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ESCOLA DE MEDICINA E SAÚDE PÚBLICA

# Pesquisa de autoanticorpos utilizados no diagnóstico de artrite reumatóide e vasculites em pacientes com tuberculose.

Tese de Doutorado

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# Pesquisa de auto anticorpos utilizados no diagnóstico de artrite reumatóide e vasculites em pacientes com tuberculose.

**Tese apresentada ao curso de Pós-graduação em Medicina e Saúde Humana da Escola Bahiana de Medicina e Saúde Pública para obtenção do título de Doutor em Medicina**

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# Índice:

# Índice de Abreviaturas

TB – Tuberculose

AR - Artrite reumatóide

ACPAs – Anticorpos antipeptídeos citrulinados

ANCA – Anticorpos anticitoplasma de neutrófilos

Anti-PR3 – Antiproteinase 3

Anti-MPO – Antimieloperoxidase

Anti-CCP – Antipeptídeo citrulinado cíclico

Anti-MCV – Antivimentina citrulinada modificada

GPA – Granulomatose com Poliangeíte

P-ANCA - ANCA padrão perinuclear

C-ANCA - ANCA padrão citoplasmático

BPI – ANCA – ANCA contra proteína que aumenta a permeabilidade da bactéria *(" bactericidal/permeability increasing protein")*

Células NK - células *Natural Killer*

PAMPs - Padrões Moleculares Associados à Patógenos

RRP - Receptores de Reconhecimento de Padrões

LB – linfócitos B

LT – linfócitos T

FR – fator reumatóide

IFI - imunofluorescência indireta

C-ANCA – ANCA com  padrão citoplasmático

ELISA - enzimaimunoensaio

P-ANCA – ANCA com  padrão perinuclear

BAAR – Bacilo álcool ácido resistente

# I.

# RESUMO

**Pesquisa de autoanticorpos utilizados no diagnóstico de artrite reumatóide e vasculites em pacientes com tuberculose**

Introdução: É reconhecida a interface entre a reumatologia, particularmente no que diz respeito às doenças autoimunes, e a infectologia, seja pela hipótese de agentes infecciosos atuando como gatilho das disfunções imunológicas, seja pelo risco infeccioso atribuído aos tratamentos imunossupressores. Adicionalmente, tem sido observada a produção de alguns autoanticorpos no curso de infecções. Por exemplo: em pacientes com tuberculose (TB), foi demonstrada a produção de anticorpos descritos como de alta especificidade para artrite reumatóide (AR) como os anticorpos antipeptídeos citrulinados (ACPAs) e, do mesmo modo, foi demonstrada a presença de anticorpos anticitoplasma de neutrófilos (ANCA) dentre os quais antiproteinase 3 (anti-PR3) e antimieloperoxidase (anti-MPO), que são marcadores de vasculites sistêmicas. Objetivos: a) revisar as publicações sobre positividade dos ACPAs em doenças infecciosas, b) pesquisar a prevalência destes anticorpos assim como do ANCA em uma população de portadores de tuberculose. Métodos: a) inicialmente foi realizada uma revisão sistemática sobre os estudos avaliando a presença de ACPAs em doenças infecciosas*;* b) posteriormente, um grupo de 50 pacientes com TB pulmonar não tratada ou com até 30 dias do início do tratamento foi avaliado quanto à presença de sintomas reumatológicos e, principalmente, quanto à positividade de anticorpos ACPAs, incluindo antipeptídeo citrulinado cíclico (anti-CCP) e antivimentina citrulinada modificada (anti-MCV) e quanto à positividade de ANCA por imunofluorescência indireta (IFI) e anticorpos anti-PR3 e anti-MPO por enzimaimunoensaio (ELISA). Resultados: a) a revisão sistemática foi publicada e encontra-se apresentada "Revisão de literatura**"** com o título *Antibodies against cyclic citrullinated peptides in infectious diseases – a systematic review*. *Clin Rheumatol 2010, Dec 29(12): 1345-51.* b) encontrou-se positividade de ACPAs em apenas dois (4%) dos 50 pacientes com TB e

não houve positividade de ANCA por IFI ou a presença de anticorpos anti-PR3 ou anti-MPO por ELISA no soro desses pacientes. Estes resultados estão apresentados em dois artigos que foram submetidos para a revista *Clinical Rheumatology* (Canadá, fator de impacto 2011: 1,996), aguardando o parecer do corpo editorial. As versões submetidas encontram-se na sessão "Artigos**"**. Conclusões: embora estudos prévios tenham relatado a presença de ACPAs e ANCA em pacientes com TB, no presente estudo a positividade dos ACPAs foi baixa e não foi observada positividade para ANCA, anti-PR3 e anti-MPO, confirmando a alta especificidade destes testes para AR e vasculites sistêmicas, respectivamente.

Palavras-chave: Tuberculose, autoanticorpos, ACPA, anti-CCP, ANCA, anti-MPO, anti-PR3, artrite reumatóide.

# II.

# INTRODUÇÃO

O sistema imunológico atua na preservação dos indivíduos frente às agressões em geral. Este complexo sistema de defesa pode ser dividido didaticamente em imunidade inata e adaptativa. A imunidade inata constitui-se na primeira linha de defesa do organismo, sendo composta por barreiras físicas como a pele e mucosas; além da atuação de células como neutrófilos, macrófagos, células dendríticas, células *Natural Killer* (NK), mastócitos, basófilos e eosinófilos. Estas células são ativadas por estímulos específicos, representados por estruturas moleculares comuns a diversos patógenos – PAMPs: Padrões Moleculares Associados a Patógenos - porém ausentes nos seres humanos, que se ligam a Receptores de Reconhecimento de Padrões (RRP) e promovem uma resposta imunológica rápida, não antígeno-específica e não duradoura. Fagocitose, liberação de mediadores inflamatórios, ativação das proteínas do sistema complemento e a produção das proteínas de fase aguda são os principais mecanismos de ação da imunidade inata, que se manifesta pelos sinais inflamatórios clássicos de calor, rubor, dor e edema. Já a imunidade adaptativa, da qual participam linfócitos B (LB), linfócitos T (LT), células dendríticas e apresentadoras de antígenos, depende de estímulos antígeno-específicos, sendo desenvolvida ao longo da vida por exposição à micro-organismos ou estímulo vacinal. É capaz de reconhecer uma imensa diversidade de patógenos e produzir uma memória imunológica, que diminui a possibilidade de, por exemplo, haver infecções em exposições subseqüentes. A imunidade inata, quando não suficiente para conter o agente estranho, acaba por gerar estímulos de ativação dos linfócitos T e B, que participam da imunidade adaptativa. Para que este complexo sistema, que envolve a interação dos elementos da imunidade inata e adaptativa, exerça a função de preservar o organismo, ele deve ter a capacidade de distinguir entre o que é próprio aos indivíduos e o que é "não-próprio",sendo anérgico aos elementos próprios. Há vários mecanismos no

sistema imune, desde os processos de maturação dos LT e LB, que visam manter a autotolerância: deleção de LB e LT auto-reativos na medula e no timo, barreiras físicas aos antígenos próprios no sistema linfóide, inativação por meio de um sinal fraco, sem co-estimulação, impedindo assim ocorrência dos processos autoimunes. A perda desta autotolerância pode estar relacionada à fatores intrínsecos e extrínsecos e associa-se à processos de adoecimento. Fatores intrínsecos como falha nos mecanismos de apoptose ou falha no *clearance* dos produtos apoptóticos, deficiência no sistema de complemento, entre outros, são apontados como causas de perda de tolerância. Fatores extrínsecos como quadros infecciosos, exposição à agentes físicos e químicos podem determinar a perda da mesma e contribuir para o desencadear de doenças autoimunes, patologias multifatoriais em que se observa a perda da capacidade de distinção entre o que é próprio e o que é "não-próprio"[1-3]. Os mecanismos que levam a perda da tolerância por agentes infecciosos são variados. Entre estes é descrito classicamente o mimetismo molecular encontrado na febre reumática, situação em que anticorpos contra proteína M do estreptococo reage contra elementos do tecido cardíaco. São descritos ainda no contexto infeccioso, a necrose celular que expõe epítopos de autoantígenos ou expõe antígenos habitualmente isolados; a ativação policlonal de LB e LT por superantígenos microbianos e a liberação de mediadores inflamatórios e de fatores de coestimulação que levam a ativação de células não envolvidas diretamente na resposta ao patógeno, como linfócitos autorreativos, constituindo-se assim mecanismos capazes de levar a perda tolerância e conseqüente adoecimento. Desta maneira, reconhece-se uma interação entre a reumatologia e a infectologia, uma vez que agentes infecciosos podem atuar como um gatilho na perda da tolerância imunológica e participar do desencadeamento de doenças autoimunes. Outros exemplos de participação de agentes infecciosos no desenvolvimento de quadros imunológicos, além

do que ocorre na febre reumática, são a vasculite leucocitoclástica secundária à crioglobulinemia[4], a poliarterite nodosa secundária à infecção pelo vírus da hepatite B [5] e o quadro compatível com síndrome de Sjogren em pacientes infectados pelo HTLV-1[6].

Tem sido observada também a presença de autoanticorpos no contexto de doenças infecciosas, mesmo na ausência de uma condição autoimune clinicamente reconhecida. O fator reumatóide (FR), por exemplo, que é um anticorpo classicamente utilizado para o diagnóstico de AR, não raramente é descrito em doenças infecciosas (chegando até a 40% nos pacientes com TB) o que configura baixa especificidade deste método para o diagnóstico da AR[7].

A AR, condição que incide em cerca de 1% da população mundial e também do Brasil, é caracterizada por poliartrite simétrica, positividade do FR na maioria dos casos, como descrito acima e presença de erosões ósseas[8;9]. Mais recentemente, tem sido feita a pesquisa de anticorpos antipeptídeos citrulinados (ACPAs) com maior sensibilidade e especificidade que o FR para o diagnóstico da AR[10]. Entretanto, estudos recentes revelaram a presença destes anticorpos em doenças infecciosas, colocando em dúvida a alta especificidade atribuída ao teste[11]. Dentre as doenças infecciosas estudadas, a TB foi a infecção que apresentou maior frequência de positividade ACPAs[12-16]. Estes anticorpos também foram pesquisados em hanseníase, hepatite B e C com positividade de ACPAs de 0 a 8,8%[17-33] Em um pequeno grupo de leishmaniose (10 pacientes) a positividade foi de 30%[34], o que não foi confirmado em dois outros trabalhos que estudaram 19 e 10 pacientes com leishmaniose e não encontraram nenhum resultado positivo para ACPAs[16;32]. Outras condições patológicas infecciosas constituíram grupos de controles - portadores de HIV, HTLV, mononucleose, Yersínia, Lyme, Schistosoma, sífilis, rubéola, malária, *parvovirus*, toxoplasma, mycoplasma, salmonela,

clamídia, legionela, *streptococcus pyogenes e* Doença de Chagas - em trabalhos que pesquisavam a performance dos ACPAs para AR, e a positividade desses anticorpos variou de 0 a 11,1%[16;18;32]. Ainda no contexto da TB, têm sido também descrita a presença de anticorpos anticitoplasma de neutrófilos (ANCA) dentre os quais antiproteinase 3 (anti-PR3) e antimieloperoxidase (anti-MPO), que são marcadores de vasculites sistêmicas primárias como granulomatose com poliangeíte (GPA), - previamente conhecida como granulomatose de Wegener -, poliangeíte microscópica e a doença de Churg Strauss[35-38].

Embora seja considerada uma doença primariamente pulmonar, a TB pode atingir qualquer órgão por disseminação linfo-hematogênica e pode cursar com sintomas ósteo-articulares, inclusive mimetizando AR, principalmente em fases iniciais da doença, período em que há maior utilidade na pesquisa dos ACPAs . Do mesmo modo, pode apresentar similaridades clínicas e radiológicas com síndromes vasculíticas, particularmente a GPA. Por essa razão, a detecção de anticorpos de alta especificidade para doenças reumatológicas como o ANCA e o ACPA em pacientes com TB, principalmente em áreas endêmicas para essa infecção, pode, virtualmente, ser responsável por diagnósticos e tratamentos equivocados.

Este dado torna-se ainda mais relevante se considerarmos que o Boletim Epidemiológico de março 2012, do Ministério da Saúde, relata a taxa de incidência de TB em 2011 de 36/100.000 habitantes/ano. O Brasil permanece entre os 22 países que concentram 82% dos casos de TB no mundo. Especificamente no estado da Bahia, foi descrita uma incidência de 35,9 casos/100.000 habitantes/ano e uma taxa de mortalidade de 2,7/100.000 habitantes/ano em 2011[39]. Na cidade de Salvador, Xavier *et al*., em 2007, publicaram os casos descritos de TB no período de 1990 a 2000. Entre os 31.903 casos relatados, 3% foram ósteo-articulares[40]. Franco *et al.,* em 2003, revisaram 275

casos de tuberculose em crianças e adolescentes tratados no Hospital Otávio Mangabeira / Salvador – Bahia, tendo encontrado 4% de tuberculose óssea[41]. Com isso, tornou-se muito importante conhecer a frequência de positividade dos autoanticorpos descritos como de alta especificidade para o diagnóstico de AR e vasculites sistêmicas nos pacientes diagnosticados com TB no Brasil , sendo esta uma iniciativa pioneira, uma vez que a única publicação nacional sobre o tema é restrita a um estudo sobre o rendimento do teste de ACPAs para o diagnóstico da AR, que incluiu um grupo controle de 10 portadores de TB[32].

# III.

# REVISÃO DA LITERATURA

12III.1 **ACPAs em doenças infecciosas:**

A publicação "*Antibodies against cyclic citrullinated peptides in infectious diseases – a systematic review*. *Clin Rheumatol 2010, Dec 29(12): 1345 -51"*[11] apresentada em "Resultados" corresponde uma ampla revisão sobre o tema.

III.2 **ANCA, incluindo anti-PR3 e anti-MPO em tuberculose**

ANCA são autoanticorpos direcionados contra componentes intracelulares de neutrófilos, usualmente descritos em vasculites de pequenos vasos como GPA, poliangeíte microscópica e vasculite de Churg-Strauss. Em geral, na GPA sua especificidade é para o antígeno PR3 e nas outras duas vasculites para a MPO[42].

Diversos estudos avaliaram a positividade do ANCA por imunofluorescência indireta (IFI) em quadros infecciosos ou induzidos por drogas. No contexto das doenças infecciosas, a TB tem particular interesse, uma vez que o quadro pulmonar pode ser de difícil distinção, tanto clínica quanto do ponto de vista histológico, quando comparado à GPA. Os achados na literatura são divergentes quanto à positividade do ANCA, anti-MPO e anti-PR3 em portadores de TB. Quatro estudos demonstram a presença de ANCA no contexto de tuberculose. Assim, Flores-Suárez *et al*. estudaram no México a prevalência de ANCA em 45 portadores de TB e compararam-nos com pacientes hígidos e com asma atópica. Foi encontrada positividade do ANCA (por IFI) em 44% dos pacientes com TB, sendo em sua maioria ANCA com padrão citoplasmático de fluorescência (C-ANCA). Também foi verificada 40% de positividade para a pesquisa direta de anticorpos anti-PR3 ou anti-MPO por enzimaimunoensaio (ELISA)[43]. Pradhan *et al.* também pesquisaram ANCA, anti-MPO e anti-PR3 em portadores de TB, em doença intersticial pulmonar e em controles hígidos. Estes autores encontraram 30% de positividade de ANCA; 47,6% de anti-MPO e 28,6% de anti-PR3 entre os portadores de TB[37]. Ghosh *et al*. compilaram os dados desse estudo com aqueles de mais dois outros trabalhos, totalizando uma população de 318 pacientes. Entre os portadores de TB, 32% foram positivos para ANCA[36]. Em publicação mais recente, Sherkat *et al*. estudaram a prevalência de ANCA, MPO e PR3 em 32 pacientes com TB ativa comparando-os com 32 controles saudáveis. A frequência de ANCA padrão perinuclear

de fluorescência (P-ANCA) foi de 25% e de ANCA padrão citoplasmático (C-ANCA) de 1,5% entre os portadores de TB. Houve uma associação significativa (p> 0,01) entre a presença de anti-MPO e TB[38]. Os autores destes quatro trabalhos concluíram que os resultados de ANCA, antiPR3 e anti-MPO devem ser interpretados com cautela em regiões endêmicas para TB , uma vez que há semelhanças do ponto de vista clínico e histológico entre esta condição e vasculites como a GPA.

Por outro lado, dois trabalhos mostraram a ausência ou pequena frequência de positividade de ANCA em portadores de TB. Teixeira *et al*., estudaram a presença destes mesmos anticorpos em 67 pacientes com TB e encontraram uma positividade de 10%, sendo que apenas um paciente foi positivo para anti-PR3[44]. Esquivel-Valerio *et al*. estudaram a presença de ANCA, anti-PR3 e anti-MPO em 68 pacientes com TB antes do tratamento, repetindo esta pesquisa em 52 pacientes após o tratamento. Previamente aos medicamentos, a positividade do ANCA foi encontrada em apenas três pacientes (4,4%), sendo um C-ANCA e dois P-ANCA. Após o tratamento, a positividade do ANCA foi observada em 28,8% dos pacientes; sendo em sua maioria contra BPI (proteína que aumenta a permeabilidade da bactéria) e não contra PR3 ou MPO[45].

# IV.

# OBJETIVOS

- Revisar sistematicamente o conhecimento atual sobre a positividade de ACPAs em doenças infecciosas, particularmente TB.
- Pesquisar a presença de ACPAs através da pesquisa de antipeptídeo citrulinado cíclico (anti-CCP) e antivimentina citrulinada modificada (anti-MCV) em uma população de 50 pacientes com TB pulmonar, tendo como grupos controles pacientes com diagnóstico de AR e doadores de sangue; e pesquisar ANCA (IFI), anti-PR3 e anti-MPO (ELISA) em uma população de 50 portadores de TB, tendo como grupo controle doadores de sangue.

# V.

# MÉTODOS

V.1 Revisão sistemática sobre os estudos avaliando a presença de ACPAs em doenças infecciosas

Foi feito levantamento bibliográfico nas seguintes bases de dados: Medline, Cochrane, SCielo e Lilacs.

Termos utilizados: anti-CCP, anti-MCV, *anti-CCP and infectious diseases*, *anti-MCV and infectious diseases, anti-CCP and virus, anti-CCP and mycobacteria, anti-CCP and tuberculosis, anti-CCP and leprosy, anti-CCP and leishmaniasis, anti-CCP and HIV, anti-CCP and HTLV, anti-CCP and Chagas Diseases, anti-CCP and Lyme Diseases, anti-MCV and virus, anti- MCV and mycobacteria, anti- MCV and tuberculosis, anti-MCV and leprosy, anti- MCV and leishmaniasis, anti- MCV and HIV, anti- MCV and HTLV, anti- MCV and Chagas Diseases, anti- MCV and Lyme Diseases.* Os termos correspondentes em português foram utilizados. Todos os artigos foram incluídos, dos quais foram extraídos as informações sobre frequência de positividade dos anticorpos anti-CCP e anti-MCV.

V.2 Realização de dois estudos avaliando um grupo de 50 pacientes com TB pulmonar não tratada ou até com 30 dias do início do tratamento quanto à presença de sintomas reumatológicos e, principalmente, quanto à positividade de ACPAs (anti-CCP e anti-MCV) (artigo VI.1) e quanto à positividade de ANCA por IFI e anticorpos anti-PR3 e anti-MPO (ELISA) (artigo VI.2).

V.2.1 ***Antibodies to citrullinated peptides in tuberculosis***

Estudo de corte transversal com grupos de comparação, cuja população foi composta por pacientes com TB pulmonar (diagnóstico baseado em critérios clínicos, radiológicos e positividade de BAAR no escarro) antes ou até há um mês do início do tratamento; portadores de AR - diagnóstico pelos critérios do Colégio Americano de Reumatologia[16] e controles hígidos (doadores de sangue).

Foram coletados dados epidemiológicos, feita avaliação reumatológica e coletado sangue para a pesquisa de anti-CCP e anti-MCV pelo método de ELISA segundo as instruções do fabricante (INOVA Diagnostics Inc.®); e pesquisa de FR também por ELISA (Orgentec®). Os exames foram realizados nos laboratórios LBPC da Fundação Gonçalo Muniz (Fiocruz) e da Faculdade de Farmácia da Universidade Federal da Bahia.

Análise estatística: utilizou-se o programa estatístico SPSS versão 18.0. As variáveis gênero e positividade de anticorpos foram apresentados sob a forma de frequências. Idade, tempo de diagnóstico e tempo de tratamento da tuberculose foram apresentados sob a forma de média $\pm$ desvio-padrão ou mediana e intervalo inter-quartil, dependendo do teste de normalidade das variáveis descritas. A comparação das medianas dos ACPAs entre os grupos foi feita pelo Teste de Kruskal-Wallis. A correlação entre os valores de anti-CCP e anti-MCV na população de AR foi feita pelo teste de Spearman.

Calculou-se a sensibilidade e a especificidade dos ACPAs e FR para o diagnóstico de AR pelo programa: http://faculty.vassar.edu/lowry/clin1.html. Os resultados foram apresentados com intervalo de confiança de 95%.

V.2.1 **ANCA, anti-PR3 and anti-MPO antibodies are not present in serum of patients with pulmonary tuberculosis.**

Estudo de corte transversal, que inclui 50 pacientes com TB pulmonar (diagnóstico baseado em critérios clínicos, radiológicos e positividade de BAAR no escarro) antes ou até há um mês do início do tratamento e controles hígidos (doadores de sangue).

Os pacientes selecionados foram submetidos a uma avaliação clínica para obtenção de dados sobre gênero, idade, tempo de diagnóstico e sintomas ósteo-articulares. A pesquisa do ANCA foi realizada por IFI (EUROIMMUN®) – vide protocolo do teste na versão do artigo. Os soros foram testados também para a presença de anti-PR3 e anti-MPO por ELISA (INOVA Diagnostics Inc.®) de acordo com as instruções do fabricante. Títulos de diluição maiores que 1/10 para ANCA e valores acima de 20U para anti-PR3 e anti-MPO foram considerados positivos. Os exames foram realizados nos laboratórios LBPC da Fundação Gonçalo Muniz (Fiocruz) e da Faculdade de Farmácia da Universidade Federal da Bahia.

*Análise Estatística:* foi utilizado o programa SSPS para Windows (versão 18.0) para a descrição das frequências das características demográficas dos grupos de pacientes estudados; bem como a freqüência de positividade dos anticorpos testados.

# VI

# RESULTADOS

# ARTIGOS

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- ***Antibodies against cyclic citrullinated peptides in infectious diseases – a systematic review. Clin Rheumatol 2010, Dec 29(12): 1345 -51[(7)].***
- ***Antibodies to citrullinated peptides in tuberculosis.***

  *Versão submetida para Clinical Rheumatology.*
- **ANCA, anti-PR3 and anti-MPO antibodies are not present in serum of patients with pulmonary tuberculosis.**

  *Versão submetida para Clinical Rheumatology.*

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REVIEW ARTICLE

# Antibodies against cyclic citrullinated peptides in infectious diseases—a systematic review

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**Abstract** Rheumatoid arthritis (RA) is a disease characterized by symmetrical polyarthritis of the large and small joints, and in the majority of patients, there is a presence of the rheumatoid factor and erosions in the X-ray of the joints. More recently, the presence of anti-cyclic citrullinated peptide antibodies (anti-CCP) in this disease has been described, with diagnostic and prognostic value. Nevertheless, these antibodies have also been described in infectious diseases. The aim of the present study was to make a systematic review of the presence of antibodies against citrullinated peptides in infectious diseases. Search was conducted in the MEDLINE (1966 to 2010), Cochrane, SCielo, and LILACS databases, using the terms: "anti-CCP, anti-MCV, and infectious diseases"; "anti-CCP, anti-MCV, and virus"; "anti-CCP, anti-MCV, and mycobacteria"; "anti-CCP, anti-MCV, and tuberculosis"; "anti-CCP, anti-MCV, and leprosy"; "anti-CCP, anti-MCV, and leishmaniasis"; "anti-CCP, anti-MCV, and HIV"; "anti-CCP and HTLV"; "anti-CCP, anti-MCV, and Chagas disease"; "anti-CCP, anti-MCV, and Lyme disease", and the corresponding terms in Portuguese. Twenty-five publications were found, which dealt with anti-CCP and infection, and only one on anti-MCV and infection. Of these, 23 were cross-sectional and three cohort studies. Anti-CCP antibodies were found in various frequencies, reaching 37% in tuberculosis. In the other infections, it was a rare finding. In only one publication, anti-MCV was found in only one patient with hepatitis. Since infectious diseases are capable of running their course with osteoarticular symptoms, sometimes difficult to differentiate from RA, additional studies are necessary to define the performance of the test for the detection of anti-CCP antibodies in populations in which the frequency of such infections is high.

**Keywords** Anti-CCP · Anti-cyclic citrullinated peptide antibodies · Autoantibodies · Infection · Tuberculosis

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## Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disease, characterized by symmetric arthritis of the large and small joints. Its prevalence is estimated to be around 1% of the adult population [1]. In Brazil, the prevalence ranges from 0.2% to 1% [2, 3]. It is a disease that has the potential to cause joint destruction that may lead to functional disability and compromise the quality of life of patients. The therapeutic approach to it is multidisciplinary, and treatment with medication must be instituted as early as possible, to avoid joint damage and dysfunction. Therefore, it is necessary to make a diagnosis in the initial stages of the disease.

The classification of a patient as having RA is based on clinical, radiological, and laboratory findings, as defined by the American College of Rheumatology [4]. More recently, research has been conducted on anti-cyclic citrullinated peptide antibodies (anti-CCP), by ELISA in patients with RA, and these antibodies would have greater specificity and positive predictive value than the rheumatoid factor (RF), and appear to be prognostic markers, as well as making diagnosis possible in the initial stages of the disease [5–7]. The experience in our service, in cooperation with the University of Calgary, Canada, confirms these findings [8].

Anti-modified citrullinated vimentin (anti-MCV) antibodies, also directed against citrullinated peptides, have been described, and their sensitivity and specificity, when compared with the anti-CCP research, have revealed variable results in the literature [9–28]. Review articles about the subject point out the heterogeneity of the publications, which makes it difficult to reach a conclusion as regards the performance of the test, but suggest that the anti-MCV could be an option to anti-CCP in the diagnosis of RA [29–31]. Curiously enough, recent studies have demonstrated the presence of anti-CCP antibodies by ELISA in infectious diseases such as tuberculosis (TB) [6, 32–36], leishmaniasis [6, 32, 37], Hansen's disease (HD) [32, 38, 39], atypical mycobacteriosis [34, 40], hepatitides B and C [32, 41–54], HIV [32], HTLV-I [32], Chagas disease [6, 32], mononucleosis [6], schistosomiasis [6, 32], Yersinia [6], and Lyme [42].

The aim of the present study is to make a systematic review of all the publications that deal with the presence of antibodies against citrullinated peptides (anti-CCP and anti-MCV) in different infections, by the ELISA method.

## Material and methods

### Inclusion/exclusion criteria of the studies

All published articles—case control, case series, and cohort studies—were included, in which research was conducted on anti-citrullinated peptide antibodies in infectious diseases, namely TB, HD, atypical mycobacteriosis, leishmaniasis, hepatitides B and C, HIV, HTLV-I, mononucleosis, schistosomiasis, Yersinia, Chagas disease, and Lyme disease. There was no restriction as regards the language of publication. Review articles were not included in the study.

### Sources of research

Search was conducted in the MEDLINE (from 1966 to January 2010), Cochrane, SCielo, and LILACS databases using the terms: "anti-CCP", "anti-MCV", "anti-CCP and infectious diseases", "anti-MCV and infectious diseases", "anti-CCP and virus", "anti-CCP and mycobacteria", "anti-CCP and tuberculosis", "anti-CCP and leprosy", "anti-CCP and leishmaniasis", "anti-CCP and HIV", "anti-CCP and HTLV", "anti-CCP and Chagas disease", "anti-CCP and Lyme disease", "anti-MCV and virus", anti-MCV and mycobacteria", "anti-MCV and tuberculosis", "anti-MCV and leprosy", "anti-MCV and leishmaniasis", "anti-MCV and HIV", "anti-MCV and HTLV", "anti-MCV and Chagas disease", "anti-MCV and Lyme disease" and the corresponding terms in Portuguese. Secondary references were additionally obtained from the included articles.

### Methodology used for review

After the search for articles was made, the authors independently evaluated whether the complete articles analyzed could be included in the review. The following information was extracted from the articles: frequency of positivity of anti-CCP or anti-MCV in different infectious diseases.

## Results

Using the above-mentioned criteria, 25 studies searching for anti-CCP antibodies in infection were identified, 15 being in hepatitis (B and/or C), six in TB, three in HD, three in leishmaniasis, two in atypical mycobacteriosis, two in Chagas disease, two in schistosomiasis, one in mononucleosis, one in Yersinia, one in HIV, and one in Lyme disease. All were cross-sectional studies, except three, which were cohort studies [6, 34, 35]. In only one, study about anti-MCV antibodies and infection was found [16].

*Anti-CCP and tuberculosis* Elkayam et al. studied 47 patients with active pulmonary TB to determine the prevalence of anti-CCP (INOVA kit; cut off, 40 U) in this population. Thirty-nine healthy individuals were their controls. From the rheumatologic point of view, in the TB group, only 4% reported arthralgia; 4% myalgia, 2% xerostomia, and 9% xerophthalmia. No patient presented arthritis. Anti-CCP was positive in 15 patients with TB (32%) and in only one healthy control (2.6%). The mean anti-CCP titer was also higher in patients with TB in comparison with the controls, 44.9 versus 20 U. In this same study, RF was positive (>6 U) in 29 patients (62%) with TB and in one healthy control (2.6%). There was no association between the presence of anti-CCP and rheumatologic symptoms. The authors questioned whether the positivity of anti-CCP in TB would not be related to the non-citrullinated epitopes of the anti-CCP test substrate [33].

Kakumanu et al. studied the presence of anti-CCP (in house; cut off, 1.7 U) and anti-CCP antibody with unmodified arginine containing peptide (anti-CAP) by ELISA in 49 patients with TB, 36 with RA, and 18 healthy controls. While anti-CCP was positive in 37% of the patients with TB (mean titer, 4 UI) and in 43% of the cases of RA (mean titer, 31 UI), the anti-CAP was positive in only one case of RA (3%) and in 13 cases of TB (27%). Anti-CCP and anti-CAP were positives in 6% of the controls. Among 25 TB patients, anti-CCP was sequentially tested, and in 11 patients, there was a transitory increase in positivity of anti-CCP in the first 2 months of treatment. Furthermore, among 11 patients negative for anti-CCP, none became positive throughout

the course of treatment, suggesting that the antibody had not been induced by drugs. Based on these findings, these authors suggested that one should search for both anti-CCP and anti-CAP antibodies to help in the differential diagnosis of RA and osteoarticular manifestations of TB [35]. Recently, Elkayam et al. also researched the presence of anti-CCP and anti-CAP in 122 patients with tuberculosis and in 12 with atypical mycobacteriosis. For this purpose two pairs of synthesized cyclic peptides were used—ELISA method (in house). Each pair contained one citrullinated and one unmodified peptide (anti-CAP): 0401cit, 0401arg, 0722cit, and 0722arg. Eighty-three patients were positive for anti-CAP (0401arg) while there were only 14 for anti-CCP (0401cit), and 61 were positive for anti-CAP (0722arg) while there were only 22 positive for anti-CCP (0722cit). The reference value for each antibody was defined by the mean plus two standard deviations of the antibody titers of 10 controls. Moreover, in this study, the sequential search for antibodies in 33 patients revealed a reduction in their titers, with the treatment. All the patients were also investigated for HIV and hepatitides B and C. There was positivity for HIV in 9%, 7% for hepatitis virus C, and 4% for virus hepatitis B. [34]. Mori et al. searched anti-CCP antibodies and IgM RF in 89 patients with active TB, 106 with recent RA, and 237 healthy controls. Patients with TB and articular symptoms, as well as those with RA with a current or previous history of TB, were excluded. The anti-CCP (Axis kit-Shield Diagnostic Limited—cut off, 5 U) was positive in 6.7% of the patients with TB (mean titer, 4 UI), in 82.1% of the cases of RA (mean titer, 159.3 UI), and in 0.4% of the healthy controls. Three patients with TB (3.4%) presented a high anti-CCP titer (>100 UI). In this same study, RF was positive in 72.6% of the cases of RA and in 18% of the patients with TB. The authors suggested that the difference in the frequency of anti-CCP in TB in comparison with the two previous publications [33, 35] could be related to the different populations studied, as well as to the different kits used. Based on the low frequency of anti-CCP in TB and on the high frequency in RA of recent onset, the authors concluded that anti-CCP is useful for differentiating RA of recent onset and patients with TB [36]. Two further studies were found [6, 32] that included patients with tuberculosis as controls in an evaluation of the performance of anti-CCP assays. Schellekens et al. found two positivity (10%) in 20 patients with tuberculosis (ELISA; cut off, 92 U)[6], whereas Silva et al. found no positive anti-CCP antibodies in 10 patients with TB[32] (ELISA; cut off, 25U).

Lim et al. studied the presence of anti-CCP and RF in 35 patients with atypical mycobacterial pulmonary infection. There was no report of osteoarticular symptoms. No patient presented positivity for anti-CCP, and five (14%) were positive for RF [40].

Table 1 summarizes the findings of the studies in which the research on anti-citrullinated peptide antibodies was conducted in patients with mycobacteriosis.

*Anti-CCP and HD* Barbosa et al. studied 64 HD patients with regard to the presence of anti-CCP antibodies (INOVA kit; cut off, 20 U). Only two (3.1%) presented these antibodies, and these patients also had antinuclear antibodies positive and RF negative [38]. Ribeiro et al. also researched anti-CCP antibodies and RF in 158 HD patients, 69 with RA, and in 89 healthy individuals. Anti-CCP antibodies (Eurodiagnostica kit; cut off, 25 U) were positive in 81% of the cases of RA and in 2.5% of HD. RF was positive in 62.3% of the cases of RA and 1.3% of HD. Among the healthy controls, there was positivity in

**Table 1** Frequency of anti-citrullinated peptide antibodies in patients with mycobacteriosis

| Author (reference) | Year | Origin | Patients×controls or RA | Anti-CCP (%) TB×RA or C | Mean titer of anti-CCP TB×RA or C | RF (%) TB×RA or C |
|---|---|---|---|---|---|---|
| Schellekens [6] | 2000 | Netherlands | 20 TB×149 RA | 10×48 | | 4×53 |
| Silva [32] | 2006 | Brazil | 10 TB×100 RA | 0×68 | 0×632 | 20×91 |
| Elkayam [33] | 2006 | Israel | 47 TB×39 C | 32×2.6* | 44.9×20* | 62×2.6 C |
| Kakumanu [35] | 2008 | Japan | 49 TB×36 RA | 37×43 | 4×31 | |
| Mori [36] | 2009 | Japan | 89 TB×106 RA | 6.7×82.1 | 15.4×159.3 | |
| Elkayam [34] | 2010 | Israel | 122 TB and 12 AM | 14 (0401 cit)[a]<br>22 (0702 cit)[a] | | |
| Lim [40] | 2010 | Korea | 35 AM | 0 | | 14 |
| Liu [16][b] | 2009 | China | 2 TB×170 RA | 0×78.2 | 0×523 | 0×72 |

AM *atypical mycobacteriosis*, *C* healthy controls

* $p=0.002$

[a] *n* patients positive

[b] anti-MCV

3.4% for both anti-CCP and RF. [39]. Silva et al. found no case of anti-CCP in 10 patients with HD [32].

*Anti-CCP and leishmaniasis* Atta et al. found anti-CCP antibodies (INOVA kit; cut off, 20 U) in the three of the 10 patients with untreated visceral leishmaniasis and in four of the 10 patients with RA. Anti-CCP was not observed in healthy controls, in patients cured of leishmaniasis, or even in inhabitants of endemic areas with Montenegro reaction negative [37]. Silva et al. and Schellekens et al. found no case of anti-CCP positive in 19 and 10 patients with leishmaniasis, respectively [6, 32].

*Anti-CCP and hepatitis* Anti-CCP antibodies in patients with hepatitis were investigated in 15 studies. Three included patients with hepatitis B [32, 46, 48] and 12 with hepatitis C [32, 41–45, 47, 49–54]. In hepatitis B, the frequency of anti-CCP ranged from 0% to 0.8%. In 12 articles that included patients with hepatitis C, the positivity of anti-CCP ranged from 0% to 8.8%; however, the kits were obtained from different manufacturers [32, 41–45, 47, 49, 50, 52–54]. In the two articles, there was an analysis according to the presence of rheumatologic symptoms: Sene et al. found 5.6% anti-CCP antibodies in virus C, all of them with rheumatologic symptoms such as arthralgia and Sjogren's Syndrome [53]. Bassyouni et al. studied 47 patients with hepatitis C (20 with osteoarticular involvement, without radiographic findings of deformities or erosions) and 30 with RA. Anti-CCP antibodies were demonstrated in 8.5% of the cases of hepatitis (and in 20% when only those who had osteoarticular symptoms were considered) and 70% of the cases of RA. [41]. Riccio et al. found a higher positivity of anti-CCP antibodies in patients with virus C [51]. This was a retrospective study that evaluated 380 patients. Thirty-eight had osteoarticular symptoms, being three with psoriatic arthritis and five with RA who were excluded in the analysis. Anti-CCP (INOVA kit; cut off, 15 U) search in the 30 patients with C virus-related arthritis was positive in 10 patients (33.3%).

Koga et al. studied the presence of RF and anti-CCP (Axis Shield kit; cut off, 5 U) in various hepatic diseases (73 cases of primary biliary cirrhosis, 55 with autoimmune hepatitis, and 45 with hepatitis C) comparing them with RA (48 cases) and healthy controls (23 individuals). Anti-CCP antibodies were absent in all the patients with hepatitis C and present in 89.5% of the cases of RA, 10.9% in autoimmune hepatitis, and 2.7% in primary biliary cirrhosis. RF and anti-CCP were negative in all the controls [45].

*Anti-CCP and Lyme* Bizarro et al. studied 20 patients with Lyme disease, as the control group in a study, and found three patients with positivity for anti-CCP antibodies [42].

*Anti-CCP and Chagas disease* Silva et al. found 33.3% positivity for anti-CCP in nine cases of Chagas disease as the control group of a study [32], whereas Schellekens et al. found one patient positive for this antibody in 10 Chagas disease patients [6].

*Anti-CCP and HIV, HTLV-I, mononucleosis, Yersinia, and schistosomiasis* Two studies evaluating the performance of anti-CCP test for the diagnosis of RA used infectious diseases as controls. Schellekens et al. found anti-CCP antibodies in two of the 76 patients with infectious mononucleosis, two of the twenty cases of Yersinia infection, and in one of the 20 cases of schistosomiasis [6]. Silva et al. observed 1% of anti-CCP positive in patients with HIV (12 tested), in 7% of the HTLV-I positive patients (28 tested), and none in the 21 cases of schistosomiasis [32]. These two studies also searched anti-CCP antibodies in syphilis (22 cases), rubella (30 cases), malaria (24 cases), parvovirosis (19 cases), mycoplasma infections (20 cases), toxoplasmosis (20 cases), salmonella (20 cases), chlamydia (14 cases), legionella (11 cases), streptococcus pyogenes (20 cases), and infectious endocarditis (17 cases), and there was no anti-CCP positivity in any of these cases.

*Anti-MCV and infection* Anti-MCV antibodies were tested in infectious diseases in only one study [16]. The authors, evaluating the role of anti-MCV antibodies in the differential diagnosis of early stage arthritis, tested eight patients with viral hepatitis and two with tuberculosis as controls. Anti-MCV antibodies were positive in only one patient with hepatitis (12.5%) and none in the patients with tuberculosis.

## Discussion

Studies on anti-citrullinated peptide antibodies were initially conducted by indirect immunofluorescence test as "antiperinuclear factor", described in 1964 by Nienhuis and Mandema [55], and by this method, these antibodies were searched in infectious diseases as HD (13 cases) and viral hepatitis (six cases), having been positive in only one of the patients with HD (7.6%) [56]. Similarly, in 1972, Keil et al. found positivity of the antiperinuclear factor in 1.1% of the 93 studied cases of TB without articular symptoms; in one of the two cases (50%) of tuberculosis who also had chronic polyarthritis, and none in patient with infectious hepatitis [57]. More recently, studies were published, which searched anti-CCP antibodies by ELISA in infectious diseases, and the finding of positivity, ranging from 0% to 37%, being the figures of TB the highest one [6, 32–36], putting in doubt the specificity of this test for the diagnosis of RA. Furthermore, these diseases may run their course with osteoarticular symptoms, and if added to

this, there is an anti-CCP positivity; one may establish a wrong diagnosis and consequently an inadequate treatment. Based on the findings of the present systematic review, it can be stated that anti-CCP antibodies may be observed in patients with infectious diseases, particularly tuberculosis; however, there is a great variability in its frequency in different studies, probably due to methodological variations. Therefore, it is necessary to conduct further studies to define the performance of these tests in populations in which the frequency of such infections is high.

The process of citrullinization is described in physiological conditions such as epidermal differentiation, pilous follicle formation, and differentiation of the myeline sheath during the formation of the central nervous system [58–60]. In a similar way, Makrygiannakis et al. found that citrullinization is a common process in inflammation. Therefore, biopsy specimens obtained from patients with inflammatory conditions such as RA, polymyositis (PM), inflammatory bowel disease (IBD), and recurrent tonsillitis were studied. Citrullinated proteins were found in 100% of the cases of RA, PM, and chronic tonsillitis and in 70% of the active IBD lesions. Unfortunately, no anti-CCP antibody search was conducted in this study [61].

Although citrullinization may be secondary to inflammation, as occurs in infectious diseases, the production of antibodies against citrullinated peptides occurs in the minority of cases. With respect to infections and particularly tuberculosis, an infection in which the highest anti-CCP antibody positivity was found, the positivity of these antibodies could be attributed to a reaction of the antibody against uncitrullinated epitopes of the peptide (positive reaction in TB would be citrulline independent)[34, 35]. Another hypothesis that must be considered is the fact that the presence of anti-CCP antibodies precedes the clinical diagnosis of RA. Would these patients with infection and positive anti-CCP antibodies suffer from RA in the future?

Anti-MCV test appears to have a similar diagnostic performance to the classical anti-CCP test. Nevertheless, its evaluation in infectious diseases is restricted to a small control group of early arthritis patients. Further studies are necessary to evaluate its performance for the diagnosis of RA including a larger number of patients with infectious diseases.

**Disclosures** None

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# Antibodies to citrullinated peptides in tuberculosis.

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# Antibodies to citrullinated peptides in tuberculosis

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## ABSTRACT

**Introduction**: Rheumatoid arthritis (RA) is characterized by symmetric polyarthritis, rheumatoid factor positivity (RF) in the majority, and bone erosions. More recently, research has been conducted into anti-citrullinated peptides antibodies (ACPAs) to which greater sensitivity and specificity have been attributed than RF for RA. However, these antibodies have also been described in infectious diseases, particularly tuberculosis, placing the high specificity of the test in doubt. The aim of the present research was to study the ACPAs in tuberculosis (TB). **Material and Methods**: Patients with bacteriologically confirmed pulmonary TB, RA (ACR criteria), and controls (C) were included. ACPAs were researched by: anti-CCP and anti-MCV, in addition to RF. **Results:** The study was conducted in 50 with TB, 50 with RA and 20 healthy controls. Anti-CCP were found in 39 (78%) of the RA, whereas anti-MCV were found in 25 (50%) of the RA. Of the patients with TB two (4%) had positivity for anti-CCP and anti-MCV and no patient in the control group tested positive for these antibodies. Sensitivity of anti-CCP for RA was 78% (CI: 63 to 88%) and specificity of 97% (CI: 89 to 99%); sensitivity of anti-MCV was 50% (CI: 35 - 64%) and specificity of 97% (CI: 89 to 99 %). RF was positive in 40 (80%) of the RA, in 30 (60%) of TB and in 1 (5%) of the controls. Thus, sensitivity of RF was 80% (CI: 65 to 89%) and specificity of 55% (CI: 43 to 67%) for RA. **Conclusion**: Our findings showed high sensitivity of anti-CCP and high specificity of both anti-CCP and anti-MCV for diagnosis of RA, even in a population with high incidence of tuberculosis. The higher frequency of positivity of ACPA in TB observed in previous studies may be attributed to methodological factors.

**Key Words:** ACPA, anti-CCP, anti-MCV, rheumatoid factor, rheumatoid arthritis, tuberculosis.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, that runs its course with symmetric arthritis of the large and small joints and affects around 1% of the adult population[1]. In Brazil, this is no different; studies have demonstrated a prevalence that ranges from 0.2 to 1%[2;3]. RA has the potential to cause joint destruction and compromise the patients' quality of life. Therefore, precise diagnosis in the initial stages of the disease is essential, so that treatment may be started as early as possible. The diagnosis of RA is based on clinical, radiological and laboratory criteria, defined and revised by the American College of Rheumatology (ACR)[4]. Advancements in laboratory tests, imagining exams and therapeutic resources have allowed the early definition of RA, which offers excellent opportunities for treatment[5]. With respect to advancements in laboratory tests, research into antibodies against citrullinated peptides (ACPAs) by ELISA test has been described, and greater specificity and positive predictive value have been attributed to these antibodies than to rheumatoid factor (RF), as markers of prognosis and enabling diagnosis in the initial stages of the disease[6-8]. The experience in our service, in cooperation with the University of Calgary, Canada, confirms these findings[9]. On the other hand, recent studies have demonstrated the presence ACPAs by ELISA in infectious diseases, such as: tuberculosis (TB)[7;10-14], visceral leishmaniasis (VL)[15], Hansen's disease (HD)[10;16;17], atypical mycobacteriosis[12;13], hepatitis B and C[10;18-31], HIV[10], HTLV-I[10], Chagas Disease[7;10], mononucleosis [7], schistosomiasis[7;10], Yersinia[7] and Lyme[19]. The aim of the present research

was to study the presence of ACPAs in patients with tuberculosis (TB) and thereby evaluate the performance of these antibodies for the diagnosis of RA in a region endemic for this infection, using two methods: anti-CCP (cyclic citrullinated peptides) and anti-MCV (modified citrullinated vimentin).

## MATERIAL AND METHODS

### Patients

This was a cross-sectional study including three groups of individuals: patients with RA (as defined by the ACR criteria)[32] being followed-up at our institution; those with pulmonary TB (diagnosed on the basis of radiologic exam and confirmed by positive sputum test for acid-fast bacillus) before or within a month of treatment, and healthy controls selected from among blood donors. All patients were over the age of 18 years, voluntarily agreed to participate in the study, and signed a term of free and informed consent. Patients with diagnosis of RA who had an associated infectious condition were excluded from the study. The selected patients were submitted to clinical evaluation to obtain data about gender, age, time of diagnosis, smoking, comorbidities, medications in use, and osteo-articular symptoms. This study was approved by the Research Ethics Committees of the institutions involved in the project.

### Laboratory Tests

All sera were tested for the presence of anti-CCP (INOVA), anti-MCV antibodies and RF by ELISA (*Orgentec)* in accordance with the manufacturers' instructions. Values higher than 20U were considered positive for anti-CCP and anti-MCV, and higher than 25U for RF.

***Statistical Analysis****:* Gender and positivity of ACPAs and RF were presented in frequencies; age, time of diagnosis and time of use of anti-TB therapy were

presented in the form of mean ± standard deviation or median plus interquartile interval (IQ), according to the test for normality of the variables. Comparison of the median of ACPAs titers among the groups was made by the Kruskal-Wallis test. Correlation between the values of anti-CCP and anti-MCV antibodies in the RA population was evaluated by Spearman's test. Sensitivity and specificity of anti-CCP, anti-MCV and RF for RA were calculated by means of the program http://faculty.vassar.edu/lowry/clin1.html and the results were presented with an confidence interval (CI) of 95%.

**RESULTS**

The study was conducted in 50 TB patients and the positivity of anti-CCP was observed in only two (4%) patients – values of 86 and 105U; a result quite similar to anti-MCV antibodies – values of 69 and 196U. These patients also presented positivity of RF: 352 and 224 U. Among all patients with TB, the RF was positive in 30 patients (60%). Of the two patients positive for ACPA, one complained of pain in the knees and a diffuse pain in the left hand, associated with a trauma in the past. These two patients were re-evaluated one year after initial evaluation and none presented manifestations compatible with RA. Fifty patients with RA were included (94% women) with a mean age of 55 years (SD: 13) and median time of diagnosis of 13 years (IQ: 6 to 17). Thirty nine (78%) were positive for anti-CCP, median titer of: 128 U (IQ: 24 to 233); twenty five (50%) were positive for anti-MCV, median titer of 21U (IQ: 10 – 218) and 40 (80%) were positive for RF median titer of 368U (IQ: 32 – 658). There was statistically significant difference between the mean ranks of values of ACPAs and RF among three studied groups. The mean ranks of anti-CCP, anti-MCV and RF were significantly higher in patients with RA when compared to those

with TB and healthy controls (figures I, II and III). Sensitivity of anti-CCP for diagnosis of RA was 78% (CI: 63 to 88%) and specificity of 97% (CI: 89 to 99%). Lower sensitivity was observed for anti-MCV: 50% (CI: 35 to 64%), nevertheless, maintaining high specificity of 97% (CI: 89 to 99%) for diagnosis of RA. Statistically significant correlation was observed between the anti-CCP and anti-MCV values (Spearman – $p > 0.001$ and $r = 0.6$) in the population with RA. As regards RF, sensitivity was 80 % (CI: 65 to 89%) and specificity of 55 % (CI: 43 to 67%). The demographic, clinical data and results of tests in the three groups of patients are presented in Table I.

**DISCUSSION**

ACPAs in RA have been investigated for a long time, and initially, this was done by means of anti perinuclear factor detection[33]. Since then, search for various antibodies against citrullinated peptides such as antikeratin, anti-filagrin, anti-Sa and anti-vimentin has been developed, and in 2000 the first kit for anti-CCP was launched [7]. In a recent study that reviewed 151 articles in which diverse methodologies were used for the detection of ACPAs, including cohort, cross-sectional and case control studies, as well as heterogeneous arthritis populations, the sensitivity of anti-CCP ranged from 40 to 93% and specificity from 70 to 100%. The authors concluded that the search of anti-CCP is useful for the diagnosis of RA, including initial stages of the disease[8]. This finding is compatible with the experience of our service in cooperation with the University of Calgary, which observed a sensitivity of 79% and a specificity of 93% for anti-CCP for the diagnosis of RA, using a population with systemic sclerosis and primary biliary cirrhosis as control[9].

On the other hand, as mentioned above, recent studies have demonstrated the presence of anti-CCP antibodies by ELISA, in infectious diseases, particularly TB. These findings may represent a disturbance to clinicians attending patients in countries such as Brazil, where there is high frequency of these diseases, mainly because osteo-articular manifestations are presented during the course of many of them. Bearing in mind this concern, we performed a systematic review on this subject which revealed studies demonstrating a positivity of anti-CCP antibodies in TB ranging from 0 to 37%[34]. In the present study there was low positivity of ACPA in TB; that is to say, only two out of 50 patients tested (4%), resulting in high specificity of the test for the diagnosis of RA. The discrepancy of our results in comparison with those of previous studies may be attributed to methodological causes, particularly due to the use of kits of different origins, or the use of "in house" ELISA. Of note, one cannot exclude the hypothesis that the positivity of ACPAs in TB or in other infectious diseases, observed in some studies, would be related to the specificity of these antibodies to non citrullinated epitopes of the substrate, and thus the choice of kit to performe this test assumes a particular importance, particularly in countries where there is high prevalence of TB. Alternatively, the diversity of the studied populations may, in part, contribute to this difference found.

In conclusion, the positivity of ACPA antibodies in TB was observed to be low. IN addition, the great diagnostic performance in the detection of ACPAs for RA was confirmed, particularly the anti-CCP test.

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**Conflict of Interest**

The authors have no conflict of interest that is directly relevant to the content of this manuscript.

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Table I. Clinical, epidemiological and laboratorial characteristics of the three groups of patients studied

| | RA (50) | TB (50) | CONTROLS (20) |
|---|---|---|---|
| Female (%) | 94 | 52 | 40 |
| Age (years) | 55 (± 13)* | 48 (± 17) * | 35 (28 – 42) ** |
| Smoking (%) | 13 | 42 | 0 |
| Time of diagnosis (years[&] or days[&&]) | 8 (2 – 18)**[&] | 8 (2-18)** [&&] | NA*** |
| Time of RIP therapy (days) | NA | 8 (2 -19)** | NA |
| Positivity of anti-CCP (%) | 78 | 4 | 0 |
| Anti-CCP Titer (%) | 128 (24 – 233)** [@] | 4 (2 – 9)** [@] | 2 (1 – 2)**[@] |
| Positivity of anti-MCV (%) | 50 | 4 | 0 |
| Anti-MCV Titer (Units) | 21 (10 - 218 )** [@] | 9 (7 – 12)** [@] | 2 (2 – 3)** [@] |
| RF Positivity (%) | 80 | 60 | 5 |
| RF Titer (Units) | 368 (32 – 658)* [@] | 30 (19 – 43)* [@] | 6 (3 – 11)* [@] |

Abbreviations: RA: rheumatoid arthritis; TB: tuberculosis; RIP: rifampicin / isoniazid / pirazinamid; RF rheumatoid factor

*Mean ± standard deviation; ** Median and interquartile interval
***NA: Not applicable,
@ $p < 0.0001$

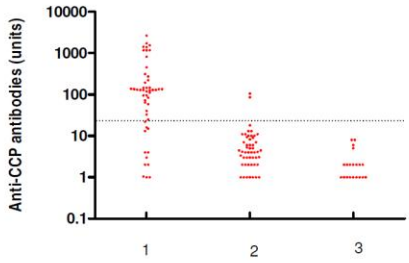


Figure I - Anti-CCP antibodie (units) in rheumatoid arthritis (1), tuberculosis (2) and controls (3)

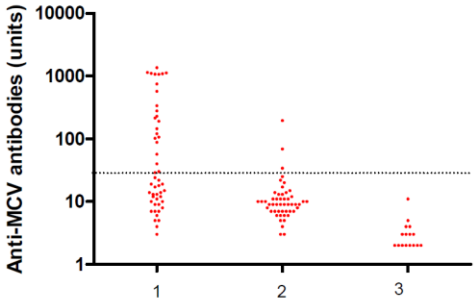


Figure II - Anti-MCV antibodies in rheumatoid arthritis (1), tuberculosis (2) and in controls (3).

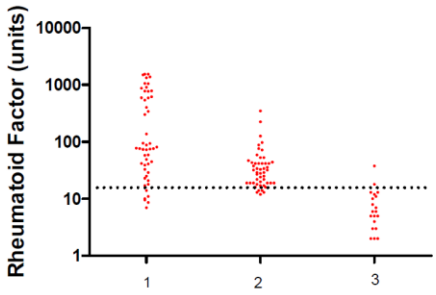


Figure III - Rheumatoid Factor in rheumatoid arthritis (1); in tuberculosis (2) and in controls (3).

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# ANCA, anti-PR3 and anti-MPO antibodies are not present in serum of patients with pulmonary tuberculosis.

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| | |

# ANCA, anti-PR3 and anti-MPO antibodies are not present in serum of patients with pulmonary tuberculosis.

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## ABSTRACT

**Objective**: Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed to intracellular components of neutrophils and are present in several vasculitic syndromes. Recently, these autoantibodies have been described in other autoimmune disorders as well as in infectious diseases such as tuberculosis (TB). As there are some clinical similarities between TB and Granulomatosis with Polyangiitis (GPA), we searched for ANCA and for antibodies to proteinase 3 (PR3) and myeloperoxidase (MPO) in a group of patients with proven TB. **Material and Methods:** Patients with TB confirmed by chest x-ray and sputum bacilloscopy either before or within 30 days after beginning treatment were included in this study. Anti-MPO and antiPR3 antibodies were detected using well-standardized ELISA kits (INOVA Diagnostics, Inc.). ANCA was also investigated by indirect immunofluorescence. **Results**: Fifty TB patients (26 females, mean age 47.34 ± 17 years) were enrolled in the present study. No patient tested positive for ANCA, MPO or PR3 antibodies. **Conclusions**: Although previous studies have shown the presence of ANCA in some infectious diseases, the findings of the present study demonstrated the absence of such antibodies in TB. The discrepancy in the prevalence of ANCA in infectious disorders among different studies may be attributed to methodological factors and/or the genetic background of the studied populations.


**Key words:** ANCA; anti-PR3; anti-MPO; tuberculosis.

## INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed to intracellular components of neutrophils, usually described in small vessel systemic vasculitis such as Granulomatosis with Polyangiitis (GPA), Microscopic Poliangiitis and Churg Strauss syndrome. In GPA their specificity is to proteinase 3 (PR3) whereas in the other two types of vasculitis the most common antigenic target is myeloperoxidase (MPO).[1]

Recently, these autoantibodies have been described in other autoimmune disorders[2-5] as well as in infectious diseases[6-13], particularly tuberculosis (TB).[14-17] As GPA shares some clinical features with TB, in areas with high prevalence of this infectious disease the positivity of these serologic tests may lead to a misdiagnosis and consequently wrong treatment. The aim of the present study was investigate the frequency of these antibodies in a group of patients with proven pulmonary TB from an endemic area in Brazil.

## PATIENTS AND METHODS

Patients either untreated or within 30 days after beginning treatment for pulmonary TB confirmed by chest x ray and sputum bacilloscopy were included in this study. All patients were older than 18 years, voluntarily agreed to participate in this study and signed a consent form. The Ethics Committee of our institution approved the project. The included patients were submitted to a complete clinical evaluation including muscle skeletal manifestations, time of the diagnosis and medicine in use.

ANCA were determined by indirect immunofluorescence (IIF) using a commercially available kit (EUROIMMUN) following the protocol summarized

below: The sera to be tested were diluted 1:10 in phosphate buffered saline (PBS) and incubated on the slides for 30 min in a moist chamber; after this period, the slides were washed with PBS. Again, the slides were incubated in the moist chamber for 30 min, this time with an anti-human IgG conjugate (goat). After washing with PBS, the slides were examined with a fluorescence microscope. Serum samples were also tested for the presence of antibodies to PR3 and MPO utilizing well standardized kits according to the manufacturer's (INOVA Diagnostics Inc.) recommendations. Values above 20U are considered positive.

Quantitative variables were presented as mean ± standard deviation or median and interquartile range, and qualitative variables were expressed as percentages. For statistical analysis a package program (SSPS for Windows version 18.0) was used.

**RESULTS**

Fifty pulmonary TB patients [twenty-six (52%) female, mean age 47.34 (± 17 years)] were enrolled in the present study. Forty-six patients were in the beginning of TB treatment with rifampicin, isoniazid and pyrazinamide and had a median treatment time of eight days (interquartile range 2-19). The majority of the patients (31) had no joint symptoms, whereas the others described some unspecific muscle skeletal pain without evidence of arthritis. The clinical and epidemiological characteristics of the patients are presented in Table I.

None of the tested samples was positive for ANCA by IIF or had antibodies to MPO or PR3 by ELISA.

**DISCUSSION**

Historically antibodies to cytoplasmic components of neutrophils detected by immunofluorescence were initially described in patients supposedly infected by *Ross River virus* in 1982.[18] These patients had several clinical features of autoimmune disorders such as fever, arthralgia, myalgia and some of them had glomerulonephritis. Later on, such antibodies were associated with GPA[19] and presently it is well known that patients with GPA have antibodies with specificity to PR3 that gives the cytoplasmatic pattern of ANCA whereas antibodies to MPO with the perinuclear pattern of ANCA (P-ANCA) are seen frequently, although not exclusively, in patients with Chung Strauss syndrome and microscopic polyangiitis.[1]

Curiously, in the last few years, studies have presented conflicting results regarding ANCA positivity in infectious diseases, particularly pulmonary TB. As this condition bears some clinical and histopathological features of GPA, it is mandatory clarify this point, as it would lead to misdiagnosis, particularly in endemic areas of TB. In this context Flores-Suárez *et al.* [20] studying 45 TB patients in Mexico found a prevalence of ANCA of 44% by IIF, mainly of the cytoplasmic pattern (C-ANCA). These authors also found an astonishing positivity of 40% in their cohort, for anti-PR3 and anti-MPO antibodies by ELISA. Pradhan *et al.* demonstrated a positivity of ANCA, anti-MPO and anti-PR3 in 30%, 47.6% and 28.6%, respectively, in their cohort of patients with TB[16]. Ghosh *et al.* gathered the findings obtained by Pradhan *et al.* with those retrieved from two other studies summing up a total of 318 TB patients. ANCA was positive in 30% of the entire studied population.[17] Recently, Sherkat *et al.* evaluated the prevalence of ANCA, anti-MPO and anti-PR3 antibodies in 32 subjects with active TB comparing with 32 healthy controls. They found P-

ANCA and C-ANCA in 25% and 3.1% of the cases, respectively, mainly due to the presence of anti-MPO antibodies. However, the mentioned study has important methodological limitations that preclude drawing any reliable conclusion from the data.[15]

On the other hand, other studies, apart from the present one, have demonstrated an absence or low frequency of positivity of these antibodies in TB. Teixeira *et al.*, found only 10% of ANCA positivity by IIF in 67 TB patients and only one had antibodies to PR3[21]. Esquivel-Valerio *et a*l. searched ANCA, anti-PR3 and anti-MPO in 68 TB patients before treatment and in 52 of them, 60 to 90 days after initiation of anti-TB therapy. In the pre-treatment samples the positivity of ANCA found was 3/68 (4.4%), being one C-ANCA and two P-ANCA. None of the samples had anti-PR3 or anti-MPO antibodies. Whereas, after initiation of treatment, ANCA was identified in 15/52 (28.8%) – twelve P-ANCA and three C-ANCA. In 11 of these 15 samples (73.3%) there was a specificity of the antibodies to bactericidal/permeability-increasing protein. PR3 and MPO antibodies were negative in all tested samples.[22]

The findings of the present study, demonstrating the negativity of ANCA by IIF, anti-PR3 and anti-MPO antibodies by ELISA in TB confirm the high specificity of these tests for the identification of vasculitic syndromes. This observation has been corroborated by the results of a previous study from our institution demonstrating very low prevalence of these antibodies in Hansen's disease[23].

The discrepancy in the prevalence of ANCA in TB observed in different studies may be attributed to methodological factors such as inclusion of patients with bacteriologically unproved TB, or at a later stage of treatment and use of

diagnostic kits from unreliable sources. Moreover the possibility of difference in the genetic background of the studied populations cannot be excluded.

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**Conflict of Interest**

The authors have no conflict of interest that is directly relevant to the content of this manuscript.

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Table I. Clinical and epidemiological features of the tuberculosis patients (n=50)

| **Characteristics** | **N(%)** |
|---|---|
| Female | 26 (52) |
| Positive bacilloscopy | 50 (100) |
| Abnormal chest x ray | 49 (98)* |
| Tabagism | 21 (42) |
| Alcoholism | 13 (26) |
| Illicit drug use | 1 (2) |
| **Tuberculosis Symptoms** | |
| Fever | 18 (36) |
| Weight Loss | 17 (34) |
| Cough | 50 (100) |
| Hemoptysis | 5 (10) |
| Chest Pain | 6 (12) |
| Dyspnoea | 9 (18) |
| **Tuberculosis Treatment** | |
| R/ I / P# | 46 (92)** |
| **Co-Morbidities** | |
| HIV | 3 (6) |
| Diabetes | 8 (16) |
| Hypertension | 10 (20) |

* One patient did not have a chest x ray

# R: rifampicin/ I: isoniazid / P: pyrazinamide

**Three patients had not started treatment and one patient discontinued it due to intolerance

# VI

# DISCUSSÃO

A etiopatogenia das doenças autoimunes é complexa, não completamente conhecida, mas sabe tratar-se de condições com causa multifatorial, que envolve uma disfunção imunológica precipitada por fator ambiental em indivíduos geneticamente predispostos. Reconhece-se que dentre os fatores ambientais, agentes infecciosos podem estar envolvidos na quebra de tolerância imunológica e, conseqüente desenvolvimento de doença autoimune. Os mecanismos através dos quais ocorre esta quebra de tolerância são diversos: necrose celular com exposição de epítopos de autoantígenos ou de antígenos originalmente não expostos; ativação policlonal de LT e LB por superantígenos microbianos; liberação de mediadores inflamatórios e de fatores de co-estimulação que ativam células não diretamente envolvidas na resposta ao patógeno; além do mimetismo molecular[1-3]. Os resultados de trabalhos recentes demonstrando uma alta frequência de ACPAs por ELISA em doenças infecciosas, particularmente TB, são intrigantes e provocativos por tratar-se de um teste diagnóstico até então considerado altamente específico para o diagnóstico de AR. Por isso, inicialmente optou-se pela realização de uma revisão sistemática sobre o tema e observou-se a grande variabilidade nos resultados dos diferentes estudos.

Elkayam *et al.* estudaram 47 pacientes com TB ativa quanto a presença de anti-CCP, que foi positivo em 15 (32%) da população estudada e em 2,6% dos controles sadios, não havendo, no entanto associação entre a presença de anti-CCP e sintomas reumatológicos[12]. Esses mesmos autores, pesquisaram a presença do anti-CCP e do anticorpo anti-arginina não modificada (anti-CAP) em 122 portadores de TB e 12 casos de micobacteriose atípica. Para tal foram utilizados dois pares de peptídeos cíclicos sintetizados – método ELISA (*in house*). Cada par continha um peptídeo citrulinado e um não modificado (anti-CAP): 0401cit, 0401arg, 0722cit, 0722arg. Oitenta e três pacientes foram positivos para anti-CAP (0401arg) enquanto apenas 14 para anti-CCP

(0401cit) e 61 foram positivos para anti-CAP (0722arg) enquanto apenas 22 positivos para anti-CCP (0722cit)[13]. Kakumanu et *al.*, utilizando um kit *in house*, também estudaram a presença de anti-CCP e do anti-CAP em 49 pacientes com TB, 36 com AR e 18 controles hígidos. O anti-CCP foi positivo em 37% dos pacientes com TB e em 43% dos casos de AR. O anti-CAP manteve alta positividade entre os portadores de TB (27%) e em apenas 3% dos casos de AR[14]. Diante destes achados, os autores sugerem que a positividade do anti-CCP observada nos pacientes de tuberculose possivelmente referia-se a sua reatividade ante a epítopos não citrulinados do antígeno.

Utilizando uma casuística própria de pacientes com TB pulmonar, observou-se no presente estudo que a positividade dos ACPAs foi baixa, ou seja, apenas dois de 50 pacientes testados (4%); e nos soros de pacientes com AR, a positividade foi de 78% para anti-CCP e 50% para anti- MCV , confirmando a alta especificidade do teste para diagnóstico de AR. Estes dois pacientes com TB e positividade para ACPSs não apresentavam sintomas compatíveis com AR, numa reavaliação clínica realizada um ano após a avaliação inicial. Porém, como esses anticorpos podem preceder a doença por anos, não se pode excluir a possibilidade de que isso ainda venha o ocorrer no futuro.

Mori et *al.* publicaram dados com achados semelhantes aos nossos: o anti-CCP foi positivo em 6,7% dos casos de tuberculose em comparação aos 82% de positividade dos casos de AR[15]. Foram encontrados ainda dois estudos, que incluíram pacientes com tuberculose como controles na avaliação de *performance* do anti-CCP. Schellekens *et al.* encontraram 2 (10%) de positividade em 20 pacientes com tuberculose (ELISA, *cut off* 92U)[16]; enquanto Silva *et al*., não encontraram nenhum anti-CCP positivo em 10 pacientes com TB (ELISA, *cut off* de 25U)[32]. O processo de citrulinização, que corresponde a modificação da arginina pela citrulina, catalisada pela enzima peptidil

arginina desaminase, é descrito em condições fisiológicas tais como: diferenciação epidermal, formação de folículos pilosos e diferenciação da bainha de mielina durante a formação do SNC[46-48]. Do mesmo modo, Makrygiannakis *et al.* constataram que a citrulinização é um processo comum na inflamação. Para tal, foram estudados espécimes de biópsias obtidas de pacientes com condições inflamatórias como AR, polimiosite (PM), doença inflamatória intestinal (DII) e amigadalites recorrentes. Proteínas citrulinadas foram encontradas em 100% dos casos de AR, PM e amigalite crônica, e em 70% das lesões ativas de DII. Infelizmente, não foi realizada a pesquisa de anticorpos anti-CCP naquele trabalho[49]. Embora a citrulinização seja um fenômeno fisiológico ou secundário à inflamação, como ocorre nas doenças infecciosas, a produção de anticorpos contra peptídeos citrulinados ocorre na minoria dos casos. Historicamente, a pesquisa dos anticorpos contra peptídeos citrulinados foi inicialmente realizada através de teste de IFI, ou seja, pesquisando-se o "fator antiperinuclear", descrito em 1964 por Nienhuis e Mandema[50]; e através desse teste esses anticorpos foram pesquisados em doenças infecciosas como: MH (13 casos) e hepatites virais (6), tendo sido positivos em apenas um dos pacientes com MH (7,6%)[51]. Similarmente, em 1972, Keil *et al.* encontraram a positividade do fator antiperinuclear em 1,1% dentre os 93 casos de TB estudados sem sintomas articulares, em um dos dois casos (50%) de tuberculose também portadores de poliartrite crônica e em nenhum portador de hepatite infecciosa[52]. Desde então, vêm sendo desenvolvidas pesquisas de diversos anticorpos contra peptídeos citrulinados – anti-ceratina, anti-filagrina, anti-Sa, anti-vimentina e em 2000 foi lançado o primeiro kit para a pesquisa de anti-CCP[16].

Em um estudo recente, que revisou 151 trabalhos que utilizaram metodologias diversas para a detecção de ACPAs, a sensibilidade do anti-CCP variou de 40 a 93% e a especificidade de 70 a 100% para o diagnóstico de AR[10]. Tal achado é compatível com

a experiência do nosso serviço em colaboração com a universidade de Calgary, que observou uma sensibilidade de 79% e uma especificidade de 93% do anti-CCP, utilizando como controle uma população de esclerose sistêmica e cirrose biliar primária[53].

Diante destes dados, não pode ser excluída a hipótese de que a positividade dos ACPAs em TB ou em outras doenças infecciosas, observada em alguns estudos, estaria relacionada à especificidade dos mesmos para epítopos não citrulinados do substrato e assim a escolha do kit para a determinação desse teste assume uma particular importância, principalmente em países onde a prevalência de TB é alta. A discrepância, portanto, dos nossos resultados em relação a estudos prévios pode ser atribuída a razões metodológicas, particularmente pela utilização de kits de diferentes origens ou a utilização de ELISA "in house", o que poderia justificar a positividade do anti-CCP em casos de TB pela sua reação aos epítopos não citrulinados do antígeno.

Outra hipótese que deve ser considerada é o fato da presença dos anticorpos anti-CCP preceder o diagnóstico clínico de AR. Não seriam estes pacientes com infecção e anti-CCP positivos futuros portadores de AR?

Ainda neste contexto, como há uma similaridade entre TB e GPA, tanto do ponto de vista clínico/radiológico em seu envolvimento pulmonar quanto histológico, foi pesquisada a presença de ANCA por imunofluorescência, antiPR3 e anti-MPO por ELISA no contexto de TB. Seriam estes anticorpos também um fator confundidor de diagnóstico de vasculites em áreas endêmicas para TB?

Historicamente, a presença do ANCA foi descrita em 1982 e relacionada a um quadro infeccioso, porém viral. Tratava-se de um grupo de 8 pacientes que apresentavam quadro clínico semelhante: artralgia, mialgia, febre, diarréia e vômitos. Adicionalmente, quatro pacientes apresentavam sintomas respiratórios como dispnéia e hemoptise; e

cinco pacientes apresentavam evidências de envolvimento renal com hematúria, edema de membros inferiores e a presença de glomerulonefrite necrotizante à biópsia. Diante da apresentação clínica, foi feita a hipótese de tratar-se de doença autoimune. No entanto, a pesquisa do fator antinuclear e outros autoanticorpos foi negativa, mas observou-se uma fluorescência positiva no citoplasma. Observou-se também que esta fluorescência desaparecia alguns dias após o início da terapia com corticóides e ou imunossupressores. Pela característica geográfica comum destes pacientes, pensou-se tratar-se de quadro relacionado a um arbovírus: *Ross River virus*, endêmico na região[54]. Posteriormente, em publicação de 1985, o ANCA foi associado à GPA com valor diagnóstico; assim como preditor de atividade de doença[55]. Por outro lado, mais recentemente algumas publicações também relataram a positividade do ANCA em quadros infecciosos. Nestes casos, em geral, a pesquisa direta por ELISA de anticorpos anti-PR3 e anti-MPO foi negativa. Revendo os dados obtidos da literatura sobre esse tema, observa-se que os mesmos são divergentes quanto à positividade destes anticorpos em portadores de TB, com alguns trabalhos mostrando uma freqüência alta de positividade de até 44% e outros mostrando a ausência ou pequena frequência da positividade de ANCA em portadores de TB[36-38;43-45], corroborando com os achados do nosso estudo. Um dos autores demonstrou que a positividade do ANCA era maior após o tratamento e estava direcionada contra a BPI-ANCA e não contra os substratos PR3 e MPO.

À semelhança das discrepâncias associadas à pesquisa de anti-CCP em tuberculose, as divergências quanto à positividade do ANCA pode estar relacionada à questões metodológicas ou à reatividade contra substratos não patogênicos.

# VII

# CONCLUSÕES

A revisão sistemática sobre a positividade de ACPAs em doenças infecciosas mostrou que há uma controvérsia no que diz respeito à positividade desses anticorpos em TB. Em relação às outras doenças infecciosas estudadas, a positividade de ACPAs não foi relevante.

A pesquisa dos ACPAs por ELISA, assim como a pesquisa do ANCA por IFI, anti-PR3 e anti-MPO por ELISA, na população estudada confirma a hipótese de alta especificidade destes testes para o diagnóstico de artrite reumatóide e vasculites sistêmicas, respectivamente.

# VIII

# ABSTRACT

**Research of autoantibodies used in diagnosis of rheumatoid arthritis and vasculitis in patients with tuberculosis**

Introduction: There is a recognized interface between rheumatology, particularly with respect to autoimmune diseases, and infectology, whether one considers the hypothesis of infectious agents acting as trigger to immunological dysfunctions, or the infectious risk attributed to immunosuppressive treatments. In addition, the production of some antibodies during the course of infections has also been observed. For example: in patients with tuberculosis (TB), the production of antibodies described as being of high specificity for rheumatoid arthritis (RA), such as anticitrullinated peptide antibodies (ACPAs) has been demonstrated, and in the same manner, the presence of antineutrophil cytoplasmatic antibodies (ANCA) has been demonstrated, among them antiproteinase 3 (anti-PR3) and antimyeloperoxidase (anti-MPO), which are markers of systemic vaculitis. Aims: a) to review publications about the positivity of ACPAs in infectious diseases, b) research the presence of these antibodies, as well as ANCA in a population of patients with TB. Methods: a) initially a systematic review was conducted of studies evaluating the presence of ACPAs in infectious diseases; b) afterwards, a group of 50 patients with pulmonary TB, untreated or at up to 30 days after the beginning of treatment, was evaluated with regard to the presence of rheumatological symptoms, an particularly with reference to positivity for ACPA antibodies, including anticitrullinated cyclic peptide antibodies (anti-CCP) and modified anticitrullinated vimentin antibodies (anti-MCV); and positivity for ANCA by immunofluorescence (IFI), and anti-PR3 and anti-MPO by enzyme immunoassay (ELISA). Results: a) the systematic review was published and is presented in the section "**Review of the Literature"** under the title *Antibodies against cyclic citrullinated peptides in infectious diseases – a systematic review*. *Clin Rheumatol 2010, Dec 29(12): 1345-51.* b) positivity for ACPAs was found in only two (4%) of the 50 patients with TB and there

was no positivity for ANCA by IFI, or presence of anti-PR3 or anti-MPO antibodies by ELISA in the serum of these patients. These results are presented in two articles that were submitted to the journal *Clinical Rheumatology* (Canada, Impact Factor 2011: 1.996), awaiting the report from the editorial body. The versions submitted are to be found in the section "**Articles"**. Conclusions: Although previous studies have related the presence of ACPAs and ANCA in patients with TB, in the present study, the positivity for ACPAs was low and no positivity was observed for ANCA, anti-PR3 and anti-MPO, confirming the high specificity of these tests for RA and systemic vasculitis, respectively.

Key Words: Tuberculosis, autoantibodies, ACPA, anti-CCP, ANCA, anti-MPO, anti-PR3, rheumatoid arthritis.

# IX
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# X
# ANEXOS

Since the introduction of EIA for the detection of ANCA and anti-GBM antibodies, standardization of test results from different EIA assays has always been a concern. In this study, we evaluated anti-PR3, anti-MPO and anti-GBM assays from Bio-Rad, Phadia and INOVA for the detection of ANCA and anti-GBM antibodies.

**Methods:** A total of 72 clinical serum samples were collected and the presence of antibodies to PR3 was determined by the Bio-Rad anti-PR3 (Cat. no: 425-2420), the Bio-Rad Kallestad anti-PR3 (Cat. no: 31023), the Phadia Veralisa anti-PR3 and the INOVA Quanta Lite anti-PR3 assays. Similarly, 94 clinical serum samples were collected and the presence of antibodies to MPO was determined by the anti-MPO assays from Bio-Rad, Phadia and INOVA. A total of 79 clinical serum samples were collected and the presence of antibodies to GBM was determined by the anti-GBM assays from Bio-Rad and INOVA. The Bio-Rad and Bio-Rad Kallestad assays were obtained from Bio-Rad Laboratories (Hercules, CA, USA). The Phadia Veralisa assays were obtained from Phadia (Freiburg, Germany). The INOVA Quanta Lite assays were obtained from INOVA (San Diego, CA, USA). All assays were performed manually according to the manufacturers' instructions. The results were reported as positive or negative according to the cut-off units recommended by each assay. The agreements between each pair of assays were calculated and compared.

**Results:** The Bio-Rad anti-PR3, anti-MPO and anti-GBM assays showed overall excellent agreements with the corresponding assays from Phadia and INOVA. For the anti-PR3 assays, the positive agreement is 100% for all comparisons. The overall agreements range from 90% to 99%. For anti-MPO assays, the positive agreements range from 98% to 100%, and the overall agreements range from 90% to 99%. For the anti-GBM assays, the positive agreement is 100% for all comparisons, and the overall agreements range from 91% to 98%.

**Conclusions:** This comparative evaluation of the anti-PR3, anti-MPO and anti-GBM assays for the detection of ANCA and anti-GBM antibodies showed good overall agreements by assays from different manufacturers, suggesting that progress has been made towards the goal of EIA standardization for ANCA and anti-GBM antibody testing.

**Declarations of interest**

All the authors are employees of Bio-Rad Laboratories, Inc.

### Anti-PR3 and anti-MPO antibodies are not present in tuberculosis, leprosy or leishmaniasis

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**Objective:** ANCA are autoantibodies directed to intracellular components of neutrophils and are present in several vasculitic syndromes. Recently, these autoantibodies have been found in other autoimmune disorders as well as in infectious diseases. As there are some similarities between pulmonary TB and WG, we searched for antibodies to PR3 and MPO in a group of patients with proven TB. For comparison, such antibodies were also tested in patients with leprosy and patients with VL.

**Methods:** Utilizing a well-standardized ELISA kit for the detection of PR3 and MPO (Inova, Inc.) we studied patients with TB confirmed by sputum bacilloscopy, leprosy confirmed by bacilloscopy and/or skin biopsy and VL confirmed by bone marrow examination.

**Results:** Fifty TB patients (24 males and 26 females, mean age 47.34 ± 17 years), 23 with VL and 20 with leprosy, were enrolled into the present study. No patient tested positive for MPO or PR3 antibodies by ELISA.

**Conclusions:** Although other studies have shown the presence of ANCA in some infectious diseases, our findings suggest that in countries where the prevalence of such infectious diseases is high, direct search for antibodies to MPO and PR3 helps to distinguish primary vasculitis syndromes from infectious diseases. The discrepancy in the prevalence of ANCA in infectious disorders among different studies may be attributed to methodological reasons and/or the genetic background of the studied populations.

**Declarations of interest**

None.

### Soluble Fms-like tyrosine kinase 1 (sFLT) is elevated in ANCA-AAV and declines during therapy

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**Objective:** Fms-like tyrosine kinase-1 (Flt-1) is one of the major receptors for VEGF. Its soluble form (sFLT) may act as a decoy receptor for VEGF in the circulation, thereby reducing VEGF bioavailability. Hence, increased levels of sFLT may cause inactivation of VEGF and therefore lead to endothelial dysfunction. Elevated serum levels of sFLT-1 have been found in sepsis and pre-eclampsia and correlated with disease severity. In WG, higher VEGF levels have been reported in patients with major disease activity compared to those with minor activity. The following study was therefore intended to determine sFLT serum level in ANCA-AAV.

**Methods:** Plasma sFLT-1 were measured with a commercially available ELISA in patients with ANCA-AAV at initial diagnosis and after 1, 3, 6 and 12 months ($n = 14$). Disease activity was assessed in accordance with the BVAS. BVAS, CRP, creatinine and ANCA titres were recorded at baseline and during follow-up.



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# ACR Invitation Acceptance Notification

1 mensagem

**acr@confex.com** <acr@confex.com> 18 de agosto de 2012 23:06
Responder a: acr@confex.com
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Dear Isabella Lima, MD,

Thank you for agreeing to participate in the 2012 ACR/ARHP Annual Meeting, in Washington, DC, November 09 - 14, 2012. We are delighted and grateful that you have agreed to serve as Poster presenter for the abstract titled "Antibodies to Citrullinated Peptides in Tuberculosis".

We have you scheduled for the following:
Session Type: Poster
Session Name: Infection-related Rheumatic Disease
Date and Session Time: Sunday, November 11, 2012: 9:00 AM-6:00 PM
Location: Poster Hall (Hall B) (WCC)
Presentation Number: 178

If you have specific questions regarding your abstract, you may contact Stacey Boyd, Annual Scientific Meeting Abstracts, at abstracts@rheumatology.org or 404-633-3777. We look forward to seeing you at this year's Annual Scientific Meeting in Washington.

Sincerely,
Chester Oddis, MD
Chair, ACR Annual Meeting Planning Committee

Linda Ehrlich-Jones, PhD, RN
Chair, ARHP Annual Meeting Program Sub-Committee