunes e a identificação de um biomarcador que contribua para melhora na condução clínica desses pacientes com a consequente redução de complicações relacionadas a DCV, melhora na sobrevida e qualidade de vida dessa população.

Objetivo da Pesquisa:

Geral: Avaliar o risco cardiovascular em pacientes com Artrite Reumatoide e a possível associação de atividade de doença, citocinas e a via Nrf2/HO-1.

Específicos:

• Analisar o perfil clínico e epidemiológico dos pacientes com Artrite Reumatoide com fatores de risco cardiovascular identificados através do escore de predição de risco cardiovascular (SCORE e mSCORE) e do estudo ultrassonográfico de coração e caróticas;

• Examinar a possível associação entre as alterações ultrassonográficas cardiovasculares e o SCORE/mSCORE dos pacientes com Artrite Reumatoide com a dosagem de citocinas inflamatórias;

• Identificar a possível associação entre as alterações ultrassonográficas cardiovasculares e o SCORE/

mSCORE dos pacientes com Artrite Reumatoide com a via Nrf2/HO-1;
• Estabelecer a possível associação entre as alterações ultrassonográficas cardiovasculares e o SCORE/mSCORE com as características clínicas e laboratoriais da doença dos pacientes com Artrite Reumatoide;
• Examinar a possível associação entre as alterações ultrassonográficas cardiovasculares e o SCORE/mSCORE com os índices de atividade da doença e outros fatores de risco de cardiovascular (hipertensão,diabetes e tabagismo) dos pacientes com Artrite Reumatoide;
• Identificar as variáveis e fatores que independentemente interferem no risco cardiovascular nos pacientes com Artrite reumatoide;
• Propor um biomarcador de risco cardiovascular na Artrite Reumatoide.

Avaliação dos Riscos e Benefícios:

Riscos: riscos mínimos como: quebra de sigilo, cansaço ou aborrecimento ao responder questionários, constrangimento ao realizar exames antropométricos e constrangimento ao se expor durante a realização dos testes ultrassonográficos. Em virtude da coleta de sangue, é lícito ressaltar que existe um risco, considerado mínimo, dos seguintes efeitos adversos: ansiedade, hematoma, sangramentos, anemia, infecção local, reflexo vaso-vagal e lesão de vasos e nervos.
Benefícios: Esta pesquisa poderá trazer maior conhecimento sobre o tema abordado, orientando condutas de tratamento. É possível que este estudo não traga benefícios diretos aos participantes, mas ao final, as informações que ele gerar, poderão trazer benefícios a outros pacientes com Artrite Reumatoide. Benefícios esperados: • Identificar indivíduos com Artrite Reumatoide e de maior risco de doença cardiovascular; • Realizar estratégias de monitoramento e manejo mais efetivas para redução do risco cardiovascular nesses pacientes; com Artrite Reumatoide; • Implementar estratégias terapêuticas custo-efetivas em prevenção primária e secundária (diagnóstico e prognóstico) das doenças cardiovasculares nos pacientes com Artrite Reumatoide.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo transversal envolvendo pacientes com AR em seguimento no Ambulatório de Reumatologia da Policlínica Bernardo Félix de Oliveira em Sobral no Ceará. Serão avaliados parâmetros epidemiológicos, clínicos, ultrassonográficos e laboratoriais. Para o cálculo amostral considerou-se uma população finita com diagnóstico de AR atendida na Policlínica de Sobral/CE em 12 meses. Realizou-se o desenho experimental em 4 fases: cálculo amostral; concordância de participação dos participantes; aplicação de critérios de exclusão; espelhamento de participantes entre os grupos. Para cada paciente com AR, um paciente sem AR com características de sexo, faixa etária e etnia

será aleatoriamente escolhido.
Estima-se cerca de 50 a 60 participantes componham cada um dos grupos finais. Revisão de prontuário, entrevista e exame físicos:participantes serão avaliados por meio de revisão de prontuários, entrevista clínica, exame físico, incluindo medidas antropométricas e cálculo dos índices compostos de atividade de doença, sendo utilizado para isso um questionário padronizado. O exame físico incluirá contagem das articulações dolorosas e edemaciadas, verificação da pressão arterial (PA), peso, altura, circunferência abdominal e índice de massa corporal (IMC). A aferição da PA será feita após 5 minutos de repouso, sendo tirada uma média entre duas medidas.
Índices de atividade de doença :Os índices compostos de atividade de doença: DAS28 (Disease Activity Score), SDAI (Simplified Disease Activity Index) e CDAI (Clinical Disease Activity Index), além de medir a atividade da doença numa escala contínua, permitem categorizar o paciente em estratos de atividade, com o uso de diferentes pontos de corte: remissão, atividade leve, moderada e alta , orientando desta forma o tratamento. Avaliação do Risco de Infarto do Miocárdio em 10 anos : serão realizados os escores SCORE e SCORE modificado na predição de risco dessa população em específico. Participantes classificados como 5% ou mais de chance de risco de evento cardiovascular fatal em 10 anos serão estratificados como alto risco. Exames laboratoriais: Os exames laboratoriais que serão avaliados por meio de revisão de prontuários serão: hemograma completo, fator reumatoide, anticorpo anti-peptídeo citrulinado cíclico, glicemia, colesterol total e frações, triglicérides, ureia, creatinina, ácido úrico, TSH. Tais exames são realizados de forma periódica durante o acompanhamento ambulatorial desses pacientes. Será coletado sangue periférico para análise do NT-proBNP por ELISA, dosagem de citocinas (TNF-, IL-6,) por ELISA e detecção por qRT-PCR da expressão dos níveis de mRNA de Nrf2 e HO-1. Exames ultrassonográficos. Os participantes serão submetidos a exames ultrassonográficos de coração e carótidas no Hospital do Coração Padre José Linhares, unidade de referência cardiológica na região norte do Estado do Ceará. Serão analisadas as características dopplerfluxométricas das artérias carótidas e avaliados parâmetros de função cardíaca.

Considerações sobre os Termos de apresentação obrigatória:
Foram apresentados de forma adequada: folha de rosto; carta de encaminhamento ao CEP; cronograma; orçamento; declaração dos pesquisadores; anuência do local onde serão realizadas as ultrassonografias e TCLE.

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Conclusões ou Pendências e Lista de Inadequações:

Sem pendências

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1633118.pdf	25/10/2020 10:40:46		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEatualizado.pdf	25/10/2020 10:39:37	Christiane Aguiar Nobre	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoCEPUFC.pdf	28/09/2020 10:25:13	Christiane Aguiar Nobre	Aceito
Declaração de Instituição e Infraestrutura	CartaInfraestruturaUNIFOR.pdf	25/09/2020 16:04:19	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaCarlosEwerton.pdf	25/09/2020 16:01:03	Christiane Aguiar Nobre	Aceito
Orçamento	Orcamentocomassinatura.pdf	25/09/2020 15:57:01	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaVicente.pdf	25/09/2020 15:56:14	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaRoberta.pdf	25/09/2020 15:55:20	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaNayara.pdf	25/09/2020 15:54:54	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaJordania.pdf	25/09/2020 15:54:43	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaJackson.pdf	25/09/2020 15:54:20	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaHelliada.pdf	25/09/2020 15:54:01	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaElisabete.pdf	25/09/2020 15:53:33	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	AnuenciaMirna.pdf	25/09/2020 15:53:18	Christiane Aguiar Nobre	Aceito
Declaração de Pesquisadores	CartaAnuenciaChristianeAguiar.pdf	25/09/2020 15:52:45	Christiane Aguiar Nobre	Aceito
Solicitação Assinada pelo Pesquisador	CartadeApreciacao.pdf	25/09/2020 15:52:19	Christiane Aguiar Nobre	Aceito

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

Continuação do Parecer: 4.491.484

Responsável	CartadeApreciacao.pdf	25/09/2020 15:52:19	Christiane Aguiar Nobre	Aceito
Cronograma	CronogramaAtulaizado.pdf	25/09/2020 15:49:57	Christiane Aguiar Nobre	Aceito
Folha de Rosto	FolhadeRostoAtualizada.pdf	25/09/2020 15:42:26	Christiane Aguiar Nobre	Aceito
Declaração de Instituição e Infraestrutura	cartadeanuenciaPoliclinica.pdf	22/09/2020 18:31:07	Christiane Aguiar Nobre	Aceito
Declaração de Instituição e Infraestrutura	CartadeanuenciaUFC.pdf	22/09/2020 18:28:23	Christiane Aguiar Nobre	Aceito
Declaração de Instituição e Infraestrutura	CartadeanuenciaHC.pdf	22/09/2020 18:27:59	Christiane Aguiar Nobre	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FORTALEZA, 07 de Janeiro de 2021

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador(a))

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

ANEXO B
TERMO DE CONCESSÃO E ACEITAÇÃO DE AUXÍLIO A PROJETO

FUNCAP

GOVERNO DO ESTADO DO CEARÁ
Secretaria da Ciência, Tecnologia e Educação Superior

TERMO DE CONCESSÃO E ACEITAÇÃO DE AUXÍLIO A PROJETO DE PESQUISA

CLÁUSULA PRIMEIRA – DA QUALIFICAÇÃO DAS PARTES

CONCEDENTE: Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico – Funcap

CNPJ/MF: 00.078.007/0001-26

BENEFICIÁRIO: Mirna Marques Bezerra Brayner **CPF/MF:** 877.081.244-68

CLÁUSULA SEGUNDA – DA FINALIDADE E DOS VALORES

FINALIDADE: Concessão de Auxílio à Pesquisa para apoio a projeto de pesquisa científica e/ou tecnológica.

TÍTULO DO PROJETO/PLANO DE TRABALHO: AVALIAÇÃO DE RISCO CARDIOVASCULAR EM PACIENTES COM ARTRITE REUMATOIDE: POSSÍVEL ASSOCIAÇÃO COM ATIVIDADE DA DOENÇA, CITOCINAS E A VIA NRF2/HO-1.

IDENTIFICAÇÃO DO PROCESSO

NÚMERO: P20-0171-00094.01.00/20 **SPU Nº:** 07897797/2020

EDITAL/CHAMADA: CHAMADA 02/2020 - Programa Pesquisa para o SUS/PPSUS-CE FUNCAP-SESA-Decit/SCTIE/MS-CNPq

AUXÍLIO FINANCEIRO

CAPITAL: R$ 41.921,24

CUSTEIO: R$ 176.186,77

BOLSA: R$ 9.600,00

VALOR TOTAL: R$ 227.708,01

Parágrafo Primeiro. Os recursos serão liberados pela Funcap em função de suas disponibilidades financeiras e orçamentárias.

Parágrafo Segundo. No caso do projeto incluir bolsas:

I. O pagamento das bolsas de longa duração será efetuado diretamente ao bolsista, mediante depósito mensal em conta-corrente, por ele indicada.
II. A vigência das bolsas não poderá ultrapassar a vigência do presente instrumento.

CLÁUSULA TERCEIRA: DA DECLARAÇÃO

3.1. Ao enviar este documento à Funcap, o BENEFICIÁRIO declara formalmente:

I – Subscrever e concordar integralmente com o referido Termo;
II – Conhecer e cumprir as normas da Funcap, ora em validade, sobre a modalidade de auxílio que lhe é concedida e que também são consideradas parte integrante deste documento;
III – Conhecer e cumprir as instruções para o dispêndio e prestação de contas dos recursos financeiros concedidos, presentes no Manual de Prestação de Contas da Funcap;
IV – Conhecer o plano de aplicação dos recursos financeiros concedidos, documento anexo deste termo, como orçamento aprovado pela Funcap, comprometendo-se a não executar qualquer dispêndio que esteja em desacordo com o plano de aplicação;
V – Possuir anuência formal da INSTITUIÇÃO de execução do projeto, seja sob a forma de vínculo empregatício ou funcional ou, na ausência deste, sob a forma de declaração de autoridade institucional

competente para a utilização de sua infraestrutura e facilidades;
VI – Dispor das autorizações legais cabíveis de instituições como Instituto Brasileiro de Meio Ambiente – Ibama, Fundação do Nacional do Índio – Funai, Comitê de Ética na Pesquisa – CEP, Comissão Nacional de Ética em Pesquisa – Conep, das Comissões de Ética em pesquisa com animais, Comissão Nacional de Energia Nuclear – CNEN e outras, no caso em que a natureza do projeto as exigir;
VII – Que manterá sob sua guarda os documentos comprobatórios, referidos nesta cláusula, por um prazo de 5 (cinco) anos após a aprovação final das contas pela Funcap;
VIII – Que conferiu as informações constantes de seu currículo Lattes e as declara corretas e atualizadas;
IX – Que tem ciência de que esta declaração é feita sob pena de caracterização dos crimes tipificados nos artigos 297-299 do Código Penal Brasileiro, que tratam sobre a falsificação de documento público e a falsidade ideológica.

3.2. No caso de o projeto incluir a concessão de bolsas, o(a) BENEFICIÁRIO(A):

I – Indicará bolsista com titulação e nível correspondentes ao da bolsa concedida, pelo tempo estipulado, por meio do formulário pertinente e que responderá integralmente pela adequação e correção desta indicação;
II – Comunicará à Funcap, por meio do formulário pertinente, a substituição do bolsista nos casos em que isso seja previsto e permitido;
III – Manterá sob sua custódia documento assinado pelo bolsista, segundo modelo disponível na página da Funcap na internet, declarando conhecer a instrução normativa que rege a modalidade de bolsa que receberá e comprometendo-se a acatá-la integralmente.

CLÁUSULA QUARTA: DA VIGÊNCIA E ALTERAÇÕES

O prazo para utilização dos recursos para bolsas e custeio/capital é de 24 (vinte e quatro) meses a partir da data de assinatura do presente Termo de Concessão e Aceitação.

Parágrafo Primeiro. O presente Termo terá vigência pelo prazo máximo constante no Edital/Chamada correspondente.

Parágrafo Segundo. Qualquer solicitação de alteração deste instrumento e/ou plano de aplicação deverá ser devidamente justificada e depende de prévia análise e deferimento da CONCEDENTE.

Parágrafo Terceiro. Não será permitido, sob hipótese alguma, o aditamento com o intuito de alterar o objeto deste instrumento.

CLÁUSULA QUINTA: DAS OBRIGAÇÕES

5.1. O BENEFICIÁRIO(A) compromete-se:

I – Dedicar-se às atividades pertinentes ao projeto de pesquisa aprovado.
II – Apresentar, nos prazos que lhe forem determinados, informações ou documentos referentes tanto ao desenvolvimento quanto à conclusão do projeto ou plano aprovado;
III – Integrar obrigatoriamente o quadro de consultores ad hoc do Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq e da Funcap. Quando solicitado, o(a) BENEFICIÁRIO(A) deverá emitir parecer sobre projeto de pesquisa apresentado ao CNPq ou à Funcap;
IV – Utilizar os recursos financeiros, exclusivamente para o desenvolvimento do projeto de pesquisa ou plano de trabalho aprovado, nos termos deste instrumento, e dentro do período previsto;
V – Propor alterações necessárias à consecução do projeto, sujeitas à prévia análise e autorização da Funcap. No caso de aprovadas alterações do plano de aplicação dos recursos financeiros, a Funcap emitirá um novo plano de aplicação que substituirá o vigente, sendo este último o documento comprobatório da autorização;
VI – Permitir e facilitar à Funcap o acesso aos locais de execução da pesquisa, o exame da documentação produzida e a vistoria dos bens adquiridos;
VII – Assumir todas as obrigações legais decorrentes de contratações eventuais necessárias à consecução do objeto, não tendo tais contratações qualquer vínculo para com a Funcap;
VIII – Apresentar relatório técnico das atividades desenvolvidas em até 60 (sessenta) dias após o término da vigência da concessão;
IX – Apresentar prestação de contas em conformidade com o disposto neste documento, especialmente na Cláusula seguinte, e no Manual de Prestação de Contas da Funcap;
X – Se necessário, solicitar prorrogação de prazo de execução do projeto, com as devidas justificativas;
XI – Enviar os dados para pagamento dos bolsistas incluídos no projeto, de acordo com os prazos e requisitos exigidos, se for o caso.

5.2. É vedado ao BENEFICIÁRIO(A):

I – Utilizar o recurso financeiro para fins distintos dos aprovados originalmente no projeto conforme estabelecido nas normas de bolsas e auxílios individuais da Funcap, convênios e/ou editais;
II – Executar despesas não previamente aprovadas pela Funcap;
III – Transferir a terceiros as obrigações ora assumidas sem prévia autorização da Funcap;
IV – Executar despesas em data anterior ou posterior à vigência do presente instrumento. Despesas realizadas fora do prazo de aplicação dos recursos serão glosadas na forma da legislação vigente.

CLÁUSULA SEXTA: DA PRESTAÇÃO DE CONTAS

Todo(a) BENEFICIÁRIO(A) de auxílio a projeto de pesquisa concedido pela Funcap está obrigado(a) a prestar contas, conforme Manual de Prestação de Contas da Funcap, parte integrante deste Termo de Concessão e disponível na página da Funcap na internet (www.funcap.ce.gov.br). Os critérios para utilização dos recursos e procedimentos de prestação de contas constam do Manual.

Parágrafo Primeiro. O saldo não utilizado deverá ser devolvido à Funcap em até 60 (sessenta) dias após o prazo previsto para a aplicação dos recursos. Caso não seja devolvido neste prazo, o valor será corrigido de acordo com a legislação vigente.

Parágrafo Segundo. Os pedidos de informações sobre prestação de contas deverão ser atendidos pelo(a) BENEFICIÁRIO(A), no prazo máximo de 15 (quinze) dias a partir da data de seu recebimento.

Parágrafo Terceiro. A aprovação da prestação de contas ficará condicionada ao atendimento dos itens exigidos no Manual de Prestação de Contas.

Parágrafo Quarto.A parcela subsequente à primeira será liberada apenas após a apresentação da prestação de contas (em conformidade com o disposto neste documento e no Manual de Prestação de Contas da Funcap) da parcela anterior.

CLÁUSULA SÉTIMA: DA AQUISIÇÃO, GUARDA E DESTINAÇÃO DOS BENS

Nos termos do artigo 13 da Lei Federal de número 13.243/2016, os bens adquiridos com recursos deste Termo de Concessão e Aceitação serão incorporados, desde sua aquisição, ao patrimônio da INSTITUIÇÃO à qual o BENEFICIÁRIO(A) estiver vinculado.

Parágrafo Primeiro. É vedada a transferência dos bens para outro local ou estabelecimento, sem prévia e expressa autorização da Funcap. Todas as despesas decorrentes da transferência dos bens e os eventuais danos causados correrão por conta e risco do(a) BENEFICIÁRIO(A) e da INSTITUIÇÃO.

Parágrafo Segundo. O(A) BENEFICIÁRIO(A) e a INSTITUIÇÃO responderão pela manutenção do bem, que deverá permanecer em perfeito estado de conservação e funcionamento.

Parágrafo Terceiro. Em caso de roubo, furto ou outro sinistro envolvendo o bem, o(a) BENEFICIÁRIO(A) ou a INSTITUIÇÃO, após a adoção das medidas cabíveis, deverá comunicar imediatamente o fato à Funcap, por escrito, juntamente com a justificativa e a prova de suas causas, como a cópia autenticada da Ocorrência Policial, se for o caso.

CLÁUSULA OITAVA: DA PROPRIEDADE INTELECTUAL E DA CRIAÇÃO PROTEGIDA

No caso das atividades realizadas originarem resultados materiais representados por inovações tecnológicas, invenções, aperfeiçoamentos e novos conhecimentos aplicáveis às atividades econômicas produtivas e propiciarem incrementos de seu desempenho, aumento da produtividade dos fatores envolvidos, otimização do uso de recursos e insumos, ou, ainda, criações intelectuais passíveis de proteção, as partes obedecerão às determinações da Lei Estadual nº 14.220, de 16 de Outubro de 2008 e, subsidiariamente, da Lei Federal nº 10.973, de 02 de dezembro de 2004, regulamentada pelo Decreto Federal nº 5.563, de 11 de outubro de 2005, atualizados pela Lei Federal nº 13.243, de 11 de janeiro de 2016 e pelo Decreto Federal nº 9.283, de 07 de fevereiro de 2018, observando-se as normas da Funcap e as demais disposições legais vigentes.

Parágrafo Único. Após análise, a CONCEDENTE decidirá sobre a necessidade de ser formalizado contrato específico, a fim de que sejam partilhados os resultados econômicos auferidos na exploração comercial da

criação protegida, inclusive na hipótese de transferência do direito de exploração a terceiros, incluindo-se a instituição onde o projeto é executado, na proporção equivalente ao montante do valor agregado.

CLÁUSULA NONA: DAS PUBLICAÇÕES E DIVULGAÇÕES

A publicação e a divulgação de trabalhos, sob qualquer forma de comunicação ou por qualquer veículo, deverão, obrigatoriamente, fazer menção expressa, no idioma da divulgação, ao apoio material e/ou financeiro da Funcap.

Parágrafo único. O material de divulgação de eventos, impressos em geral, publicações e a publicidade relativa a eles, quando disserem respeito a trabalhos e atividades apoiadas ou financiadas pela da CONCEDENTE, deverão trazer a logomarca desta em lugar visível, de fácil identificação em escala e tamanho proporcionais à área de leitura.

CLÁUSULA DÉCIMA: DA DENÚNCIA, SUSPENSÃO E RESCISÃO

Quando o(a) BENEFICIÁRIO(A) desistir da execução do projeto antes do seu início, os recursos serão devolvidos à Funcap, com justificativa plausível da desistência, no prazo de 30 (trinta) dias de seu recebimento. A não observância desse prazo implicará a correção do valor originalmente concedido, na forma da legislação pertinente.

Parágrafo Primeiro. O(A) BENEFICIÁRIO(A) deverá, formalmente, comunicar à Funcap qualquer descontinuidade do plano de aplicação, do projeto de pesquisa ou do programa do evento, acompanhada da devida justificativa, do relatório técnico e da prestação de contas.

Parágrafo Segundo. A liberação dos recursos do apoio financeiro ao projeto de pesquisa, bem como de quaisquer outros benefícios concedidos pela Funcap será suspensa quando ocorrer uma das seguintes impropriedades:

I – Não comprovação da utilização adequada da parcela anteriormente recebida, na forma da legislação pertinente;
II – Verificação de desvio de finalidade na utilização dos recursos ou dos bens patrimoniais adquiridos no projeto;
III – Atrasos não justificados no cumprimento das etapas ou fases programadas do plano de trabalho/projeto de pesquisa;
IV – Quando for descumprida qualquer cláusula ou condição deste instrumento.

Parágrafo Terceiro. Este Termo poderá ser suspenso, denunciado ou rescindido quando:

I – Verificado o desvio de finalidade na utilização dos recursos ou dos bens patrimoniais adquiridos no projeto;
II – O desempenho do(a) BENEFICIÁRIO(A) for considerado insatisfatório;
III – Ocorrer a interrupção das atividades constantes do plano de atividades;
VI – Deixar de subsistir recursos para pagamento do auxílio;
V – Houver a solicitação, por parte do(a) BENEFICIÁRIO(A), mediante apresentação de justificativa;
VI – Por falecimento do(a) BENEFICIÁRIO(A);
VII – O Conselho Executivo da Funcap, justificadamente, assim decidir.

Parágrafo Quarto. O(A) BENEFICIÁRIO(A), cuja prestação de contas e relatório técnico final não forem aprovados, será considerado(a) inadimplente e terá suspensos os pagamentos, bem como a concessão de novas modalidades de apoio, sem prejuízo de outras medidas julgadas necessárias pela Funcap e previstas na lei.

Parágrafo Quinto. Quando da denúncia, rescisão ou extinção do benefício, os saldos financeiros remanescentes deverão ser devolvidos à Funcap no prazo improrrogável de 30 (trinta) dias do evento, sob pena de instauração de tomada de contas especial.

CLÁUSULA DÉCIMA PRIMEIRA: DAS DISPOSIÇÕES GERAIS

O disposto neste Termo refere-se a projeto de pesquisa/plano de aplicação a ser financiado com recursos da Funcap. Se financiado com recursos de outras fontes, poderão prevalecer disposições específicas constantes em editais, convênios e outros regulamentos pertinentes.

Parágrafo Primeiro. A concessão objeto do presente instrumento não gera vínculo de qualquer natureza ou

relação de trabalho, constituindo doação com encargos feita ao(à) BENEFICIÁRIO(A).

Parágrafo Segundo. O pessoal envolvido na execução do projeto de pesquisa não possuirá vínculo de qualquer natureza com a Funcap e desta não poderá demandar quaisquer pagamentos, sendo estes de inteira responsabilidade do(a) BENEFICIÁRIO(A)/INSTITUIÇÃO sede do projeto, que os tiverem empregado na execução dos trabalhos.

Parágrafo Terceiro. Se eventualmente a Funcap for demandada pelo pessoal utilizado nos trabalhos, o(a) BENEFICIÁRIO(A) do PROJETO e a INSTITUIÇÃO ressarcirão a Funcap das despesas que realizar, atualizadas monetariamente.

Parágrafo Quarto. A licitação é dispensável na aquisição de bens ou na contratação de serviços destinados ao desenvolvimento da pesquisa objeto do apoio individual. O(A) BENEFICIÁRIO(A) deverá observar as regras contidas no Decreto Estadual nº 28.088/2006 (DOE 12.01.06), bem como o princípio do menor preço, sem deixar de considerar, igualmente, os aspectos de qualidade e de rendimento que possam comprometer o resultado da pesquisa, possibilitando assim o melhor aproveitamento dos recursos públicos.

Parágrafo Quinto. O processo somente será encerrado após as aprovações do relatório técnico final e da prestação de contas e desde que cumpridas todas as condições previstas neste instrumento e nas normas aplicáveis.

Parágrafo Sexto. O descumprimento de qualquer condição constante deste Termo e a inobservância de dispositivos legais aplicáveis a esta concessão implicará o cancelamento/interrupção imediato da concessão e rescisão do Termo e obrigará o(a) BENEFICIÁRIO(A) a ressarcir integralmente a Funcap de todas as despesas realizadas, atualizadas nos termos da legislação, sem prejuízo da aplicação de outras penalidades cabíveis.

Parágrafo Sétimo. A recusa ou omissão do(a) BENEFICIÁRIO(A) quanto ao ressarcimento de que trata este item, ensejará a consequente abertura de tomada de contas especial, a inscrição do(a) BENEFICIÁRIO(A) e do débito nos cadastros de inadimplência do Tesouro Estadual e o protocolo de ação judicial cabível.

Parágrafo Oitavo. O(A) BENEFICIÁRIO(A) reconhece que à Funcap compete exercer a autoridade normativa de controle e fiscalização sobre a execução do projeto, bem como assumir ou transferir a responsabilidade pelo mesmo, no caso da paralisação ou de fato relevante que venha a ocorrer, de modo a evitar a descontinuidade das atividades.

Parágrafo Nono. As partes e as testemunhas que assinam adiante reconhecem que o presente termo possui plena eficácia e força de título executivo extrajudicial, nos termos do artigo 784 do Código de Processo Civil.

Parágrafo Décimo. Fica eleito o foro da Justiça Comum da Comarca de Fortaleza para dirimir qualquer divergência decorrente da execução deste instrumento, com exclusão de qualquer outro.

Fortaleza, 15 de março de 2021.

Luiz Drude de Lacerda
Diretor Científico da Funcap

Mirna Marques Bezerra Brayner
Coordenador(a) do Projeto

UNIVERSIDADE FEDERAL DO CEARÁ - UFC
Instituição de execução do projeto

Testemunha: José Clauber Matos Brayner
CPF: 411.521.663-87

Testemunha: Walber Serra Nóbrega
CPF: 360.165.603-59

ANEXO A - PLANO DE APLICAÇÃO

CAPITAL

Material Permanente

1 pHmetro de Bancada
1 Balança
1 Banho tipo Bloco Seco
1 Notebook
3 Kits Pipetas
2 Agitadores

TOTAL: R$ 41.921,24
TOTAL CAPITAL: R$ 41.921,24

CUSTEIO

Material de Consumo

Kits de citocinas humano
Produtos químicos, reagentes, soluções, tampões, placas, descartáveis
Ponteiras
Filme óptico

TOTAL: R$ 159.186,77

OST - Pessoa Jurídica

Realização dos exames
Manutenção/consertos de equipamentos

TOTAL: R$ 17.000,00
TOTAL CUSTEIO: R$ 176.186,77

BOLSAS

BTT6

Descrição	Qtde	Duração	Valor Mensal	Total
BTT6	1	12	800,00	9.600,00

TOTAL: R$ 9.600,00
TOTAL BOLSAS: R$ 9.600,00

Total Capital:	**R$**	**41.921,24**
Total Custeio:	**R$**	**176.186,77**
Total Bolsa:	**R$**	**9.600,00**
Total do Projeto:	**R$**	**227.708,01**

Fortaleza, 15 de março de 2021.

Mirna Marques Bezerra Brayner

Coordenador(a) do Projeto

ANEXO C
ARTIGO PUBLICADO EM REVISTA INDEXADA

Clinical Immunology 278 (2025) 110541

Contents lists available at ScienceDirect

Clinical Immunology

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

Full Length Article

ProBNP, cytokines, and the Nrf2/HO-1 signaling pathway: A cross-sectional study on cardiovascular risk in rheumatoid arthritis

Christiane Aguiar Nobre [a,b], Carlos Ewerton Maia Rodrigues [c,d,e,*], Natacha Xavier Cavalcante [b], Thácilla Siqueira Eugênio Nascimento [b], João Gabriel Marques Brayner [b], Giovanna Azevedo Sousa [b], Nayara Alves de Sousa [b], Regislane Pinto Ribeiro [b], Vanessa Maria Eufrásio de Fiqueirêdo [f], Giulia Albuquerque Paiva [f], José Jackson do Nascimento Costa [f], Paula Goes [g,h], Hellíada Vasconcelos Chaves [i], Ticiana Mont'Alverne Parente Feijão [j], Mirna Marques Bezerra [a,b]

[a] Postgraduate Program in Translational Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil
[b] School of Medicine, Federal University of Ceará, Sobral, Ceará, Brazil
[c] Graduate Program in Medical Sciences, Medical School, University of Fortaleza (Unifor), Fortaleza, Brazil
[d] Department of Internal Medicine, Federal University of Ceará, Fortaleza, Brazil
[e] Rheumatology Division, Hospital Geral de Fortaleza, Fortaleza, Brazil
[f] School of Medicine, University Center INTA – UNINTA, Sobral, Ceará, Brazil
[g] Postgraduate Program in Morphological Science, Department of Morphology, School of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil
[h] Department of Pathology and Legal Medicine, Medical School, Federal University of Ceará, Fortaleza, Ceará, Brazil
[i] School of Dentistry, Federal University of Ceará, Sobral, Ceará, Brazil
[j] School of Medicine, Luciano Feijão College, Sobral, Ceará, Brazil

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ABSTRACT

We evaluated associations between clinical/laboratory findings and serum cytokines, Nrf2/HO-1 pathway expression and cardiovascular risk in both RA patients and controls. Sixty RA patients and 60 controls were included in the study. Serum cytokine and proBNP levels were assessed by ELISA, while serum Nrf2 and HO-1 mRNA levels were quantified by qRT-PCR. The RA group (91.7 % women) and the control group (90 % women) were aged 52 ± 12 and 52 ± 13 years, respectively. ProBNP levels were higher in the RA group than in controls ($p = 0.009$). Nrf2 mRNA levels were higher ($p < 0.001$) and HO-1 mRNA levels were lower ($p = 0.030$) in the RA group than in controls. CDAI scores were significantly associated with serum IL-6 levels ($p = 0.033$). This study found a significant dysregulation in Nrf2/HO-1 pathway activity in RA patients, although without association with cardiovascular risk, RA-related clinical and laboratory variables. Moderate/high disease activity was positively associated with IL-6 levels.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by symmetrical polyarthritis compromising the bones, with an overall prevalence of 0.5–1 % [1]. RA not only affects the joints, but is associated with several comorbidities of which cardiovascular disease (CVD) is the main cause of mortality [2]. RA patients tend to experience cardiovascular events earlier and have lower life expectancy than the general population [3]. Evidence suggests that inflammatory response to RA is an independent cardiovascular risk (CVR) factor accelerating rapid atherosclerosis [4,5].

In addition to inflammatory activity, the pathogenesis of RA also

* Corresponding author at: Graduate Program in Medical Sciences, University of Fortaleza (Unifor), Rua Gontran Giffoni 366, Apt 301, Torre 1, Patriolino Ribeiro, CEP: 60810-220, Fortaleza, Ceará, Brazil.
E-mail addresses: chrisan.0805@gmail.com (C.A. Nobre), carlosewerton@hotmail.com (C.E.M. Rodrigues), natachaxc@alu.ufc.br (N.X. Cavalcante), thacillaenascimento@alu.ufc.br (T.S.E. Nascimento), nayara.alves@sobral.ufc.br (N.A. de Sousa), jackson.costa@uninta.edu.br (J.J. do Nascimento Costa), paulagoes@ufc.br (P. Goes), helliadachaves@ufc.br (H.V. Chaves), mirna@ufc.br (M.M. Bezerra).

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involves oxidative stress [3]. In order to maintain homeostasis, cells employ adaptive protective mechanisms, such as the nuclear factor-erythroid 2-related factor 2 (Nrf2) signaling pathway, which regulates the transcription of several genes involved in redox balance, detoxification and inflammation [6]. Nrf2 activation results in the production of heme oxygenase-1 (HO-1), one of the most important cytoprotective mechanisms activated during cellular stress [7]. Recent studies have shown that the Nrf2/HO-1 pathway allows for the formation of bioactive metabolites protective against oxidative stress and inflammatory response in RA [8,9]. Moreover, reduced Nrf2 expression is known to contribute to the pathophysiology of cardiovascular disease in conditions such as obesity, diabetes mellitus, hypertension and atherosclerosis [10]. However, little is known about the Nrf2/HO-1 pathway and how it relates to cardiovascular protection in RA patients.

To better understand the possible modulatory role of the Nrf2/HO-1 signaling pathway in RA, in this study we evaluated associations between clinical/laboratory findings and serum cytokines, Nrf2/HO-1 pathway expression and CVR in both RA patients and controls.

2. Methods

2.1. Approval of study protocol

This cross-sectional study was conducted at a secondary-level outpatient service in Northeastern Brazil between October 2021 and October 2022. The protocol complied with the tenets of the Helsinki Declaration [11], all procedures being performed according to well-established standards and guidelines, and was approved under file #110729/2020 by the research ethics committee of the Federal University of Ceará (CEP/UFC/PROPESQ). All participants gave their informed written consent prior to study entry.

2.2. Patients and controls

Initially, 123 participants were recruited and assigned to one of two groups: A control group of healthy volunteers and an RA group. RA was diagnosed according to the 2010 American College of Rheumatology criteria [12]. One RA patient was excluded due to mitral valve disease caused by rheumatic fever, and two other participants were excluded from the control group (one did not complete the study protocol, the other was diagnosed with hypertensive heart disease and non-dialysis-dependent chronic kidney disease), leaving a final sample of 120 participants (60 in each group). The non-RA participants were sex and age-paired residents from the local community. The exclusion criteria were: age under 18 years, pregnancy, history of congestive heart failure, coronary artery disease, cerebrovascular disease, atrial fibrillation, cancer, chronic kidney disease and/or other collagen diseases such as systemic lupus erythematosus, Sjögren syndrome, scleroderma and inflammatory myopathy.

2.3. Clinical and anthropometric evaluation

The physical examination included tender and swollen joint count (RA group), and arterial blood pressure, waist circumference (WC) and body mass index (BMI) (both groups). WC was measured with a tape held horizontally between the last rib and the iliac crest. BMI was obtained by dividing weight (kg) by height squared (m^2). Individuals with BMI in the range 25–29.9 were considered overweight, while BMI >30 was interpreted as obesity [13]. The 10-year CVR was estimated based on the Systematic Coronary Risk Evaluation (SCORE), the modified SCORE [14], and the Framingham Risk Score [15]. The modified SCORE (mSCORE) was obtained by multiplying SCORE by 1.5 in RA patients meeting at least two of the following criteria: i) disease duration >10 years, ii) positivity for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP), and iii) presence of extra-articular manifestations [16]. Disease activity was quantified with the Clinical Disease Activity Index (CDAI), the Simple Disease Activity Index (SDAI), and the Disease Activity Score-28 (DAS28). CDAI scores were interpreted as follows: <2.8 clinical remission, >2.8–10 low activity, >10–22 moderate activity, and > 22 high activity. SDAI scores were interpreted as follows: <3.3 clinical remission, >3.3–11 low activity, >11–26 moderate activity, and > 26 high activity. Based on C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR), DAS28 scores were interpreted as follows: <2.6 clinical remission, >2.6–3.1 low activity, >3.2–5.0 moderate activity, and > 5.1 high activity [17].

2.4. Laboratory evaluation

Laboratory data, including complete blood count, rheumatoid factor (RF), anti-CCP, glycemia, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and uric acid, were obtained from patient records.

For the quantification of proBNP and cytokines (TNF-α, IL-1β, IL-6, IL-10) by ELISA, as well as the assessment of Nrf2 and HO-1 mRNA expression by qRT-PCR, a 25 mL peripheral blood sample was collected from each participant.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Following collection, blood samples were anticoagulated, diluted in phosphate-buffered saline (PBS), and carefully layered over a Ficoll-Paque solution, then centrifuged at 4000*g* for 15 min at 4 °C, without brake. The PBMC layer was collected, washed twice with PBS (300 g, 10 min, 4 °C), and resuspended in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS) for subsequent experiments.

An aliquot of 1–2 × 10^6 PBMCs was used for cytokine assays or RNA extraction, while the remaining cells were stored at −80 °C for future analyses.

ProBNP levels were estimated with a clinically validated automated immunoassay (Cobas® e 801, Roche Diagnostics, Basel, Switzerland). The controls used the manufacturer's acceptability limits, with measurement intervals of 5–35,000 pg/mL. No cross-reactivity with atrial natriuretic peptide, N-terminal atrial natriuretic peptide or BNP was detectable.

The levels of TNF-α, IL-1β, IL-6 and IL-10 were measured with the commercial kit DuoSet ELISA (R&D Systems Inc., MN, USA), along with standard curves. All kits were used following the manufacturer's directions, and the results were expressed in pg/mL.

The qRT-PCR analysis of the peripheral RNA samples was performed as follows: the total RNA was extracted from peripheral blood collected in EDTA tubes using the reagent Trizol (Invitrogen, São Paulo, Brazil). The reverse transcription was performed with SuperScript IV (Invitrogen, São Paulo, Brazil), according to the manufacturer's directions. A StepOne Real-Time PCR thermocycler (Applied Biosystems, Warrington, UK) with SYBR Green Master Mix (Applied Biosystems, Warrington, UK) was employed for the qRT-PCR reaction, following the manufacturer's directions. The relative gene expression was determmined with the 2-ΔΔCt method [18]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as endogenous control to normalize the mRNA expression. The specificity of each pair of primers was confirmed by dissociation curve analysis of the PCR products. The primer sequences for real-time PCR were: Nrf2 (Forward 5′-3′: GTCCCAGCAGGACATGGAT; Reverse 5′-3′: GCTCATACTCTTTCCGTCGC; GenBank serial number: HM446346.1), HO-1 (Forward 5′-3′: GGCCTAAACTTCAGAGGGGG; Reverse 5′-3′: CAGCTGCCACATTAGGGTGT; GenBank serial number: NM_002133.3) and GAPDH, the reference gene (Forward 5′-3′: GTGGCTGGCTCAGAAAAAGG; Reverse 5′-3′: GGTGGTCCAGGGGTCTTACT; GenBank serial number: AY340484.1).

2.5. Statistical analysis

Clinical and demographic variables were expressed as mean ± standard deviation (normally distributed continuous variables) or as

absolute numbers (n) and percentages (%) (categorical variables) and submitted to Pearson's chi-squared test (χ^2). Medians (minimum-maximum) were calculated for continuos variables not normally distributed.The Shapiro-Wilk test was used to confirm the normal distribution of each quantitative data set. Non-parametric data were analyzed with the Mann-Whitney test or the Kruskal-Wallis test, as required, followed by Dunn's post-test. Spearman's correlation test was employed to calculate the correlation coefficient (r) and the determination coefficient (r^2) of the quantitative variables. All analyses were performed with the software SPSS for Windows (v. 20.0), with the level of significance set at $p < 0.05$.

3. Results

3.1. Epidemiological, clinical and laboratory profile

The participants ($n = 120$) were predominantly female (90 %). In the RA group 91.7 % were female. The mean age was 52 ± 13 years (controls) and 52 ± 12 years (RA group).

Table 1 shows the traditional CVR factors and the clinical and laboratory findings. Higher frequencies of the following cardiovascular risk factors were observed in controls compared to RA patients: dyslipidemia (55 % vs 33 %, $p = 0.027$), WC (91.67 ± 10.63 vs. 87.65 ± 10.99, $p = 0.017$), BMI (28.58 ± 4.17 vs. 26.83 ± 5.22, $p = 0.010$), glycemia (109.95 ± 30.86 vs. 93.92 ± 26.35, $p < 0.001$),role and triglycerides (154.27 ± 93.00 vs. 114.37 ± 54.96, $p = 0.004$).

The mean disease duration was 10.43 ± 7.55 years, and 63.3 % were positive for RF and/or anti-CCP. Disease activity was quantified and each RA patient was classified as either 'in remission/low activity' or 'moderate/high activity'. The latter category was assigned to 38.3 % (DAS-28 PCR), 50 % (DAS28 ESR), 53.3 % (SDAI) and 45 % (CDAI).

Almost all RA patients (98.3 %) were in treatment, receiving corticosteroids (26.7 %) and/or synthetic disease-modifying anti-rheumatic drugs DMARDs (91.7 %), the most common of which was methotrexate (72.9 %). DMARDs were not associated with other classes of antirheumatic drugs in 38.3 %. Biologic DMARDs were used in 21.7 %, targeted DMARDs in 11.7 %.

3.2. Cardiovascular risk

The two groups did not differ significantly with regard to 10-year CVR according to the SCORE/mSCORE ($p = 0.261$) and Framingham scores ($p = 0.069$) (Table 2). On the other hand, proBNP levels were significantly higher in the RA group (86.77 ± 76.03) than in the control group (60.12 ± 52.28) ($p = 0.009$) (Table 2).

3.3. Serum and Nrf2/HO-1 pathway cytokines

The RA group displayed significantly higher levels of IL-10 ($p = 0.004$) and IL-6 ($p = 0.002$) and lower levels of IL-1β ($p = 0.001$) than the control group (Fig. 1A, B and C, respectively). No significant difference was observed between the groups for TNF-α ($p = 0.073$) (Fig. 1D).

Nrf2 mRNA levels were significantly higher in the RA group (4.79 ± 6.72) than in the control group (3.88 ± 11.46) ($p < 0.001$) (Fig. 1E). Conversely, HO-1 mRNA levels were significantly lower in the RA group (4.18 ± 5.15) than in the control group (7.33 ± 31.67) ($p < 0.030$) (Fig. 1F).

RA = rheumatoid arthritis. Data expressed as mean ± standard deviation.

3.4. Analysis of association

In this study we evaluated the association between different disease activity indices and CVR. A significant association ($p = 0.018$) was found between remission on DAS28 ESR and Framingham CVR. No other disease activity index (SDAI, CDAI, DAS 28 PCR) was significant (Table 3).

When evaluating the association between disease activity and serum cytokines levels, we found a significant positive association between moderate/high activity on CDAI and IL-6 ($p = 0.03$). Spearman correlation between CDAI and IL-6 was 0.17, $p = 0.197$. No other cytokine (IL-10, IL-1β, TNF-α) was significantly associated with any of the disease activity indices (CDAI, SDAI, DAS 28 ESR, DAS 28 PCR) ($p > 0.05$) (Table 4).

Finally, the cytokines (IL-6, IL-10, IL-1β, TNF-α) and the Nrf2/HO-1 signaling pathway were not significantly associated with RF/anti-CCP

Table 1
Traditional cardiovascular risk factors and clinical and laboratory findings of subjects with and without rheumatoid arthritis (RA).

Variable	Controls ($n = 60$) (n/%)	RA (n = 60) (n/%)	p-value
Family history of cardiovascular disease			
Yes	6 (10.0)	9 (15.0)	0.582[a]
No	54 (90.0)	51 (85.0)	
Smoking (current)			
Yes	6 (10.0)	1 (1.7)	0.114[a]
No	54 (90.0)	59 (98.3)	
Smoking (previous)			
Yes	10 (16.7)	14 (23.3)	0.360[a]
No	50 (83.3)	46 (76.7)	
Systemic arterial hypertension			
Yes	21 (35.0)	21 (35.0)	1.000[a]
No	39 (65.0)	39 (65.0)	
Diabetes mellitus			
Yes	11 (18.3)	5 (8.3)	0.178[a]
No	49 (81.7)	55 (91.7)	
Dyslipidemia			
Yes	33 (55.0)	20 (33.3)	**0.027**[a]
No	27 (45.0)	40 (66.7)	
Sedentary lifestyle			
Yes	39 (65)	47 (78.3)	0.152[a]
No	21 (35)	13 (21.7)	
Physical examination			
Systolic blood pressure	123.33 ± 18.66	120.50 ± 9.46	0.905[b]
Diastolic blood pressure	81.50 ± 11.02	80.67 ± 5.48	0.602[b]
Waist circumference (cm)	91.67 ± 10.63	87.65 ± 10.99	**0.017**[b]
Body mass index	28.58 ± 4.17	26.83 ± 5.22	**0.010**[b]
Body mass index			
Low weight + ideal weight	13 (21.7)	20 (33.3)	0.234[a]
Overweight	27 (45)	28 (46.7)	
Obesity	20(33,3)	12 (20.0)	
Laboratory tests			
Blood glucose	109.95 ± 30.86	93.92 ± 26.35	**<0.001**[b]
Uric acid	3.29 ± 0.97	3.20 ± 1.29	0.371[b]
Total cholesterol	197.60 ± 43.63	192.05 ± 29.60	0.785[b]
HDL	56.60 ± 12.04	59.77 ± 14.17	0.301[b]
LDL	110.15 ± 40.83	108.43 ± 28.96	0.795[b]
TGs	154.27 ± 93.00	114.37 ± 54.96	**0.004**[b]

Data expressed as absolute numbers (n) and percentages (%) or mean ± standard deviation of the mean or absolute number (%).

[a] Pearson's chi-square test.

[b] Mann-Whitney test. Bold type = significant at the level of 5 % ($p < 0.05$). HDL = high density lipoprotein); LDL = low density lipoprotein; TG = triglycerides.

Table 2
Prediction of cardiovascular risk in SCORE/mSCORE and Framingham scores, and proBNP serum levels in subjects with and without rheumatoid arthritis (RA).

Variable	Controls (n = 60) (n/%)	RA (n = 60) (n/%)	*p*-value
SCORE/mSCORE			
Low (<1 %)	31 (55.4)	25 (43.9)	
Moderate (1 % to <5 %)	25 (44.6)	32 (56.1)	0.261[a]
Framingham			
Low (≤10 %)	41 (68.3)	51 (85)	0.069[a]
Moderate (>10–20 %)	14 (23.3)	5 (8.3)	
High (≥20 %)	5 (8.3)	4 (6.7)	
ProBNP serum levels	60.12 ± 52.28	86.77 ± 76.03	**0.009**[b]

Data expressed as absolute numbers (n) and percentages (%) or mean ± standard deviation of the mean or absolute number (%).
[a] Pearson's chi-square test.
[b] Mann-Whitney test. Bold type = significant at the level of 5 % ($p < 0.05$).
ProBNP=N-terminal prohormone brain natriuretic peptide.

positivity or disease duration ($p > 0.05$) (Table 5).

4. Discussion

To our knowledge, this is the first study to investigate to what extent serum cytokines and the Nrf2/HO-1 signaling pathway are associated with clinical and laboratory variables and CVR in RA patients. We found the Nrf2 gene expression to be stronger and the HO-1 expression weaker in RA patients than in healthy controls, although the tested serum cytokines and the Nrf2/HO-1 pathway could not be shown to be associated with RF/anti-CCP positivity, disease duration or disease activity. We also observed increased serum proBNP levels in RA patients and higher disease activity in patients with high serum levels of IL-6.

CVR scores have been used to make predictions for the general population, subsidize preventive measures and avoid unfavorable outcomes [16]. In this study, however, SCORE/mSCORE and Framingham scores were statistically similar for RA and non-RA subjects, while the traditional CVR factors (WC, BMI, glucose, triglycerides) were more prevalent among controls. This finding raises the hypothesis that CVR was increased in RA due to the pathogenesis of the condition, equating the deleterious effects of the more prevalent CVR factors observed in the control group [19].

Serum proBNP levels were increased in RA patients. A diagnostic biomarker of heart failure and dysfunction in clinical practice, high proBNP levels have been independently associated with CVD and mortality [20,21]. Matching our findings, a study involving patients with early RA and no history of DMARD or glucocorticoid use reported high levels of proBNP [22]. Likewise, proBNP was a predictor of mortality in the EURIDISS cohort study, which included RA patients with up to 4 years of disease duration followed up for 10 years, suggesting that proBNP is a simple, robust and non-invasive CVR indicator in RA patients which can help identify and stratify risk in this population [23].

In the present study, RA patients had significantly higher serum IL-10 and IL-6 levels and significantly lower IL-1β levels than healthy controls, but no difference was found for TNF-α levels. Other authors have found raised serum IL-10 levels in RA patients [24], possibly indicating a continuous effort of the organism to restore local homeostasis. However, increased levels of other inflammatory mediators in RA patients are known to counteract this effort, even when the clinical management is adequate [25,26].

Interestingly, in our RA patients the increase in IL-6 levels was associated with higher CDAI scores. Likewise, some authors [27] have reported serum IL-6 levels almost three times higher in RA patients than in controls and a positive correlation between IL-6 and DAS28 ESR, suggesting IL-6 may be a biomarker of disease activity. An earlier study [28] also identified significantly higher serum IL-6 levels in RA patients vs. non-RA controls, in addition to significant associations between IL-6, CRP and duration of morning stiffness. Others found serum IL-6 levels to be significantly correlated with RA activity and severity, suggesting IL-6 may be an important treatment target in RA [29].

Our RA patients displayed significantly lower levels of serum IL-1β than our healthy controls. Increased serum IL-1β levels are generally expected as a result of the presence of erosions in the early stages of RA. In fact, high serum IL-1β levels in RA patients correlate well with disease activity [30], but the predictive value of this marker may be compromised by the use of DMARDs [31,32]. The lower IL-1β levels observed in our study are therefore likely due to disease control achieved by long-term treatment of RA. Importantly, one study showed that RA patients with high disease activity appear to have the highest risk of developing CVD. These findings support the implementation of tight control (treat-to-target) strategies in daily clinical practice to achieve low disease activity or remission, also aiming to reduce CVD risk in this population [33]. Moreover, a systematic review and meta-analysis of observational and controlled trials-including 28 studies and 236,525 RA patients-found that DMARDs (particulary methotrexate) were associated with a 28 % reduction in cardiovascular events (relative risk (RR) 0.72, 95 % confidence interval (CI) 0.57 to 0.91) and TNF inhibitors with a 30 % reduction(RR 0.70, CI 0.54 to 0.90), with specifically protective effects observed for myocardial infarction (RR 0.59,CI 0.36 to 0.97) and stroke (RR 0.57, CI 0.35 to 0.92) [34].

When analyzing the association between CVR and disease activity, we observed that patients with RA in remission on DAS 28 ESR had lower Framingham scores, matching the literature which shows that inflammation in RA increases CVR [33]. These findings reflect the multifactorial nature of CVR in RA, considering ~30 % of the risk to be due to disease-related chronic inflammatory activity and ~ 70 % to be explained by traditional CVR factors, with emphasis on arterial hypertension and smoking [33].

Notably, the gene expression of Nrf2 and HO-1 was significantly higher and lower, respectively, in the RA group than in the control group, indicating compromised antioxidant protection in RA. This finding aligns with previous studies reporting disturbances in the Nrf2 signaling pathway in this condition [35]. It has been demonstrated that RA synovial tissues exhibit abnormally low levels of Nrf2 targets heme oxygenase 1 (HO-1) mRNA and protein [36]. A possible explanation is that chronic inflammation and oxidative stress in RA may induce to compensatory upregulation of Nrf2 expression as a cellular response to oxidative damage [8]. However, despite increased Nrf2 mRNA levels, its downstream transcriptional activity may be impaired due to several factors, such as epigenetic modifications, post-translational alterations (e.g., excessive Keap1-mediated degradation), or disrupted nuclear translocation of the Nrf2 protein [37]. These mechanisms may prevent adequate upregulation HO-1 expression, even in the presence of elevated Nrf2 levels. Moreover, pro-inflammatory cytokines associated with RA have been shown to suppress HO-1 transcription through epigenetic mechanisms, including promoter hypermethylation, histone deacetylation, and microRNA regulation [38].

The discrepancy between elevated Nrf2 mRNA levels and reduced HO-1 expression observed in RA patients suggests that additional regulatory mechanisms may disrupt the canonical Nrf2-HO-1 axis. Previous studies have reported increased Nrf2 expression in experimental models of RA, likely as a compensatory response to persistent inflammation and oxidative stress [35,36,38]. However, this transcription upregulation does not necessarily translate into activation of downstream target genes. Supporting this notion, Ray et al. [39] demonstrated that HO-1 expression requires histone H3 phosphorylation at serine 10, indicating that permissive chromatin remodeling is a prerequisite for transcriptional activation. In addition, Reichard et al. showed that the transcriptional repressor BACH1 must be inactivated for Nrf2 to effectively induce HO-1 expression [40]. More recently, Yoo et al. reported that TonEBP (NFAT5) suppresses HO-1 transcription by preventing Nrf2 recruitment to its promoter, even in the context of elevated Nrf2

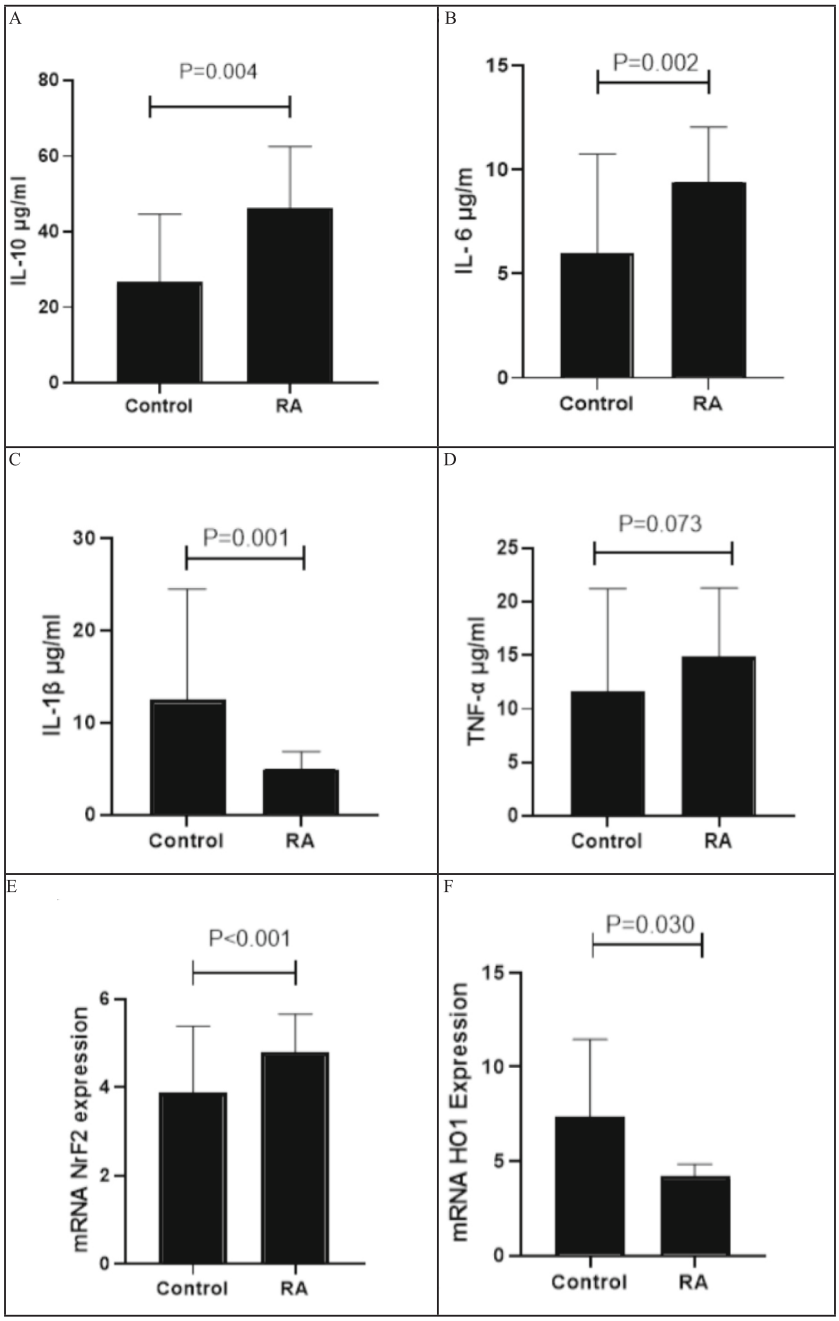

Fig. 1. Serum levels of the cytokines IL-10 (A), IL-6 (B), IL-1 beta (C) and TNF-alpha (D), and serum levels of mRNA-Nrf2 (E) and mRNA-HO-1 (F) in subjects with and without rheumatoid arthritis.

expression [41]. Collectively, these findings underscore the importance of transcriptional co-regulators and epigenetic modifications in enabling HO-1 induction, suggesting that Nrf2 abundance alone may be insufficient in the absence of a permissive epigenetic environment.

Little information on the role of the Nrf2/HO-1 pathway in the cardiovascular system is available, but one study suggests that a compromised Nrf2/HO-1 pathway can negatively affect the redox balance and mediate cardiovascular changes such as those observed in patients with obesity, diabetes mellitus and atherosclerosis [10]. Also, long-term hyperlipidemia reduces Nrf2/HO-1 pathway activation, inducing oxidative stress in endothelial cells [42], and Nrf2 reduces oxidative damage to endothelial cells and prevents endothelial dysfunction in cardiovascular diseases [43].

Overexpression of HO-1 has been shown to reduce the formation of atherosclerotic lesions by inhibiting lipid peroxidation, indicating HO-1 may have an intrinsic antioxidant function [44]. Likewise, under acute oxidative stress and inflammation (as in sepsis), induction of the Nrf2/HO-1 pathway reportedly improves cardiovascular function in rats [45].

The complexity of the pathogenesis of RA, which involves immune response, genetics, inflammation and oxidative stress, makes it difficult to identify the proinflammatory molecules causing vascular endothelial damage, but it should be considered that dysfunction of the Nrf2/HO-1 pathway likely contributes to vascular damage and disease-related cardiovascular risk by inducing oxidative stress and inflammation.

Our study was to some extent limited by i) the cross-sectional study design which did not allow us to longitudinally observe cardiovascular outcomes and their possible relationship with oxidative stress, ii) the higher prevalence of traditional CDR factors in the control group, iii) the potential bias associated with the recruitment of patients from a secondary-level outpatient service, with an arguably smaller proportion

Table 3
Disease activity scores vs. Framingham scores in patients ($n = 60$) with rheumatoid arthritis (RA).

Variable	Framingham score			*p*-value
Disease activity scores	Low (n/%)	Moderate (n/%)	High (n/%)	
DAS 28 CRP				
Remission	26 (51.0)	4 (80.0)	1 (25.0)	0.163
Low	5 (9.8)	0 (0.0)	1 (25.0)	
Moderate	19 (37.3)	1 (20.0)	1 (25.0)	
High	1 (2.0)	0 (0.0)	1 (25.0)	
DAS 28 ESR				
Remission	21 (41.2)	1 (20.0)	0 (0.0)	**0.018**
Low	4 (7.8)	3 (60.0)	1 (25.0)	
Moderate	16 (31.4)	1 (20.0)	1 (25.0)	
High	10 (19.6)	0 (0.0)	2 (50.0)	
SDAI				
Remission	15 (29.4)	1 (20.0)	1 (25.0)	0.261
Low	8 (15.7)	3 (60.0)	0 (0.0)	
Moderate	17 (33.3)	1 (20.0)	2 (50.0)	
High	11 (21.6)	0 (0.0)	1 (25.0)	
CDAI				
Remission	20 (39.2)	1 (20.0)	1 (25.0)	0.258
Low	7 (13.7)	3 (60.0)	1 (25.0)	
Moderate	18 (35.3)	1 (20.0)	1 (25.0)	
High	6 (11.8)	0 (0.0)	1 (25.0)	

Data expressed as absolute numbers (n) and percentages (%). Pearson's chi-square test. Bold type = significant at the level of 5 % ($p < 0.05$). DAS28 = Disease Activity Score-28; ESR = erythrocyte sedimentation rate; CRP=C-reactive protein; SDAI=Simplified Disease Activity Index; CDAI=Clinical Disease Activity Index.

of severe cases and lower CVR, and iv) we cannot determine whether reduced HO-1 expression might also result from impaired Nrf2 nuclear translocation or post-translational modifications, as the current study focused solely on mRNA expression in peripheral blood samples and did not include protein quantification or subcellular localization assays. Nevertheless, our study provide a rationale for future investigations into the nuclear-cytoplasmic dynamics of Nrf2, which may further clarify the mechanisms underlying Nrf2-HO-1 uncoupling in RA.

5. Conclusion

In conclusion, we identified higher Nrf2 expression and lower HO-1 expression in the RA group, though not associated with CVR, RA-related clinical and laboratory variables, and serum cytokine levels. Moderate/High disease activity was positively associated with IL-6 and high levels of proBNP were found in patients with RA compared to controls. This study found a significant dysregulation in Nrf2/HO-1 pathway activity in RA patients, providing clinical evidence to support future research on the role of this pathway in RA pathogenesis.

Abbreviations

Anti-CCP	Anti-cyclic citrullinated peptide
BMI	Body mass index
BNP	Brain natriuretic peptide
CDAI	Clinical Disease Activity Index
CEP/UFC/PROPESQ	Research ethics committee of the Federal University of Ceará
CRP	C-reactive protein
CVD	Cardiovascular disease
CVR	Cardiovascular risk
DAS28	Disease Activity Score-28
DMARD	Disease-modifying anti-rheumatic drugs
ESR	Erythrocyte sedimentation rate

(*continued on next column*)

Table 4
Disease activity indices vs. serum cytokines and Nrf2/HO-1 gene expression in patients (n = 60) with rheumatoid arthritis (RA).

Variable	SDAI		
	Remission / Low	Moderate / High	*p*-value
Interleukins			
Median (Minimum-Maximum)			
IL-10	0.00 (0.00–693.14)	0.00 (0.00–580.10)	0.754[b]
IL-6	1.00 (0.00–80.96)	2.21 (0.00–85.69)	0.258[b]
TNF-alpha	0.00 (0.00–114.02)	0.00 (0.00–343.25)	0.771[b]
IL-1 beta	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.339[b]
Gene expression			
Median (Minimum-Maximum)			
Nrf2	3.81 (0.02–26.77)	2.37 (0.24–59.8)	0.477[b]
HO-1	1.65 (0.30–21.86)	1.08 (0.31–20.08)	0.900[b]

Variable	CDAI		
	Remission / Low	Moderate/ High	p-value
Interleukins			
Median (Minimum-Maximum)			
IL-10	0.00 (0.00–693.14)	1.95 (0.00–580.10)	0.405[b]
IL-6	0.00 (0.00–80.96)	3.96 (0.00–85.69)	**0.03**
TNF-alpha	0.00 (0.00–343.28)	0.00 (0.00–88.88)	0.986
IL-1 beta	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.819
Gene expression			
Median (Minimum-Maximum)			
Nrf2	3.59 (0.02–26.77)	2.72 (0.24–35.11)	0.567
HO1	1.14 (0.30–21.86)	2.00 (0.31–20.08)	0.789

Variable	DAS 28 CRP		
	Remission / Low	Moderate/ High	p-value
Interleukins			
Median (Minimum-Maximum)			
IL-10	0.00 (0.00–693.14)	1.95 (0.00–580.10)	0.638
IL-6	0.50 (0.00–80.96)	3.96 (0.00–85.69)	0.063
TNF-alpha	0.00 (0.00–343.28)	0.00 (0.00–88.88)	0.986
IL-1 beta	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.839
Gene expression			
Median (Minimum-Maximum)			
Nrf2	3.99 (0.02–35.11)	2.02 (0.24–23.00)	0.251
HO-1	1.31 (0.30–21.86)	0.83 (0.37–16.31)	0.988

Data expressed as absolute numbers (n) and percentages (%). Mann-Whitney test. Bold type = significant at the level of 5 % ($p < 0.05$). DAS28 = Disease Activity Score-28; ESR = erythrocyte sedimentation rate; CRP=C-reactive protein; SDAI=Simplified Disease Activity Index; CDAI=Clinical Disease Activity Index; Nrf2 = Nuclear factor erythroid 2-related factor 2; HO-1 = heme oxygenase 1.

(*continued*)

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HDL	High-density lipoprotein
HO-1	Heme Oxigenase-1
LDL	Low-density lipoprotein
mSCORE	Modified Systematic Coronary Risk Evaluation
Nrf2	Nuclear factor-erythroid 2-related factor 2
ProBNP	N-terminal prohormone of brain natriuretic peptide
r^2	r-squared
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SCORE	Systematic Coronary Risk Evaluation
SDAI	Simplified Disease Activity Index
TC	Total cholesterol
TG	Triglyceride
WC	Waist circumference

(*continued on next page*)

Table 5
Serum cytokines and Nrf2/HO-1 gene expression vs. FR/Anti-CCP positivity and disease duration in patients ($n = 60$) with rheumatoid arthritis (RA).

Variable	RF/Anti-CCP+	RF or Anti-CCP+	RF/Anti-CCP-	*p*-value
Interleukins Median (Minimum-Maximum)				
IL-10	0.00 (0.00–115.48)	0.00 (0.00–693.14)	0.00 (0.00–580.10)	0.978
IL-6	2.13 (0.00–81.77)	0.54 (0.00–80.96)	1.00 (0.00–85.69)	0.791
TNF-alpha	0.00 (0.00–88.88)	0.03 (0.00–114.02)	0.00 (0.00–343.28)	0.609
IL-1 beta	0.00 (0.00–28.07)	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.779
Gene expression. Median (Minimum-Maximum)				
Nrf2	2.49 (0.04–23.00)	1.92 (0.00–9.88)	3.74 (0.02–35.12)	0.584
HO-1	0.85 (0.31–16.32)	3.49 (0.37–21.86)	2.01 (0.30–20.08)	0.997

Variable	Disease duration			
	1–5 years	5–10 years	>10 years	*p*-value
Interleukins Median (Minimum-Maximum)				
IL-10	1.39 (0.00–115.48)	3.06 (0.00–693.14)	0.00 (0.00–580.10)	0.671
IL-6	1.09 (0.00–37.38)	3.54 (0.00–81,77)	0.94 (0.00–85.69)	0.633
TNF-alpha	0.00 (0.00–49.94)	0.03 (0.00–114.02)	0.00 (0.00–343.28)	0.819
IL-1 beta	0.00 (0.00–28.07)	0.00 (0.00–87.35)	0.00 (0.00–59.80)	0.249
Gene expression. Median (Minimum-Maximum)				
Nrf2	2.49 (0.04–23.00)	1.92 (0.00–9.88)	3.74 (0.02–35.12)	0.504
HO-1	0.85 (0.31–16.32)	3.49 (0.37–21.86)	2.01 (0.30–20.08)	0.359

Data expressed as absolute numbers (n) and percentages (%). Mann-Whitney test. Bold type = significant at the level of 5 % ($p < 0.05$). RF = rheumatoid factor; Anti-CCP = anti-cyclic citrullinated peptide; Nrf2 = Nuclear factor erythroid 2-related factor 2; HO-1 = heme oxygenase 1.

(*continued*)

χ^2	Pearson's chi squared
RR	relative risk
CI	confidence interval

CRediT authorship contribution statement

Christiane Aguiar Nobre: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carlos Ewerton Maia Rodrigues:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Natacha Xavier Cavalcante:** Data curation. **Thácilla Siqueira Eugênio Nascimento:** Writing – review & editing. **João Gabriel Marques Brayner:** Writing – review & editing. **Giovanna Azevedo Sousa:** Data curation. **Nayara Alves de Sousa:** Methodology. **Regislane Pinto Ribeiro:** Methodology. **Vanessa Maria Eufrásio de Fiqueirêdo:** Methodology. **Giulia Albuquerque Paiva:** Methodology. **José Jackson do Nascimento Costa:** Methodology, Formal analysis. **Paula Goes:** Writing – review & editing, Writing – original draft. **Hellíada Vasconcelos Chaves:** Writing – review & editing. **Ticiana Mont'Alverne Parente Feijão:** Methodology. **Mirna Marques Bezerra:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data underlying this article will be shared following a reasonable request to the corresponding author.

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ANEXO D
ARTIGO SUBMETIDO EM REVISTA INDEXADA

Internal and Emergency Medicine

Subclinical diastolic dysfunction and its association with disease activity in patients with rheumatoid arthritis

--Manuscript Draft--

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Corresponding Author:	CARLOS EWERTON MAIA RODRIGUES, PhD Universidade de Fortaleza Fortaleza, CE BRAZIL
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidade de Fortaleza
Corresponding Author's Secondary Institution:	
First Author Secondary Information:	
First Author:	Christiane Aguiar Nobre, MD
Order of Authors:	Christiane Aguiar Nobre, MD
	CARLOS EWERTON MAIA RODRIGUES, PhD
	Luzia Keyne Sousa Carneiro Frota, MD
	Natacha Xavier Cavalcante, medical student
	Thácilla Siqueira Eugênio Nascimento, medical student
	João Gabriel Marques Brayner, medical student
	Giovanna Azevedo Sousa, medical student
	Paula Goes, PhD
	Vicente de Paulo Teixeira Pinto, PhD
	Hellíada Vasconcelos Chaves, MD
	Mirna Marques Bezerra, PhD
Order of Authors Secondary Information:	
Funding Information:	Funcap (02/2020) — Not applicable
Abstract:	Rheumatoid arthritis (RA) is a chronic inflammatory disorder associated with cardiovascular risk (CVR), regardless of traditional risk factors. In this study we investigated the association between disease activity, CVR and cardiac and carotid ultrasound (US) findings in 60 RA patients and 60 age and sex-matched healthy controls. The clinical variables included epidemiological and laboratory data and disease activity scores (DAS28-PCR). CVR was stratified using SCORE/mSCORE and Framingham scores. The US methods employed (transthoracic echocardiography and Doppler carotid ecography) allowed to evaluate carotid intima-media thickness (CIMT), atherosclerosis plaque, and cardiac function. The female sex was predominant (RA=91.7%; controls=90%) and the mean age was 52 ± 12 years and 52 ± 13 years, respectively. The mean disease duration was 10.43 ± 7.55 years. Serum testing for

	rheumatoid factor and anti-CCP was double-negative in 36.7%. The DAS28-PCR scores identified 61.7% as ‘remission/low activity’ and 31.3% as ‘moderate/high activity’. CVR was similar in the two groups (p=0.261). When the echocardiographic data was stratified, diastolic dysfunction was significantly more prevalent in subjects with higher disease activity (p=0.04). In addition, high clinical CVR scores were associated with greater CIMT and carotid plaque (p<0.05). Our results suggest that inflammatory activity may play a major role in subclinical cardiovascular dysfunction in RA.
Suggested Reviewers:	Jozelio Freire de Carvalho, PhD Professor, Universidade Federal da Bahia jotafc@hotmail.com RESEARCHER'S AREA OF EXPERTISE
	KIRLA WAGNER POTI GOMES, MD Hospital Geral de Fortaleza kirlawpg@gmail.com RESEARCHER'S AREA OF EXPERTISE
	Andrea Rocha, PhD Professor, Universidade de Fortaleza andrearoc@yahoo.com Expertise area

Subclinical diastolic dysfunction and its association with disease activity in patients with rheumatoid arthritis

Christiane Aguiar Nobre[a,b,c], Carlos Ewerton Maia Rodrigues[d,e,f,*], Luzia Keyne Sousa Carneiro Frota[g], Natacha Xavier Cavalcante[b], Thácilla Siqueira Eugênio Nascimento[b], João Gabriel Marques Brayner[b], Giovanna Azevedo Sousa[b], Paula Goes[h,i], Vicente de Paulo Teixeira Pinto[b], Hellíada Vasconcelos Chaves[j], Mirna Marques Bezerra[a,b]

[a] Postgraduate Program in Translational Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil.

[b] School of Medicine, Federal University of Ceará, Sobral, Ceará, Brazil.

[c] School of Medicine, University Center INTA - UNINTA, Sobral, Ceará, Brazil.

[d] Graduate Program in Medical Sciences, Medical School, University of Fortaleza (Unifor), Fortaleza, Brazil,

[e] Department of Internal Medicine, Federal University of Ceará, Fortaleza, Brazil

[f] Rheumatology Division, Hospital Geral de Fortaleza, Fortaleza, Brazil.

[g] Hospital do Coração, Sobral, Ceará, Brasil.

[h] Postgraduate Program in Morphological Science, Department of Morphology, School of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil.

[i] Department of Pathology and Legal Medicine, Medical School, Federal University of Ceará, Fortaleza, Ceará, Brazil.

[j] School of Dentistry, Federal University of Ceará, Sobral, Ceará, Brazil.

* Corresponding author:
Carlos Ewerton Maia Rodrigues, PhD
Rua Gontran Giffoni 366, Luciano Cavalcante, Fortaleza, Ceará, Brazil, CEP 60810-220.
Phone +55 85 988911796.
E-mail carlosewerton@hotmail.com

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disorder associated with cardiovascular risk (CVR), regardless of traditional risk factors. In this study we investigated the association between disease activity, CVR and cardiac and carotid ultrasound (US) findings in 60 RA patients and 60 age and sex-matched healthy controls. The clinical variables included epidemiological and laboratory data and disease activity scores (DAS28-PCR). CVR was stratified using SCORE/mSCORE and Framingham scores. The US methods employed (transthoracic echocardiography and Doppler carotid ecography) allowed to evaluate carotid intima-media thickness (CIMT), atherosclerosis plaque, and cardiac function. The female sex was predominant (RA=91.7%; controls=90%) and the mean age was 52 ± 12 years and 52 ± 13 years, respectively. The mean disease duration was 10.43 ± 7.55 years. Serum testing for rheumatoid factor and anti-CCP was double-negative in 36.7%. The DAS28-PCR scores identified 61.7% as 'remission/low activity' and 31.3% as 'moderate/high activity'. CVR was similar in the two groups (p=0.261). When the echocardiographic data was stratified, diastolic dysfunction was significantly more prevalent in subjects with higher disease activity (p=0.04). In addition, high clinical CVR scores were associated with greater CIMT and carotid plaque (p<0.05). Our results suggest that inflammatory activity may play a major role in subclinical cardiovascular dysfunction in RA.

Key words: Rheumatoid arthritis; Disease activity; Cardiovascular risk; Ultrasonography; Echocardiogram; Subclinical diastolic dysfunction.

Introduction

A chronic inflammatory condition affecting the joints, rheumatoid arthritis (RA) is considered an independent cardiovascular risk factor (CVRF) [1,2]. As shown in the literature, RA patients are at a significantly higher risk of cardiovascular events and mortality than the general population [3,4], mostly due to persistent systemic inflammation.

Disease activity in patients with RA is directly related to cardiovascular risk, and this risk is proportional to the intensity and persistence of systemic inflammation, which contributes to endothelial dysfunction, lipid alterations, and a higher prevalence of subclinical atherosclerotic plaques [5-7].

The repercussion of RA on the cardiovascular system are multifaceted and generally involve structural and functional changes to the heart[8-10]. Functional changes often include diastolic dysfunction and reduced ejection fraction [11,12].

An understanding of the negative impact of cardiovascular disease (CVD) on overall health, quality of life and life expectancy, as well as of the early detection of subclinical signs, is essential for risk stratification and management of RA patients. Ultrasonography (US) is usually employed to this end, but the ideal method has not yet been established [13]. US modalities like transthoracic echocardiography and Doppler carotid ecography are low-cost, non-invasive and sensitive tools in cardiovascular screening [9,14]. In fact, a study on 104 RA patients found carotid US to be more sensitive than coronary artery calcium scores in the detection of subclinical atherosclerosis in RA [15].

The combination of traditional clinical scores with cardiac and carotid imaging methods is recommended to enhance cardiovascular risk assessment. The populations that benefit most from integrating traditional clinical scores with cardiac and carotid imaging methods for cardiovascular risk assessment are asymptomatic adults classified as low to intermediate risk by traditional scores (such as Framingham, SCORE), particularly those aged 40 to 65 years, without known cardiovascular disease[16-18].

In view of the known association disease activity in patients with RA and increased CVRF, the clinical benefit of early risk stratification, this study we evaluated the association between disease activity and subclinical diastolic dysfunction in RA in Northeastern Brazil.

Methods

Patients

This cross-sectional study was conducted at a secondary-level health care facility in Northeastern Brazil between October 2021 and October 2022. The study protocol complied with the principles of the Declaration of Helsinkki [19] and was approved under #10729/2020 by the research ethics committee of the Federal University of Ceará (CEP/UFC/PROPESQ). All procedures followed well-established research standards and guidelines, and all participants gave their informed written consent prior to entering the study.

The sample consisted of 120 participants, of which half were RA patients and half were sex and age-matched healthy controls recruited from the local community. All RA patients met the 2010 diagnostic criteria of the American College of Rheumatology[20]. The exclusion criteria were age under 18 years, pregnancy, previously diagnosed CVD, chronic kidney failure, and association with collagen disorders such as systemic lupus erythematosus, Sjögren syndrome, inflammatory myopathy, and sclerodermia.

Sample size calculation

The sample size was calculated considering carotid intima-media thickness (CIMT) as primary variable outcome. In an earlier study [21], patients with recent-onset RA had an average CIMT of 0.64 ± 0.13 mm, compared to 0.58 ± 0.09 mm in healthy controls. Based on this expected difference, we estimated that a statistical power of 80% at the 5% level of significance (α=0.05) would require at least 55 participants per group. In this study, each group included 60 subjects, matching the design adopted in another cross-sectional study [22].

Clinical and anthropometric evaluation

The collected information included epidemiological, clinical and laboratory data, such as blood count, C-reactive protein, rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), glycemia, total cholesterol, high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), and triglycerides.

The 10-year cardiovascular risk was assessed using the systematic coronary risk evaluation (SCORE), the modified SCORE (mSCORE), and the Framingham score [23,24]. To calculate mSCORE, we multiplied the SCORE index by 1.5 in RA patients meeting at least 2 of the following 3 criteria: disease duration >10 years, positivity for FR and/or anti-CCP, and extra-articular manifestations [25].

Upon physical examination, information was collected on the number of painful and swollen joints, arterial blood pressure, waist circumference (WC), and body-mass index (BMI). WC was measured with a tape positioned horizontally between the iliac crest and the lowest rib. BMI was calculated by dividing the weight by the height squared (kg/m²). Overweight was defined as BMI 25.0-29.9 kg/m² and obesity was defined as ≥30.0 kg/m².

Disease activity was scored with the composite index DAS28-CRP, using the following interpretation: <2.6=clinical remission; 2.6-3.1=low activity; 3.2-5.0=moderate activity; >5.1=high activity [26].

Ultrasound evaluation

Both groups of participants were submitted to cardiac and carotid US by a cardiologist (LKSCF) trained and experienced in the respective US techniques. Carotid US in B-mode with spectral Doppler allowed to conduct a morphological and hemodynamic analysis, with emphasis on CIMT measurement and the detection of atherosclerosis plaque—a well established marker of subclinical atherosclerosis [27,28]. Cardiac function was evaluated by 2-dimensional transthoracic echocardiography while the left ventricle ejection fraction (LVEF) was determined with the Teicholz method, following the guidelines of the American Society of Echocardiography [29].

Statistical analysis

Clinical and demographic variables, when quantitative and continuous, were expressed as means ± standard deviation (SD) and medians + interquartile range, or, when categorical, as absolute numbers (n) and percentages (%). The main groups (RA vs. controls) and the CVRF subgroups were compared with the chi-squared test (categorical variables) or the Mann-Whitney and Kruskal-Wallis test (non-parametric and continuous variables). All statistical analyses were performed with the software SPSS v. 26.0 (IBM Corp., Armonk, NY, USA) at the 5% level of significance (p<0.05).

Results

The female sex was predominant in both the RA group (91.7%) and the control group (90%). The mean age was 52 ± 12 years and 52 ± 13 years, respectively.

In the case-control analysis, when comparing the groups with regard to traditional CVRFs (family history of CVD, current or previous smoking, arterial hypertension, diabetes mellitus, sedentary lifestyle, systolic and diastolic blood pressure, total cholesterol, HDL and LDL), no significant difference was observed ($p>0.05$). Likewise, the two groups were statistically similar with regard to IMC and the distribution of low weight, overweight and obesity, but the prevalence of dyslipidemia was significantly higher in the control group (55.0%) than in the RA group (33.3%) ($p=0.027$). Moreover, the controls displayed higher WC (91.67 cm vs. 87.65 cm; $p=0.017$) and BMI values (28.58 vs. 26.83; $p=0.010$). As for laboratory parameters, the controls had significantly higher levels of glycemia (109.95 mg/dL vs. 93.92 mg/dL; $p<0.001$) and triglycerides (154.27 mg/dL vs. 114.37 mg/dL; $p=0.004$).

The mean disease duration was 10.43 ± 7.55 years, distributed as follows: 1-5 years=30%, 5-10 years=18.3%, and >10 years=51.7%. The mean time since diagnosis was 8.57 ± 6.84 years. Positivity for either RF or anti-CCP was 20%, for both 43.3%, and for neither 36.7%. According to the DAS-28 PCR scoring system, 61.7% were classified as remission or low activity. The remainder (31.3%) had moderate or high activity.

With regard to therapy, 59 of the 60 patients (98.3%) were treated with disease-modifying antirheumatic drugs (DMARDs), primarily csDMARDs (n=55; 91.7%) used alone (n=39; 66.1%) or in combination with biological DMARDs (n=13; 22.0%) or targeted DMARDs (n=7; 11.9%). Current use of corticosteroids was reported by 26.7%, while methotrexate was used by 72.9%.

Table 1 shows the CVRFs of participants with and without RA expressed in SCORE/mSCORE and Framingham scores. According to the SCORE/mSCORE index, low risk was assigned to 45% of the RA patients and to 55.4% of the controls. Moderate risk was higher among RA patients (55% vs. 44.6%), but the difference was not significant ($p=0.261$). The mean Framingham score was slightly higher in the control group (8.34 ± 8.95%) than in the RA group (6.33 ± 6.78%), though not significantly ($p=0.208$). When arranged by category, low CVR (≤10%) was assigned to 68.3% of the controls and to 85% of the RA patients. Moderate risk (10-20%) and high risk (≥20%) were less frequent in the RA group, though not significantly.

Cardiac and carotid US findings were statistically similar in the two groups with regard to CVR parameters (Table 1).

Table 1: Cardiovascular risk factors (CVRFs) determined by ultrasound scanning of subjects without (controls) and with rheumatoid arthritis (RA).

Variables	Controls (n=60)	RA (n=60)	*p*-value
Ultrasound findings			
Intima-media thickness R (mm)	0.65 ± 0.15	0.66 ± 0.14	0.346 [b]
Intima-media thickness L (mm)	0.65 ± 0.15	0.68 ± 0.19	0.474 [b]
Plaque D			
Yes	8 (13.3%)	6 (10%)	0.777 [a]
No	52 (86.7%)	54 (90%)	
Plaque L			
Yes	4 (6.7%)	8 (13.3%)	0.362 [a]
No	56 (93.3%)	52 (86.7%)	
Teicholz ejection fraction	65.92 ± 5.55	64.34 ± 5.35	0.097 [b]
Diastolic dysfunction			
Yes	14 (23.3%)	8 (13.3%)	0.239 [a]
No	46 (76.7%)	52 (86.7%)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Mann-Whitney test.

Within the RA group, the DAS28-PCR scores were statistically similar for remission/low activity and moderate/high activity, left and right-side CIMT, and atherosclerosis plaque (Table 2), but diastolic dysfunction was more prevalent in patients with moderate/high activity (33.3%) than in patients with remission/low activity (5.7%) (p=0.044).

Table 2: DAS28-PCR scores *versus* US findings in subjects with rheumatoid arthritis (RA) (n=60).

Variables	DAS28-PCR		*p*-value
	Remission/Low	Moderate/High	
Echocardiographic findings			
Intima-media thickness R (mm)	0.66 ± 0.14	0.67 ± 0.15	0.837[b]
Intima-media thickness L (mm)	0.63 ± 0.11	0.75 ± 0.25	0.058[b]
Plaque R (n/%)			
Yes	4 (10.8)	2 (8.7)	1.000[a]
No	33 (89.2)	21 (91.3)	
Plaque L (n/%)			
Yes	5 (13.5)	3 (13.0)	1.000[a]
No	32 (86.5)	20 (87.0)	
Teicholz ejection fraction	63.33 ± 4.56	65.96 ± 6.18	
Diastolic dysfunction (n/%)			
Yes	2 (5.7)	8 (33.3)	**0.044[a]**
No	35 (94.3)	16 (66.7)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Mann-Whitney test. Bold type=significant at 5% (p<0.05).

When comparing low and moderate CVR in RA patients expressed in SCORE/mSCORE, some echocardiographic parameters were significantly different (Table 3). Thus, left and right-side CIMT values were significantly higher for moderate risk (0.69 mm and 0.71 mm; p=0.048) than for low risk (0.62 mm and 0.60 mm; p<0.001). Atherosclerosis plaque in the right carotid artery was also more common for moderate risk (14.5%) than for low risk (3.4%) (p=0.049). Finally, diastolic dysfunction was significantly more frequent among participants with moderate risk (32.1% vs. 5.2%; p<0.001).

Table 3: Association between echocardiographic findings and cardiovascular risk expressed in SCORE/mSCORE in subjects with rheumatoid arthritis (RA) (n=60).

Variables	SCORE/mSCORE scores		*p*-value
	Low	Moderate	
Echocardiographic findings			
Intima-media thickness R (mm)	0.62 ± 0.12	0.69 ± 0.16	**0.048**[b]
Intima-media thickness L (mm)	0.60 ± 0.11	0.71 ± 0.18	**<0.001**[b]
Plaque R (n/%)			
Yes	2 (3.4)	8 (14.5)	**0.049**[b]
No	56 (96.6)	47 (85.5)	
Plaque L (n/%)			0.089[a]
Yes	2 (3.4)	7 (12.7)	
No	56 (96.6)	48 (87.3)	
Teicholz ejection fraction	64.92 ± 5.77	64.92 ± 5.77	0.538[b]
Diastolic dysfunction (n/%)			
Yes	3 (5.2)	17 (32.1)	**<0.001**[b]
No	55 (94.8)	36 (67.9)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Mann-Whitney test. Bold type=significant at 5% (p<0.05).

The Framingham scores assigned to the echocardiographic findings of the RA patients (Table 4) reflected major differences in mean CIMT, which was significantly greater for moderate/high risk on the right side (0.70 mm) and the left side (moderate 0.91 mm; high 0.74 mm) than for low risk (both 0.65 mm) (p=0.042 and p<0.011, respectively). Atherosclerosis plaque in the carotids was also significantly more frequent in high-risk patients. Despite these structural changes, the ejection fraction and the prevalence of diastolic dysfunction were similar in the two groups (p>0.5).

Table 4: Association between echocardiographic findings and cardiovascular risk expressed in Framingham scores in subjects with rheumatoid arthritis (RA) (n=60).

Variables	Framingham scores			*p*-value
	Low	Moderate	High	
Echocardiographic findings				
Intima-media thickness R (mm)	0.65 ± 0.15	0.7 ± 0.1	0.7 ± 0.03	**0.042[b]**
Intima-media thickness L (mm)	0.65 ± 0.17	0.91 ± 0.26	0.74 ± 0.19	**<0.011[b]**
Plaque L (n/%)				
Yes	2 (3.9)	2 (40.0)	2 (50.0)	**0.009[b]**
No	49 (96.1)	3 (60.0)	2 (50.0)	
Plaque L (n/%)				**0.020[a]**
Yes	5 (9.8)	3 (60.0)	0 (0.0)	
No	46 (90.2)	2 (40.0)	4 (100.0)	
Teicholz ejection fraction	64.22 ± 5.57	65.8 ± 2.28	64 ± 5.89	0.505[b]
Diastolic dysfunction (n/%)				
Yes	7 (14.0)	0 (0.0)	1 (25.0)	<0.867[b]
No	43 (86.0)	4 (100.0)	3 (75.0)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Mann-Whitney test. Bold type=significant at 5% (p<0.05).

No significant association was observed between the echocardiograhic parameters and disease duration (Table 5), nor between US changes and positivity for RF and/or anti-CCP (Table 6).

Table 5: Association between echocardiographic findings and disease duration in subjects with rheumatoid arthritis (RA) (n=60).

Variables	Disease duration			*p*-value
	1-5 years	5-10 years	>10 anos	
Echocardiographic findings				
Intima-media thickness R (mm)	0.63 ± 0.12	0.70 ± 0.11	0.67 ± 0.16	0.263[b]
Intima-media thickness L (mm)	0.64 ± 0.11	0.67 ± 0.11	0.70 ± 0.24	0.569[b]
Plaque R (n/%)				
Yes	1 (5.6)	1 (9.1)	4 (12.9)	0.849[b]
No	17 (94.4)	10 (90.9)	27 (87.1)	
Plaque L (n/%)				
Yes	1 (5.6)	2 (18.2)	5 (16.1)	0.596[a]
No	17 (94.4)	9 (81.8)	26 (83.9)	

Teicholz ejection fraction	64.18 ± 4.98	63.64 ± 6.67	64.68 ± 5.21	0.733[b]
Diastolic dysfunction (n/%)				
Yes	2 (11.1)	4 (36.4)	4 (12.9)	0.352[a]
No	16 (88.9)	7 (63.6)	27 (87.1)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Kruskal-Wallis test followed by Dunn's post test.

Table 6: Association between serum profile (rheumatoid factor and anti-CCP) and echocardiographic findings in subjects with rheumatoid arthritis (RA) (n=60).

Variables	RF+ & anti-CCP+	RF+ or anti-CCP+	RF- & anti-CCP-	*p*-value
Echocardiographic findings				
Intima-media thickness R (mm)	0.70 ± 0.16	0.63 ± 0.11	0.64 ± 0.14	0.307[b]
Intima-media thickness L (mm)	0.68 ± 0.18	0.62 ± 0.11	0.71 ± 0.22	0.537[b]
Plaque R (n/%)				
Yes	2 (7.7)	1 (8.3)	3 (13.6)	0.856[b]
No	24 (92.3)	11 (91.7)	19 (86.4)	
Plaque L (n/%)				
Yes	3 (11.5)	2 (16.7)	3 (13.6)	1.000[b]
No	23 (88.5)	10 (83.3)	19 (86.4)	
Teicholz ejection fraction	65.05 ± 5.38	63.58 ± 6.01	63.91 ± 5.09	0.660[b]
Diastolic dysfunction (n/%)				
Sim	5 (19.2)	1 (8.3)	3 (14.3)	0.895[a]
Não	21(80.3)	11 (91.7)	18 (85.7)	

Data expressed as means ± standard deviation of the mean, or absolute numbers (n) and percentages (%). mm=millímeters. [a] Pearson's chi-squared test. [b] Kruskal-Wallis test followed by Dunn's post test.

Discussion

In this study we investigated cardiac and carotid functional and structural changes in RA patients and their association with disease activity, clinical findings and cardiovascular risk. The clinical SCORE/mSCORE and Framingham scores were statistically similar for RA patients and controls, but RA patients with high scores displayed changes on US, such as increased CIMT and prevalence of carotid atherosclerosis, suggesting subclinical vascular impairment.

Despite the absence of statistical significance between RA patients and controls with regard to overall cardiac and carotid US parameters, when we stratified the data, the results proved to be relevant. Thus, the fact that diastolic dysfunction was significantly more frequent in RA patients with moderate/high disease activity suggests an association between systemic inflammation and functional cardiac impairment. Moreover, subjects with moderate CVR also tended to have more severe arterial thickening, carotid plaque and diastolic dysfunction. These findings are consistent with published evidence that 30% of cardiovascular risk in RA patients is due to the disease, while the remaining 70% is explained by traditional CVRFs like hypertension and smoking [30].

In our RA patients, the highest risk scores coincided with US changes suggestive of subclinical atherosclerosis. Clinical CVR scores like Framingham and SCORE are widely used in clinical practice to estimate the 10-year risk of cardiovascular events, but evidence shows that these models are of limited usefulness in patients with autoimmune disease, such as RA, because they do not adequately take into account the role of chronic inflammation in the development of atherosclerosis [31]. Even the correction factor of 1.5 proposed by EULAR seems insufficient to assess the actual risk in this patient population [32]. On the other hand, the evaluation of subclinical atherosclerosis on carotid US—a non-invasive, low-cost, reproducible and sensitive method capable of early detection of structural changes, such as increased CIMT and plaque formation—has been shown to compensate for this shortcoming. In short, combining clinical scores and US findings allows for a more accurate and low-cost risk stratification of RA patients, hence earlier and more targeted interventions [33].

The prevalence of diastolic dysfunction in RA patients is reported to be between 31% and >50%, depending on population profile and follow-up time. The change is usually detected on Doppler echocardiography, even in patients with preserved ejection fraction and without evident cardiovascular symptoms [34-38]. Disease activity, as expressed by increased DAS28 and CDAI scores and levels of CRP and ESR, is independently correlated with the presence and severity of diastolic dysfunction [34-41]. Patients with active RA are known to be at a significantly higher risk of heart failure, especially diastolic, than patients with RA in remission or low activity [39,40]. The fact that the prevalence of diastolic dysfunction was similar in healthy controls and in RA patients with low disease activity suggests that careful control of inflammation can mitigate subclinical CVR [41]. The finding in the present study of an association between diastolic dysfunction and high RA activity supports this notion.

The correlation in RA patients between high clinical CVR scores and US findings suggestive of subclinical atherosclerosis has been pointed out elsewhere, but the literature on this specific topic is very limited [42]. Importantly, the association established in our study between cardiovascular changes (diastolic dysfunction, increased CIMT, atherosclerosis plaque) and high clinical CVR scores highlights the need for an integrated approach to CVR in this patient population, combining clinical stratification, US scanning, and monitoring of inflammatory activity [15-17].

No association was found between disease duration and cardiovascular changes. Classically, disease duration has been regarded as an independent predictor of arterial stiffness, since aging of the arteries is more accelerated in RA than in healthy controls [43], and since the cumulative damage caused by RA is greater in patients with ≥10 years of disease, even in the absence of traditional CVRFs [44]. Interestingly, neither did we observe any association between positivity for antibodies and changes on US. Cohort studies with long follow-up have shown that both RF and anti-CCP are associated with an increased incidence of cardiovascular events in patients with established RA, including acute coronary syndrome, stroke, and cardiovascular death [45,46]. The risk is particularly high in subjects with high anti-CCP titers. On the other hand, and supporting our findings, a large cohort study concluded that, if traditional CVRFs and systemic inflammation are adjusted for, positivity for RF or anti-CCP is in itself not a robust, independent predictor of cardiovascular events, with inflammatory activity and disease severity being the main risk determinants [47].

Considering that systemic inflammation is a major factor in the acceleration of atherosclerosis in RA, the absence in our study of a significant association between disease duration, serum positivity and structural and functional cardiovascular changes may in part be explained by the fact that over 60% of our sample displayed RA in remission/low activity due to efficient clinical control.

The strengths of this study include i) our integrated approach (combining echocardiography and carotid US) to improve the analysis of structural and functional cardiovascular changes and subclinical atherosclerosis, and ii) the combination of US findings with CVR scores to improve the correlation between these parameters and cardiac screening

in RA. On the other hand, the study was limited by i) the small sample size, ii) the single-center design, iii) the cross-sectional observational design, and iv) the lack of multivariate analysis to identify independent predictors of CVR in RA patients submitted to US.

Conclusion

In conclusion, our findings show that inflammatory activity played a major role in subclinical cardiovascular dysfunction in a sample of RA patients. Hence, US screening of the heart and carotids should be considered for earlier detection and intervention, especiallly in subjects with active RA or additional CVRFs. Furter investigations are needed to explore the methods of early detection of CVRFs in RA.

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Availability of data and materials

The data that support the finding of this study are available on a reasonable request from the corresponding author.

Conflict of interest

None to declare**.**

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Authors' contributions

Christiane Aguiar Nobre: Writing – review & editing, Writing – original draft, Data curation, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis. **Carlos Ewerton Maia Rodrigues:** Writing – review & editing, Writing- original draft, Formal analysis, Data curation, Conceptualization, Methodology. Investigation, Formal analysis. **Luzia Keyne Sousa Carneiro Frota:** Data curation. **Natacha Xavier Cavalcante**: Writing – review & editing. **Thácilla Siqueira Eugênio Nascimento:** Methodology**. João Gabriel Marques Brayner:** Writing – review & editing. **Giovanna Azevedo Sousa:** Data curation. **Paula Goes:** Writing – review & editing, Writing – original draft. **Vicente de Paulo Teixeira Pinto:** Methodology, Data curation. **Hellíada Vasconcelos Chaves:** Writing – review & editing. **Mirna Marques Bezerra:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis.

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Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links	IAEM-D-25-01784	Subclinical diastolic dysfunction and its association with disease activity in patients with rheumatoid arthritis	15 Nov 2025	30 Dec 2025	Under Review

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ANEXO E
ARTIGO SUBMETIDO EM REVISTA INDEXADA

Lower educational level is associated with higher disease activity and increased cardiovascular risk in rheumatoid arthritis from northeastern Brazil : A cross-sectional study

Christiane Aguiar Nobre[1,2], Mirna Marques Bezerra[1,2], Natacha Xavier Cavalcante[2], Thácilla Siqueira Eugênio Nascimento[2], João Gabriel Marques Brayner[2], Giovanna Azevedo Sousa[2], Valder Cavalcante Maia Mendonça Filho[2], Paula Goes[3,4], Hellíada Vasconcelos Chaves[5], Carlos Ewerton Maia Rodrigues[6,7,8]

1.Postgraduate Program in Translational Medicine, Federal University of Ceará, Sobral, Ceará, Brazil.

2.School of Medicine, Federal University of Ceará, Sobral, Ceará, Brazil.

3.Postgraduate Program in Morphological Science, Department of Morphology, School of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil.

4.Department of Pathology and Legal Medicine, Medical School, Federal University of Ceará, Fortaleza, Ceará, Brazil.

5.School of Dentistry, Federal University of Ceará, Sobral, Ceará, Brazil.

6.Graduate Program in Medical Sciences, Medical School, University of Fortaleza (Unifor), Fortaleza, Brazil

7.Department of Internal Medicine, Federal University of Ceará, Fortaleza, Brazil .

8.Rheumatology Division, Hospital Geral de Fortaleza, Fortaleza, Brazil.

Corresponding author: E-mail: carlosewerton@hotmail.com(orcid.org/0000-0003-1367-6782);
Contributing authors:
chrisan.0805@gmail.com(orcid.org/0009-0009-6700-1757); mirna@ufc.br(orcid.org/0000-0003-2146-4456); natachaxc@alu.ufc.br (orcid.org/0009-0002-0184-0741);
thacillaenascimento@alu.ufc.br (orcid.org/0009-0006-8791-9483); joaobrayner@alu.ufc.br (orcid.org/0009-0002-0184-0741); giovannaasousa@alu.ufc.br (orcid.org/0009-0006-8791-9483); valderfilho10@gmail.com (orcid.org/0000-0002-3204-5903), paulagoes@ufc.br (orcid.org/0002-9704-2412); helliadachaves@ufc.br(orcid.org/0000-0002-7718-9900).

Abstract

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with increased cardiovascular risk (CVR) and greater morbidity. Evidence suggests that socioeconomic determinants, such as education level, may influence inflammatory control and cardiometabolic risk, but few studies have simultaneously evaluated these outcomes in populations from Northeast Brazil. This study investigated the relationship between educational level, CVR, and disease activity in patients with RA. **Methods**: A cross-sectional study was conducted between October 2021 and October 2022 with participants treated at a referral outpatient clinic in the interior of Ceará, Brazil. Sociodemographic and clinical information was collected through interviews and physical examinations. CVR was estimated using the SCORE and mSCORE scores, and disease activity was assessed using the DAS28-CRP and DAS28-ESR, categorized as remission/low activity and moderate/high activity. Educational level was converted into years of schooling according to the classification of the Brazilian Institute of Geography and Statistics (IBGE). Data

were analyzed using chi-square, Mann-Whitney, and Kruskal-Wallis tests, adopting $p<0.05$. **Results**: The study evaluated 120 participants, equally distributed between the groups with and without RA. Both groups presented similar characteristics regarding age, sex, and ethnicity. The RA group presented a lower educational level ($p=0.013$). Lower education was significantly associated with moderate CVR ($p<0.001$), with a higher proportion of individuals with 0–8 years of education in the increased-risk group. A similar relationship was observed with disease activity, by both DAS28-CRP ($p=0.038$) and DAS28-ESR ($p=0.028$), with a predominance of low education among those with higher inflammatory activity. Patients with moderate/high activity presented a longer average disease duration, while age, sex, and ethnicity did not differ between the groups. **Conclusion**: Patients with lower education levels presented higher CVR and greater disease activity, with education highlighted as a relevant social determinant in RA. The findings reinforce the need for care strategies adapted to the patient's educational level, focusing on health literacy, therapeutic adherence, and cardiovascular prevention.

Keywords: rheumatoid arthritis, educational level, disease activity, cardiovascular risk

1. Background

Rheumatoid arthritis (RA) is characterized by persistent systemic inflammation and a set of articular and extra-articular manifestations that increase morbidity throughout the course of the disease. In addition to functional limitations and a direct impact on quality of life, patients with RA have a higher cardiovascular risk (CVR) than the general population, a consequence of

traditional cardiovascular risk factors and inflammatory mechanisms that accelerate the atherosclerotic process [1-3]. In this context, understanding the factors that modulate this risk is fundamental to improving care and guiding preventive strategies.

Among the determinants involved in the clinical behavior of RA, educational level stands out as a social and possibly clinical marker. Patients with lower education tend to have less awareness of the severity of the disease and more barriers to adopting self-care practices [1-2]. These differences may result in more unfavorable outcomes over time, due to the interaction between social vulnerabilities, less access to health information, and more prevalent risk behaviors. From a public health and clinical care perspective, these findings reinforce the need to recognize education as a structuring element in therapeutic planning. Simplified educational strategies, health literacy interventions, the use of accessible instructional materials, and multidisciplinary follow-up can contribute to reducing inequalities and improving adherence. Previous studies suggest that well-structured educational interventions can modify behaviors and promote better clinical outcomes, especially in socially vulnerable populations [1-3]. However, there is still a lack of specific investigations into patients with RA and low education, representing an important gap for future research.

Education has also been associated with the degree of inflammatory activity in RA [3]. Lower educational levels are associated with higher DAS28 scores and a lower probability of achieving remission, regardless of demographic characteristics or the use of disease-modifying therapies [4-6].

Despite advances in the literature, investigations that simultaneously assess the influence of education on CVR and inflammatory activity in the same population are still scarce. Considering that Northeastern Brazil presents clear socioeconomic inequalities and specific patterns of access to health care, it becomes particularly relevant to explore how education relates to these outcomes in the regional context. Thus, the objective of this study was to analyze the association between educational level, CVR, and disease activity in RA patients followed up at a specialized service in Northeastern Brazil.

2. Methods

2.1 Patients

This cross-sectional study was conducted between October 2021 and October 2022 in a secondary-level outpatient service in Northeastern Brazil. All participants signed the Informed Consent Form (ICF), and the study protocol was conducted in accordance with the principles of the Declaration of Helsinki. The research was approved by the Research Ethics Committee of the Federal University of Ceará (CEP/UFC/PROPESQ), under number 110729/2020.

The study evaluated 120 participants, equally distributed between the groups with and without RA. A control group of healthy volunteers were recruited from local community and controls that had any history of chronic and autoimmune disease were excluded. The classification of patients with RA followed the 2010 criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [7].

Exclusion criteria included: aged under 18 years, pregnant, established cardiovascular disease such as heart failure, coronary artery disease, cerebrovascular disease, atrial fibrillation, as well as neoplasms, chronic renal failure, and association with other connective tissue diseases, such as inflammatory myopathies, scleroderma, systemic lupus erythematosus, and Sjögren's syndrome.

2.2 Interview and clinical assessment

The information collected included epidemiological and clinical data. The conversion of schooling categories into years of study followed the methodology used by the Brazilian Institute of Geography and Statistics (IBGE) in its population surveys [8]. For analytical purposes, the categories were converted as follows: illiteracy = 0 years, primary education = 8 years, secondary education = 11 years, and completed higher education = 15 years [8].

Cardiovascular risk over 10 years was assessed using the Systematic Coronary Risk Evaluation (SCORE) and the modified SCORE (mSCORE) scores [9]. To calculate the mSCORE, the original SCORE value was multiplied by 1.5 in RA patients who met at least two of the following criteria: disease duration greater than 10 years, positivity for RA and/or anti-CCP, and presence of extra-articular manifestations [9].

From the physical examination information was obtained on the number of painful and swollen joints. Disease activity was assessed using the DAS28 composite index, using the following interpretation: <2.6 = clinical remission; 2.6–3.1 = low activity; 3.2–5.0 = moderate

activity; >5.1 = high activity [10]. Participants were stratified into 2 groups according to disease activity using DAS 28-CRP and DAS 28-ESR scores: remission/low activity and moderate/high activity [10,11].

2.3 Sample size calculation

The sample size was estimated for a finite population of RA patients seen over 12 months in Northeastern Brazil, adopting p=q=0.5, a 5% error, and 95% confidence, also considering an adherence rate of 70% and expected exclusions (~20%), resulting in 60 individuals.

2.4 Statistical analysis

Quantitative variables were expressed as mean ± SD or median and interquartile range, and categorical variables as absolute numbers and percentages. Comparisons between groups used the chi-square test (categorical variables) and the Mann-Whitney or Kruskal-Wallis tests (continuous variables). The analyses were conducted using SPSS v.26.0, adopting a significance level of 5% ($p<0.05$).

3. Results

The study included 120 participants, equally distributed between the RA and control groups. As shown in Table 1, the groups showed similar profiles regarding age (p=0.832), sex (p=1.000), and ethnic-racial composition (p=0.131). A significant difference was observed in

relation to education: the RA group had a lower educational level, with a higher proportion of individuals without formal years of study and a lower frequency of participants with 15 years or more of study (p=0.013). Among RA patients, 11.7% had no years of study, 41.7% had 8 years, and another 41.7% had 11 years of study, while only 5.0% had 15 years of completed study.

Table 1. Demographic data of participants without RA (control) and with RA.

Variables	Control (n=60)	RA (n=60)	p-value
Sex			
Male	6 (10.0)	5 (8.3)	1.000 [a]
Female	54 (90.0)	55 (91.7)	
Ethnicity			
White	0 (0.0)	2 (3.3)	
Mixed-race	56 (93.3)	50 (83.3)	0.131 [a]
Black	4 (6.7)	8 (13.3)	
Years of education (n/%)			
0 years	4 (6.7)	7 (11.7)	
8 years	27 (45.0)	25 (41.7)	**0.013** [a]
11 years	15 (25.0)	25 (41.7)	
15 years	14 (23.3)	3 (5.0)	
Age	52±13	52±12	0.832 [b]

Data expressed as absolute number (n) and percentage (%). a. Pearson's Chi-Square Test. . b. Mann-Whitney Test . Value in bold is significant when $p<0.05$.

Table 2 presents the assessment of the education level of participants classified as low and moderate CVR, according to the SCORE/mSCORE scores. The analysis demonstrated a significant association between lower education and classification of moderate CVR ($p < 0.001$). In the moderate risk group, 68.8% of individuals belonged to the lower education categories, while in the low-risk group this percentage was only 28%. Furthermore, no participant without formal years of study was identified in the low-risk group.

Table 2: Distribution of education level according to cardiovascular risk stratification by mSCORE/SCORE.

	mSCORE/SCORE		
Years of education (n/%)	**Low**	**Moderate**	**p-value**
0 years	0 (0.0)	7 (21.9)	
8 years	7 (28.0)	15 (46.9)	**<0.001**
11 years	17 (68.0)	8 (25.0)	
15 years	1 (4.0)	2 (6.3)	

Data presented as an absolute number (n) of participants followed by the percentage (%). Pearson's Chi-Square test. Value in bold is significant when $p<0.05$.

In the stratification of participants according to disease activity, assessed by the DAS28-CRP and DAS28-ESR scores (Tables 3 and 4), a significant association was found between lower education and higher levels of disease activity. This was observed in both the DAS28-CRP ($p = 0.038$) and DAS28-ESR ($p = 0.028$) analyses. Furthermore, it was found that participants with moderate/high disease activity had a longer mean RA duration compared to those in remission/low activity, as measured by the DAS28-CRP ($p = 0.047$) and DAS28-ESR ($p = 0.012$) indices. In contrast, no statistically significant differences were identified between the groups regarding age, sex, or ethnicity, regardless of the score used.

Table 3: DAS 28 CRP value versus epidemiological and clinical parameters of participants with RA.

	DAS 28 CRP		
Variables	**Remission / Low**	**Moderate / High**	**p-value**
Sex (n/%)			
Male	4 (10.8)	1 (4.3)	
Female	33 (89.2)	22 (95.7)	0.640[a]
Ethnicity (n/%)			
White	2 (5.4)	0 (0.0)	
Mixed-race	29 (78.4)	21 (91.3)	0.430[a]
Black	6 (16.2)	2 (8.7)	
Years of education (n/%)			
0 years	2 (5.4)	5 (21.7)	
8 years	14 (37.8)	11 (47.8)	
11 years	20 (54.1)	5 (21.7)	**0.038[a]**
15 years	1 (2.7)	2 (8.7)	
Age (years)	51±13	53±11	0.513[b]
Clinical data			
Time of disease (years) (mean±sd)	9.51±8.33	11.89±5.96	**0.047[b]**
Time of disease (n/%)			
1-5 years	15 (40.5)	3 (13.0)	0.082[a]
5-10 years	6 (16.2)	5 (21.7)	
> 10 years	16 (43.2)	15 (65.2)	

Data presented as mean ± standard deviation of the mean or absolute number (n) of participants followed by the percentage (%). a Pearson's Chi-Square Test. b Mann-Whitney Test. Value in bold is significant when $p<0.05$.

Table 4: DAS 28 ESR value versus epidemiological and clinical parameters in participants with RA.

	DAS 28 ESR		
Variables	**Remission / Low**	**Moderate / High**	**p-value**
Sex (n/%)			
Male	4 (13.3)	1 (3.3)	
Female	26 (86.7)	29 (96.7)	0.353[a]
Ethnicity (n/%)			
White	1 (3.3)	1 (3.3)	
Mixed-race	26 (86.7)	24 (80.0)	0.850[a]
Black	3 (10.0)	5 (16.7)	
Years of education (n/%)			
0 years	2 (6.7)	5 (16.7)	
8 years	9 (30.0)	16 (53.3)	
11 years	18 (60.0)	7 (23.3)	**0.028[a]**
15 years	1 (3.3)	2 (6.7)	
Age (years)	50±13	53±12	0.267[a]
Clinical data			
Time of disease (years) (média±sd)	8.88±8.61	11.97±6.08	**0.012[b]**
Time of disease (n/%)			
1-5 years	14 (46.7)	4 (13.3)	**0.017[a]**
5-10 years	5 (16.7)	6 (20.0)	
> 10 years	11 (36.7)	20 (66.7)	

Data presented as mean ± standard deviation of the mean or absolute number (n) of participants followed by the percentage (%). [a] Pearson's Chi-Square Test. [b] Mann-Whitney Test. Value in bold is significant when $p<0.05$.

4. Discussion

Several studies have demonstrated that lower education levels are independently associated with a higher risk of developing RA and worse clinical outcomes throughout the course of the disease. Although part of the relationship is mediated by modifiable factors - such as smoking, higher BMI, and unfavorable socioeconomic conditions - the impact of education persists even after these adjustments, suggesting more complex mechanisms, possibly related to lower health literacy, environmental exposure, and trajectories of vulnerability since childhood. In this context, this study simultaneously evaluated the influence of education on CVR and inflammatory activity in patients with RA. Interestingly, the results show that individuals with lower educational levels tend to perform worse in these two outcomes, suggesting that social factors may interact directly with the progression of the disease, and these findings are in agreement with data from the literature, which describes the relevance of social determinants in risk behavior and the management of chronic diseases [1-3].

The association between low education and higher CVR observed by the SCORE/mSCORE scores reinforces a pattern already documented in the literature: individuals with lower education have lower levels of knowledge and perception about CVR, as well as lower adherence to preventive practices [1]. In the study by Boo et al., conducted with 150 patients with RA, participants with lower education had a 35% lower cardiovascular knowledge score (mean 11.3 vs. 17.4 points; $p<0.001$) and a 28% lower risk perception when compared to patients with higher education [1]. In addition, only 22% of individuals with low education correctly recognized

their risk factors, in contrast to 54% among those with Higher education level, a difference that directly impacts the adoption of preventive measures.

In the context of RA, characterized by persistent systemic inflammation and increased atherosclerotic risk, this vulnerability tends to intensify [2-3] A review by Dijkshoorn et al. (2022) demonstrated that the cardiovascular risk in RA patients is 50% to 70% higher than in the general population, and even higher in groups with socioeconomic disadvantages, including lower education levels [2]. In parallel, the multicenter cohort of Crowson et al. revealed that sociodemographic factors, including reduced education, are associated with a significant increase in the risk of major cardiovascular events (HR 1.46, 95% CI: 1.12–1.90), regardless of inflammatory activity or the use of disease-modifying therapies [3].

It is also plausible that differences in lifestyle reinforce this relationship. Patients with lower levels of education have a higher prevalence of harmful habits, such as smoking (up to 20% to 30% more frequent) and less regular physical activity, as well as less engagement in self-care measures, such as blood pressure control and adherence to treatment. These factors, added to the greater intrinsic inflammatory risk of RA, create a scenario in which social and clinical determinants synergistically combine, increasing cardiovascular risk and worsening prognoses.

Regarding disease activity, the pattern identified in this study corroborates the literature that points to social determinants as components in the management and prognosis of RA. In the COMORA study, which evaluated more than 4,000 patients with RA in 17 countries, Putrik et al. Studies showed that individuals with lower levels of education have significantly higher DAS28 scores and a lower probability of achieving clinical remission, even after adjusting for age, sex,

comorbidities, and type of healthcare system [4]. Patients belonging to the lowest educational stratum have a higher risk of remaining with moderate/high disease activity compared to those with higher educational levels, demonstrating that social inequalities persist even in similar therapeutic contexts.

Similar results were observed in longitudinal cohorts. In the study by Movahedi et al., involving more than 14.000 patients in real-life follow-up, it was observed that individuals with lower levels of education exhibited a greater probability of unfavorable inflammatory trajectories for up to 5 years, with a 30% to 40% higher risk of remaining in moderate/high activity profiles [5]. These findings suggest that lower health literacy may influence inflammatory control.

On the other hand, there are investigations that do not identify a direct association between education and inflammatory activity, as reported in the Swedish cohort analyzed by Jiang et al [12]. In this context, it is possible that the homogeneity of access to care, solid public health policies, and structured health education programs mitigate the impact of socioeconomic inequalities. Thus, differences between health systems - especially between high-income countries and regions with severe social disparities - may modulate this effect.

In the present study, conducted in a region characterized by socioeconomic inequalities and relevant variations in access to health information, the influence of education on inflammatory activity proved to be more evident, corroborating the literature that points to social determinants as components in the management and prognosis of RA.

Another relevant point in this study was the longer disease duration observed among individuals classified with moderate/high activity. Although the cross-sectional design prevents

causal inferences, greater chronicity may indicate a cumulative inflammatory process with prolonged periods of suboptimal control or even difficulties in accessing timely treatment in the early stages of RA. These factors are consistent with evidence from longitudinal cohorts. In the study by Movahedi et al., patients with longer disease progression were more likely to remain on unfavorable inflammatory trajectories, with a 30% to 40% higher risk of maintaining moderate/high levels of activity for up to five years of follow-up [5]. Thus, the combination of longer disease duration and socio-educational barriers suggests a cumulative and persistent impact of these inequalities on the clinical evolution of RA, reinforcing the need for interventions targeted at more vulnerable groups. Another relevant point is that variables such as age, sex, and ethnicity did not show significant differences between the groups. This suggests that education level may function as an independent marker of clinical vulnerability.

Some limitations should be considered. The cross-sectional design prevents inferring causality, and the small sample size, predominantly female and from a single center, limits generalizability. Furthermore, the absence of socioeconomic variables such as income, occupation, and health literacy measurements restricts a broader understanding of the impact of social determinants. The use of SCORE/mSCORE scores, developed for European populations, may also not fully reflect CVR in Latin American contexts.

5. Conclusion

Lower levels of education were associated with higher CVR and greater disease activity in patients with RA. These findings reinforce the role of education as a relevant social determinant

of health in RA and suggest the need for educational and care strategies adapted to vulnerable groups, aiming to optimize inflammation control and reduce unfavorable cardiovascular outcomes.

6. List of abbreviations

- RA - Rheumatoid arthritis
- CVR - Cardiovascular Risk
- ICF - Informed Consent Form
- ACR/EULAR - American College of Rheumatology/European League Against Rheumatism
- IBGE - Brazilian Institute of Geography and Statistics
- SCORE - Systematic Coronary Risk Evaluation
- mSCORE - Modified Score

7. Declarations

Ethics approval and consent to participate

All participants signed the Informed Consent Form (ICF), and the study protocol was conducted in accordance with the principles of the Declaration of Helsinki. The research was approved by the Research Ethics Committee of the Federal University of Ceará (CEP/UFC/PROPESQ), under number 110729/2020.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article. On reasonable request, the datasets used and/or analysed during the current study are available from the corresponding author

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

CAN: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

MMB: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

NXC: Data curation.

TSEN: Writing – review & editing.

JGMB: Writing – review & editing.

GAS: Data curation.

VCMMF: Data curation.

PG: Writing – review & editing, Writing – original draft.

HVC: Writing – review & editing.

CEMR: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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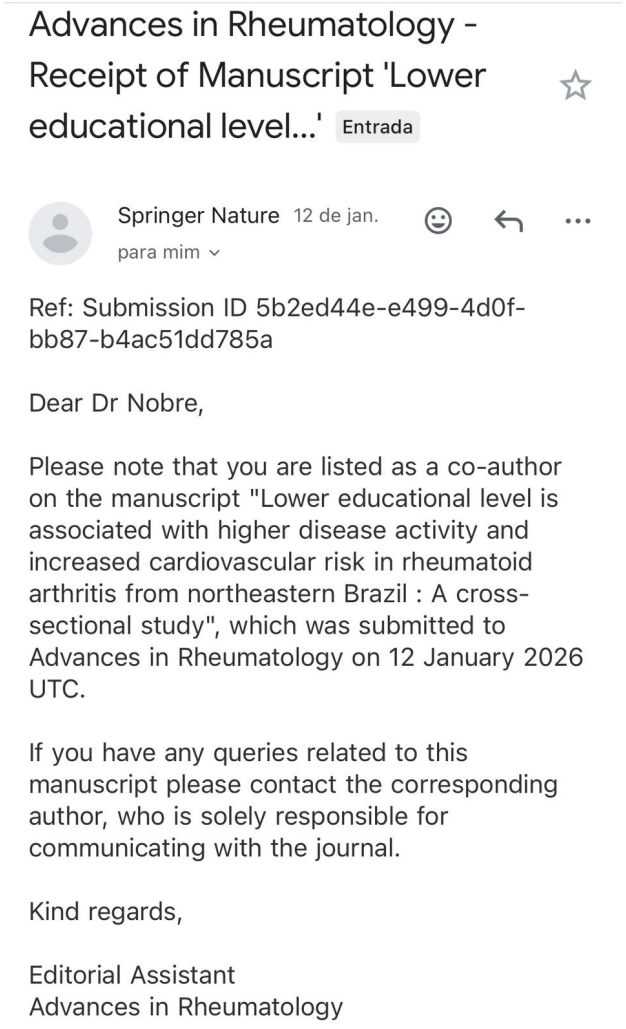

Advances in Rheumatology - Receipt of Manuscript 'Lower educational level...' Entrada

Springer Nature 12 de jan.
para mim

Ref: Submission ID 5b2ed44e-e499-4d0f-bb87-b4ac51dd785a

Dear Dr Nobre,

Please note that you are listed as a co-author on the manuscript "Lower educational level is associated with higher disease activity and increased cardiovascular risk in rheumatoid arthritis from northeastern Brazil : A cross-sectional study", which was submitted to Advances in Rheumatology on 12 January 2026 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Editorial Assistant
Advances in Rheumatology

ANEXO F
DEVOLUTIVA SOCIAL: CORDEL EDUCATIVO

ARTRITE REUMATOIDE:
A MULESTA DAS JUNTA QUE ADOECE O CORAÇÃO

FICHA TÉCNICA

TEXTO

Profa. Christiane A. Nobre
Curso de Medicina - UFC - Campus Sobral
Curso de Medicina - Uninta.
Doutoranda do Programa de Pós-Graduação em Medicina Translacional

Acadêmica Giovanna A. Sousa
Curso de Medicina - UFC - Campus Sobral

Acadêmico João Gabriel M. Brayner
Curso de Medicina - UFC - Campus Sobral

Acadêmica Natacha X. Cavalcante
Curso de Medicina - UFC - Campus Sobral

Acadêmica Thácilla S. E. Nascimento
Curso de Medicina - UFC - Campus Sobral

ORIENTAÇÃO

Prof. Carlos Ewerton M. Rodrigues
Curso de Medicina - UFC - Campus Fortaleza
Pós-graduação em Ciências Médicas da Unifor

Profa. Mirna M. B. Brayner
Curso de Medicina - UFC - Campus Sobral

REVISÃO

Acadêmico Aridênio D. da Silva
Curso de Medicina - UFC - Campus Sobral

CAPA, ILUSTRAÇÃO E DIAGRAMAÇÃO

Acadêmica Mainara de Souza e Lima
Curso de Design - UFC - Centro de Tecnologia

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
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Capa, ilustração e diagramação de Mainara de Souza e Lima
1. Artrite Reumatóide. 2. Cardiologia - Doença 3. Cordel I. Título

CDD 616.7227

Sobral, CE - 2023

DEDICATÓRIA

Esse Cordel ilustrado foi feito por muitas mãos e teve o esforço e contribuição de outras tantas que dele participaram, direta ou indiretamente. Dedicamos os nossos imensos agradecimentos aos pacientes e funcionários da Policlínica Bernardo Félix da Silva pela compreensão e disponibilidade.

Agradecemos também pelo apoio e conselho dos nossos colegas colaboradores dessa poesia: Dra. Luzia Keyne Sousa Carneiro Frota (Médica Cardiologista e Ecocardiografista do Hospital do Coração de Sobral), a Dra. Ticiana Parente Feijão (do Laboratório LACLISO), Profa. Maria Elisabete Amaral de Moraes (NPDM), Profa. Hellíada Vasconcelos Chaves (UFC - Campus Sobral), Profa. Roberta Jeane Bezerra Jorge (NPDM), Dra. Paola Tôrres (Hematologista e Cordelista) e a nossa

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Dedicamos também aos que muito pouco sabem como lidar com a dor, mas depositam a sua esperança, sono e qualidade de vida no trabalho feito pela ciência médica e que confiam em nós, profissionais, e no SUS.

OBRIGADO [A]!

SOBRE

Esta cordel foi criado para a orientação dos pacientes com artrite reumatoide acerca dos riscos cardiovasculares dessa doença, com o objetivo de informar sobre prevenção e cuidados, além de retornar à comunidade os investimentos feitos para pesquisa e inovação em saúde.

É fruto de um projeto intitulado: "Avaliação de Risco Cardiovascular em pacientes com Artrite Reumatóide: possível associação com atividade da doença, citocinas e a via NRF-2/HO-1", que foi financiado pelo Programa de Pesquisa para o SUS - PPSUS (Chamada 02/2020).

O PPSUS tem como objetivo apoiar as atividades de pesquisa científica, tecnológica e de

inovação relacionadas àt área da saúde, desen- volvidas em instituições de ensino superior e/ou de pesquisa no estado do Ceará.

Esse projeto teve como Instituição Executora a Universidade Federal do Ceará (Curso de Medicina, Campus Sobral - CE e Núcleo de Pesquisa e Desenvolvimento de Medicamentos - NPDM, Campus do Porangabuçu, Fortaleza - CE) e como Instituições Colaboradoras a Universidade de Fortaleza (UNIFOR, Fortaleza - CE), Hospital do Coração de Sobral - CE e Policlínica Bernardo Félix de Oliveira, Sobral - CE.

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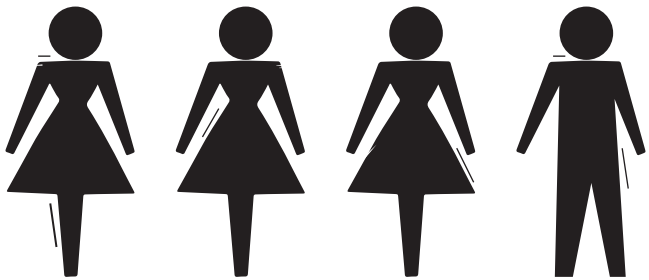
BÓ TRATÁ E PREVENIR! ····· P. 9

DE ONDE VEM ESSA MULESTA?

A famosa dor de junta
Inflama e não se aquieta
Pega três muié pra cada hômi
e dois santo em cada reza

Não mata, mas atormenta
e não tem essa de idade
Vai se aprumando nos vasos
Uma chaga de verdade!



E se sua mãe ou irmã tiver
se atente se a junta inchar
Então não fique bobeando
que pode ser familiar

Uma coisa os dotô sabem
o cigarro é bom tirar
Não faz bem pra mulesta
e a sua dor pode piorar

E COMO ELA É?

O que faz essa mulesta?
Você pode perguntar
Ela vai pegar mão e punho
Podendo o pé agarrar

Se na junta aparecer
dureza quando acordar
uma dor graúda e inchaço
O dotô é bom buscar

Ela morde dos dois lados
E além das mãos deformar
Ela busca o coração
Gosta é dele provar

Dela pode vir um AVC
E a sua boca entortar
Mas disso não tenha medo
O dotô pode ajudar


12
3
6
9
4

COMO O DOTÔ VAI SABER O QUE É?

Ao procurar ajuda
Diga como começou
Pro dotô deve dizer
Como dói e se inchou

Muito ele vai perguntar
Cansaço de esmorecer?
Você tem dor quando acorda?
E melhora ao mexer?

Conte como é a dor
Se ela na mão aparecer
Pra descobrir o que é
A gente tem que saber

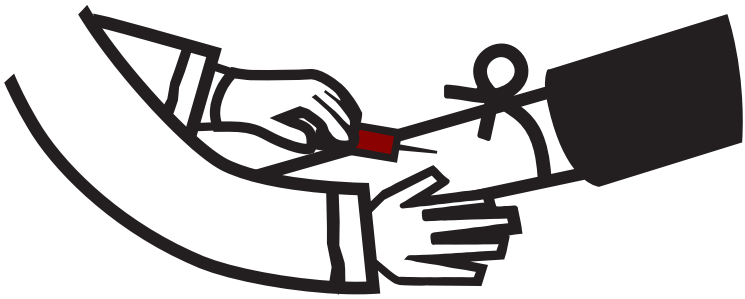
Descreva por onde dói
Se é de queimar ou arder
Se ela é pontuda ou não
É importante dizer


SOBRAL
PREFEITURA

O sangue vai querer ver
E buscar inflamação
Os nomes são diferentes
É uma perturbação

Um é fator reumatoide
Importante de se ver
Outro é um anti-CCP
Que não se deve esquecer

E depois de olhar tudo isso
Ele vai poder dizer
É artrite reumatoide
O que você deve ter




HEMOGRAMA

BÓ TRATÁ E PREVINIR!

Se mais cedo cê agir
Mais fácil é de tratar
Menor a chance de agravo
E a junta não vai entortar

Mas não há lugar pra tristeza
Porque existe solução
Agora tem de atentar
Pra essa nossa explicação

Isso não afeta só as junta
Então você fique atento
O coração também sofre
Siga bem o tratamento

O remédio da pressão
Você não deve esquecer
Pra ela não descontrolar
E o corpo não sofrer

Eu falo da prednisona
É uma amiga de respeito
Reduz o inchaço das junta
Um remédio que dá jeito

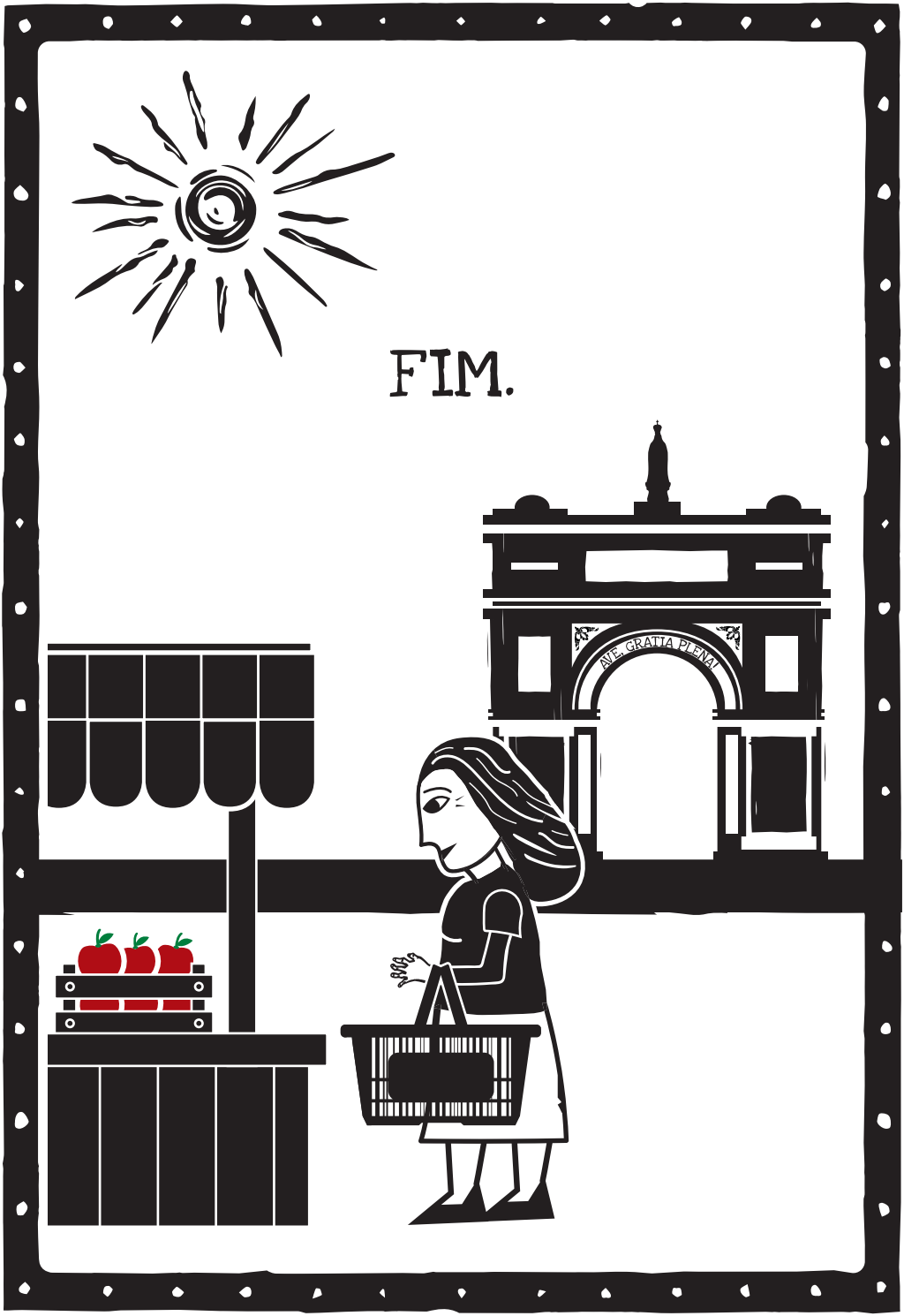
O metotrexato acode
Mas se ele não resolver
Não fique desesperado
Temos mais a te dizer

Tem de maneirar no sal
E mais salada incluir
Além de se exercitar
E do cigarro fugir

O açúcar diminuir
Pra diabetes baixar
Beber com moderação
E o colesterol tratar

As juntas e o coração
Tem que seguir esse esquema
Se a saúde for acordo
A artrite não é problema

E num se deixe abater
Na lida dessa mulesta
Ainda tem muita vida
Pra fazer muita festa


FIM.
AVE GRATIA PLENA

QUER SABER MAIS SOBRE A ARTRITE REUMATOIDE?

Aponte sua câmera do celular para o QR code abaixo: