Increased serum IL-6 is predictive of coronary artery disease and long-term cardiovascular events in high-risk patients submitted to coronary angiography

IL6 sérica elevada é fator preditivo de doença arterial coronariana e eventos cardiovasculares em pacientes de alto risco encaminhados para cateterismo cardíaco

TESE DE DOUTORADO

MÁRCIO MOSSMANN

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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IL6 sérica elevada é fator preditivo de doença arterial coronariana e eventos cardiovasculares em pacientes de alto risco encaminhados para cateterismo cardíaco: estudos observacionais.

Autor: Márcio Mossmann

Orientador: Prof. Dr. Marco Vugman Wainstein

Co-orientador: Prof. Dr. Marcello Casaccia Bertoluci

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LISTA DE ABREVIATURAS E SIGLAS

IC: Insuficiência Cardíaca

DCE: Depuração de Creatinina Estimada

TCLE: Termo de Consentimento Livre e Esclarecido

CK-MB: creatine kinase-myocardial band

IAM: Infarto Agudo do Miocárdio

DAC: Doença Arterial Coronariana

PCR: Proteína C Reativa

AI: Angina Instável

AE: Angina Estável

SCA: Síndrome Coronariana Aguda

SAA Amiloid sérico A

CAC : Escore de Cálcio Coronariano

IL-6: Interleucina-6

NYHA: New York Heart Association

RESUMO

IL-6 é uma citocina inflamatória relacionada a aumento de risco para eventos cardiovasculares. No primeiro estudo, foi avaliado a correlação entre doença arterial coronariana (DAC) com os níveis séricos de IL-6. Em uma amostra de 48 pacientes com suspeita clínica de DAC, aqueles com valores de IL-6>1pg/mL (27% da amostra) todos apresentavam estenoses coronarianas de pelo menos 30%, contra 64% daqueles com Il-6<1pg/mL. No segundo estudo, coorte de pacientes encaminhados para cateterismo cardíaco por risco cardiovascular aumentado devido a dor torácica ou isquemia identificada em exame de imagem, foi dosado IL-6 sérica no momento do procedimento e avaliado desfechos primários cardiovasculares ao longo de semanas. Com uma média de tempo-evento para desfechos primários de 297 semanas, pacientes com níveis mais elevados de IL-6 tiveram (com média de 5,7 anos) maior prevalência de eventos cardiovasculares (HR 2.72 IC 1.32-5.59 p 0.02); aqueles com dosagens menores apresentaram um importante valor preditivo negativo (82%) para eventos cardiovasculares. Em subanálise não se identificou correlação com ter ou não diabetes melito, obesidade e hipertensão arterial sistêmica. Em conclusão, IL-6 demonstrou tanto ser um marcador importante da presença de DAC quanto apresentar valor prognóstico independente para ocorrência de desfechos cardiovasculares adversos em pacientes submetidos à cinecoronariografia.

PALAVRAS-CHAVE: IL-6; doença arterial coronariana; eventos cardiovasculares; cateterismo cardíaco**;** diabetes

ABSTRACT

IL6 is an inflammation-related cytokine associated with elevated cardiovascular risk events. In first we conducted an observational study to correlate the presence of coronary artery disease with serum levels of IL6. A sample of 48 patients in suspicious of clinical relevant coronary disease, 27% of these had serum levels of IL6>1pg/mL and all of them confirmed the presence of significant coronary disease of 30% stenosis at least. In whom IL6<1pg/mL only 64% had significant coronary disease. In the second observational study of elevated risk patients referred to cardiac catheterization for chest pain or ischemia induced in imaging tests, serum IL6 was obtained at this time and correlated with long time cardiovascular outcomes. With a median overall time-to-event for the primary outcome of 297 weeks, elevated serum IL6 patients presented higher prevalent outcomes (HR 2.72 IC 1.32-5.59 p 0.02); lower serum IL6 patients had important negative predicted value for cardiovascular outcomes (82%). Secondary analysis did not presented correlations with diabetes, obesity and hypertension. In conclusion, IL-6 demonstrated to be an important marker for the presence of CAD as well as had an independent prognostic impact to predict the occurrence of adverse cardiovascular events in patients submitted to coronary angiography.

KEYWORDS: IL6, coronary artery disease, cardiovascular outcomes, cardiac catheterization, diabetes

Introdução:

Doença cardiovascular é a principal causa de morbidade e mortalidade em todo mundo. Apesar do avanço nas terapias empregadas em doenças que levam aos desfechos cardiovasculates, como na medicina preventiva contra infarto do miocárdio e acidente vascular cerebral, muitos eventos têm ocorrido a despeito do emprego de diretrizes atualizadas. Recentemente o processo de inflamação na progressão das doenças tem chamado atenção da comunidade científica.

Citocinas liberadas por células imunológicas são mediadoras desta inflamação cardíaca e levam a progressão das doenças cardiovasculares (infarto, aterosclerose, hipertensão arterial sistêmica, insuficiência cardíaca hipertrófica). Alem disso, várias comorbidades associadas a doença cardiovascular, como diabetes, obesidade etc, exacerbam o processo inflamatório do sistema cardiovascular resultando no final em insuficiência cardíaca. Atividade de moleculas pró-inflamatórias como a IL6 na placa aterosclerótica podem estar relacionados a eventos cardíacos futuros. [1]

Por isso que estudos com citocinas inflamatórias tem sido realizados; inicialmente nos eventos agudos e mais recentemente na doença estável.

Nas complicações agudas da doença aterosclerótica, principalmente no infarto do miocárdio, existe conhecimento, por diversos estudos, de que níveis elevados de IL6 estão correlacionados com desfechos piores, com maior área de necrose e consequentemente menor fração de ejeção ventricular esquerda.[2]

Em pacientes com doença aterosclerótica coronariana conhecida estável e com fatores de risco graves para complicações, também se detectou que altos níveis séricos de IL6 conferem piores desfechos clínicos.[3]

No nosso primeiro artigo do nosso grupo demonstramos que pacientes com níveis elevados de IL6 apresentavam maior probabilidade de ter aterosclerose coronariana maior que 30%; neste estudo os pacientes não poderiam ter diabetes nem outras complicações e/ou doenças crônicas graves ou agudizadas, com intuito de tentar diminuir processo inflamatório por outras causas e assim diminuindo viéses de seleção.

O nosso segundo estudo versa sobre o papel da IL6 no prognóstico a longo prazo em pacientes com doença coronariana estável, com sintomas de angina e/ou isquemia em testes não invasivos a nível ambulatorial, sem fatores de risco graves e não compensados que possam interferir nos resultados e prognóstico. Excluímos pacientes com Infarto a menos de 60 dias, IC em clase IV, doença renal crônica com DCE menor que 45 ml/m/1.73m^2 , doença pulmonar obstrutiva crônica, portadores de HIV, hepatites B ou C, doenças reumatológicas crônicas ou usuários de medicações que alterassem o estado inflamatório. Como no primeiro estudo, no dia do cateterismo cardíaco havia o convite para fazer parte dele, assim como assinatura do TCLE e coleta de amostra sérica para posterior dosagem laboratorial.

Não tinhamos conhecimento à época de estudo prospectivo avaliando níveis de IL6 e desfechos cardiovasculares na doença estável. Definimos como desfechos; 1- novo infarto agudo do miocárcio, 2- internação por insuficiência cardíaca, 3- novo acidente vascular cerebral, 4- necessidade de nova revascularização, 5- morte por causa cardiovascular e 6- morte por qualquer causa, o que apresentasse primeiro.

Revisão da Literatura

Marcadores de lesão miocárdica como a Troponina e a creatine kinase-myocardial band (CK-MB) são ferramentas validadas principalmente para determinação de risco nos pacientes com infarto agudo do miocárdio (IAM). No entanto, pacientes sem evidência de necrose miocárdica podem estar em risco para evoluir com este desfecho. Por esta razão muitos têm focado no mecanismo inflamatório da estabilidade da placa aterosclerótica para melhor compreender a fisiopatologia da doença.

As células adiposas produzem citocinas, especialmente Interleucina-6 (IL6), que induz a síntese de Proteína C Reativa (PCR) no fígado. Esta estimula a captação de LDL-colesterol pelos macrófagos, induz a ativação do complemento que leva a dano celular arterial e aumenta no monócito a produção de fator tecidual, que eleva o risco de trombose. Isto explica a suspeita de que as lesões ateroscleróticas ricas em células inflamatórias e citocinas são mais propensas a eventos agudos, espasmos ou tromboses.[4]

Na década de 90, estudos observando fibrinogênio demonstravam que seus níveis séricos estavam correlacionados com risco e severidade da cardiopatia isquêmica (CI), pois refletiam numa maior inflamação da parede endotelial e consequentemente maior produção de citocinas inflamatórias, como IL6, IL1-β, TNF-α, entre outros.[5]

Então no início deste século já havia muita evidência corroborando a hipótese de que tanto infecção quanto inflamação faziam parte da gênese da aterosclerose.[6] IL6 seria um ativador para os monócitos na parede endotelial para deposição de fibrinogênio e um inativador da lipase lipoproteica, fazendo os macrófagos captarem mais lipídeos para formação da placa aterosclerótica. Outro conhecimento seria que a IL6 estimularia o eixo hipotálamo-pituitaria-adrenal, levando a obesidade, hipertensão e resistência insulínica, fatores de risco conhecidos na aterogênese.[7]

INFARTO AGUDO DO MIOCÁRDIO

No final do século passado foram publicados alguns estudos mostrando a importância da IL-6 no evento agudo. Dosagens de IL6 e IL8 de espécimes da placa aterosclerótica, de trombos coronarianos e da aorta em pacientes com infarto agudo do miocárdio

foram correlacionadas com pacientes controles, não vítimas de infarto. Resultados mostraram diferenças importantes entre os grupos (15.3 ± 4.5 vs. 3.8 ± 1.2 pg/ml; $P < 0.01$), aumentando muito mais a suspeita de que uma cascata inflamatória elevada resultaria em um estado pró-trombótico exacerbado e com consequente placas ateromatosas propensas a ruptura – mecanismo das sindromes coronarianas agudas.[8]

Em outros trabalhos, achados diferentes de valores de IL6 foram encontrados a despeito do quadro clínico. Comparando controles com pacientes com síndrome coronariana aguda (SCA) e outros com angina estável, outros marcadores inflamatórios eram elevados em ambos grupos com doença comparados aos controles; no entanto, a IL6 só estava elevada quando em SCA (SCA 10.8±1.8 x AE 1.8 ± 0.8 x C 1.2±0.6, P < 0.0001), demonstrando sua atividade aguda na doença.[9] Mas com um número reduzido de 117 participantes totais pode não ter obtido diferença entre grupos AE e controle.

Quando avaliaram picos de IL6 ou de seus receptores alfa ou beta na SCA, eles se correlacionavam com elevações também de ANP e BNP nos pacientes que complicavam com disfunção miocárdica severa, podendo o nível de IL6 ser um marcador de pior prognóstico nos pacientes que evoluiam com Insuficiência Cardíaca aguda.[10]

Em 2001, o grupo FRISC II publicou trabalho que a IL6 era um poderoso marcador prognóstico independente de mortalidade em pacientes com SCA. Demonstraram que aqueles com IL6 elevada se beneficiavam de tratamento invasivo precoce com redução relativa de mortalidade de 65% em 1 ano (0.17-0.74; p=0.004). Inversamente, os com IL6 baixa não se beneficiavam de tratamento invasivo precoce comparado ao tratamento clínico (0.44-1.65; p=0.64). No entanto, os pacientes com maiores benefícios foram aqueles com marcadores de necrose (troponina) elevados e infradesnivelamento do segmento ST no eletrocardiograma da chegada, fatores já conhecidos como de alto risco para eventos cardiovasculares na SCA.[11] Com o conhecimento atual podemos interpretar tal informação não como um viés de confusão somente, pois quanto maior a inflamação, provavelmente maior será a carga isquêmica (ou vice-versa) e necrose, com aumento de troponina e alteração eletrocardiográfica correspondente.

No SOLID-TIMI 52, em 4939 pacientes nos primeiros 30 dias pós-infarto, quando tiveram sua inclusão para uso ou não de Darapladib (inibidor da fosfolipase A2), também

realizaram a dosagem de IL-6. Os pacientes que apresentaram eventos após o infarto índice tinham em média valores de IL-6 significativamente mais elevados do que os livres de eventos em 2.5 anos (excluindo os com AVC, que pode não ter sido significativo pelo baixo número ocorrido). Também, ao dividir os pacientes em quartis pela IL-6, verificou-se aumento do número de eventos com o aumento do quartil (p<0.0001), incluindo morte, infarto e internação por IC. Novamente não foi significativo a ocorrência de AVC.[12]

Funayama e cols em 2004 avaliaram a presença de IL6 em 36 pacientes com infarto do miocárdio com supra desnível do segmento ST (IAMCSST) diretamente da coronária culpada pelo evento, da aorta e de sangue periférico. Grupo controle com 10 pacientes foi utilizado para dosagem de IL6 de sangue periférico. A concentração de IL6 na coronária (14.4pg/ml) foi significantemente maior que na aorta (8.0pg/ml) e veia periférica (6.5pg/ml); todas três foram significativamente maior que o grupo controle (1.77pg/ml). Pelos resultados podemos inferir que a IL6 esta envolvida na vulnerabilidade para ruptura da placa aterosclerótica, com maior ativação de macrófagos envolvidos no processo trombo-oclusivo do infarto.[13]

Muito semelhante fez Mayer e cols que dosaram IL-6, amiloide sérico A (SAA) e PCR diretamente da coronária ocluida e da aorta, revelando no mesmo momento maiores valores locais que sistêmicos de IL-6 (8.9 x 5.0 ng/L, p <0.0001) e SAA (24.3 x 22.1 mg/L, p<0.0001), mas não de PCR (2.5 x 3.0 mg/L, p 0.0001). Resultados confirmam que tanto IL-6 quanto SAA são citocinas com efeitos locais de inflamação enquanto que a PCR tem efeito mais sistêmico.[14]

Suzuki e cols, com 122 pacientes com IAMCSST e controles, também demonstraram elevação dos níveis de IL6, entre outros marcadores, dosados diretamente da coronária responsável pelo infarto nas primeiras 12 horas do evento do que no sangue periférico, mas não após esse período de 12hs. Talvez por a citocina estar envolvida na vulnerabilidade para ruptura da placa e não mais após o processo de cicatrização desta.[23] Heinisch e cols também acharam resultados semelhantes da IL-6 aumentada somente no momento do evento agudo, não em 15 dias ou 30 dias após este, demonstrando importância dela na instabilização aguda, possivelmente só da placa aterosclerótica.[15]

Em contrapartida, Brueckman e cols analisaram marcadores inflamatórios (alfa-FNT, IL6, IL1-ra e ligante CD40) em pacientes com SCA e controles e não identificou diferença dos níveis destes marcadores se coletados do sangue periférico ou do seio coronário, apesar de elevados no infarto. Em sua conclusão refere o estado inflamatório de todo organismo como responsável pela instabilização aguda, não efeito local na coronária. Mas era um trabalho com número muito pequeno de participantes, 13 em cada grupo, o que gera falta de significância estatística.[16]

Já Lee e cols pesquisaram o valor prognóstico (ocorrência de morte, novo infarto ou revascularização) de alguns marcadores, dentre eles a IL6, em 30 dias e 1 ano após SCA(n=156), comparando a pacientes com angina estável (n=36) e controles (n=40), dosando níveis séricos nas primeiras 24-48hs do ínicio dos sintomas. Aqueles que apresentavam com novo evento, tanto em 30 dias quanto em 1 ano, tinham dosagens mais elevadas de IL6 no evento incial. No entanto, esses mesmos pacientes já se apresentavam com escore de *TIMI risk* mais elevado, o que poderia ser um viés de confusão; ao ajustar para esse subgrupo nos pacientes com IAMCSST, mantiverem resultados como preditores de eventos em 30 dias, o que não ocorreu nos com IAMSSST-AI.[17]

Monakier e cols, visto o efeito inflamatório até então demonstrado por inúmeros trabalhos na gênese da aterosclerose no infarto, tentaram demonstrar se o tratamento com Rofecoxib, inibidor da COX-2, na SCA diminuiria os valores de marcadores inflamatórios como PCR, IL6 e FNT-R1 solúvel após 1-3meses e melhora da função endotelial. Randomizaram 36 pacientes, mas só conseguiram demonstrar que houve diminuição da PCR aos 1 e 3 meses em relação ao valor basal e de IL6 após 1mês. Este foi um dos primeiros trabalhos recentes com antiinflamatórios na tentativa de identificar mecanismo de supressão de atividade inflamatória ou retardo de aterosclerose. Mesmo com resultados não muito consistentes pela limitada amostra, deixava um indicativo de que a redução inflamatória poderia se refletir em diminuição de eventos futuros, ajudando a confirmar também o papel da atividade inflamatória na piora de prognóstico dos pacientes com aterosclerose.[18]

Trabalho algo semelhante realizou Bogaty e cols com Rofecoxib em pacientes com pelo menos dois episódios anteriores de SCA a mais de 3 meses. Com 35 pacientes

randomizados e tratados com Rofecoxib ou placebo por 6 meses (além de AAS 100mg/dia), os níveis de IL-6 e outros marcadores diminuíram significativamente no grupo Rofecoxib: IL-6 de 2,3 para 1,7 (p 0,0002) x 2,7 para 3,5 no placebo. Apesar dos autores concluirem à época que o uso de inibidores da COX-2 poderia ser benéfico e/ou sem efeito deletério em pacientes de alto risco para eventos cardiovasculares, não foram avaliados desfechos no estudo pra ter conclusões sobre benefícios, a não ser subtendido pela redução da IL-6, e alguns participantes tiveram complicações durante os 6 meses, algo que com um número de participantes muito pequeno não consegue ter poder para determinar.[19]

No mesmo sentido, Luo e cols avaliaram o efeito da Sinvastatina na redução de parâmetros inflamatórios (PCR e IL-6) e dos lipídeos (Colesterol Total, LDL e HDL, Triglicerídeos e Glicose) em pacientes com SCA [n =50, IAM (n = 20) e AI (n = 30)], AE (n = 34) e Controles (n = 30). Todos receberam medicação padrão para a época. Após randomização dentro dos grupos e 3 semanas de tratamento com Sinvastatina 20mg por dia ou placebo, houve redução significativa ($p<0.001$) nos níveis de PCR e IL-6 nos grupos de SCA comparado aos seus controles dentro dos grupos. O mesmo ocorreu com os níveis de colesterol total e LDL, mas não houve correlação entre a diminuição dos lípides com dos parâmetros inflamatórios, mostrando que a Sinvastatina apresenta poder antiinflamatório independente da diminuição lipídica.[20]

Ridker avaliou se o tratamento com Metotrexate 15mg semanal poderia alterar desfechos cardiovasculares em pacientes com infarto prévio ou doença coronariana grave, tendo três quartos realizado previamente revascularização, e diabetes ou sindrome metabólica, por 5 anos. Após randomização de 4168 pacientes, o uso de metotrexate não modificou nem desfechos nem níveis plasmáticos de IL-6, PCR-us e LDL-colesterol. Analisaram níveis basais destes marcadores em todos pacientes e separaram em quartis. Comparação interquartis demonstrou que quanto mais elevado o nível sérico desses marcadores mais alta probabilidade de evento cardiovascular.[21]

ANGINA INSTÁVEL

Na Angina Instável (AI) a IL6 se mostrava um marcador independente, com poder discriminatório comparando com a Angina Estável (AE). Ainda, essa diferença se mantinha caso os pacientes se apresentavam com quadro de angina em repouso nas últimas 48hs.

Um mês após a Angioplastia coronariana de pacientes com AI não havia mais diferença importante dos níveis de IL6 comparando com pacientes com AE e controles, mostrando que a IL6 se correlaciona com a instabilidade da placa aterosclerótica e que a angioplastia com stent pudesse ser uma parte do processo de estabilização e reendotelização da placa.[22]

Resultados similares também foram encontrados em pacientes com Angina Instável que apresentavam complicações durante as primeiras 48 horas de internação, apresentando valores de IL6 e IL1-ra crescentes após 2 dias (57% e 37%, respectivamente); diferentemente daqueles sem complicações após 48horas, com níveis decrescentes de IL6 e IL1-ra (13% e 12%, respectivamente). Observação seja feita: os que tiveram eventos hospitalares já tinham níveis mais elevados das citocinas (7.3 x 4.7pg/mL), então já eram pacientes mais propensos a complicações agudas. E, apesar de todos receberem o mesmo tratamento clínico, quem respondesse com queda de marcadores inflamatórios teriam melhor desfecho clínico.[23]

Mas também avaliaram a elevação da IL-6 em pacientes com Insuficiência Cardíaca de etiologia isquêmica ou hipertensiva, tentando descobrir correlação com gravidade dos sintomas através da classe funcional de NYHA. Dos 80 pacientes avaliados, houve elevação dos níveis de IL-6 com a piora da classe funcional, chegando a 58% apresentando valores elevados de IL-6 na classe IV.[24]

Estudando o prognóstico na Angina Instável, com desfecho de mortalidade coronariana em 17 meses, Koukkunem et al mostraram que aqueles com amostras na admissão nos terços mais elevados de PCR e IL6 tinham risco 6 vezes maior de morte, assim como 3.5 vezes se nos tercis mais elevados de fibrinogênio e FNT-alfa.[25]

Santoro e cols avaliaram a relação de IL6 e IL10 na ocorrência de complicações na internação, morte, reinternação e recorrência de Síndrome de Takotsubo após em média 178 dias após evento índice. Dos 56 pacientes, 23% apresentaram complicações

na internação e 20% eventos adversos no seguimento. Os que dosaram níveis mais elevados de IL6 e IL10 na admissão apresentaram mais eventos adversos (120 ± 294 *vs.* 22 ± 40 pg/ml, $p<0.05$; 13 ± 35 *vs.* 2 ± 3 pg/ml, $p=0.05$, respectivamente) e maiores taxas de mortalidade (Log-Rank $p<0.001$, HR 20.8).[26]

Hojo e cols publicaram em 2000 estudo em 32 pacientes submetidos a ACTP (por balão, aterectomia ou stent) em que dosagens de IL6 coletadas antes e após o procedimento demonstraram elevações significativas do marcador com o tratamento da estenose indiferente da modalidade utilizada e que naqueles em que após 6 meses apresentaram com reestenose também tiveram maior variação de IL-6 no procedimento index ($r = 0.73$, $p <0.001$).[27] Provavelmente um dos primeiros estudos avaliando prognóstico, mesmo que seja um desfecho com menor importância clínica e sem poder fazer distinção entre tipos de tratamentos, que hoje em dia não são realizados, como a angioplastia sem implante de stent, devido número restrito de participantes.

Dongfane Su e cols seguiram prospectivamente 718 pacientes com DAC para examinar a associação de IL-6 com risco de morte por qualquer causa ou morte cardiovascular. Após uma média de 2.3 anos de seguimento, com 71 eventos no total, detectou uma associação positiva e crescente entre IL-6 e desfecho (HR 2.93 e 2.04).[28] No entanto, pacientes recrutados foram todos "hospitalizados por DAC" no momento da inclusão, não inferindo qual patologia responsável pela internação; sabemos que pacientes em quadro instável apresentam valores maiores de marcadores inflamatórios e também pior prognóstico, podendo refletir um grande viés de seleção.

NA DOENÇA ESTÁVEL

Neste sentido, Rifai e cols compararam 100 pacientes com cardiopatia isquêmica documentada em cateterismo cardíaco com 100 controles ajustados para idade, sexo e tabagismo e sem cardiopatia isquêmica para avaliar marcadores inflamatórios, dentre eles a IL-6. Confirmou que os com CI apresentavam níveis mais elevados de IL-6 (2.3 x 1.7, $p < 0.013$), mesmo ajustando para idade, diabetes, hipertensão, tabagismo e

colesterol. Não houve diferença neste estudo com a severidade da doença coronária e argumentaram à época que a inflamação representaria a difusidade da doença e que por esta razão não teria correlação com a severidade dela. No entanto, sem avaliar localmente cada coronária aterosclerótica e entender o quanto cada uma acrescenta em inflamação no todo, principalmente para avaliar o potencial de instabilização que cada uma contém, não teria significativa relação. Uma placa com maior risco de instabilidade levaria a maior nível inflamatório; placas estáveis sem risco de ruptura, mesmo que múltiplas, liberariam pouca inflamação sistêmica.[29]

Nos pacientes com infarto agudo do miocardio, os trabalhos já apontavam que essas citocinas estavam muito mais presentes, o que demonstrava a importância de melhor entendermos o papel deles tanto na doença aguda quanto na estável.[30]

Erren e cols, avaliando diferentes pacientes, uns com cardiopatia isquêmica, outros com doença arterial periférica, com ambas doenças e controles, verificou que os níveis de IL6 e PCR eram significativamente mais elevados naqueles com CI em comparação com os controles, e que a severidade da doença também era correlacionável. No entanto, juntando ambas doenças a correlação era muito mais elevada, demonstrando possivelmente que quanto maior a extensão da aterosclerose (coronária x sistêmica), provavelmente maior seria a elevação dos parâmetros inflamatórios. Uma possível explicação levantada seria que quanto mais difusa a aterosclerose, maior a chance de ter múltiplas placas instáveis.[31]

Avaliar prospectivamente o valor prognóstico da IL-6 primeiramente foi por Ridker e cols, que em 2000 publicou no *Circulation* trabalho com mais de 14.000 participantes. Com 202 pacientes apresentando IAM e 202 controles, aqueles apresentavam valores basais de IL-6 significantemente maiores que seus controles (1.81 versus 1. 46 pg/mL; P=0.002), independente de quando o evento ocorreu no tempo, de 2 até 6 anos após, e independente de quantos fatores de risco para doença aterosclerótica apresentava.[32]

Muito semelhante estudo publicado no JAMA em 2002, primariamente para avaliar a relação da reposição hormonal pós-menopausa e efeitos na PCR, sabidamente relacionada a doença cardiovascular. Em 75.343 participantes sem doença cardíaca ou câncer prévios documentados, 304 apresentaram infarto ou morte de origem

coronariana em 2.9 anos em média e foram comparados com 304 controles ajustados por idade, etnia, tabagismo e tempo no estudo. Níveis de IL-6 na entrada do estudo foram significativamente mais elevados nos casos: 1.81 x 1.47 pg/mL, p<0.001. Avaliação do uso ou não de terapia de reposição hormonal não demonstrou interferência nos resultados com a IL-6, mas sim nos valores de PCR como esperado.[33]

Em 2004 no NEJM estudo que incluiu mulheres do "Nurses' Health Study" e homens do "Health Professionals Follow-up Study " avaliou retrospectivamente a relação da IL-6 e da PCR em pacientes livres de doença cardíaca que após 8 anos e 6 anos, respectivamente, tiveram eventos coronarianos (infarto fatal ou não fatal). Após ajustado para tabagismo e idade, ambos se mostraram preditores de eventos. No entanto, após ajuste para outros fatores de risco coronarianos clássicos, a associação se desfez, mostrando que ainda não tinhamos conhecimento sobre o papel exato desses marcadores na cardiopatia isquêmica.[34]

Fraser e cols avaliaram coorte com 3581 mulheres entre 60-79 anos ao longo de em média 4,6 anos em que 198 apresentaram evento coronariano neste período. Avaliando em tercis da IL-6 com o risco de eventos, em ajuste somente para idade, verificou-se correlação entre os dados do maior tercil com evento comparado ao menor tercil (HR 1.89; 1.26 – 2.82, p<0.05); entretanto, ajustando para os demais fatores de risco, novamente se perdeu a significância. Grande parte da perda de associação deveu-se ao tabagismo e a doença pulmonar crônica.[35]

Li Tan Kuo e cols avaliaram 135 pacientes com aterosclerose coronariana estável por dor torácica e compararam a complexidade da placa aterosclerótica com os níveis de IL-6 e PCR. Foram ao todo 77 pacientes com estenoses menores que 50%, 15 pacientes com estenoses maiores de 50% e placas simples e 43 pacientes com estenoses maiores de 50% e placas complexas (segundo classificação de Ambrose). Comparando estenoses menores que 50% com as placas complexas, estas apresentaram valores séricos de IL-6 e PCR significantemente mais elevados (p= 0.026 e p = 0.0001, respectivamente), mostrando a potencial influência da inflamação na constituição das placas ateroscleróticas, deixando rastros do processo em seus níveis séricos.[36]

Nishida e cols realizaram trabalho semelhante ao nosso. Em 121 pacientes com multiplos farores de risco e/ou doença coronariana prévia realizaram dosagens de IL-6 e PCR e foram seguidos por 2.9 anos em média; 50 apresentaram evento cardiovascular (AVC/AIT, IAM, oclusão arterial e revascularização cardíaca). Somente a IL-6 apresentou relação significativa com desfechos comparados aos controles sem desfechos: 3.9 ± 2.6 e 3.0 ± 2.2 pg/mL, *P* = 0.04. Analisando a IL-6 em tercis, quanto mais alto menos chance de ficar livre de evento. A PCR não apresentou correlaçao alguma com desfechos, demonstrando que a IL-6 provavelmente seja um preditor mais forte de eventos do que a PCR.[37]

O Cardiovascular Health Study foi um trabalho comunitário com 5.806 participantes com mais de 65 anos e que foram dosados PCR e IL-6 no recrutamento e os individuos foram acompanhados semestralmente por em média 13.1 anos para avaliar correlação desses marcadores com risco de morte súbita. Na população geral, o risco de morte súbita aumentou gradualmente com o aumento dos marcadores, indo de 2.6/1000 pessoas-ano no primeiro quintil de IL-6 para 6.5/1000 pessoas-ano no último quintil ($p< 0.0001$), o que se mostrou também significativo quando ajustado para fatores de risco cardíacos e também para quem já tinha no recrutamento doença coronariana conhecida.[38]

Tentaram também avaliar a relação de marcadores inflamatórios, dentre eles a IL-6, com o Escore de Cálcio Coronariano (CAC), marcador sabidamente importante na predição de eventos cardiovasculares. Quando havia alguma relação entre IL-6 e CAC, ela se desfazia quando se acrescentava outros fatores de risco tradicionais ao cálculo.[39] Zhao e cols avaliaram 303 pacientes sem doença coronariana conhecida que realizaram Tomografia Coronariana e dividiram em 3 grupos de acordo com os valores de IL-6. Aqueles no maior tercil de IL-6 apresentaram maior carga de aterosclerose comparado ao menor tercil, tendo lesões mais longas e em mais segmentos. Ainda, quem estava no maior tercil apresentou em período de 3 anos mais eventos cardiovasculares e maior mortalidade.[40]

Diferentemente, no subestudo do VADT (Veterans Affairs Diabetes Trial) quando fizeram comparação semelhante ao acima, só que em pacientes todos diabéticos, demonstraram que existe associação positiva e significativa entre IL-6 e CAC, mesmo após ajuste para os fatores de risco tradicionais para DAC. Resultado positivo não se deu

por influência do diabetes, pois essa correlação foi inversa de acordo com níveis de hemoglobina glicosilada.[41]

No estudo PRIME envolvendo 3 centros europeus, seguiram ao longo de 10 anos 9971 homens, sem doença coronariana a princípio, e 664 apresentaram evento coronariano neste período (inclusive 50 casos de morte súbita). Usaram como controles 2 pacientes livres de eventos para cada um dos casos. Dos marcadores dosados somente a IL-6 foi preditor de maior risco para morte súbita, com um aumento de risco de 3 vezes (IC 95%, 1.2 – 7.81; p 0.02) do maior tercil para o menor. No entanto, os pacientes que sofreram morte súbita eram mais hipertensos, tabagistas e diabéticos, o que provavelmente os conferiram valores maiores de IL-6, prejudicando assim uma conclusão mais fidedigna.[42]

Comprovando que a IL-6 seja preditor de eventos cardiovasculares talvez medidas que a diminuem, com real redução de seus níveis séricos, possa ser também preditor de melhor prognóstico. É de conhecimento que o exercício físico tem papel importante na melhora dos fatores de risco para DAC, podendo ser por redutor da atividade inflamatória, assim como demonstrou trabalho conduzido por Goldhammer, onde 28 pacientes com média de idade de 64 anos realizaram um programa de treinamento aeróbico por 12 semanas (esteira, bicicleta ergométrica, bicicleta de braço) , 3 vezes por semana, 45 minutos cada treino e atingindo 70-80% da frequência cardíaca máxima individual. Todos indivíduos tinham doença coronariana comprovada e estável, sem sinais de descompensação por outras patologias também. Após as 12 semanas, não houve redução significativa de peso, IMC, níveis séricos de lipídeos ou valores pressóricos. No entanto, valores de IL-6 reduziram significativamente de 2.50 ± 1.50 para 1.44 ± 0.57 pg/ml, p=0.002; o mesmo aconteceu com a PCR, IL-1 e FNTα.[43]

Trabalho publicado recentemente por Ferencik e cols no *JACC Cardiovascular Imaging* demonstrou que em pacientes com dor torácica estável e IL-6 acima da média do grupo (1.8ng/L), esta foi preditora de eventos cardiovasculares (morte, infarto e angina instável; HR: 1.9 [95% CI: 1.1-3.3]; P = 0.03). Usaram também no trabalho análise com troponina ultra-sensível, que também foi preditora de eventos na análise univariada (HR: 2.1 [95% CI: 1.3-3.6]; P = 0.006). Resultados demonstraram que isquemia e inflamação provavelmente agem em conjunto na formação da placa aterosclerótica e na piora da doença cronicamente.[44]

Então, têm-se o conhecimento de que a inflamação enfraquece a placa aterosclerótica e a deixa suscetível a ruptura, levando ao fenômeno trombo-embólico e/ou trombo-oclusivo. A detecção de quais pacientes estariam em risco cardiovascular deste fenômeno elevou a procura de marcadores inflamatórios com valores preditivos com significância.[45]

Justificativas:

Visto todos esses estudos, ainda não sabemos ao certo qual o real papel da IL6 na avaliação de risco e prognóstica de pacientes estáveis e com potencial maior risco cardiovascular, principalmente pelos inúmeros fatores que possam tanto elevar seus valores quanto o risco de complicações, confundindo análises do papel desta de forma prospectiva. Por isso, decidimos avaliar a relação desta interleucina com a presença de aterosclerose coronariana e prospectivamente esse marcador de inflamação em pacientes de alto risco cardiovascular, mas sem obesidade importante ou outras doenças descompensadas que poderiam influenciar o seu resultado.

Objetivos:

Os objetivos dos nossos estudos seriam: 1- avaliar a correlação entre os níveis séricos de IL6 em pacientes com suspeita de cardiopatia isquêmica estável e a presença de aterosclerose coronariana; e 2- avaliar a correlação entre os níveis séricos de IL6 em pacientes com suspeita de cardiopatia isquêmica estável e desfechos cardiovasculares ao longo de semanas, avaliando também a correlação de obesidade, diabetes melito e hipertensão arterial nos níveis de IL6 com os desfechos clínicos apresentados.

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ARTIGO 1 PARA REVISTA DIABETOLOGY AND METABOLIC SYNDROME

Elevated Serum Interleukin-6 is Predictive of Coronary Artery Disease in Intermediate Risk Overweight Patients Referred for Coronary Angiography

Marco V. Wainstein[1,2,3,4], Márcio Mossmann[1], Gustavo N. Araujo[1,2,4], Sandro C. Gonçalves[1,2], Gabriela L. Gravina[4], Marlei Sangalli[4], Francine Veadrigo[4], Roselene Matte[2], Rejane Reich[2], Fernanda G. Costa[2], Michael Andrades[5], Antônio Marcos V. da Silva[6], Marcello C. Bertoluci[3,4,7].

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[1]Programa de Pós-Graduação em Medicina:Cardiologia; Universidade Federal do Rio Grande do Sul, Brazil; [2.]Serviço de Cardiologia do Hospital de Clínicas de Porto Alegre, Brazil; [3]Serviço de Medicina Interna do Hospital de Clínicas de Porto Alegre, Brazil; [4]Faculdade de Medicina da Universidade Federal do Rio Grande do Sul, Brazil; [5]Unidade de Análises Moleculares e de Proteínas (UAMP) do Hospital de Clinicas de Porto Alegre, Brazil; [6]Departamento de Fisioterapia e Reabilitação, Universidade Federal de Santa Maria, Brazil; [7]Programa de Pós-Graduação em Medicina:Clinica Médica da Universidade Federal do Rio Grande do Sul, Brazil

Corresponding Author:

Marcello C. Bertoluci, MD

Hospital de Clínicas de Porto Alegre - Ramiro Barcelos 2350, 90035-003.

Porto alegre, RS, Brazil.

E-mail: mcbertoluci@gmail.com

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ABSTRACT

Background: Interleukin-6 (IL-6) plays a central role in atherosclerosis and inflammation. It may improve risk prediction in patients at low to intermediate cardiovascular risk.

Objective: To analyze the impact of serum IL-6 in predicting early angiographic coronary artery disease in patients at low-intermediate cardiovascular risk with chest pain.

Methods: In a cross-sectional study, low-intermediate-risk patients referred for coronary angiography due to suspected coronary artery disease (CAD) were included. Coronary artery disease was defined as the presence of at least 30% stenosis in one or more coronary arteries. Severity of CAD was classified by the anatomic burden score. Performance of IL-6 was compared with ACC/AHA ASCVD risk score and hs-CRP through ROC curves.

Results: We have included 48 patients with a mean 10-year ASCVD risk of 10.0±6.8%. The prevalence of CAD was 72.9%. The presence of CAD was associated with higher mean levels of IL-6 (p=0.0252) Patients with CAD were significantly more overweighed than subjects without CAD. In 27% of the patients, IL-6 was >1.0 pg/ml and 100% of these patients had CAD, while only 64% in those with IL-6<1.0 pg/ml, corresponding to a positive predictive value of 1.0 (p=0.035). The area under the ROC curve of IL-6, hs-CRP and ASCVD were respectively 0.72, 0.60 and 0.54. Low-intermediate risk patients with IL-6>1.0 pg/ml were further reclassified as ASCVD high risk due to the presence of coronary lesions.

Conclusion: In low-intermediate risk patients referred for coronary angiography a serum

IL-6 level above 1 pg/ml is predictive of significant CAD.

KEY WORDS: Interleukin-6; Risk factors; Risk scores; Coronary angiography; Inflammation; Coronary Artery Disease

Running Title: Interleukin-6 and coronary disease

INTRODUCTION

Coronary artery disease (CAD) is the main cause of death globally, with an increasing prevalence in the Western world [1, 2]. The precision in estimating the risk for future CAD is crucial for treatment decision and prevention strategies. Improving accuracy in risk prediction is especially important among patients at intermediate cardiovascular risk, where calibration is limited with global risk score calculators [3]. The 2013 ACC/AHA guidelines currently suggest the use of 10 year ASCVD risk score to estimate the risk of future coronary events and to define strategies for primary prevention [4]. Although this approach is important, the precision in patients at intermediate coronary risk is less accurate than higher and lower risk strata [5].

The prevalence of coronary disease in patients who are referred for coronary artery angiography due to chest pain is usually high [6]. However, chest pain may have been managed with distinct approaches according to specific assistance protocols in different health care systems. In general, middle-aged non-diabetic individuals who have few cardiovascular risk factors are considered at intermediate-high risk, and the presence of chest pain in these patients poses an important challenge for the health care team. The incidence of false positives for coronary disease tends to be higher in this category than in patients at high-risk. Therefore, the use of emergent biomarkers may help in risk reclassification, being an interesting approach.

There is extensive evidence supporting a role of inflammatory response in the pathophysiology of acute coronary syndromes (ACS) and in the natural history of

atherosclerosis [7-9]. Cardiovascular events are more common in patients with high circulating levels of several inflammatory markers, and treating based on inflammatory parameters, such as hs-C reactive protein (hs-CRP), have been proved to reduce outcomes [10]. Interleukin-6 (IL-6), however, is a central mediator of the acute-phase response and a primary determinant of hepatic production of C-reactive protein (CRP) [11]. IL-6 is associated with increased incidence of myocardial infarction and mortality among patients with ACS [12, 13]. As hs-CRP has been classically linked to coronary events [14], it is reasonable also to address the role of IL-6, an even more precocious biomarker of inflammation than hs-CRP [15], in improving the detection of CAD and the severity of disease in this population.

The present study aimed to analyze the predictive value of IL-6 for diagnosing the presence of early CAD through coronary angiography in intermediate risk patients with chest pain. We hypothesized that IL-6 could improve the accuracy of traditional risk scores such as ASCVD in patients at intermediate risk.

METHODS

Study Design and Patients

We conducted a cross-sectional study with patients referred for coronary angiography due to non-acute chest pain in a reference cardiology center at Hospital de Clínicas of Porto Alegre, Brazil. Between 2013 and 2014 all patients referred to elective coronary angiography for non-acute chest pain were screened. Patients who fulfilled inclusion and exclusion criteria and accepted to participate were included in the study. All participants signed in the written informed consent from the local Ethics Committee that approved the study protocol.

Inclusion criteria were: age between 40 to 70 years old and chronic chest pain. We excluded patients with a known history of diabetes, previous acute coronary syndrome or stroke, previous coronary artery revascularization, estimated glomerular filtration rate below 45ml/min/1.73m^2, presence of class IV-NYHA congestive heart failure, chronic obstructive pulmonary disease, body mass index above 44 kg/m^2, previous organ transplantation, current evaluation for any organ transplantation and

those with presence of rheumatic, endocrine or infectious chronic diseases. We also excluded patients using any medication that could modify glucose-insulin metabolism such as insulin, metformin, sulfonylureas and patients using corticosteroids, HIV anti-retrovirals, carbamazepine, phenytoin, drugs for cancer, immunosupressor drugs, nitrofurantoin, anti-malarics, lithium and anti-psicotic drugs.

Biochemical investigation:

Blood samples were collected between 12-24h after coronary angiography. For IL-6 measurement, a custom Luminex® assay was employed (Invitrogen®, #LHB0001CM) following the manufacturer orientation. Briefly, 50 µL of undiluted sample was added in a well containing buffers and magnetic beads. After 2 hours of incubation (550 rpm, room temperature), the wells were washed and the detection antibody was added for further 1 hour. After washing, streptavidin-RPE was added for further 30 min, the wells were washed again and the beads were suspended in 125 µL of the wash buffer. Beads were read in Luminex® x-Map 200 and a minimum of 100 events were recorded for each bead. The limit of detection was defined by the lowest standard value (0.08, pg/mL). Values were expressed as pg/mL.

Serum high sensitive C-reactive protein (hs-CRP) measurements were determined through the turbidimetric immunoassay method (Roche®). Serum creatinine (Jaffé method), lipid profile, glycated hemoglobin (HPLC) were also measured.

ASCVD risk score

The atherosclerotic cardiovascular disease (ASCVD) risk score was calculated based on AHA/ACC 2013 guidelines to estimate the 10-year risk score for men and women from 40 to 79 years of age for a first hard ASCVD event. The variables to estimate each patient risk included age, gender, race, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, actual treatment for high blood pressure, diabetes and current smoking status [3]. The ASCVD calculator was obtained at: http://tools.acc.org/ASCVD-Risk-Estimator/. We considered intermediate risk when the 10 years score was between 7.5%-20%.

Coronary Artery Angiography Parameters

Coronary angiography was performed using the Axiom Artis Siemens® equipment (Germany) in all patients. Two experienced interventional cardiologists who were blinded to all other clinical variables made all angiographic measurements. Angiographic analyses were made by visual (non-quantitative) estimates of luminal narrowing in at least two different orthogonal projections.

The presence of CAD was defined as any lesion causing 30% or more in reduction of diameter stenosis in any epicardial vessel. We considered not significant coronary disease (NO CAD) when lesions were undetectable or below to 30% stenosis.

Severity of CAD was assessed through the "anatomic burden score" obtained from the COURAGE trial [16]. This score consisted in a grading scale of 17 progressive degrees of severity starting from zero, corresponding to complete absence of coronary disease with stenosis above 50%, to 17, which corresponds to severe 3-vessels disease, including lesions at proximal left anterior descending artery, plus left circumflex artery and right coronary involvement. To meet criteria, each lesion must represent at least 50% diameter stenosis. We divided patients into 3 groups: NONE for patients with zero score; MILD-MODERATE, corresponding to patients with score 1 to 5, corresponding to isolate lesions in right coronary artery, left circumflex artery and left anterior descending artery, increasing score in this order; and SEVERE, in patients with score between 6 and 17, corresponding to two-vessel and three-vessel artery disease.

Statistical analysis:

Continuous variables with parametric distribution were expressed as mean ± standard or error deviation, whereas non-parametric variables levels were expressed as median (95% confidence interval) and analyzed using Mann-Whitney's test. Categorical data were expressed as frequencies and their differences were analyzed using the chi-square test in the general characteristic table 1. ROC curves were used to evaluate the discriminatory power of IL-6 to determine CAD. The severity of CAD (Burden Score) analysis was performed through ANOVA with Bonferroni posttests. Comparison of ROC curves was performed by comparing area under the curves through trapezoid method

and C statistics. We used the following cut-offs values for analysis: IL-6: 1.0pg/mL, hs CRP and ASCVD score. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois).

RESULTS:

After exclusion criteria and missing at random losses, 48 patients were included in the study. The prevalence of CAD was 72.9%, with a mean age of the whole group was 57.1 (±7.1) years old. Groups CAD and NCAD were similar in respect of age, gender, smoking status, hypertension, metabolic syndrome, renal function, lipid profle, HbA1c, HOMA-IR and statin use. The CAD group had a higher prevalence of overweight and obesity (p = 0.001), a greater mean BMI (p = 0.037) and abdominal circumference (p= 0.059). Clinical characteristics of patients are shown in Table 1.

The presence of CAD was associated with higher mean levels of IL-6 (1.37 vs 0.29, p= 0.0252) (Fig. 1a), IL-6 >1.0 pg/mL was present in 12 (25%) of patients. According to anatomic CAD burden score, from a total of 48 patients included, 24 (50%) had zero score (NONE), 15 (31%) patients had score 1–5 (MILD–MODERATE), and 9 (19%) patients had score 6–17 (SEVERE) CAD. The SEVERE group had progressively increased IL-6 levels compared to both MILD–MODERATE (p = 0.013) and NONE groups (p = 0.007) (Fig. 1b)

Among 12 patients with high IL-6 levels (IL-6 >1.0 pg/ mL), all (100%) had CAD while 23 (63.8%) among 36 patients with IL-6 <1.0 pg/mL had CAD. (Chi-squared 5.943, p = 0.0148). The positive predictive value (PPV) in patients with IL-6 >1.0 pg/mL was 100%. The mean ASCVD risk score in 10 years of the entire group was 10.0 ± 6.8%, without differences between groups. According to the ASCVD score, among the 48 patients included, 21 (43.7%) were at a low-risk, 24 (50%) were at intermediate risk and 3 (6.2%) were at high-risk. Of the 45 patients at low-intermediate ASCVD risk score, 12 (26.6%) with IL-6 >1.0 pg/mL were re-classified into a high-risk category

The area under the receiver operating characteristic curve (AUC), compares the performance of IL-6 and ASCVD risk score and indicates increased accuracy with IL-6. Mean AUC for IL-6, ASCVD and hs-CRP were respectively: 0.74 (95% CI: 0.57–0.84), 0.54

(95% CI: 0.38–0.68) and 0.60 (95% CI: 0.44–0.74). This represents an increase of 38% in accuracy with IL-6 compared to ASCVD, while only 12% when hs-CRP is compared to ASCVD (Fig. 2)

DISCUSSION:

The present study indicates that in low-intermediate risk patients referred for coronary angiography a serum IL-6 level above 1 pg/ml is highly predictive of CAD. IL-6 is also an indicator of the severity of atherosclerotic disease. This preliminary study is, to our knowledge, the first to evaluate the association of high IL-6 to the burden of atherosclerotic disease in the intermediate-risk population.

Usually, hs-CRP levels are used to re-classify patients at an intermediate ASCVD risk. In this scenario, a single determination of IL-6 above 1pg/ml increased in 38% the accuracy of ASCVD score alone for detecting significant CAD, changing 30% of low-intermediate risk according to ASCVD score, to a higher risk condition due to the presence of CAD at angiography. In this regard, IL-6 had a better performance when compared to hs-CRP, as seen by the increment of the area under the curve in the ROC curve.

Previous studies have observed the association between IL-6 gene polymorphisms and coronary artery disease. A recent metanalysis [17] found associations between IL-6 levels and CAD severity, coronary events, mortality and progression to heart failure [12, 13, 18, 19]. Shirai *et al.* have shown that patients with pre-existing CAD had higher levels of IL-6 compared to patients without CAD, which were not affected by the pharmacotherapy used for treating CAD [20]. None of these studies, however, found a direct association between IL-6 levels and CAD extension.

Inflammation plays a pivotal role in atherosclerosis. Several inflammatory biomarkers have been extensively studied and have found to predict the development of CAD [14, 21, 22]. Remarkably, hs-CRP is the most related inflammatory biomarker with an increased risk of CAD development. IL-6, however, seems to be a more likely causal factor of CAD compared to hs-CRP [23, 24]. In our analysis, the area under the

ROC curve of IL-6 was larger than hs-CRP to predict CAD, indicating that it may be more accurate. Tis may be explained due to the fact that IL-6 plays an earlier and more central role in pro-inflammatory regulation process.

Among several mechanisms by which statins reduce cardiovascular outcomes, reducing inflammatory response is probably one of the most accepted. Oka et al. [25] have found that patients who received atorvastatin for 12 weeks had lower IL-6 levels compared to control group. Another study in patients with rheumatoid arthritis found that tocilizumab (a monoclonal antibody that competitively inhibits IL-6 receptor) improved endothelial dysfunction in patients receiving this drug [26]. These are all indirect evidence suggesting that IL-6 plays a role in the development of CAD.

Another important finding in our study was the strong positive predictive value of high IL-6 levels to detect the presence of CAD in patients referred to coronary angiography. Our data suggest that a high IL-6 serum level in non-diabetic overweight patients, at intermediate ASCVD risk can be useful to indicate a higher risk condition and the need for a more invasive approach. Lindmark et al. [27] have tested this hypothesis in patients with unstable coronary artery disease and found that high circulating IL-6 identifies patients who benefit most from a strategy of early invasive management. Differently than IL-6, hs-CRP has a strong negative predictive value in patients with chest pain in the emergency room [28]. Testing the combination of these two inflammatory markers could bring interesting results.

An important strength of the present study was the highly homogeneous intermediate-risk population obtained from an otherwise presumed high-risk population, which resulted in a high powered study ($\beta=0.93$, $\alpha < 0.05$, two-tailed). Tis allowed conclusions regarding the impact of high IL-6 levels in a lower risk population usually presenting to coronary angiography. Although we have not studied the occurrence of clinical cardiovascular outcomes, there is evidence demonstrating that minor degrees of coronary stenosis (such as 20%) may be predictive of long-term mortality, when compared to the absence of any epicardial coronary stenosis [29].

The main limitations of the present study are the small sample of 48 patients and its cross-sectional design, which does not allow cause-and-effect relationship

inferences, as it may suffer impact from covariates not measured in the study. We believe, however, that the high power obtained with a small sample may be an indication that IL-6 may become an important predictor for CAD in future larger longitudinal studies.

In conclusion, an elevated IL-6 above 1 pg/mL in intermediate cardiovascular risk population submitted to coronary angiography may be highly predictive of CAD, being associated with the burden of atherosclerosis. IL-6 may be a useful biomarker for detecting signifcant CAD and to reclassify patients at intermediate ASCVD risk score into a higher risk category. Further long-term clinical trial with IL-6 studies are than warranted to confirm these findings.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Baseline characteristics of patients with and without coronary artery disease.

CHARACTERISTIC	**NCAD (N=13)**	**CAD**	***P***

		(N=35)	
Male sex(%)	4(30.7)	17(48.5)	0.29
Age (years)	59.6±6.0	56.9±7.5	0.24
BMI (kg/m^2)	24.52±4.93	27.92±4.64	0.03
BMI>25(%)	4(30.7)	28(82.3)	0.001
Smoking (%)	6(46.1)	8(22.8)	0.13
AC(cm)	88.23±11.78	95.59±8.71	0.05
Presence of hypertension (%)	7(53.8)	9(25.7)	0.25
Metabolic Syndrome n (%)	3(23.0)	15(44.1)	0.18
ASCVD Risk Score (mean)	10.58±7.62	9.72±6.54	0.70
Previous AMI	1(7.69)	5(14.2)	0.54
Systolic Blood Pressure (mmHg)	134.94±16.60	138.87±22.52	0.57
Diastolic Blood Pressure (mmHg)	73.08±16.53	80.80±11.69	0.08
Serum Creatinine (mg/dl)	0.68±0.12	0.81±0.22	0.05
UA/Ucre (mg/g)	13.5±24.5	24.9±39.8	0.16
Fasting Plasma Glucose (mg/dl)	93.31±10.59	92.82±8.8	0.87
HbA1c (%)	5.60±0.37	5.68±0.33	0.49
Fasting Insulin (µU/mL)	9.21±4.20	12.04±6.64	0.13
HOMA-IR	2.18±1.16	2.84±1.65	0.25
Total Cholesterol (mg/dl)	173.21±63.13	179.97±39.19	0.66
HDLc (mg/dl)	49.62±11.4	43.39±10.6	0.09
Triglycerides (mg/dl)	107.15±56.64	132.79±82.84	0.31
US-CRP (mg/L)	4.59±5.74	5.74±6.01	0.56
Alanina Transferase (ALT) (mg/dl)	19.15 ±3.80	24.82±26.54	0.45
On ASA(%)	6(46.1)	22(70.9)	0.19
On Statin(%)	7(53.8)	24(77.4)	0.09

NCAD patients without coronary artery disease. CAD patients with coronary artery disease. UAlb/Ucre was the ratio between urinary albumin concentration and urinary creatinine concentrations. Data are expressed as mean ± SD for all continuous and parametric variables. UAlb/Ucre (mg/g) data were expressed as median and 95% confdence interval BMI body mass index (Kg/cm2), ASCVD atherosclerotic cardiovascular disease, AMI acute myocardial infarction, HbA1c glycated hemoglobin, HOMA-IR homeostasis model assessment resistance, HDLc high density lipoprotein cholesterol (mg/dL), GPT alanine aminotransferase, ASA acetyl salicilic acid, Ins insulin, HbA1c glycated haemoglobin, hs-CRP high-sensitive C-reactive protein (mg/L)

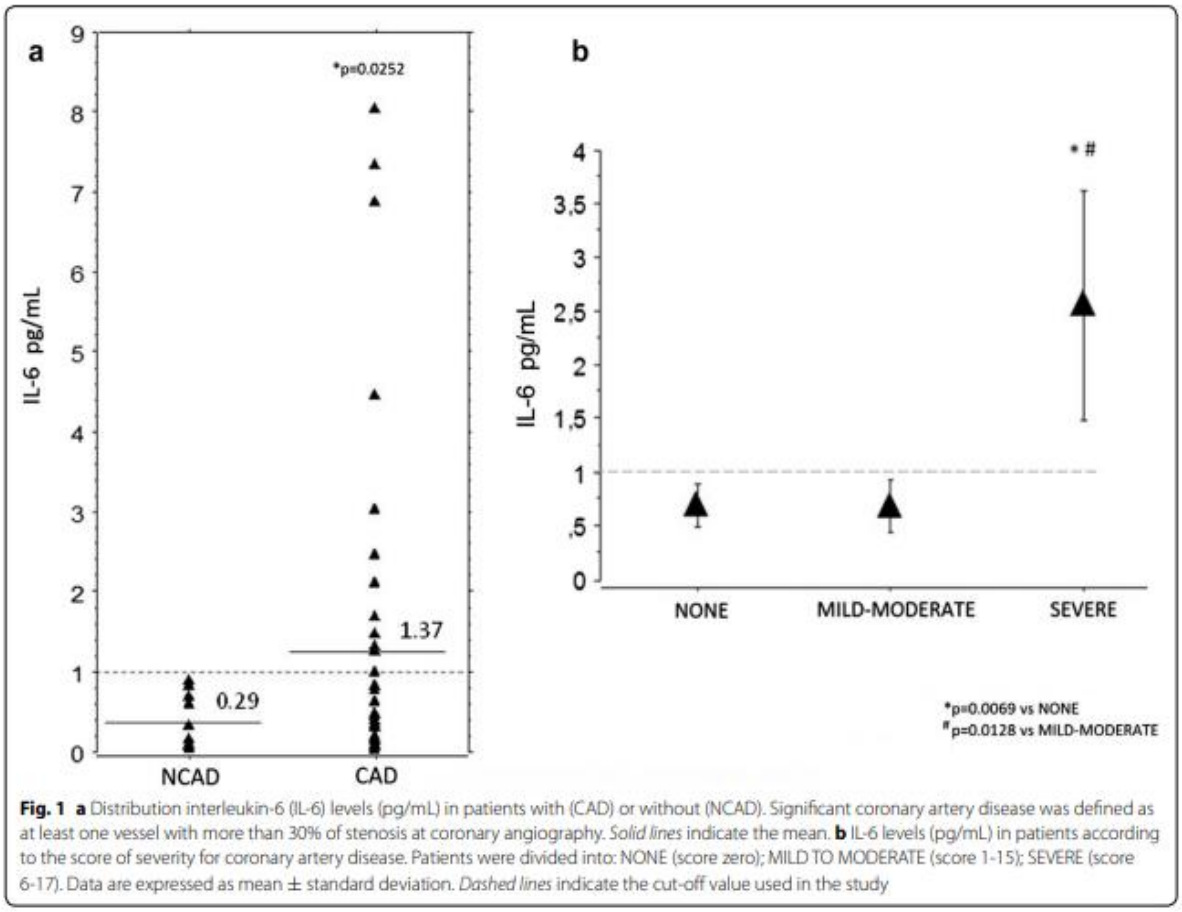

Fig. 1 **a** Distribution interleukin-6 (IL-6) levels (pg/mL) in patients with (CAD) or without (NCAD). Significant coronary artery disease was defined as at least one vessel with more than 30% of stenosis at coronary angiography. *Solid lines* indicate the mean. **b** IL-6 levels (pg/mL) in patients according to the score of severity for coronary artery disease. Patients were divided into: NONE (score zero); MILD TO MODERATE (score 1-15); SEVERE (score 6-17). Data are expressed as mean ± standard deviation. *Dashed lines* indicate the cut-off value used in the study

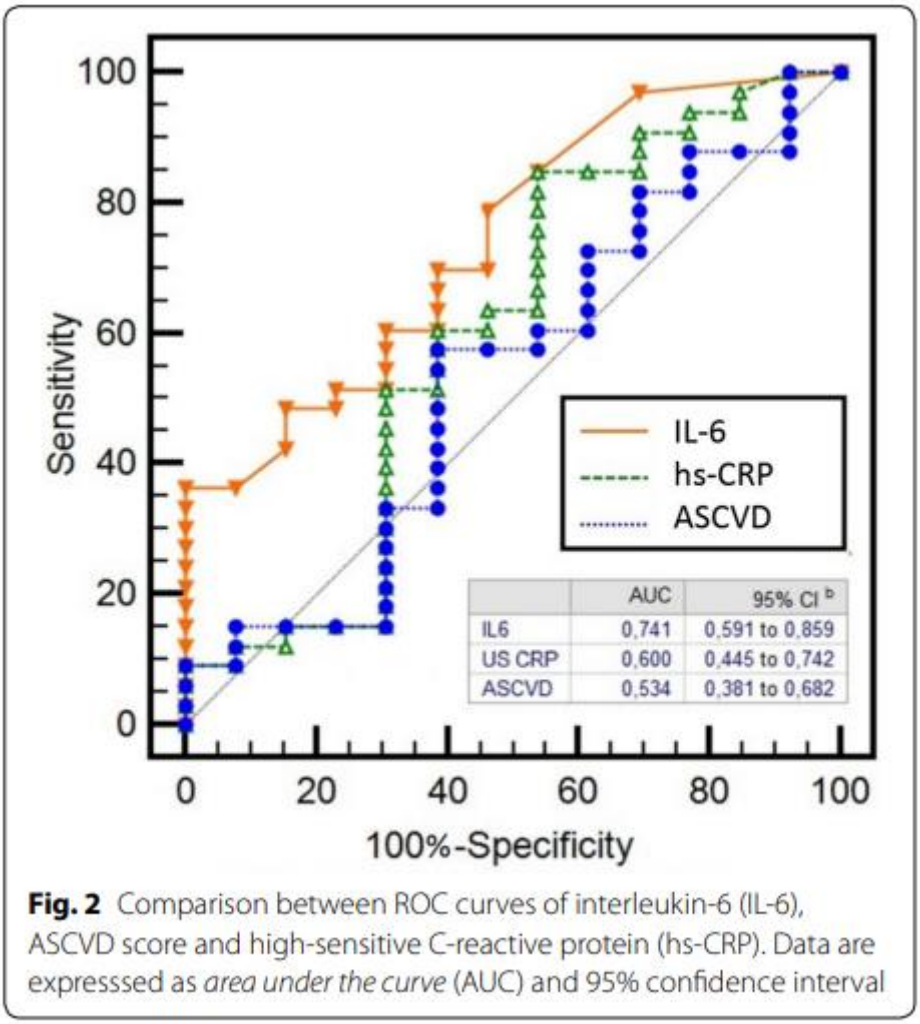

Fig. 2 Comparison between ROC curves of interleukin-6 (IL-6), ASCVD score and high-sensitive C-reactive protein (hs-CRP). Data are expresssed as *area under the curve* (AUC) and 95% confidence interval

ARTIGO 2 PARA REVISTA DIABETOLOGY AND METABOLIC SYNDROME

Increased serum IL-6 is predictive of long-term cardiovascular events in high-risk patients submitted to coronary angiography: an observational study

Márcio Mossmann MD, MSc[1]; Marco Vugman Wainstein MD, PhD[1,2,4]; Stéfani Mariani MD[1]; Guilherme Pinheiro Machado MD, MSc[1]; Gustavo Neves de Araújo MD, PhD[1]; Michael Andrades[5], Sandro Cadaval Gonçalves MD, PhD[1,2]; Marcello Bertoluci MD, PhD[3,4]

ABSTRACT

BACKGROUND: Interleukin-6 (IL-6) is an inflammation-related cytokine associated with an elevated risk of cardiovascular events. In a previous study, we demonstrated that increased IL-6 was predictive of sub-clinical atherosclerotic coronary disease in intermediate-risk patients undergoing coronary angiography. In the present study, we investigated whether increased serum IL-6 is predictive of cardiovascular events in high-risk patients.

METHODS: In this observational study, consecutive patients referred for elective coronary angiography due to stable chest pain/myocardial ischemia had IL-6 measured immediately before the procedure. Long-term follow-up was performed by phone call or e-mail, and their clinical registries were revised. The primary outcome was a composite of new myocardial infarction, new ischemic stroke, hospitalization due to heart failure, new coronary revascularization, cardiovascular death, and death due to all causes.

RESULTS: From the 141 patients submitted to coronary angiography and IL-6 analysis, 100 had complete follow-up data for a mean of 5.7 years. The median age was 61.1 years, 44% were men, and 61% had type-2 diabetes. The median overall time-to-event for the primary outcome was 297 weeks (95% confidence interval [CI] = 266.95–327.16). A receiver operator characteristic curve defined the best cut-off value of baseline serum IL-6 (0.44 pg/mL) with sensitivity (84.37%) and specificity (38.24%) to define two groups. High (>0.44 pg/mL) IL-6 levels were predictive of cardiovascular events (*p* for interaction = 0.015) (hazard ratio = 2.81; 95% CI = 1.38–5.72, p=0.01). The subgroup analysis did not find interactions between patients with or without diabetes, obesity, or hypertension.

CONCLUSION: In conclusion, levels of interleukin-6 higher than 0.44 pg/mL obtained just before the coronary angiography for elective reasons were associated with a poorer prognosis after a mean of 5,7-year. A pre-procedure IL-6 below 0.44 pg/ml, on the other hand, has a very good negative predictive value, indicates a good prognosis, and may be useful to better indicate coronary angiography in high-risk patients.

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Keywords: Interleukin-6, coronary artery disease, diabetes, high-sensitive C-reactive protein, inflammation**.**

BACKGROUND

Interleukin-6 (IL-6) is an acute-phase protein that plays a significant role in the inflammatory response, vascular inflammation, and atherosclerosis process [1]. It contributes to the remodeling of connective tissue by increasing metalloproteinase gene expression [2]. Focal overexpression of activated metalloproteinase may promote destabilization and degradation of the plaque's fibrous cap, leading to plaque instability

during the atherosclerotic process [3]. In a study including patients with unstable coronary artery disease (CAD), very high IL-6 levels (>5 pg/mL) were strongly associated with mortality, which was independent of many risk factors, including age, sex, diabetes, previous myocardial infarction (MI), and high cholesterol levels [4]. In a nested case-control study of patients with previous MI, the risk of future MI increased progressively with increasing quartiles of baseline IL-6 concentration [5].

IL-6 is also predictive of cardiovascular events in patients with stable coronary disease. In a sub-study from the *Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy Trial* (STABILITY), higher levels of IL-6 were independently associated with the risk of major adverse cardiovascular events, cardiovascular and all-cause mortality, MI, heart failure, and cancer mortality [6]. Recently, our group demonstrated an association between serum IL-6 concentrations and subclinical CAD, in which high levels of serum IL-6 level (>1 pg/mL) were predictive of coronary stenosis ≥ 30% in intermediate-risk patients referred for coronary angiography [7].

Although there is a growing body of evidence associating IL-6 with cardiovascular disease, most are indirect observations from case-control studies or sub-group analyses. IL-6 has been inadequately studied prospectively concerning its predictive value. Much less is known about the role of IL-6 in patients referred for elective coronary angiography. Moreover, obesity and non-cardiovascular inflammatory diseases considerably interfere with serum IL-6 levels, which may lead to a confusing bias. In the present study, we aimed to prospectively analyze the impact of higher and lower levels of IL-6 on cardiovascular events in patients with high or very high cardiovascular risk, excluding severely obese patients and patients with previously known inflammatory conditions.

METHODS

Study design

This is an observational study divided into two phases: an initial cross-sectional phase and an observational prospective cohort phase. The inclusion period was from October 2012 to August 2016. We screened potential participants who were referred to the cardiology division catheterization laboratory (Cath Lab) of Hospital de Clínicas, a large tertiary care university hospital in southern Brazil.

We considered for inclusion every patient referred for elective coronary angiography due to non-acute chest pain or chronic myocardial ischemia confirmed by non-invasive investigation and who did not have any exclusion criteria (figure 1).

Inclusion and exclusion criteria were checked immediately before the procedure by the investigators through a hospital registry review and direct personal interview. If the patients were eligible and agreed to participate in the study, a signed consent, anthropometric data, and a fasting blood sample for IL-6 and blood chemistry were obtained, and blood pressure was measured in the sitting position. Tis study was approved by the Institutional Research Ethics Committee (IRB number 18-0221).

After the procedure, patients were discharged from the unit and were referred to their respective assistant physicians. From March 2020 to August 2020, all patients were contacted by one of the investigators through multiple phone calls and e-mail, and their hospital and city obituary registries were reviewed.to obtain the most recent clinical information available.

Inclusion and exclusion criteria

We selected patients between the ages of 30–80 years with were suspected of CAD due to a history of chronic chest pain or stable myocardial ischemia confirmed through a non-invasive test. Considering current guidelines and clinical trials [8], in our practice, we indicated coronary angiography and stenting for patients with large areas of ischemia; left main disease on coronary-CT; reduced ejection fraction, and/or three-vessel disease. We considered high-risk patients defined by the presence of at least 3 traditional risk factors, or the presence of sub-clinical atherosclerosis (less than 50% of stenosis) presented at coronary angiography. Patients with previous myocardial infarction events, coronary revascularization, or more than 50% coronary stenosis were considered at very high risk.

We excluded patients with known congestive heart failure, class-IV New York Heart Association, recent acute coronary syndrome, defined as any confirmed acute coronary syndrome occurring in coronary in the last 60 days; clinically significant renal disease defined as a glomerular filtration rate of less than 45 mL/min/1.73 m^2; any known inflammatory conditions such as chronic pulmonary obstructive disease confirmed by thorax X-Ray and spirometry; known active chronic infectious diseases such as tuberculosis (defined by typical lesion on thorax X-ray), HIV infection (detected by antibody against HIV), chronic rheumatic disease (rheumatoid arthritis and systemic erythematous lupus detected by antinuclear antibodies); chronic B or C hepatitis (detected by HBsAg and HCV antibodies), hyper or hypothyroidism determined by a low or high TSH (less than 0,01mUI/L or above 5 mUI/L), a history of any organ transplantation or patients undergoing evaluation for transplantation, and any known type of cancer. We also excluded severely obese patients with a body mass index (BMI)

> 35 kg/m^2 and those taking medications that might interfere with the inflammatory status of the patient, such as corticosteroids, HIV-antiretroviral, carbamazepine, phenytoin, any drug for cancer, immunosuppressant, nitrofurantoin, anti-malaria, lithium, and anti-psychotic drugs. We did not exclude patients with diabetes or hypertension.

Coronary artery angiography procedure

Coronary angiography was always performed in the morning, in the fasting state. We used the Axiom Artis Siemens® equipment (Germany) in all patients. Two experienced interventional cardiologists, who were blinded to all other clinical variables, made all the angiographic measurements. Angiographic analyses were made by visual (non-quantitative) estimates of luminal narrowing in at least two different orthogonal projections. The presence of CAD was defined as any lesion causing >30% reduction in the diameter of an epicardial coronary artery.

Clinical and biochemical investigation

Blood Pressure Measures

Baseline blood pressure was measured at the Cath Lab before coronary angiography, after 15 min of rest, in the sitting position, in the right arm. Three sequential measurements were made using an automatic aneroid sphygmomanometer (OMRON Comfort III Visomat Incoterm, Germany). We considered the lowest blood pressure reading as the final measure.

IL-6 measures

Blood samples were collected at the Cath Lab just before the beginning of coronary angiography. For serum IL-6 measurement, a custom Luminex® assay was employed (Invitrogen®, #LHB0001CM) following the manufacturer's instructions. Briefly, 50 µL of the undiluted sample was added to wells containing buffers and magnetic beads. After 2 h of incubation (550 rpm), the wells were washed and the detection antibody was added further for 1 h. After washing, streptavidin-phycoerythrin was added further for 30 min, the wells were washed again, and the beads were suspended in 125 µL of wash buffer. Beads were read in Luminex® x-Map 200, and a minimum of 100 events was recorded for each bead. The limit of detection was defined as the lowest standard value (0.08, pg/mL). Values are expressed as pg/mL.

Other assays

Blood samples for high-sensitivity C-reactive protein (hs-CRP) were also collected simultaneously and aliquoted. Serum hs-CRP levels were determined using the turbidimetric immunoassay method (Roche®). Serum creatinine (Jaffé method), lipid profile, glycated hemoglobin (high-performance liquid exchange chromatography), and glucose (colorimetric assay) were also measured.

Follow-up phase

After discharge, all included patients were contacted through phone calls by one of the investigators from March to August 2020, following a specific protocol. Patients were required to confirm their clinical outcomes through medical registries. In-hospital registries were also obtained from those who continued to visit the hospital. Information regarding death was confirmed by a family member who attended the call, and their city obituary data were confirmed. Patients who could not be contacted after

several attempts and had no further clinical hospital registry information after discharge were considered missing at random.

Outcomes

We analyzed a composite of six cardiovascular outcomes: 1. new acute coronary syndrome or MI, according to the Universal Definition of Myocardial Infarction updated, including unstable angina; 2. hospitalization due to heart failure, with elevated levels of B-type natriuretic peptide (BNP) and/or NYHA class of symptoms III or IV; 3. Hospitalization due to ischemic stroke, with new-onset neurological deficit with corresponding imaging lesion on CT or MRI scan; 4. New coronary revascularization, new symptoms with positive non-invasive ischemia assessment; 5. Cardiovascular death, and 6. Death due to all causes. Only the first event after the coronary angiography was considered.

Time-to-event was expressed in weeks and confirmed using medical records and phone calls to the patients. The follow-up period was defined as the period between the date of discharge from the Cath Lab and the date of the first outcome reported or the last contact if no outcomes occurred.

Statistical analysis

Continuous variables were expressed as mean ±standard deviation [SD]) or median (interquartile range) based on their symmetrical or asymmetrical distribution, respectively. The normality of the distribution of each variable was assessed using the Shapiro-Wilk test. Categorical variables were represented by their relative and absolute frequencies.

Patients were divided according to their baseline IL-6 levels into 2 groups – above and below 0.44 pg/mL. This cut-off value was chosen from the ROC curve analysis

as the best cut-off point for sensitivity and specificity. Patient groups were compared using the independent samples Student's t-test or Kruskal-Wallis test, as appropriate, for continuous variables and Fisher's exact tests for categorical variables. The Kaplan-Meier analyses and comparison using the log-rank test were performed using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium). All remaining statistical analyses were conducted using SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA).

The sample size was based on a previous paper from our group [7] in which we compared IL-6 in individuals with and without coronary artery disease. In that study, we observed IL-6 serum levels of 1.37 pg/mL in the group with coronary artery disease and IL-6 0.29 pg/mL in the non-CAD group, a difference of 1.08. In the present study, as all patients had coronary artery disease we arbitrarily decided to considerer a smaller difference between groups (0.5 pg/mL). To accept an alpha error of 0.05 we estimated a sample size of 100 patients, which would generate power of 0.73.

All patients provided written informed consent (the ethics committee of the Hospital de Clínicas approved the study protocol).

RESULTS

A detailed flowchart of the inclusion process is depicted in **Figure 1**. A total of 4792 cardiac catheterizations were performed at the Cath Lab between October 2012 and August 2016. During this period, 141 patients were selected according to the inclusion and exclusion criteria. Of these, 100 were analyzed with complete follow-up data.

Baseline clinical and anthropometric characteristics are shown in **Table 1**. Forty-four percent were men and the median age of all patients was 61.1 years. CAD,

that is > 30% stenosis, was present in 81% of the patients and was similar between both sub-groups of IL-6. No one patient had left main coronary disease. There were a higher proportion of patients with more severe coronary disease, including two-vessel and multi vascular disease, in the higher IL-6 group. The proportion of sub-clinical coronary atherosclerosis was similar between both IL-6 groups. There was also a higher proportion of patients with hypertension and type-2 diabetes (T2DM) and a trend toward a higher proportion of obese patients in the group with higher levels of IL-6. The mean hs-CRP level, as expected, was increased in the group with higher levels of IL-6.

The median overall time-to-event for the primary outcome was 297 weeks (95% confidence interval [CI] = 266.95 - 327.16). The outcomes are shown in detail in **Table 2**. Overall, 32 cardiovascular events occurred during the follow-up period, being 26 in the higher IL-6 group and 6 in the lower IL-6 group. **Figure 2** shows the Kaplan-Meier curves with the hazard ratios (HRs) of primary outcome between high and low IL-6 groups. There was a significantly higher cumulative incidence of the primary outcome during the follow-up period in the group of patients with increased baseline IL-6 (HR = 2.81; 95% CI = 1.38. 5.72, *p* for interaction = 0.015).

The area under the ROC curve of IL-6 for the combined outcome was 0.585 (95% CI = 0.482 – 0.683; *p* = 0.156; **Figure 3**) with a sensitivity of 81.25 (95% CI = 63.6– 92.8), specificity of 38.24 (95% CI = 26.7 – 50.8), positive predictive value of 38.2 (95% CI = 26.7 – 50.8), negative predictive value of 81.2 (95% CI = 63.6 – 92.8) and a power of 0.73.

To examine if IL-6 correlates with cardiovascular risk, we considered hypertension, type 2 diabetes, smoking, male gender, age above 60 years, and the presence of CHD as isolated risk factors and correlated with IL-6. As IL-6 has a skewed

distribution we used the Spearman correlation test. We found a Spearman rho of 0.428 with a p-value <0.0001, indicating a definite correlation between cardiovascular risk and IL-6 levels here (data not shown).

Although the proportion of patients with T2DM was higher in the group with higher IL-6 levels **(table 1),** a sub-group analysis between patients with and without T2DM examining the presence or absence of the primary outcome **(Figure 4)** did not show any interaction. Moreover, the subgroups analysis of patients with and without hypertension and with or without BMI>30kg/m^2, regarding cardiovascular outcomes, did not show interaction as well.

DISCUSSION

The present study shows that serum IL-6 is predictive of long-term cardiovascular events in symptomatic patients with stable coronary disease who have a high cardiovascular risk. Serum IL-6 measurements above 0.44 pg/mL increased the risk of cardiovascular events by 2.8 times. Although there was an increased proportion of T2DM, hypertension, and obesity in the group with increased levels of IL-6 there was no interaction among these variables considering the primary outcome. Although the correlation between IL-6 and coronary events is widely known, this study highlights its strong negative predictive value, with potential clinical application in indicating coronary angiography in high-risk patients.

A previous study based on the analysis of two population-based cohorts [10] suggested that circulating serum IL-6 levels could be associated with increased coronary risk (defined as nonfatal MI or fatal coronary heart disease [CHD]). In that study, stored blood samples of patients who later developed non-fatal MI or died of CHD were used

for baseline measurements. Patients who developed CHD had greater levels of IL-6 compared with controls with no history of CHD. The odds ratio for CHD, adjusted for several established risk factors, was 1.46 (95% CI = 1.29 – 1.65) per 2 SDs of increase in baseline IL-6 values.

IL-6 has been associated with increased cardiovascular risk in some populations. In a meta-analysis of 29 population-based prospective studies [11], the adjusted relative risk for non-fatal MI or CHD death was 1.25 for every point of higher baseline SD in IL-6. However, in this meta-analysis, there was considerable heterogeneity (I^2=53.6%, p=0.001), and not all studies included indicated a clear risk prediction for IL-6. One possible reason is that many co-variables may have impacted the results in some studies.

In the present study, we observed that the predictive cut-off value of IL-6 was relatively lower (0.44 pg/mL) compared to that in other studies. In the sub-analysis of the STABILITY trial [6], the risk of cardiovascular death and major adverse cardiovascular events in 3–4 years started to increase progressively when IL-6 levels were above 1.5 ng/L. In the FRISC II trial [4], the highest predictive value was obtained when IL-6 levels were above > 5 pg/mL. We attribute our findings to the fact that we were able to exclude patients with chronic inflammatory diseases and severe obesity, which are major confounders when studying sub-clinical vascular inflammation as there is a strong relationship *(rho*=0.85; p<0.00001) between IL-6 levels and BMI [12]. We believe that the strict selection criteria improved the accuracy of IL-6, also increasing the negative predictive value.

There is a clear plausibility for IL-6 levels to be associated with a higher risk of cardiovascular events. Experimental studies indicate that vascular endothelial and smooth muscle cells from normal and aneurysmal arteries can produce IL-6 [13,14].

Moreover, IL-6 gene transcripts are expressed in atherosclerotic lesions [15], confirming local production. IL-6 has procoagulant effects [16], and elevated levels have been reported among patients with acute coronary syndromes [17]. Considering that atherosclerosis is a chronic inflammatory disorder, IL-6 levels are expected to be increased among individuals with sub-clinical atherosclerosis who are at greater risk for future MI. However, a cause-and-effect relationship between IL-6 and cardiovascular events cannot be clearly defined so far, as few randomized trials are targeting IL-6 treatment.

In the *Canakinumab Anti-inflammatory Thrombosis Outcome Study* (CANTOS), a randomized, double-blind, placebo-controlled trial involving stable patients with previous myocardial infarction, the human monoclonal antibody, canakinumab, that targets the interleukin-1β innate immunity pathway, led to a significant decrease of recurrent cardiovascular events compared to placebo, independently of lipid-level lowering [18]. In this trial, patients had a clinical atherosclerotic disease and a high mean baseline IL-6 (2,54 pg/mL), consistent with an inflammatory state. After 48 months, canakinumab 150 mg reduced significantly the relative event rate by 15%. In a sub-analysis of the CANTOS trial (19) patients who achieved IL-6 levels lower than 1.65pg/mL experienced a 32% reduction in cardiovascular events, a 52% reduction in cardiovascular mortality, and a 48% reduction in all-cause mortality. These results suggest the existence of a clear association between lower levels of IL-6 and reduced incidence of cardiovascular events.

Despite the substantial IL-6 fall seen in the CANTOS study, the mean IL-6 levels at 3 months seen in that study, continued high even in the group with the lowest IL-6 (1.93 (1.45-2.63 pg/mL). The mean IL-6 achieved in the present study, however, was

much lower than in CANTOS. In our study, the mean IL-6 in the group with low levels was 0.09 pg/mL (95% CI 0.04-0.14) and was associated with a low incidence of cardiovascular events.

In the present study, because we selected patients excluding most common inflammatory confounders that could raise IL-6, we were able to include patients with even normal levels of IL-6, despite the established coronary disease. This allowed us to evaluate the negative predictive value of IL-6. We observed that levels of IL-6 below 0.44pg/mL were predictive of a better prognosis in 82%, an indication of a lower inflammatory status seen in that group. This may be a useful tool to evaluate the cardiovascular risk of candidates to coronary angiography.

The first limitation of this study is the sample size. Thus, the borderline difference in combined outcomes found between groups may be subject to beta error. Yet, the high-risk patients and long follow-up with a considerable incidence of combined outcomes may have balanced the small sample size. Second, as an observational study, we could not confirm a cause-effect relationship between IL-6 levels and the incidence of cardiovascular events. It is also possible that IL-6 may be a marker of cardiovascular disease rather than a risk factor per se. However, the study confirms the strong body of literature on this subject and highlights IL-6 applicability in a specific context. Third, we were not able to make adjustments to covariates due to the relatively low incidence of cardiovascular events. Nonetheless, this is a sample of patients at high cardiovascular risk that frequently has many comorbidities.

CONCLUSIONS:

In conclusion, levels of interleukin-6 higher than 0.44 pg/mL obtained just before the coronary angiography for elective reasons were associated with a poorer prognosis after a mean of 5,7-year. A pre-procedure IL-6 below 0.44 pg/ml, on the other hand, has

a very good negative predictive value, indicates a good prognosis, and may be useful to better indicate coronary angiography in high-risk patients.

Abbreviations:

CAD: coronary artery disease

IL-6: interleukin-6

MI: myocardial infarction

Cath Lab: catheterization laboratory

T2DM: type-2 diabetes mellitus

CI: confidence interval

HR: hazard ratio

CHD: coronary heart disease

SD: standard deviation

ROC: Receiver operator characteristic

CT: Computed Tomography

MRI: Magnetic Resonance Imaging

CKD: Chronic Kidney Disease

DECLARATIONS:

Ethics approval and consent to participate:

All patients provided written informed consent, and the ethics committee of the Hospital de Clínicas approved the study protocol.

Competing interests:

The authors have no conflicts of interest to declare and report no financial relationships regarding the content.

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Availability of data and materials: The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication:

not applicable

Authors' contributions:

SM, MM, GNA and SCG recruited patients and collected data; GPM analyzed statistical data; MM, MVW and MCB wrote the original manuscript, MVW and MCB raised funds, and MCB reviewed the last form of the manuscript.

Authorship declaration:

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors agree with the manuscript.

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FIGURE LEGENDS

Figure 1 – Flowchart of the inclusion process

Figure 2 – Time-to-event curves for composite outcome according to IL-6 levels

Event rates are calculated with the use of Kaplan-Meier methods and compared with the use of the log-rank test.IL-6: interleukin-6

Figure 3 – Receiver operator characteristic (ROC) graph showing areas under the curve IL-6 for the composite outcome IL-6: interleukin-6

Figure 4 – Forrest plot of subgroup analysis for the presence or absence of T2DM, hypertension, and obesity T2DM: type-2 diabetes mellitus

Figure 1. Flowchart

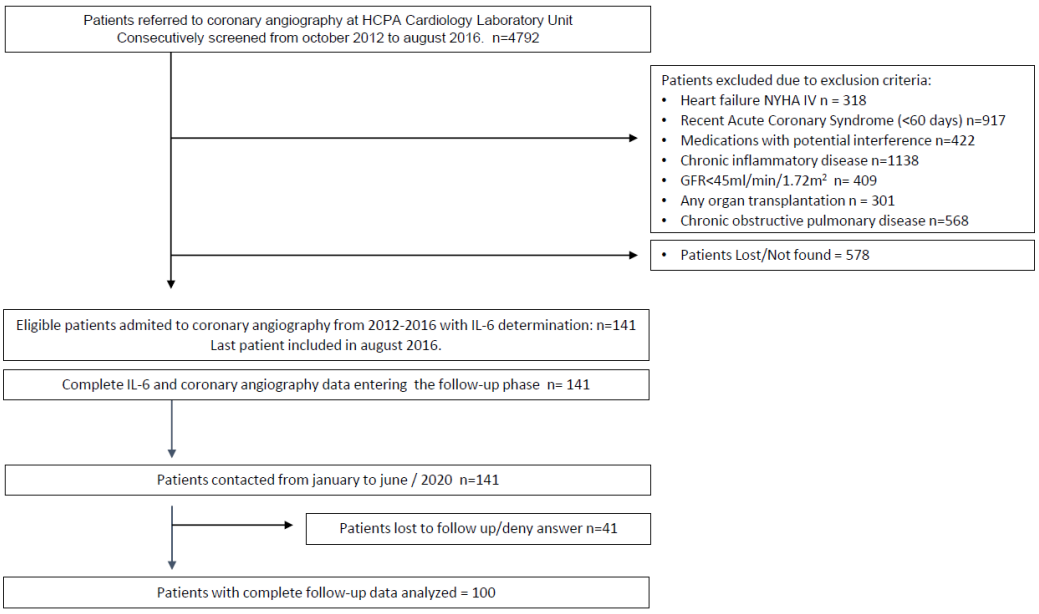

Figure 2.

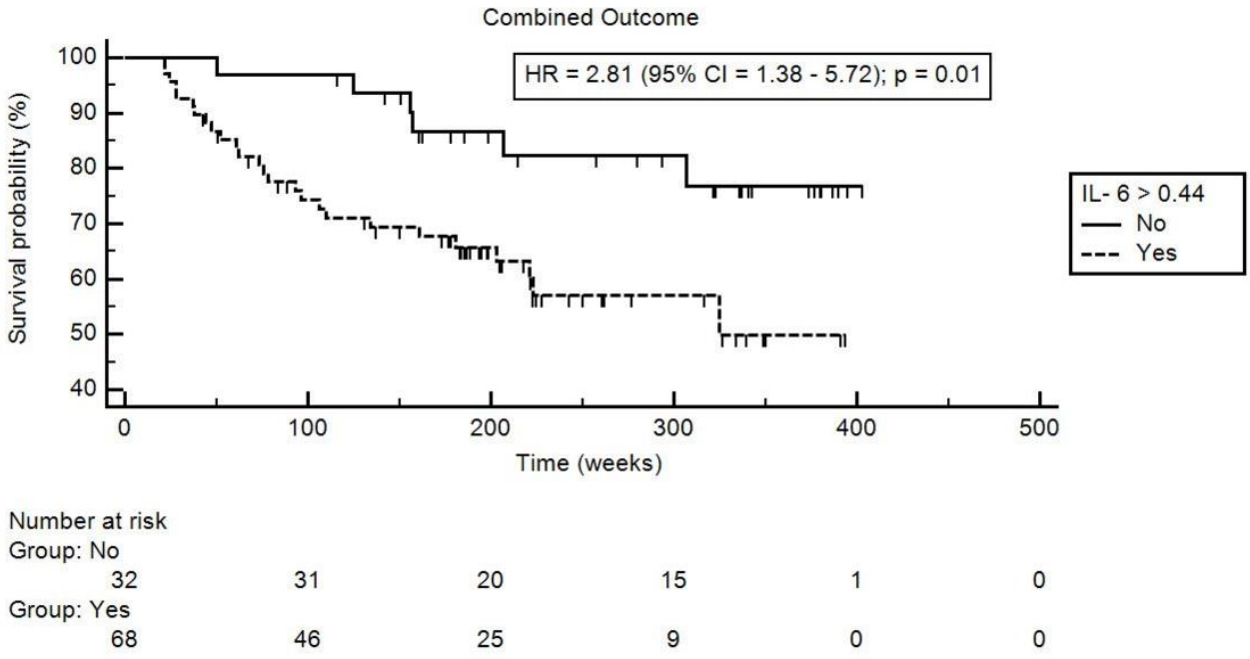

Figure 3.

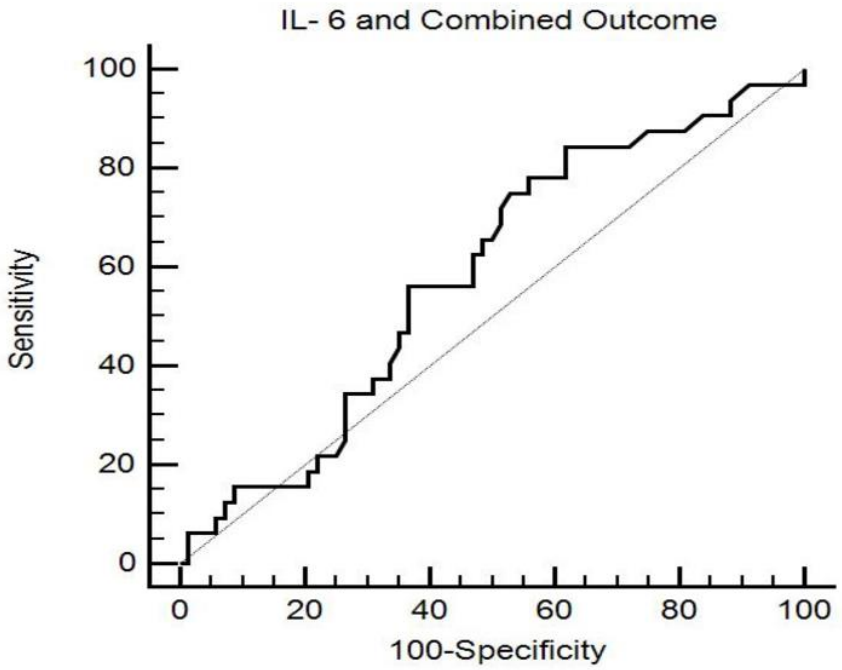

Figure 4.

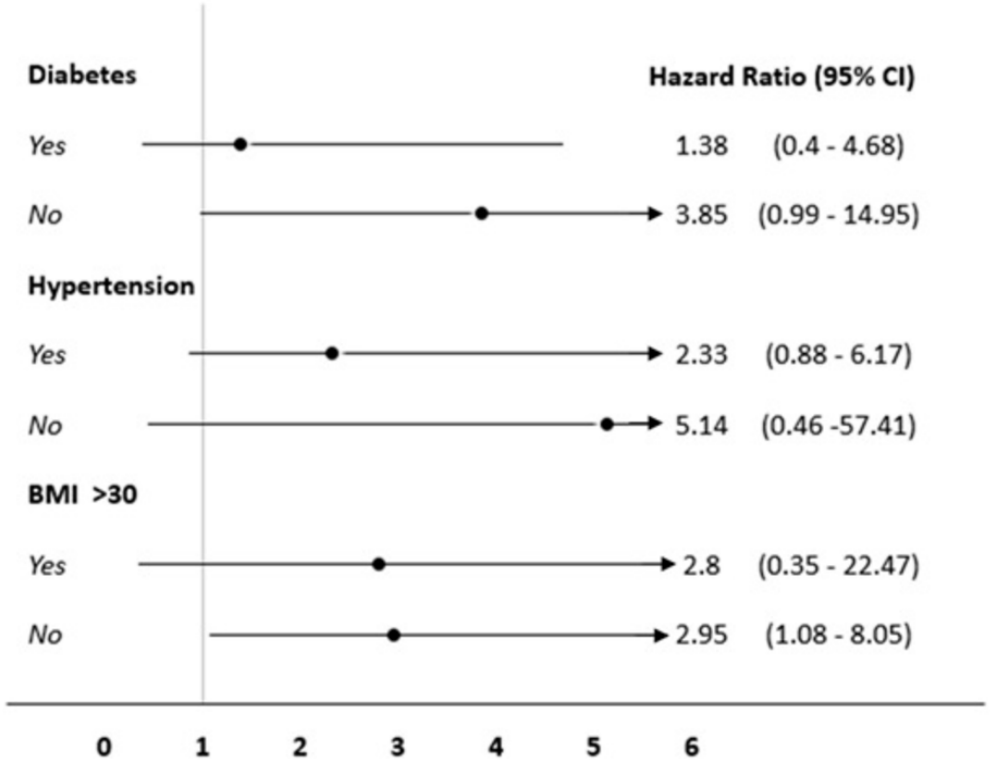

Table 1 - Overall baseline characteristics according to IL-6 group.

	Overall	**IL-6 ≤ 0.44**	**IL-6 > 0.44**	*p*
N	100	32	68	
Age (years)	61.1 (8.7)	58.4 (7.1)	62.8 (9.2)	
Male	44 (44.0)	11 (34.4)	34 (50.0)	0.196
BMI (kg/m^2)	28.7(5.6)	27.1 (5.4)	29.5 (5.6)	0.053
AC (cm)	100.7 (14.3)	95.7 (12.1)	103.1 (14.7)	0.015
SBP (mmHg)	140(22)	136.4 (20.7)	142.1(23.2)	0.236
DBP (mmHg)	76.9 (12.6)	75.1 (10.40)	77.8 (13.5)	0.328
% of hypertension	85 (85.0)	22(68.8)	63 (92.6)	0.005
CAD >30%	81 (81.0)	23 (71.9)	58 (85.3)	0.170
n of T2DM (%)	61 (61.0)	10 (31.3)	51 (75.0)	<0.0001
n of current smoking (%)	20 (20.0)	5(15.6)	15(22.1)	0.595
n with decreased eGFR (%)	45 (45.0)	11 (34.4)	34 (50.0)	0.196
n with previous AMI (%)	28 (28.0)	6 (19.4)	22(32.8)	0.231
n with heart failure (%)	17 (17.0)	2 (18.2)	15 (29.4)	0.712
HbA1c (%)	6.8 (1.50)	6.3 (1.4)	7.1 (1.5)	0.02
Blood glucose (mg/dL)	112 (92-154)	97 (88 – 127)	117 (99 – 164)	0.016
Total cholesterol (mg/dL)	163 (133.5-194.5)	179 (134-200)	158 (132 – 182)	0.075
Serum creatinine (mg/dL)	0.82 (0.2)	0.72 (1.5)	0.86 (0.2)	0.001
IL-6 (pg/mL)	1.10 (1.6-2.10)	0.09 (0.04-0.14)	1.5(0.96-2.40)	<0.001
hs-CRP	2.6 (1.2 -7.9)	1.63 (1.0 -2.8)	3.9 (1.5-8.7)	0.008
% using aspirin	71.0	67.7	74.6	0.478
% using clopidogrel	16.0	27.3	25.5	1.000
% using ACEi	35.0	54.5	56.9	1.000
% using ARB	19.0	18.2	33.3	0.478
% using beta blockers	48.0	72.7	78.4	0.700
% using statins	83.0	80.6	86.6	0.548

Values are expressed as mean ± standard deviation, median with interquartile range or proportion (%); **BMI:** body mass index; **AC:** abdominal circumference; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure; **CAD**: coronary artery disease; **T2DM:** type 2 diabetes mellitus; **eGFR:** estimated glomerular filtration rate (below 90 mL/min/1.73 m^2 - CKD-EPI formula); **AMI:** acute myocardial infarction; **HbA1c:** hemoglobin A1c; **IL-6:** interleukine-6; **hs-CRP**: high-sensitive C-reactive protein; **ACEi:** angiotensin converting enzyme inhibitors; **ARB:** angiotensin II receptor blocker;

Table 2. Outcomes

All Combined (%)	**32 (32.0)**	**6(18.8)**	**26 (38.2)**	**0.06**
Myocardial Infarction (%)	1 (1.0)	0 (0.0)	1 (1.5)	1.000
Heart Failure hospitalization (%)	10 (10.0)	1 (3.1)	9 (13.2)	0.162
CAD hospitalization (%)	8 (8.0)	2 (6.3)	6 (8.8)	1.000
CV death (%)	2 (2.0)	0 (0.0)	2 (2.9)	1.000
New re-vascularization (%)	10 (10.0)	3 (9.4)	7 (10.3)	1.000
All-cause mortality (%)	1 (1.0)	0 (0.0)	1 (1.5)	1.000

Data are the total number of events and the percent within each group.

Conclusões

Níveis séricos de IL-6 estão elevados tanto em pacientes com síndrome coronariana aguda como nos de risco mais elevado para eventos cardiovasculares.

A noção de que as proteínas inflamatórias sejam não somente a expressão de dano celular como a causada no processo de injúria-isquemia miocárdica, mas também o mecanismo patogênico que leva a desfechos cardiovasculares é importante e deve ser muito estudada ainda.

A diminuição do risco cardiovascular passa por mecanismos multifatoriais - desde o reconhecimento de que o indivíduo apresenta um risco maior, identificado tanto por suas patologias de base quanto a presença de marcadores de pior prognóstico, como por exemplo a IL-6 tem se demonstrado – como o tratamento medicamentoso, não medicamentoso e mudanças no estilo de vida. A melhora nos níveis de colesterol, triglicerídeos, glicemia, peso corporal são alguns que já temos conhecimento que diminuem a probabilidade de eventos; confirmar agora que os marcadores inflamatórios estão relacionados a pior prognóstico e que medidas que ocasionam sua redução contribuem para diminuir esse risco é etapa essencial a se confirmar.

Em nosso primeiro trabalho, demonstramos que em pacientes com risco intermediário para cardiopatia isquêmica, a IL-6 elevada tinha maior valor preditivo positivo para presença de aterosclerose coronária. Ainda, a curva ROC para DAC nesses pacientes era maior pela IL-6 do que pela PCR e pelo escore de ASCVD (0.72, 0.60 e 0.54, respectivamente).

Posteriormente, no segundo estudo, a IL6 se demonstrou um importante marcador prognóstico em pacientes de alto risco cardiovascular, pois aqueles com valores elevados apresentaram desfechos cardiovasculares importantes ao longo de 5,7 anos. Por outro lado, os com IL6 baixa demonstraram um valor preditivo negativo significativo para talvez reclassificarmos o paciente em seu risco de eventos. Ainda, não identificamos correlação entre obesidade, hipertensão ou diabetes na análise de subgrupos. Apesar de alguns trabalhos demonstrarem que uma parte da produção de IL6 seja no tecido adiposo e que a IL6 se elevaria com o aumento dos níveis de adiposidade, em nosso trabalho não se confirmou, podendo ser reflexo do nosso baixo

número de participantes ou pela possível variabilidade circadiana que a IL6 possa apresentar, ainda a confirmar.

Em resumo, nossos dois estudos foram capazes de demonstrar que níveis elevados de IL-6 estão associados com a presença de DAC e com um aumento do risco de eventos cardiovasculares adversos em pacientes que foram submetidos à cinecoronariografia. Outro achado relevante foi de que níveis normais de IL-6 apresentam um ótimo valor preditivo negativo para eventos clínicos adversos nessa população.