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RELAÇÃO ENTRE NÍVEIS CIRCULANTES DE PROTEÍNA C-REATIVA E *STATUS* COGNITIVO EM PACIENTES ATENDIDOS NO AMBULATÓRIO DE CORONARIOPATIA DO HCPA-UFRGS

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Trabalho de Conclusão de Curso apresentado como parte dos requisitos para obtenção do grau de Biomédico pela Universidade Federal do Rio Grande do Sul.

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"Toda nossa ciência, comparada com a realidade, é primitiva e infantil – e, no entanto, é a coisa mais preciosa que temos."

Albert Einstein (1879 – 1955)

AGRADECIMENTOS E DEDICATÓRIA

Agradeço e dedico o presente trabalho à minha família, meu porto seguro, sempre e em qualquer lugar:

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Por fim, parafraseando Sir Isaac Newton: *"Se enxerguei mais longe foi porque estava sobre os ombros de gigantes".*

Muito obrigada!

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LISTA DE ABREVIATURAS

AVC – Acidente Vascular Cerebral

CC – Comprometimento Cognitivo

DA – Doença de Alzheimer

IL-1 – Interlecina-1

IL-6 – Interlecina-6

LDL - *Low Density Lipoprotein*

MEEM – Mini Exame do Estado Mental

mg/L- Miligrama por Litro

PCR – Proteína C-Reativa

RESUMO

Níveis séricos elevados de proteína C-reativa (PCR) vem sendo associados à leucoaraiose cerebral em indivíduos com idade avançada. Por sua vez, diversos estudos indicam que a leucoaraiose está associada a um maior risco de comprometimento cognitivo (CC). No entanto, desconhece-se quanto do efeito da PCR sobre a cognição é mediado pela leucoaraiose. Assim, este trabalho investiga a relação entre os níveis séricos de PCR, a presença de leucoaraiose e de CC em uma população de 135 indivíduos coronariopatas com mais de 50 anos. Este estudo se baseia na mensuração de níveis séricos de PCR através de análise turbidimétrica, na presença de leucoaraiose detectada através de tomografia computadorizada de encéfalo e no desempenho cognitivo avaliado através do mini exame do estado mental (MEEM), sendo que todas as análises foram ajustadas para idade, sexo e escolaridade. A aplicação dos recursos acima descritos mostrou que os níveis de PCR explicaram 7,18% (p: 0.002) da variância do MEEM, sendo que o ajuste para a presença de leucoaraiose pouco modificou esta variância (5,98%; p: 0.005), indicando que apenas uma pequena parcela da influência da PCR sobre a cognição foi mediada via leucoaraiose. Quarenta indivíduos (29,6%) apresentaram níveis de PCR $\geq$ 5,0 e 34 sujeitos (25,2%) foram considerados portadores de CC. Portadores de níveis de PCR $\geq$ 5,0 tiveram uma chance 2,9 (CI: 1,26–6,44) vezes maior de apresentarem CC (p: 0.012). Por outro lado, os níveis de PCR entre portadores de CC foram significativamente maiores (5,82 ± 3,21) do que entre controles (4,33 ± 2,02; p: 0.002). De modo geral, os resultados obtidos agregam conhecimento ao estudo da PCR e contribuem para o entendimento da patologia envolvida no processo demencial.

Palavras-Chave: Proteína C-Reativa, Comprometimento Cognitivo, Leucoaraiose.

1. INTRODUÇÃO COMPREENSIVA

1.1 Proteína C-reativa - Estrutura

A denominada proteína C-reativa (PCR) foi descrita pela primeira vez no ano de 1930, segundo estudos de Tillett e Francis, na Universidade de Rockefeller - NY, os quais isolaram a fração "C" de pacientes com pneumonia pneumocócica (Ridker, 2009). Dez anos após Avery e Mccarty descreveram a PCR como sendo um reagente de fase aguda que aumenta no soro de pacientes com estímulos inflamatórios. Mais tarde, outros pesquisadores demonstraram o aumento da PCR após infarto agudo do miocárdio, relacionando este biomarcador a aterotrombose (Koenig, Sund *et al.*, 1999). E no final da década de 70, a PCR foi caracterizada como uma proteína plasmática, sintetizada pelo fígado e altamente conservada filogeneticamente (Volanakis e Wirtz, 1979).

A PCR é membro de uma família de proteínas chamada Pentraxina, possui arranjo pentamérico cíclico, com cinco protômeros idênticos e não-glicosilados, de aproximadamente 23 kDa cada. Possui sítios de ligação dependentes de cálcio (**Figura 1**) e para partículas de colesterol de baixa densidade (LDL) (Pepys e Hirschfield, 2003).

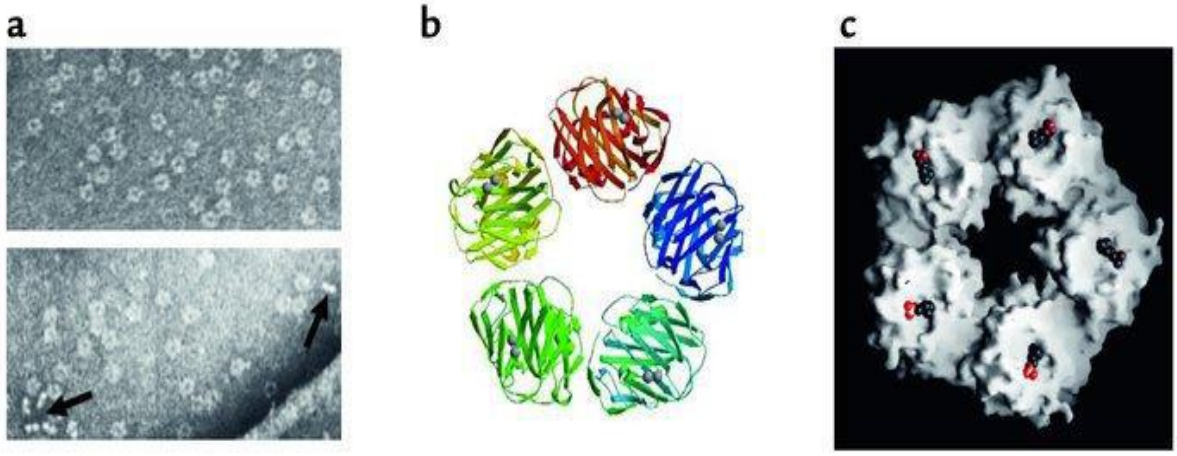

Figura 1. Estrutura molecular e morfologia da PCR humana. (a) Micrografia eletrônica mostrando o disco típico pentamérico (setas). (b) Diagrama da estrutura cristalina, mostrando a alça de lectina e os

dois átomos de cálcio (esferas) no sítio de ligação de cada protômero. (c) Modelo com preenchimento espacial da molécula de PCR mostrando uma única molécula de fosfocolina localizada no sítio de ligação de cada protômero. Fonte: (Pepys e Hirschfield, 2003) p. 1807.

A PCR é produzida nos hepatócitos por meio do estímulo de citocinas inflamatórias como a interleucina-6 (IL-6), a interleucina-1 (IL-1) e o fator de necrose tumoral. Sua meia vida plasmática é de 19 horas e sua concentração plasmática é determinada pela taxa de síntese (Hutchinson, Noble *et al.*, 1994). Em indivíduos saudáveis a PCR é uma proteína traço na circulação sanguínea, com aproximadamente 1 mg/L. No entanto, como membro prototípico da família de proteínas de fase aguda, a sua concentração plasmática pode ter um aumento de até mil vezes ou mais em casos de injúrias, inflamações ou infecções. Essas propriedades fazem com que a PCR plasmática seja muito útil para a investigação diagnóstica de doenças inflamatórias e infecciosas (Di Napoli, Schwaninger *et al.*, 2005).

1.2 Proteína C-reativa - Inflamação e Aterogênese

O papel fisiológico da PCR, ainda não está completamente elucidado, no entanto, sabe-se que mais do que simplesmente um marcador inflamatório, ela possui um papel pró-aterogênico e está diretamente envolvida na aterogênese (Di Napoli, Schwaninger *et al.*, 2005). Estudos indicam que a elevação modesta da concentração da PCR, em indivíduos aparentemente saudáveis é um forte preditor de eventos cardiovasculares futuros. O aumento plasmático da PCR também está relacionado com o aumento de risco de eventos cerebrovasculares e de eventos cardiovasculares fatais e não fatais em pacientes com AVC (acidente vascular cerebral) isquêmico (Emsley, Smith *et al.*, 2003). A PCR é aprovada pela Food and Drug Administration como preditora de aumento de risco cardiovascular (Kuo e Lipsitz, 2004).

Essa proteína atua em diversos mecanismos contribuidores para o processo aterosclerótico, como, por exemplo: na ativação da via clássica do sistema complemento, na quimiotaxia de monócitos, na ativação de citocinas, na opsonisação

e mediação da captação de LDL pelos macrófagos facilitando a formação de células espumosas, na ativação da migração e da proliferação de células vasculares musculares lisas para o local da lesão endotelial, na expressão de moléculas de adesão como a E-selectina, na atenuação da produção de óxido nítrico fazendo com que ocorra vasoconstricção, na estimulação da adesão leucocitária e da agregação plaquetária, causando danos como oxidação, trombose e inflamação vascular, acelerando dessa forma a progressão da aterosclerose (Kuo e Lipsitz, 2004; Di Napoli, Schwaninger *et al.*, 2005).

Avanços na biologia vascular têm mostrado que a inflamação possui um papel importante no desenvolvimento da doença aterosclerótica. O acidente vascular cerebral é a segunda causa de morte em todo o mundo, excedida apenas pela doença cardíaca (Murray e Lopez, 1997). As formas de AVC, cuja principal causa é a aterotrombose, podem ser anóxico-isquêmicas ou hemorrágicas, os subtipos isquêmicos são lacunares, ateroscleróticos e embólicos, e os hemorrágicos são intraparenquimatosos e subaracnóideos (Chaves, 2000). Extensivos estudos na PCR têm demonstrado que a sua mensuração prediz riscos cardiovasculares não refletidos pelos fatores de risco tradicionais, adicionando informação prognóstica à avaliação destes e predizendo riscos cardiovasculares no longo prazo em indivíduos sem evidências primárias de cardiopatias (Ridker, 2004). A relação entre a concentração basal da PCR do paciente e risco cerebrovascular futuro tem sido consistente e provado ser independente de idade, sexo, tabagismo, colesterol, pressão arterial e diabetes, os maiores riscos avaliados na prática clínica diária (Ridker, 2001).

Na fase aguda do AVC, a inflamação contribui para os danos cerebrais iniciados pela isquemia (Del Zoppo e Hallenbeck, 2000). A cascata inflamatória é mediada por pelo aumento da concentração de citocinas locais, moléculas de adesão, proteínas de fase aguda, macrófagos e lecucócitos e a força dessa resposta está relacionada aos desfechos clínicos iniciais e tardios (Chamorro, 2004). Dessa forma, a PCR aumentada no plasma tem demonstrado ser colaboradora do dano cerebral, induzido por isquemia, pois contribui com o aumento de infartos cerebrais. Além do mais, alguns estudos têm demonstrado positiva associação entre valores de PCR e severidade do AVC e inabilidade neurológica (Smith, Emsley *et al.*, 2004).

1.3 Proteína C-reativa - Declínio Cognitivo

Um declínio nas funções cognitivas é um processo inevitável que acontece normalmente de acordo com o envelhecimento, mas a gradação da deterioração varia entre as pessoas saudáveis idosas. Esse processo ainda não é bem entendido, mas já se sabe que alguns fatores podem influenciar ou conferir risco à performance cognitiva como: a idade, o nível educacional, a constância de atividades físicas, o gênero, a hipertensão, entre outros. Dessa forma, o conhecimento de marcadores bioquímicos correlacionados com o envelhecimento cognitivo normal pode ser útil para o entendimento tanto do envelhecimento cognitivo normal quanto do patológico (Teunissen, Van Boxtel *et al.*, 2003), apesar de não haver um único marcador sorológico conclusivo estabelecido até os dias atuais.

Mecanismos inflamatórios têm sido sugeridos estarem envolvidos com prejuízo cognitivo e demência (Mcgeer e Mcgeer, 2001). Além disso, a inflamação está ligada à patogênese da doença cardiovascular, obesidade e resistência à insulina, as quais, por sua vez, também estão associadas ao risco de declínio cognitivo (Pearson, Mensah *et al.*, 2003). A associação desses marcadores, incluindo a PCR, para alterações nas funções cognitivas tem sido base de diversos estudos epidemiológicos (Yaffe, Lindquist *et al.*, 2003), no entanto, os resultados ainda não são consistentes. Exames *post mortem* realizados em cérebros de pacientes com doença de Alzheimer (DA) revelaram presença abundante de mediadores inflamatórios, entre os quais está a PCR, que tem sido detectada nas placas senis e nos emaranhados neurofibrilares cerebrais (Duong, Nikolaeva *et al.*, 1997).

A hipótese de que a inflamação está relacionada ao prejuízo cognitivo, apesar de ser relativamente nova, é bastante consistente. A PCR tem mostrado ter neurotoxicidade direta *in vitro.* Como é um importante componente do sistema imune inato, a PCR atua como uma opsonina e ativa a via clássica do sistema complemento, age na resposta aguda como componente pró-inflamatório e adquire potencial destrutivo, se a inflamação persistir. Além da resposta pró-inflamatória que causa danos neuronais diretamente, concentrações aumentadas de PCR, atuando como fator de risco cardiovascular ou causando aterosclerose cerebral, também pode resultar em macroangiopatias ou microangiopatias cerebrais. Ambos os tipos de lesão

rompem a integridade dos circuitos frontais-subcorticais e são responsáveis pelo desenvolvimento de declínio cognitivo, demência ou desordens depressivas (Kuo e Lipsitz, 2004).

O aumento dos níveis plasmáticos de proteínas pró-inflamatórias antes do início dos sinais clínicos de casos demenciais sugere que a inflamação periférica está envolvida no processo patológico que culmina em demência (Engelhart, Geerlings *et al.*, 2004). Primeiro, há associação entre o uso de anti-inflamatórios e a diminuição do risco de demência, pois além de terem efeitos cerebrais, agem suprimindo o sistema imune periférico (In T' Veld, Ruitenberg *et al.*, 2001). Segundo, proteínas periféricas são capazes de atravessar a barreira hematoencefálica e aumentar a permeabilidade da mesma, pelo fato da concentração de proteínas inflamatórias no plasma estarem aumentadas, além de amplificar a formação de depósitos cerebrais beta-amilóides. Terceiro, aterosclerose, a idéia de que essa patologia seja a ligação entre inflamação periférica e demência é suportada pela observação de associações relativamente fortes entre a PCR e a demência vascular (Engelhart, Geerlings *et al.*, 2004).

Por outro lado, elevadas concentrações de proteínas pró-inflamatórias no plasma podem ser conseqüência do processo patofisiológico demencial. Inflamação periférica pode resultar de deposição cerebral de beta-amilóide, que induz a produção local de proteínas inflamatórias, como IL-1 e IL-6 (Akiyama, Barger *et al.*, 2000). Estas citocinas produzidas no cérebro induziriam então o aumento dos níveis de proteínas pró-inflamatórias periféricas, por atravessarem a barreira hematoencefálica e também por estimularem a proliferação de outras proteínas, como foi demonstrado em modelos animais (De Simoni, De Luigi *et al.*, 1993). Logo, se a ativação do sistema imune periférico é a causa e a consequência do processo demencial, a cascata patológica irá ocorrer. Cascata essa que inclui a formação de depósito beta-amilóide, que leva à inflamação local cerebral, resultando na ativação do sistema imune periférico, que então leva ao aumento da deposição de beta-amilóide (Engelhart, Geerlings *et al.*, 2004).

Estudos com a PCR durante o envelhecimento e em pacientes demenciados são relativamente escassos e contraditórios (Teunissen, Van Boxtel *et al.*, 2003). Entretanto, a grande maioria relaciona a PCR com risco de demência, principalmente nos subtipos de demência vascular (até 3 vezes mais que os outros subtipos) e de doença Alzheimer, sendo que uma parte da associação entre PCR e demência

vascular é mediada por aterosclerose subclínica. Também se considera o fato de a PCR aumenta a probabilidade de todas as formas de vasculopatias, particularmente a doença dos pequenos vasos cerebrais, que morfologicamente é correlacionada com demência vascular, porém tem mostrado também ter um papel na doença de Alzheimer (Schmidt, Schmidt *et al.*, 2002).

1.4 Proteína C-reativa - Leucoaraiose

A doença cerebral da substância branca, também conhecida como doença cerebral de pequenos vasos ou ainda leucoaraiose, é frequentemente reportada em exames de imagem, predominantemente em pacientes idosos. É considerada uma das mais comuns desordens degenerativas de vasos em cérebros humanos senescentes, cuja fisiopatologia ainda não está elucidada, porém a disfunção endotelial é considerada a primeira etapa do desenvolvimento da isquemia dos pequenos vasos. Leucoaraiose pode ser uma consequência de isquemia crônica ou de disrupção da barreira hematoencefálica com vazamento de substâncias potencialmente tóxicas no cérebro (componentes plasmáticos que normalmente não conseguiriam ultrapassar a barreira hematoencefálica), ou pela combinação desses dois mecanismos. Essas áreas de dano aparecem como lesões na substância branca em exames de imagem e cortes histológicos (Grueter e Schulz, 2012).

As lesões são constatadas quando afetam significante parte do cérebro, com o aumento da idade e em indivíduos com hipertensão, e estão relacionadas com um risco aumentado de comprometimento cognitivo, AVC e morte (Grueter e Schulz, 2012). Estudos indicam que processos inflamatórios sistêmicos, que incluem altos níveis de PCR, provavelmente estão relacionados à patogênese da doença cerebral dos pequenos vasos, em particular, no desenvolvimento de lesões na substância branca cerebral e infartos lacunares (Van Dijk, Prins *et al.*, 2005). O aumento da PCR na microvasculatura do encéfalo pode indicar progressão arteriolosclerótica, promovendo o estreitamento do lúmen vascular e insuficiência da autorregulação cerebral, resultando em lesão isquêmica da substância branca e da substância cinzenta subcortical cerebral (Van Dijk, Prins *et al.*, 2005; Umemura, Kawamura *et al.*, 2011).

Contudo, estudos indicam que provavelmente os níveis de PCR são relacionamos às lesões na substância branca e aos infartos lacunares por via de

diferentes mecanismos (Van Dijk, Prins *et al.*, 2005). Infartos lacunares ocorrem devido à oclusão de um vaso perfurante e são outras manifestações da patologia dos pequenos vasos, apesar da sua fisiopatologia diferir daquela da leucoaraiose (Grueter e Schulz, 2012). Assim, foi demonstrado que a mensuração da PCR poderia ter melhor valor como marcador preditivo de eventos vasculares do que a progressão da doença de pequenos vasos, embora diferenças raciais possam influenciar na associação distinta dos níveis de PCR com a presença e a progressão da leucoaraiose (Umemura, Kawamura *et al.*, 2011).

2. ARTIGO CIENTÍFICO*

Title

INVERSE CORRELATION BETWEEN C-REACTIVE PROTEIN LEVELS AND COGNITIVE FUNCTION IN CORONARY PATIENTS IS UNRELATED TO THE DEGREE OF LEUKOARAIOSIS: A CASE CONTROL STUDY

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Abstract

Background: Elevated serum levels of C-reactive protein (CRP) have been associated with leukoaraiosis in elderly brain. However, several studies indicate that leukoaraiosis is associated with an increased risk of cognitive impairment. Is unknown how the effect of CRP on cognition is mediated by leukoaraiosis. The purpose of this study is to assess the relationship between serum levels of CRP, the presence of leukoaraiosis and cognitive impairment in a population of coronary patients over 50 years old.

Methods: 135 coronary patients with more than 50 years old were assessed. Serum levels of CRP were measured by immunoturbidimetric analysis, the presence or absence of leukoaraiosis was detected by computational tomography scan (CT scan) and the cognitive performance was evaluated by Mini Mental State Examination (MMSE). Patients who scored less than minus two (≤ - 2) points the expected MMSE score were considered as cognitively impaired for the purpose of this study. It was used a multivariate logistic regression analysis. All analyzes were adjusted for age, sex and educational level.

Results: CRP levels explained 7.18% (p: 0.002) the variance of the MMSE. The adjustment for the presence of leukoaraiosis little changed this variance (5.98%, p: 0.005), indicating that only a small portion of the CRP influence on cognition was mediated via leukoaraiosis. Patients with CRP levels ≥ 5.0 had 2.9 (CI: 1.26 - 6.44) times more chance to present cognitive impairment (p: 0.012). Moreover, CRP levels between patients with cognitive impairment were significantly higher (5.82 ± 3.21) than among controls (4.33 ± 2.02, p: 0.002).

Conclusion: We found that elevated serum levels of CRP were associated with increased risk of cognitive impairment in elderly and it was not mediated by presence of leukoaraiosis.

Keywords: C-reactive protein, Cognitive Impairment, Leukoaraiosis.

Background

Inflammation has been increasingly recognized as component in cerebrovascular [1] and neurodegenerative diseases [2, 3]. In addition, biological aging of the brain is partly attributable to aging of the cerebrovascular circulation and the effects of vascular changes on the brain [4]. Inflammation has been linked to the pathogenesis of cardiovascular disease, obesity and insulin resistance, which are so related to cognitive impairment [5]. The hypothesis that inflammation is related to cognitive impairment, although new, is consistent [2]. Therefore, few studies evaluated that circulating inflammatory proteins are associated with increased risk of dementia [6], cognitive impairment [7] and cerebral white matter lesions (WML), common referred to leukoaraiosis [8-10].

CRP, composed of five 23 kDa subunits, is a hepatically derived pentraxin that has important role in the human immune system [11]. That protein is a sensitive nonspecific marker of systemic low-grade inflammation [5] and increased serum concentrations of CRP has been associated with impaired cognition, stroke and depression [2, 6, 8]. Beyond pro-inflammatory response, that causes neuronal damage directly, increased concentrations of CRP acting as cardiovascular risk factor - approved predictor by Food and Drug Administration - or causing brain atherosclerosis, can result in cerebral macro or microangiopathies. Both lesions can disrupt the integrity of frontal-subcortical circuits and are responsible for the development of cognitive impairment, dementia or depressive disorders [12]. There are some evidences that elevated serum CRP levels may be a useful biomarker to identify individuals at an increased risk for cognitive impairment [7].

With the availability of improved brain imaging techniques, the high prevalence and clinical importance of cerebral small vessel disease have been increasingly recognized in recent years. WML are often found incidentally on image exams, predominantly in elderly people [13]. These WML reflect multiple physiologic and pathologic changes, including ischemic lesions, loss and deformation of myelin sheath, damage to the walls of small vessels, gliosis, micro-hemorrhages, and breaches of the cerebro-spinal fluid brain barrier [14, 15]. All these damages may lead to an increase in the clinical consequences namely: cognitive

impairment, decreased mobility and increased stroke risk [16]. Vascular risk, hypertension and inflammation, which increase with age, may contribute to white matter deterioration and proliferation of WML, nonetheless, much white matter volume variance remains unexplained [10, 17]. Growing evidence shows that it is cause of cognitive impairment and functional loss in the elderly population.

Whether inflammatory processes, excluded from their involvement in large-vessel disease, are implicated in the pathogenesis of cerebral small vessel disease remains unclear. Blood markers of vascular dysfunction reflect the underlying pathology and provide an independent measure of pathology based on biology. Some studies showed that systemic inflammatory processes, which include high levels of CRP, are related to the pathogenesis of cerebral small vessel disease at the development of cerebral WML and lacunar infarcts [9, 10, 17]. The increase of CRP at the microvasculature of the brain may act in synergy to promote arteriolosclerotic progression by different mechanisms like: activation of classic complement system, mediation of low density lipoprotein uptake by macrophages, promotion of foam-cell formation, endothelial dysfunction, low nitric-oxide production, stimulation monotype recruitment and vascular smooth muscle proliferation and migration [2]. All these processes cause narrowing of vascular lumen and failure of cerebral self-regulation, resulting in cerebral microangiopathies that may interrupt the integrity of the frontal-subcortical circuit and thus result in cognitive impairment [2, 9, 18]. The association of blood markers of vascular dysfunction with subclinical brain changes is still unclear.

Considering that high serum levels of CRP have been associated with leukoaraiosis and that several studies indicated that leukoaraiosis is associated with cognitive impairment, the aim of this study was to examine how much is the effect of CRP on cognition and if it was mediated by leukoaraiosis or not in a sample of coronary patients. We hypothesize that increased levels of the CRP biomarker would be related to brain leukoaraiosis, as evaluated by CT, and cognitive impairment.

Methods

Study Design

Case-control study.

Study Population and sample

The study population comprised outpatients attended at the cardiology ambulatory of the Porto Alegre Clinics Hospital of the Federal University of Rio Grande do Sul, located in the southernmost state of Brazil.

The total sample was composed by 149 coronary patients, who were enrolled at the baseline examination and were assessed at the referred ambulatory. Subjects who obtained MMSE < 15 or CRP ≥ 13 were excluded (n= 14), totalizing 135 patients for the final sample. Patients with cognitive impairment were considered the cases (n= 34) and those without cognitive impairment were the controls (n= 101).

Inclusion and Exclusion Criteria

Patients with cognitive impairment were included in this study. All patients were 50 years or older.

People who met the following criteria: dementia, stroke, Parkinson's disease or other neurological diseases that potentially cause cognitive impairment, were excluded at the baseline.

Possible confounders and mediators

All analyses were adjusted for age, gender, and educational level. These variables were chosen for the analyses based on their known association with cognition as well as the association with CRP in the present study. Cognitive impairment increases exponentially with aging, and aging reflects many biologic

changes, including increase of inflammatory processes. People that obtained MMSE < 15 or CRP ≥ 13 were considered outliers to lower the probability that the associations could be attributed to subjects with apparent dementia.

Variables and data collection

Sociodemographic variables

A questionnaire was applied in order to assess the following sociodemographic data: income, age, sex and years of schooling.

CRP quantification

Patients were submitted to fasting venipuncture and the level of inflammatory protein CRP was assessed in serum samples, which were stored at -80°C until analyses. CRP concentration was determined with immunoturbidimetric assay (Siemens Healthcare Diagnostics Inc.) performed on a ADVIA® 1800 Chemistry System (Siemens) that is based on the agglutination of latex particles when the CRP in the sample is coated with anti-human CRP antibodies. The degree of agglutination is proportional to the concentration of CRP in the sample and can be measured by turbidimetry (517 nm). This process is based on optical detection of very small particles suspended in liquid medium. When anti-human CRP antibody and sample are mixed they form immunocomplexes. Dilution acquires turbidity, which is proportional to the amount of antigen. The technicians were blinded to the clinical status of study participants and the samples.

Diagnosis of Leukoaraiosis by computed tomography scans (CT scan)

From each participant brain CT scan was performed using a CT imaging scanning (Philips Medical Systems). Trained neurologists and radiologists, who were blinded to patients, laboratorial and clinical data, assessed the existence, location and extension of the leukoaraiosis lesions on CT scan. Images were analyzed by using a semiquantitative rating scale devised by Fazekas *et al* [19]. This method scored

subcortical and deep WML on a four-point scale of increasing severity as follows: 0: No lesions; 1: Isolated hypodensities; 2: Initially confluent hypodensities; 3. Difuse and extense hypodensities.

Assessment of cognitive function

To assess the cognitive function, the Mini Mental State Examination (MMSE) [20] was performed to screen all subjects, translated and validated for Brazilian population [21]. The MMSE is a tool that can be used to systematically and thoroughly assess mental status. It is a scale that tests seven areas of cognitive function: tempo-spacial orientation, registration, attention/calculation, recall, language and visual constructive capacity. The test was designed as a screening instrument for cognitive impairment and dementia and is widely used in both clinical practice and scientific studies. The score range from 0 to 30, with a higher score indicating better performance. A score of 23 or lower is indicative of cognitive impairment. Patients who scored less than minus two ($\leq$ - 2) points the expected MMSE score, corresponding one standard deviation, were considered as cognitively impaired for the purpose of this study.

Assessment of geriatric depression

To assess depression symptoms, the Geriatric Depression Scale (GDS), developed by Yesavage, et al. [22], was applied to the subjects. The GDS was translated and validated for Brazilian population [23]. It was used the short form GDS consisting of 15 questions. Questions from the long form GDS which had the highest correlation with depressive symptoms in validation studies were selected for the short version. The short form is more easily used to patients who have short attention spans and/or feel easily fatigued. Presence of significant depressive symptomatology was considered for all subjects who scored $\geq$ 6 points in the scale.

Statistical Analysis

We examined the association between CRP and leukoaraiosis lesions by multivariate logistic regression analysis. The level of inflammatory protein was entered into the model through a linear term, in which the regression coefficient was expressed per standard deviation increase. A linear regression equation predicted the expected score of each person based on age, sex, and years of schooling (all $p < 0.05$). Student T-test was used for independent variables. Analysis of covariance (ANCOVA) was used for adjusted means. Chi-square was used for categorical variables. All analyses were performed using SPSS (Statistical Package for Social Sciences), version 17.0.

Ethical Aspects

This study was approved by the Ethics Committee in Research of Porto Alegre Clinics Hospital (HCPA), project number 09-349. All participants provided informed consent.

Results

The sociodemographic and clinical characteristics of the sample are summarized at **Table 1** for numeric variables. The **Table 2** shows characteristics for categorical variables. The mean age of the participants was 66,6 ± 8,7 years old and majority were men (60%).

Foreseen MMSE was performed using the equation MMSE: 27.086 – {[age x (-0.06)] + [education level x 1.594] + [gender x 0.876]} and presented a variance of 35,6%. Who obtained the difference between MMSE evaluated and foreseen ≤ -2 (ΔMMSE ≤ -2) was considered with cognitive impairment, what means one standard deviation on this study. Thus, we analyzed 34 individuals with cognitive impairment (25,2%) and 101 individuals without cognitive impairment (controls).

Table 1. Student T test for numeric variables.

	Cognitive Impairment (n= 34) Mean ±SD	Controls (n=101) Mean ±SD	P value
Age	66,59 ± 8,1	66,56 ± 8,9	0.050
Years of Schooling	2,06 ± 1,3	2,39 ± 1,2	0.047
GDS	4,68 ± 3,4	3,87 ± 3,1	0.034
Income	4,97 ± 1,3	4,95 ± 1,3	0.091
CRP (mg/L)	5,81 ± 3,2	4,33 ± 2,02	0.001
Leukoaraiosis Score	0,88 ± 0,69	0,89 ± 0,88	0.003

GDS: Geriatric Depression Scale; CRP: C-reactive Protein.

Table 2. Chi-Square for categorical variables.

	Cognitive Impairment (n= 34) n (%)	Controls (n=101) n (%)	P value
SEX			
Male	19 (55,8%)	62 (61,4%)	0.057
Female	15 (44,2%)	39 (38,6%)	
GDS			
≤ 5	21 (61,7%)	68 (67,3%)	0.055
≥ 6	13 (38,3%)	33 (32,7%)	
CRP			
< 5.0 mg/L	18 (52,9%)	77 (76,2%)	0.001
≥ 5.0 mg/L	16 (47,1%)	24 (23,8%)	
Leukoaraiosis			
No	9 (26,4%)	40 (39,6%)	0.002
Yes	25 (73,6%)	61 (60,4%)	

GDS: Geriatric Depression Scale; CRP: C-reactive Protein.

At first, we analyzed the degree of leukoaraiosis with ΔMMSE and degree of leukoaraiosis with CRP. We found no significant association between these items as is showed on **Figure 1**. It suggests that the effects on the relationship between CRP and cognitive impairment are not completely mediated via leukoaraiosis

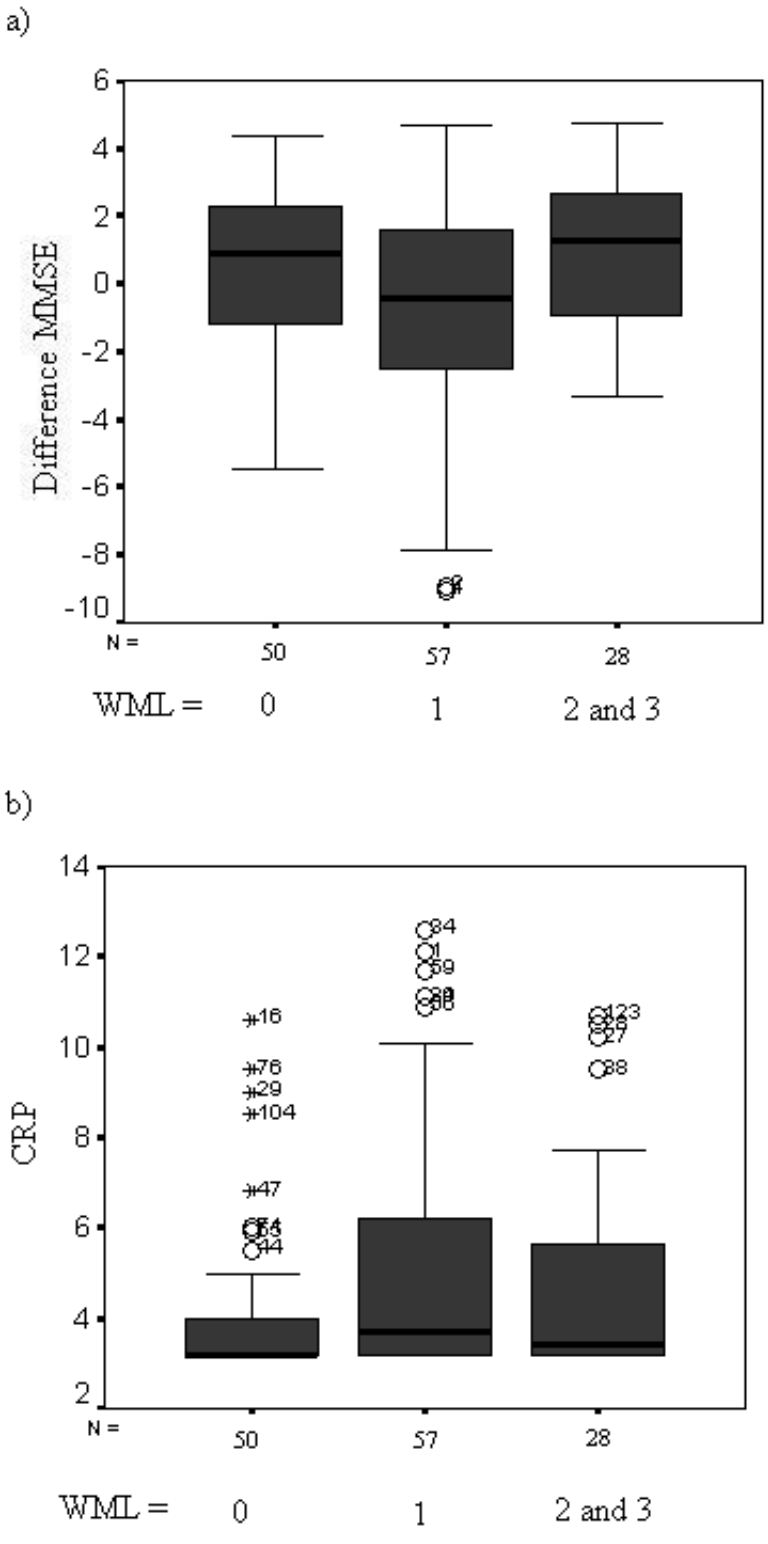

Figure 1. ANOVA between groups using a linear term a) p: 0.470 b) p: 0.200.

CRP: C-reactive protein; MMSE: Mini Mental State Examination; WML: White Matter Lesions; 0: No lesions; 1: Isolated hypodensities; 2: Initially confluent hypodensities; 3: Difuse and extense hypodensities.

When leukoaraiosis was analyzed as absence lesions or presence lesions we found 50 patients without lesions and 85 patients with lesions on CT scan. Those without lesions obtained the mean on ΔMMSE 0.480±2.7 and those with lesions obtained the mean on ΔMMSE -0.200±3.24 with p value 0.250. The patients without lesions obtained the mean on CRP 4.080±1.82 and those with lesions obtained the mean on CRP 5.070±2.71 with p value 0.025.

At second, we analyzed the linear regression of the relationship between the ΔMMSE and of CRP levels (mg/L), what means that high CRP levels are related with lower difference in the variation of MMSE (**Figure 2**).

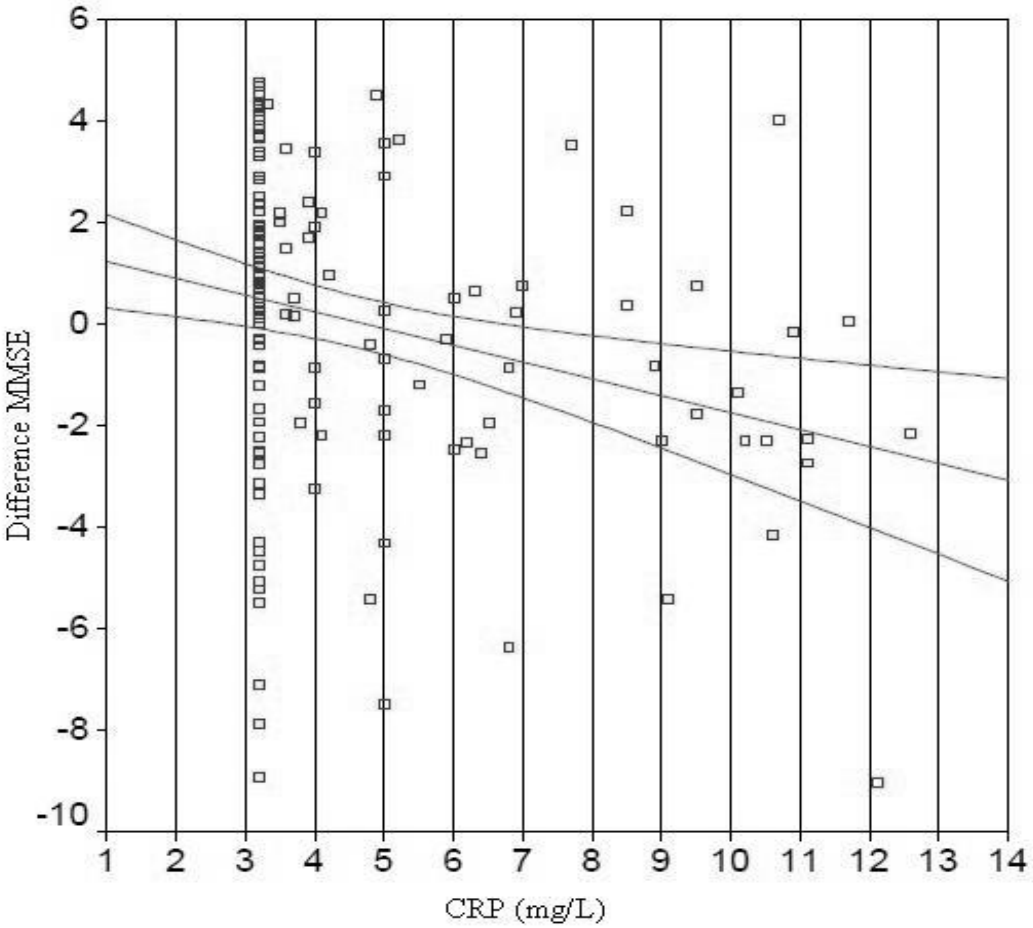

Figure 2. Linear regression showing the relationship between ΔMMSE and of CRP levels (mg/L) ($p<0.001$).

CRP: C-reactive protein; MMSE: Mini Metal State Examination.

With these results we found that the CRP levels can explain 7.18% (p: 0.002) of the variance of ΔMMSE, and adjusting for leukoaraiosis this variance little changed (5.98%; p: 0.005), showing that little CRP influence on cognition was mediated by leukoaraiosis, as evaluated by CT scan. Adjusted logistic regression analysis reveled that people with high levels of CRP had 2.9 (CI 95%: 1,26 – 6,44) higher chance to present cognitive impairment (**Figure 3**).

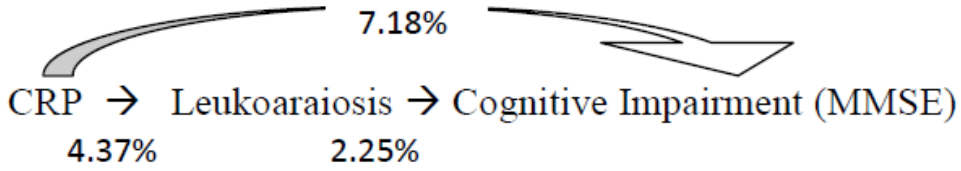

Figure 3. Relationship between the variance on cognition mediated by CRP levels (7.18%; p: 0.002) and adjusted or leukoaraiosis (5.98%; p: 0.005).

CRP: C-reactive protein; MMSE: Mini Metal State Examination.

We found 34 individuals with cognitive impairment, corresponding 25.3% sample. People that obtained CRP serum levels ≥ 5.0 mg/L were considered with a high CRP levels; we found 40 individuals with high CRP levels, corresponding 29,8% sample. (**Figures 4 and 5**).

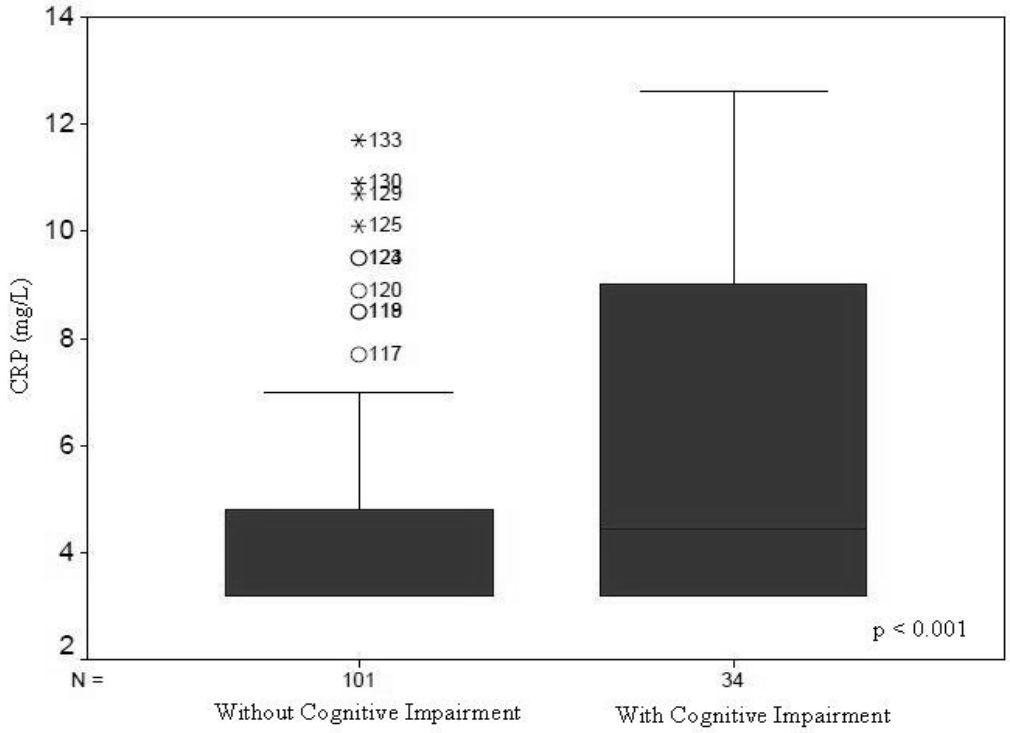

Figure 4: CRP level distribution in those without and with cognitive impairment. Mean CRP with cognitive impairment: 5,81; mean CRP adjusted for covariates: 5,7; p<0.001. Mean CRP without cognitive impairment: 4,33; mean CRP adjusted for covariates: 4,7; p<0.001.

CRP: C-reactive protein.

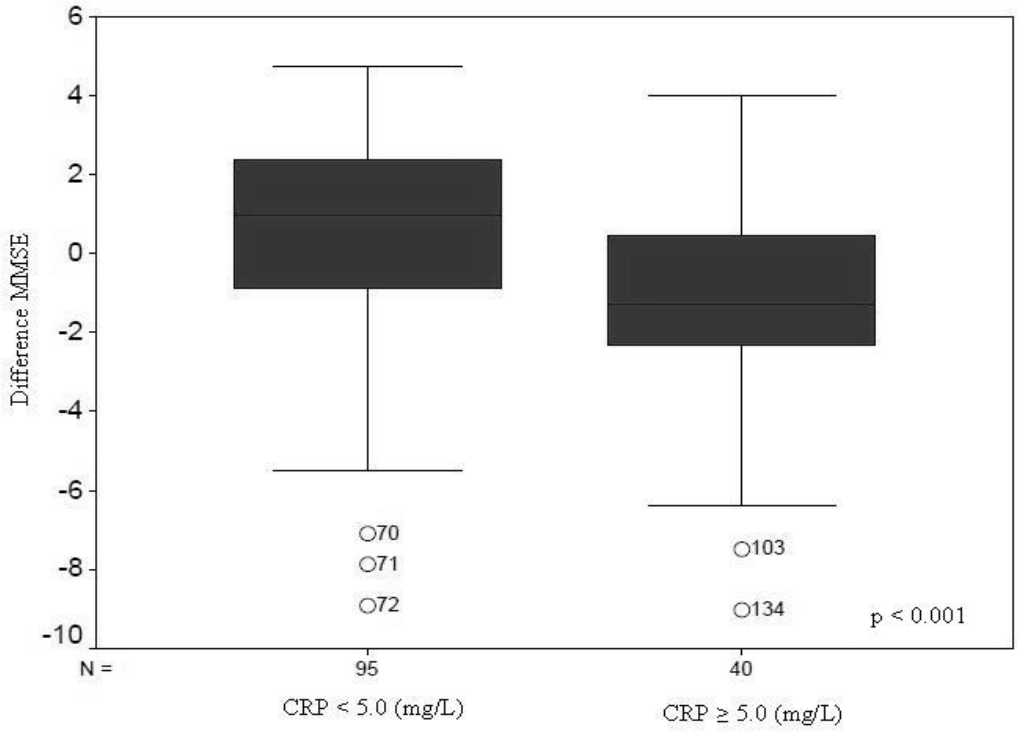

Figure 5: ΔMMSE distribution in those with high (≥ 5.0 mg/L) and normal (< 5.0 mg/L) CRP levels. Mean Δ MMSE (CRP ≥ 5.0 mg/L) = **-1,19** p: 0.003 and adjusted for covariates: **-1,13** p: 0.031. Mean Δ MMSE (CRP < 5.0 mg/L) =**+0.52** p: 0.003 and adjusted for covariates: **+0.30** p: 0.031.

CRP: C-reactive protein; MMSE: Mini Metal State Examination.

CRP levels between patients with cognitive impairment were significantly higher (5.82 ± 3.21) than among controls (4.33 ± 2.02; p: 0.002). Assessing Pearson's partial correlation coefficients, controlling for age, sex and educational level, we found negative correlation between CRP and MMSE (r: -0.268; p: 0.002), and positive correlation between CRP and presence leukoaraiosis lesions on CT (r: 0.209; p: 0.017). These results agree with the hypothesis that high levels of CRP are associated with cognitive impairment and that patients with WML have elevated serum levels of CRP.

Discussion

The present study found an inverse linear association between CRP marker and cognitive performance in a sample of patients with ischemic heart disease. We found that elevated serum levels of CRP were associated with worse cognitive function and an increased risk of cognitive impairment in people aged 50 years and older. CRP levels in patients with cognitive impairment were significantly higher (5.82 ± 3.21) than among controls (4.33 ± 2.02; p: 0.002). Analyzing the degree of leukoararіosis with ΔMMSE and with CRP we found no significant difference, what suggests that the effects on the relationship between CRP and cognitive impairment are not completely mediated via leukoaraiosis. These results are in accord with the hypothesis that high levels of CRP were associated with cognitive impairment and that patients with white matter lesions have elevated serum CRP levels. Moreover, the variance of CRP serum levels upon cognition (7.18%; p: 0.002) was independent of the degree of leukoaraiosis, because these variance little changed after adjustment for the later (5.98%; p: 0.005). These results remained significant even after accounting for confounders like age, sex and educational level.

Thereby, our findings are in line with other studies that found that elevated CRP levels are related to cognitive impairment [24, 25]. A study found that elevated CRP levels predate in 25 years the clinical onset dementia, suggesting that inflammatory process occur long before clinical symptoms appear [8]. However, in some other studies no association between CRP and cognition was found [26-28].

The mechanisms underlying WML are not fully understood, but the observation that CRP, as a marker of inflammation, may be involved in the pathophysiology of cerebral small vessel disease is in accord with studies that link hypertension and diabetes to vascular dementia and small vessel subtypes [6]. Several studies have reported that some inflammatory proteins and cytokines are related to an increased risk of WML through endothelial dysfunction [9, 10, 29-31], whereas others have found no difference in the degree of WML according to inflammatory status [30, 32-34]. Diversity in the methods utilized may explain some of this variation among different studies.

Cerebral small vessel disease is one of the most common degenerative vessel disorders in the ageing human brain, together with cerebral atherosclerosis and cerebral amyloid angiopathy [35]. Endothelial dysfunction is thought to play a important role on it and could implicate on further complications [36], like Alzheimer's disease or vascular dementia. Therefore, elevated serum levels of CRP as endothelial biomarker dysfunction might contribute to the development of those pathologies or be a consequence of their injury. Finally, the disruption of subcortical neural circuits that control executive cognitive functioning leads to damage on: short-term memory, organization, mood, regulation of attention, ability to act or make decisions, and appropriate behavior.

The population-based Rotterdam Scan Study evaluated 1033 nondemented elderly individuals and showed that higher CRP levels were associated with presence and progression of leukoaraiosis, independent of cardiovascular risk factors and the degree of carotid atherosclerosis [9]. This finding was later confirmed not only in whites but also among blacks as well in the Cardiovascular Health Study [29]. However, several studies failed to find associations between CRP and WML, especially in Asian populations [32, 33]. Asian populations seem to have low levels of CRP, which may reflect their low prevalence of coronary heart disease in comparison with those of Western populations [33]. Our analysis demonstrated that only a small portion of the CRP influence on cognition was mediated via leukoaraiosis. Studies showed that if the level of CRP is associated with cerebral small vessel disease, this protein might be utilized as a useful marker for monitoring the risk of cerebral small vessel disease-related brain lesions [33].

A meta-analysis that evaluated associations between CRP and cognitive deficit found that older men are more susceptible to elevated concentrations of CRP than elderly women [37]. Another study found that men are generally more susceptible to the deleterious effects of inflammation than women as suggested by the finding that CRP was associated with a 12% reduction in survival time and a one-year reduction in expected lifespan in men but not in women [38]. Therefore, gender may be an important variable to consider when studying the association between inflammation and cognition.

The levels of CRP in the brain are generally more than 100 times lower than in plasma [6, 39]. Increased plasma levels of pro-inflammatory proteins before the onset of clinical signs of dementia suggests that peripheral inflammation is involved in the disease process, which culminates in dementia. On the other hand, high concentrations of pro-inflammatory proteins in plasma may be consequence of the dementia pathophysiology, because amyloid plaques induces the expression of cytokines, like IL-1 and IL-6, which increases the levels of peripheral pro-inflammatory proteins. This was demonstrated in animal models [40]. Thus, peripheral immune system activation might be both a cause and a consequence of the dementing process. This cascade includes the formation of beta-amyloid deposits and leads to local inflammation on the brain. This results in peripheral immune system activation, which, in turn, foster increased deposition of beta-amyloid [6].

In this study, CRP levels are higher among patients with cognitive deficits and individuals with CRP levels ≥ 5.0 had 2.9 (CI: 1.26 - 6.44) times more chance to present cognitive impairment (p: 0.012). However, because this was a cross-sectional study, it is not possible to determine if elevated CRP levels occur before the development of dementia, or are consequence of the disease. That is, the cross-sectional design limits causal inferences and does not enable cause-effect inferences.

Other limitations of the study need to be taken into account. Measurement of the CRP was performed at only one time. Even though it is well known that CRP levels have few short-term fluctuations [41], within-person variability and measurement error may have resulted in dilution of the associations. Another limitation of our as well as of most other studies is that the inflammatory parameters measured in the circulation do not necessarily reflect local inflammation in the brain. Although CT scan is less sensitive than magnetic resonance image (MRI) for both the detection and quantification of leukoaraiosis, it is a more readily accessible method in developing countries than is MRI.

Strengths of our study include simultaneous measurement of inflammatory biomarker CRP and white matter damage as assessed by CT scan in a population of high risk for small-vessel cerebrovascular disease. The degree of leukoaraiosis was conducted by a blind rater using a previously validated method. Even having used low sensitive methods like CT scan and MMSE, our findings were statistically

significant. This probably means that if we have utilized more sensitive techniques found associations would be even stronger.

Longitudinal studies would expect to find steeper rates of neural and cognitive impairment in people carrying higher levels of pro-inflammatory proteins, like CRP. Certain risk factors for cognitive impairment appear modifiable, and CRP represents a potentially modifiable inflammation marker that may be associated with an increased risk of cognitive impairment. Further research on the relationships among biomarkers, cognition, and structural brain changes in older adults are necessary in order to clarify the longitudinal associations between these variables.

One approach could be the use of functional image methods, like positron emission tomography scan (PET-scan), in order to evaluate the relationship between high CRP levels, often-associated endothelial dysfunction, and chronic brain hypoperfusion that may lead to cognitive impairment before WML due to small vessel disease can be noted on MRI. In particular, future studies need to investigate the mechanisms by which CRP is related to WML and worse cognition.

Conclusion

We found that CRP levels are inversely associated with cognitive performance in coronary patients, and this relation was independent of age, sex, educational attainment, and degree of leukoaraiosis. Patients with CRP levels ≥ 5.0 had 2.9 (CI: 1.26 - 6.44) times more chance to present cognitive impairment (p: 0.012) than controls.

Competing interests

There are no actual or potential conflicts of interest.

Author's Contributors

MRSC made substantial contributions to conception and design of the study, analysis and interpretation of data; LR was involved in acquisition of data and in drafting the manuscript, revising it critically for important intellectual content. All authors read and approved the final manuscript

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3. CONCLUSÕES E PERSPECTIVAS

O presente trabalho agrega conhecimento aos escassos e contraditórios estudos referentes à PCR e auxilia na caracterização da atuação dessa proteína pró-inflamatória em relação ao declínio cognitivo senil. Como resultado das análises feitas nas amostras coletadas, encontrou-se uma relação linear inversa entre níveis séricos de PCR e o desempenho no exame MEEM dos sujeitos avaliados. Portanto, ao final deste trabalho, conclui-se que níveis séricos elevados de PCR estão associados com o aumento do risco de comprometimento cognitivo em pacientes coronariopatas. Também se observou que os efeitos mediados pela PCR em relação à cognição não estão relacionados com a presença de lesões na substância branca, apesar da leucoaraiose também estar relacionada com comprometimento cognitivo em indivíduos de idade avançada.

Considerando que mecanismos inflamatórios estão relacionados com a progressão de déficits cognitivos com o avançar da idade, pode-se supor que este é um dos mecanismos que acelera o aparecimento de processos demenciais. Assim, este estudo também agrega conhecimento à fisiopatologia de doenças como: demência vascular, doença de Alzheimer e outras formas de comprometimento cognitivo; o que poderá contribuir para possíveis intervenções e prevenções nessas patologias.

Alguns fatores de risco para disfunção cognitiva são potencialmente modificáveis e a PCR representa um possível marcador de inflamação que pode ser modulado a fim de diminuir o risco de comprometimento cognitivo. Este estudo encoraja mais pesquisas em relação a possíveis biomarcadores inflamatórios, ao processo cognitivo e seu declínio com a idade e também a alterações estruturais cerebrais em adultos. Tais estudos poderão auxiliar na descoberta de mecanismos que auxiliem na prevenção de patologias relacionadas, através de triagens clínicas e laboratoriais, fornecendo benefícios à sociedade.

As perspectivas em relação a este estudo consistem na realização deste trabalho com métodos mais sensíveis a fim eliminar as possíveis limitações. Também, realizar mensurações seriadas da PCR para que os níveis da proteína

inflamatória não resultem de variações individuais ou erros de mensuração. Dessa forma, estudos futuros necessitam investigar de forma mais aprofundada os mecanismos pelos quais a PCR é relacionada com leucoaraiose cerebral e déficits cognitivos, a fim de elucidar os mecanismos relacionados a essas patologias que ainda estão subentendidos.

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