UNIVERSIDADE FEDERAL DE SÃO CARLOS - UFSCar
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE - CCBS
PROGRAMA DE PÓS-GRADUAÇÃO EM GERONTOLOGIA - PPGGERO DEPARTAMENTO DE GERONTOLOGIA - DGERO
LABORATÓRIO DE BIOLOGIA DO ENVELHECIMENTO - LABEN

MARIA PATRÍCIA OLIVEIRA MONTEIRO E PEREIRA DE ALMEIDA

A DESINTEGRINA E METALOPRTEINASE 10 (ADAM10) COMO BIOMARCADORA PARA A DOENÇA DE ALZHEIMER: UMA REVISÃO SISTEMÁTICA

SÃO CARLOS
2022

MARIA PATRÍCIA OLIVEIRA MONTEIRO E PEREIRA DE ALMEIDA

A DESINTEGRINA E METALOPRTEINASE 10 (ADAM10) COMO BIOMARCADORA PARA A DOENÇA DE ALZHEIMER: UMA REVISÃO SISTEMÁTICA

Dissertação apresentada ao Programa de Pós-Graduação em Gerontologia do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, para obtenção do título de Mestre em Gerontologia.

Orientadora: Profa. Dra. Márcia Regina Cominetti

SÃO CARLOS
2022

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Gerontologia

Folha de Aprovação

Defesa de Dissertação de Mestrado da candidata Maria Patrícia Oliveira Monteiro e Pereira de Almeida, realizada em 08/08/2022.

Comissão Julgadora:

Profa. Dra. Márcia Regina Cominetti (UFSCar)

Prof. Dr. Vicente Paulo Alves (UCB)

Profa. Dra. Carla Helena Augustin Schwanke (PUCRS)

O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Gerontologia.

Dedico este trabalho às pessoas que sofrem de Alzheimer e aos seus cuidadores que dedicam suas vidas ao cuidado destes.

AGRADECIMENTOS

Agradeço a Deus, pela graça de realizar este sonho de tantos anos, que foi fazer o Mestrado e construir o meu aprendizado sobre a Doença de Alzheimer.

Aos meus pais José Augusto e Maria Teresa, e aos meus irmãos Leonardo José e Anna Paula, por todo apoio, durante esse tempo de estudos longe de casa e nesse período da pandemia. À minha amiga Nathalia, pela força dada desde o início do mestrado, me encorajando e me acompanhando em todos os momentos.

Agradeço à minha orientadora Márcia, por ter partilhado comigo seu conhecimento em uma área com muitos desafios, foi muito bom trabalhar com uma excelente pesquisadora como você. Também agradeço à Renata, por toda ajuda dada neste momento de construção do meu trabalho.

Agradeço aos professores do Programa de Pós-Graduação em Gerontologia, por todo conhecimento partilhado, à FAPESP pelo financiamento desta pesquisa e aos membros da banca por contribuírem para este trabalho, com observações pertinentes.

AGRADECIMENTO ÀS AGÊNCIAS DE FOMENTO

O presente trabalho foi realizado com apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; processo nº 2019/26444-9), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Código de Financiamento 001) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq.

As opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade dos autores e não necessariamente refletem a visão da FAPESP, CAPES e CNPq

RESUMO

Introdução: Estudos têm demonstrado que A Desintegrina e Metaloproteinase 10 (ADAM10) é a principal a-secretase na clivagem não amiloidogênica da proteína precursora do amiloide (APP). A ADAM10 evita a produção do peptídeo β-amiloide, uma das características patológicas da doença de Alzheimer (DA). **Objetivo:** O objetivo deste trabalho foi investigar a ADAM10 presente no líquido cefalorraquidiano (LCR), plaquetas e plasma/soro como um potencial biomarcador para DA. **Métodos:** Foi realizada revisão sistemática nas bases de dados Sistema Online de Busca e Análise de Literatura Médica (MEDLINE/PubMed), na Base de dados de informações de bibliografias e citações (Web of Science), na Base de Dados Excerpta Medica (Embase) e no Banco de dados bibliográfico para resumos e citações de artigos de periódicos acadêmicos (Scopus), bem como a busca de citações de forma manual, utilizando os termos e operadores booleanos: "Alzheimer" AND "ADAM10" AND "biomarker". Os critérios de inclusão foram estudos originais sobre ADAM10 em sangue ou LCR de pacientes com DA. O risco de viés foi avaliado usando a Ferramenta de Avaliação de Qualidade para Coorte Observacional e Estudos Transversais. O protocolo da revisão foi registrado no banco de dados PROSPERO (CRD42021274239). **Resultados:** Dos 97 estudos recuperados, 17 foram incluídos. Há fortes evidências de níveis mais baixos de ADAM10 em plaquetas de pessoas com DA, em comparação com participantes cognitivamente saudáveis. Por outro lado, no plasma, foram encontrados níveis mais elevados de ADAM10. Em relação ao LCR, foram encontrados resultados controversos com níveis mais baixos e mais altos de ADAM10 em pessoas com DA, em comparação com idosos saudáveis. **Conclusão:** Evidências mostram que os níveis de ADAM10 estão alterados em plaquetas, plasma, soro e LCR de pessoas com DA. A alteração foi evidente em todos os estágios da doença e, portanto, a proteína pode representar um biomarcador complementar para a DA. No entanto, mais estudos devem ser realizados para estabelecer valores de corte para os níveis de ADAM10 para discriminar participantes com DA de idosos sem comprometimento cognitivo.

Palavras-chave: ADAM10, Alzheimer, Biomarcador, LCR, Plasma

ABSTRACT

Introduction: Studies have shown that Disintegrin and Metalloproteinase 10 (ADAM10) is the main α-secretase in the non-amyloidogenic cleavage of amyloid precursor protein (APP). ADAM10 prevents the production of β-amyloid peptide, one of the pathological features of Alzheimer's disease (AD). **Objective:** The objective of this work was to investigate ADAM10 present in cerebrospinal fluid (CSF), platelets and plasma/serum as a potential biomarker for AD. **Methods**: A systematic review was carried out in the Online System of Search and Analysis of Medical Literature databases (MEDLINE/PubMed), in the Database of bibliographic and citation information (Web of Science), in the Excerpta Medica Database (Embase) and in the bibliographic database for abstracts and citations of articles from academic journals (Scopus), as well as the manual search for citations, using the Boolean terms and operators: "Alzheimer" AND "ADAM10" AND "biomarker". Inclusion criteria were original studies on ADAM10 in blood or CSF of patients with AD. Risk of bias was assessed using the Quality Assessment Tool for Observational Cohort and Cross-sectional Studies. The review protocol was registered in the PROSPERO database (CRD42021274239). **Results**: Of the 97 studies retrieved, 17 were included. There is strong evidence for lower levels of ADAM10 in platelets from people with AD compared to cognitively healthy participants. On the other hand, in plasma, higher levels of ADAM10 were found. Regarding CSF, controversial results were found with lower and higher levels of ADAM10 in people with AD compared to healthy elderly people. **Conclusion:** Evidence shows that ADAM10 levels are altered in platelets, plasma, serum and CSF of people with AD. The alteration was evident in all stages of the disease and, therefore, the protein may represent a complementary biomarker for AD. However, further studies should be carried out to establish cut-off values for ADAM10 levels to discriminate participants with AD from elderly people without cognitive impairment.

Keywords: ADAM10, Alzheimer, Biomarker, CSF, Plasma

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE SIGLAS E ABREVIATURAS

ADAM - A disintegrin and metallopeptidase (Uma desintegrina e metalopeptidase)

Aβ - Amyloid-beta (Peptídeo beta-amiloide)

APP - Amyloid Precursor Protein (Proteína precursora amiloide)

BACE - Beta-site amyloid precursor protein cleaving enzyme (Enzima de clivagem de APP do sítio β)

CSF - Cerebrospinal fluid (Fluido cerebrospinal)

DA - Doença de Alzheimer

ELISA - Enzyme-linked immunonoabsorbent assay (Ensaio imunonoabsorvente ligado a enzimas)

HEK - Human embryonic kidney cells (Células renais embrionárias humanas)

LC- MS/MS -Liquid chromatography-mass spectrometry (Espectrometria de massa por cromatografia em fase líquida)

LCR - Líquido cefalorraquidiano

MCI - Mild cognitive impairment (Comprometimento cognitivo leve)

mRNA - Messenger RNA (Mensageiro do ácido ribonucleico) NIA-AA - National Institute of Aging and the Guidelines of Alzheimer's Association (Instituto Nacional do Envelhecimento e as Diretrizes da Associação de Alzheimer)

NINCDS-ACRDA - National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (Instituto nacional de desordens neurológicas e comunicativas e critérios da associação de infarto e doenças relacionadas ao Alzheimer)

PRISMA - Preferred reporting items for systematic reviews and meta-analyses (Itens de relatório preferidos para revisões sistemáticas e meta-análises)

RT-qPCR – Quantitative reverse-transcriptase polymerase chain reaction (Reação quantitativa em cadeia da polimerase com transcriptase reversa)

SDS-PAGE - Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Eletroforese em gel de poliacrilamida com dodecil sulfato de sódio)

TNCL - Transtorno neurocognitivo leve

SUMÁRIO

INTRODUÇÃO

O século XX marcou definitivamente a importância do estudo do envelhecimento, fruto de um lado, da natural tendência de crescimento do interesse nas pesquisas e estudos sobre o processo de envelhecimento, que já se anunciava em anos anteriores - e de outro - da própria necessidade de prevenir e tratar as doenças comuns desta fase da vida. O aumento do número de idosos em todo o mundo exerceu pressão passiva sobre o desenvolvimento dos estudos relacionados ao envelhecimento (Netto.,2016). Segundo a Organização Mundial da Saúde (OMS) a expectativa de vida aumentou em quase todos os povos do planeta, sendo muito comum atualmente a maioria das pessoas viver com 60 ou mais. Espera-se que em 2050, a população mundial com 60 anos ou mais chegue a 2 bilhões (Neto *et al.*, 2016).

Acompanhando o aumento da expectativa de vida, a prevalência das demências também aumenta progressivamente com o envelhecimento, sendo a idade o maior fator de risco para a doença. O tipo mais comum de demência é a doença de Alzheimer (DA). O diagnóstico da DA é feito fundamentalmente através de critérios clínicos preestabelecidos juntamente com a exclusão de outras possíveis causas para a demência, feita através de um conjunto composto pelo exame clínico, por exames laboratoriais e pela neuroimagem cerebral (Yassuda *et al.* 2009).

Os sintomas neuropsiquiátricos característicos da DA causam grande sofrimento para pessoas acometidas e seus cuidadores, e contribuem para a institucionalização precoce (Lanctôt *et al.*, 2017). A DA corresponde a 60% dos quadros demenciais, sendo a mais prevalente no mundo. Atualmente, 35,6 milhões de pessoas convivem com a doença e a estimativa é de que esse número praticamente dobre a cada 20 anos, chegando a 65,7 milhões em 2030 (Teixeira *et al.*, 2015).

Considerando que o diagnóstico precoce é crucial para melhores estratégias prognósticas e terapêuticas para a DA, várias abordagens têm sido empregadas com esse objetivo, incluindo exames de imagem, teste do LCR e, mais recentemente, avaliação sanguínea de marcadores específicos da DA (Zetterberg e Burnham 2019, Koychev, Jansen et al. 2020, Manzine, Vatanabe

et al. 2020).

Os biomarcadores de DA baseados no sangue são vantajosos em relação a outros métodos de diagnóstico devido a vários aspectos, incluindo seu uso como um método de triagem pouco invasivo, que pode ser aplicado em larga escala e de baixo custo (Blennow e Zetterberg, 2018).

A Desintegrina e Metaloproteinase 10 (ADAM10) é a principal -secretase que realiza a clivagem não amiloidogênica da proteína precursora do amiloide (APP), impedindo a agregação e acúmulo do peptídeo β-amiloide (Aβ) em placas senis, o que leva à inflamação, tauopatia e consequente perda sináptica característica da DA (Manzine, Vatanabe et al. 2020). Os primeiros estudos sobre a ADAM10 e sua relação com a DA foram publicados há pouco mais de 20 anos, quando foi demonstrada a função *in vitro* dessa proteína como a secretase que cliva a APP em células renais embrionárias humanas (HEK 293) (Lammich, Kojro et al. 1999), fornecendo evidências de que o aumento de sua expressão e atividade pode ser benéfico para o tratamento da DA.

A ADAM10 foi confirmada como a principal α-secretase em neurônios (Vincent *et al.*, 2016). Para além de sua implicação bem estabelecida no chamado processamento não-amiloidogênico de clivagem da APP e seu provável papel protetor contra DA, esta metaloprotease também cliva muitos outros substratos, implicando em vários processos fisiológicos e patológicos como câncer e inflamação (Vincent *et al.*, 2016). Logo depois, outros estudos identificaram outra proteína, desta vez com atividade de β-secretase (BACE) para a clivagem de APP (Skovronsky, Moore et al. 2000). Os pesquisadores identificaram a co-expressão de APP e BACE *in vivo* em camundongos e em cérebros humanos (Marcinkiewicz e Seidah 2000).

Apenas dois anos depois foram publicados estudos demonstrando níveis mais baixos de ADAM10 no sangue (plaquetas) e no líquido cefalorraquidiano (LCR) em pacientes com DA, em comparação com controles cognitivamente saudáveis (Colciaghi, Pastorino et al. 2001), demonstrando o potencial da ADAM10 periférica como uma biomarcadora para a DA.

Neste sentido, a ADAM10 foi identificada como uma candidata a biomarcadora para a DA.

Esta proteína demonstrou níveis reduzidos nas plaquetas de pacientes com DA em comparação com indivíduos cognitivamente saudáveis (Colciaghi, Pastorino et al. 2001, Colciaghi, Marcello et al. 2004, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013).

Além disso, os níveis de ADAM10 em plaquetas demonstraram aumentar ao longo do envelhecimento cognitivamente saudável (Schuck, Wolf et al. 2016). Por outro lado, os níveis plasmáticos de ADAM10 solúvel foram encontrados aumentados no transtorno neurocognitivo leve do tipo amnéstico (TNCL) e em pacientes com DA em comparação com idosos cognitivamente saudáveis (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021).

Considerando todos os aspectos mencionados sobre o potencial da ADAM10 como biomarcadora para DA, esta revisão sistemática teve como objetivo investigar se seus níveis no LCR e plasma/soro poderiam ser utilizados como potencial biomarcador para a DA.

JUSTIFICATIVA

O processo de envelhecimento, bem como sua consequência natural, a velhice, continuam sendo preocupações da humanidade desde os seus primórdios. A história relata que as concepções e as preocupações sobre a velhice são tão antigas quanto a própria humanidade (Camarano et al, 2016). Os problemas relacionados ao envelhecimento humano, como doenças a ele associadas e que resultam em incapacidade funcional têm despertado a atenção da sociedade em geral, bem como dos pesquisadores da área (Camarano *et al.*, 2016).

Dentre as doenças comuns do envelhecimento estão as demências e dentre elas, a DA corresponde a 60% dos quadros demenciais, sendo o tipo mais prevalente no mundo. Atualmente, 35,6 milhões de pessoas convivem com a doença e a estimativa é de que esse número praticamente dobre a cada 20 anos, chegando a 65,7 milhões em 2030 (Teixeira *et al.*, 2015). Sendo assim, é intensa a busca de biomarcadores periféricos, de baixo custo e que permitam avaliações no formato de triagem em larga escala da população, para que a DA possa ser diagnosticada o quanto antes possível.

OBJETIVO

O objetivo deste estudo foi investigar, através de uma revisão sistemática na literatura recente, se ADAM10 presente no líquido cefalorraquidiano (LCR), plaquetas ou no plasma/soro poderia ser considerada como uma potencial biomarcadora para a DA.

Esta dissertação está apresentada no formato alternativo estabelecido pelo Programa de Pós-Graduação em Gerontologia da UFSCar, que permite o envio do artigo completo. Portanto,

a seguir está apresentada a revisão sistemática submetida à revista *Révue Neurologique* para apreciação por pares.

ARTIGO SUBMETIDO À REVISTA

ADAM10 as a biomarker for Alzheimer's disease: A systematic review

Maria Patrícia Oliveira Monteiro e Pereira de Almeida[1#], Renata Valle Pedroso[1#], Marina Mantellatto Grigoli[1], Tamires Vicente Silva[1], Patrícia Regina Manzine[1], Márcia Regina Cominetti[1*]

[1]Department of Gerontology, Federal University of Sao Carlos, Brazil. Rod. Washington Luis, Km 235. Monjolinho, CEP 13565-905. Tel.: (+55) 16 33066663

[#]Both authors equally contributed to this work

*Corresponding author: Prof. Dr Marcia Regina Cominetti, Department of Gerontology, Federal University of São Carlos. Rod. Washington Luis, km 235 - São Carlos / SP – Brazil. Postal code: 13565-905 Phone: +55 16 33066663; e-mail: mcominetti@ufscar.br

Abstract

Background: Studies have shown that A Disintegrin and Metalloproteinase 10 (ADAM10) is the main a-secretase in the non-amyloidogenic cleavage of the amyloid precursor protein, avoiding the production of amyloid-b peptide, one of the pathological hallmarks of Alzheimer's disease (AD). **Objective**: To investigate ADAM10 from cerebrospinal fluid (CSF) and plasma/serum as a potential biomarker for AD. **Methods**: A systematic review was carried out in the MEDLINE/PubMed, Web of Science, Embase and Scopus databases, using the terms and Boolean operators: "Alzheimer" AND "ADAM10" AND "biomarker". Citation searching was also adopted. The inclusion criteria were original studies of ADAM10 in blood or CSF of patients with AD. The risk of bias was assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. The analysis methods were registered in the PROSPERO database (#CRD42021274239). **Results:** Of the 97 records screened, 17 were included. There is strong evidence for lower levels of ADAM10 in platelets of persons with AD, compared to cognitively healthy participants. On the other hand, higher levels of ADAM10 were found in plasma. Regarding CFS, controversial results were found with lower and higher levels of ADAM10 in persons with AD, compared to healthy older adults. The differences found may be related to diverse reasons, including different sample collection and processing and different antibodies, highlighting the importance of standardizing the experiments and choosing the appropriate antibodies for ADAM10 detection. **Conclusion:** Evidence shows that ADAM10 levels are altered in platelets, plasma, serum, and CSF of persons with AD. The alteration was evident in all stages of the disease, and therefore, the protein may represent a complementary biomarker for the disease. However, more studies must be performed to establish cut-off values for ADAM10 levels to discriminate AD participants from cognitively unimpaired older adults.

Keywords: ADAM10, Alzheimer, Biomarker, CSF, Plasma

Introduction

A Disintegrin and Metalloproteinase 10 (ADAM10) is the main a-secretase performing the non-amyloidogenic cleavage of amyloid precursor protein (APP), preventing the aggregation and accumulation of β-amyloid (Aβ) into senile plaques, which leads to inflammation, tauopathies, and consequent synaptic loss characteristic of Alzheimer's disease (AD) (Manzine, Vatanabe et al. 2020).

The first studies of ADAM10 and its relationship to AD were published just over 20 years ago when the *in vitro* function of this protein as an a-secretase cleaving APP was demonstrated in human embryonic kidney cells (HEK 293) (Lammich, Kojro et al. 1999), providing evidence that the increase of its expression and activity might be beneficial for the AD treatment. Soon after, other studies identified ADAM10 and a competitor with β-secretase (BACE) for the APP cleavage (Skovronsky, Moore et al. 2000) and its co-expression with APP and BACE *in vivo* in mice and in *ex vivo* in human brains (Marcinkiewicz and Seidah 2000). Only two years later, studies demonstrating lower blood (platelets) and cerebrospinal fluid (CSF) levels of ADAM10 in AD patients, compared to cognitively healthy controls, were published (Colciaghi, Pastorino et al. 2001), demonstrating the potential of peripheral ADAM10 as an AD biomarker.

Considering that early diagnosis is crucial for better prognostic and therapeutic strategies for AD, several approaches have been employed with this aim, including imaging, CSF testing and, more recently, blood evaluation of specific AD markers (Zetterberg and Burnham 2019, Koychev, Jansen et al. 2020, Manzine, Vatanabe et al. 2020). Blood-based AD biomarkers are advantageous over other diagnostic methods due to several aspects, including their use as a low-invasive, large-scale, and low-cost screening method (Blennow and Zetterberg 2018). In this regard, ADAM10 has been identified as an AD biomarker candidate. This protein has been further shown to be reduced in the platelets of AD patients compared to cognitively healthy individuals (Colciaghi, Pastorino et al. 2001, Colciaghi, Marcello et al. 2004, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013). Moreover, platelet ADAM10 levels have been shown

to increase throughout the cognitively healthy aging (Schuck, Wolf et al. 2016). On the other hand, plasma soluble ADAM10 levels were raised early in amnestic mild cognitive impairment (aMCI) and in AD patients compared to cognitively unimpaired older adults (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021).

Considering all the aspects mentioned above regarding ADAM10 potential as a biomarker for AD, this systematic review aimed to investigate ADAM10 from cerebrospinal fluid (CSF) and plasma/serum as a potential biomarker for AD.

Methods

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page, McKenzie et al. 2021). The selected search strategy and analysis methods were registered in the PROSPERO database (#CRD42021274239).

Search Strategy

The literature search was performed until January 2022 using the following electronic databases: MEDLINE/PubMed, Web of Science, Embase and Scopus. The following search terms and Boolean operators were used in all databases: [Alzheimer] AND [ADAM10] AND [biomarker]. No additional filters were adopted. Citation searching was also employed as a supplementary search for any other possibly relevant investigations.

Eligibility criteria

Original studies that evaluated ADAM10 in patients with AD were considered eligible. Investigations were excluded if 1) research that did not examine ADAM10 in blood or CSF of patients with AD; (2) did not contain an Alzheimer's disease cohort and a control cohort; (3) studies involving experimental models; (4) research not available in English, Spanish or Portuguese; (5)

studies whose full texts were not available; (6) the investigation was a review, a case report, a letter to the editor or abstract from a congress. There were no further restrictions on publication dates other than the date range specified in the previous subsection for each electronic database.

Study selection

Two authors screened the titles and abstracts of the identified studies for eligibility. After independently reviewing the selected studies for inclusion, these were compared by both authors to reaching an agreement. Once the agreement had been reached, a full-text copy of every potentially relevant study was obtained. If it was unclear whether the study met the selection criteria, advice was sought from a third author and a consensus was reached.

Data extraction

Two investigators screened the results of the systematic search. Data were extracted using a structured form containing information on the authors; publication date; title; the country in which every study was carried out; sample size; methods regarding ADAM10 evaluation; results; reported strengths and limitations and key conclusions by the authors. All discrepancies were reviewed, and an agreement was reached by discussion.

Quality appraisal and risk of bias

Two investigators evaluated the methodological quality of the selected studies. Both authors compared the results to reach an agreement. In case of doubt, advice was required from another author. The studies were evaluated using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (NHLBI 2014), which consist of 13 elements of quality assessment: the clarity of the research objective; the definition, selection and composition of the study population; the definition and assessment of exposure and outcome variables; the measurement of exposures before outcome assessment; the study timeframe and follow-ups;

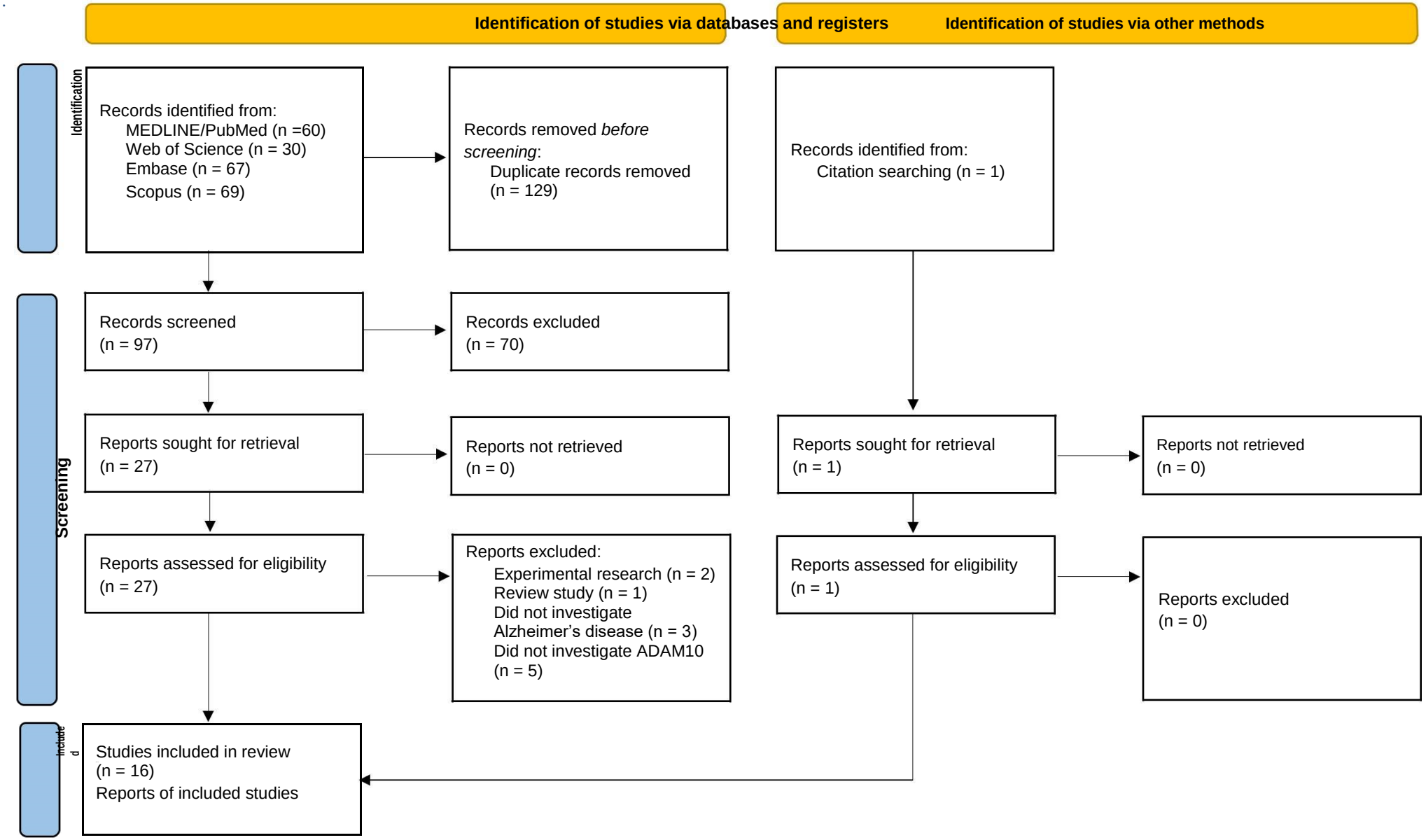
study analysis and power; and other factors. Each question can be answered as “yes,” “no,” “cannot determine,” “not applicable”, or “not reported”. All responses other than “yes” indicate a risk of bias. Inherent to the design, cross-sectional studies automatically score “not applicable” on criteria 8, 10 and 13. The quality of each study was classified as poor, fair, or good (NHLBI 2014).

Results

Study selection

The initial bibliographic search in the databases resulted in 226, 129 were removed due to duplicity. After screening the remaining 97 studies, 70 were excluded according to the pre-established inclusion criteria. Finally, 27 articles were fully read, of which 16 met the selection criteria. In addition, one record was identified through other sources, totalling 17 articles included in this review (Figure 1).

Figure 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers, and other sources.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Characteristics of studies

Table 1 presented a summary of the main characteristic of the included studies. Most of them were conducted in South America (n = 8) (Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Manzine, Marcello et al. 2015, Sarno, Talib et al. 2017, de França Bram, Talib et al. 2019, de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021) and Europe (n = 5) (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Bermejo-Bescós, Martín-Aragón et al. 2013, Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020), and other studies were conducted in Asia (n = 3), (Vinothkumar, Krishnakumar et al. 2018, Wongchitrat, Pakpian et al. 2019, Yu, Liu et al. 2021) and North America (n=1) (Tang, Hynan et al. 2006).

Studies were published between 2002 (Colciaghi, Borroni et al. 2002) to 2021 (Vatanabe, Peron et al. 2021, Yu, Liu et al. 2021). All of them compared AD and control, of these, 5 compared different stages of dementia (Colciaghi, Marcello et al. 2004, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, de Oliveira, Erbereli et al. 2020), 5 studies included a comparison with individuals with MCI (Bermejo-Bescós, Martín-Aragón et al. 2013, Manzine, Marcello et al. 2015, de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021, Yu, Liu et al. 2021) and one included two individuals with ADAM10 tyr167*mutation (Agüero, Sainz et al. 2020).

Concerning the sample characteristics, a total of 1128 individuals were involved, comprising 510 older adults with AD, 133 older adults with MCI, 445 older adults as a control group and 40 young as a young control. Mean age of AD and Control groups ranged from 79.7 years (Bermejo-Bescós, Martín-Aragón et al. 2013) to 67.7 years (Colciaghi, Marcello et al. 2004), and from 80.4 (Bermejo-Bescós, Martín-Aragón et al. 2013) to 61.1 (Colciaghi, Borroni et al. 2002), respectively. Among AD, studies included individuals in different stages of dementia: mild (n = 3) (Colciaghi, Marcello et al. 2004, Sogorb-Esteve, García-Ayllón et al. 2018, Vatanabe, Peron et al. 2021), mild and moderate (n = 2) (Sarno, Talib et al. 2017, de França Bram, Talib et al. 2019), mild and severe

(n = 1) (Tang, Hynan et al. 2006), mild, moderate and severe (n = 5) (Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Manzine, Barham et al. 2013b, Manzine, Marcello et al. 2015, de Oliveira, Erbereli et al. 2020), and not reported (n = 6) (Colciaghi, Borroni et al. 2002, Bermejo-Bescós, Martín-Aragón et al. 2013, Vinothkumar, Krishnakumar et al. 2018, Wongchitrat, Pakpian et al. 2019, Agüero, Sainz et al. 2020, Yu, Liu et al. 2021).

Table 1. Characteristics of participants, sample source, ADAM10 measurement, antibody and the results of the studies included in the systematic review.

Authors (Year); Country	AD and MCI participants (n; mean age; % female; stage of dementia)	Control participants (n; mean age; % female)	Diagnosis of AD	Sample source	ADAM10 measurement	Antibody	Results
Vatanabe et al. (2021); Brazil	**CSF***: AD* (n = 7; 72.7 ± 7.0 years old; 100% female; mild dementia; *aMCI* (n = 7; 71.1 ± 6 years old; 71 % female). **Plasma:** *AD* (n = 31; mean age and % female NR; mild dementia); *aMCI* (n= 28; mean age and % female NR).	**CSF:** *Control* (n = 8; 63.7 ± 3.7 years old; 100% female). **Plasma:** *Control* (n = 29; mean age and % female NR).	Aβ42 and tau levels in CSF + memory cognitive impairment.	Plasma and CSF	SDS-PAGE and western blotting	N-terminal ADAM10 antibody (cat. No. 2051, ProSci Poway; and ab39153, Abcam)	-Higher sADAM10 levels in CSF and plasma of AD, compared to Control. -sADAM10 presented no activity in CSF and plasma samples of all groups.
Yu et al. (2021); China	*AD* (n = 9; 73.1 ± 5.2 years old; 5% female; stage dementia NR); *MCI* (n = 10; 72.5 ± 2.4 years old; 60% female).	*Control* (n = 9; 72.6 ± 2.6 years old; 55% female).	NIA-AA	Platelets	TMT-LC-MS/MS, SDS-PAGE and western blotting	ADAM10 antibody (AF5294, Affinity)	-Lower ADAM10 levels in AD and MCI, compared to Control. using the TMT-LC-MS/MS technique -The western blotting analysis did not show differences between groups
Oliveira et al. (2020); Brazil	**Cohort 1**: *mild AD* (n = 10; age = 75 (66-84); 80% female); *moderate AD* (n = 8; age = 79 (69-89); 63% female); *severe AD* (n = 7; age = 71 (60-90); 43% female); *MCI* (n = 10; age = 69 (60-78); 50% female) **Cohort 2:** *AD* (n = 9; age = 73 (59-81); 78% female); *MCI* (n = 7; age = 71 (65-81); 71% female)	**Cohort 1:** *Control* (n = 10; age = 73 (65-86); 80% female). **Cohort 2**: *Control* (n = 8; age = 64 (57-69); 100% female)	NINCDS-ACRDA	**Cohort 1:** Plasma; **Cohort 2:** Serum and CSF	**Cohort 1:** ELISA Assay; **Cohort 2:** DμP Assay	----------	**Cohort 1:** - Higher sADAM10 levels in plasma of AD at different stages of dementia and MCI, compared to Control **Cohort 2:** - Higher sADAM10 levels in serum and CSF of AD and MCI, compared to Control
Agüero et al. (2020); Spain	*AD* (n = 10; mean age, % female and dementia stage NR); *ADAM10 tyr167*mutation* (n=2; mean age, % female and dementia stage NR)	*Control* (n = 12; mean age, % female and dementia stage NR)	Aβ42, T-tau, P-tau levels in CSF	CSF	SDS-PAGE and western blotting	ADAM10 ectodomain antibody (OAGA02442, Aviva)	-Lower ADAM10f and sADAM10 levels in CSF of both mutation carriers and AD group, compared to Control
Bram et al. (2019); Brazil	*AD (*n = 20; 76.2 ± 7.2 years old; 70% female; mild and moderate dementia)	*Control* (n = 20; 74.9 ± 4.5 years old; 75% female)	DSM-IV and NINCDS-ACRDA	Platelets and leukocytes	SDS-PAGE and western blotting	C-terminal ADAM10 antibody (ab124695, Abcam)	-Lower ADAM10f levels in platelets of AD, compared to Control -No differences ADAM10f levels in leukocytes -Lower mRNA ADAM10 level in AD, compared to both Control groups. -Positive correlation between mRNA ADAM10 levels and TMSE scores
Wongchitrat et al. (2019); Thailand	*AD* (n = 39; 78.2 ± 1.3 years old; 64% female; stage dementia NR)	*Older Control* (n = 40; 68.5 ± 1.1 years old; 65% female); *Young Control* (n = 40; 28.8 ± 0.7 years old; 73% female)	Thai clinical practice guidelines for dementia	Plasma	RT-qPCR	----------	
Vinothkumar et al. (2018); India	*AD* (n=10; age > 70 years old; 40% female; stage dementia NR)	*Control* (n = 30; ages between 20-49 yeares old; 46 % female)	NR	Platelets	SDS-PAGE and western blotting	C-terminal ADAM10 antibody (bs-3574R, Bioss Inc)	-Lower ADAM10f levels in platelets of AD, compared to Control

Sogorb-Esteve et al. (2018); Spain	*AD* (n = 27; 71 ± 1 years old; 74% female; mild dementia)	*Control* (n = 26; 70 ± 2 years old; 30% female)	Aβ42, T-tau, P-tau levels in CSF and NIA-AA	CSF	SDS-PAGE and western blotting	ADAM10 ectodomain antibody (OAGA02442, Aviva); N-terminal ADAM10 antibody (ab39153, Abcam) and C-terminal ADAM10 antibody (ab124695, Abcam)	-Lower ADAM10f and sADAM10 levels in CSF of AD, compared to Control
Sarno et al. (2017); Brazil	*AD* (n = 23; 73.1 ± 6.9 years old; 74% female; mild and moderate dementia)	*Control* (n = 38; 72.3 ± 6.6 years olde; 68% female)	DSM-IV and NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	C-terminal ADAM10 antibody (bs-3574R, Bioss Inc)	-Lower ADAM10f levels in platelets of AD, compared to Control
Manzine et al. (2015); Brazil and Italy	**Total blood RNA**: *AD* (n = 47; age = 77 (60-69); 68% female; all stages of dementia). *MCI* (n = 21; age = 72 (60-84); 67% female). **Platelets RNA**: *AD* (n = 21; age = 70 (65-75); 71% female). *MCI* (n = 16; age = 69 (61-74); 50% female; all stages of dementia)	**Total blood RNA**: *Control* (n = 32; age = 74 (64-86); 68% female). **Platelets RNA:** *Control* (n = 19; age = 67 (63-71); 68% female)	NINCS–ADRDA	Total blood and platelets	RT-qPCR	----------	-No difference in ADAM10 mRNA expression between AD, MCI and Control
Bermejo et al. (2013); Spain	*AD* (n = 45; 79.7 ± 0.9 years old; 64% female; stage dementia NR); MCI (n = 34; 76.6 ± 1.4 years old; 58% female)	*Control* (n = 28; 80.4 ± 2.1 years old; 60% female)	NINDS-ADRDA	Platelets	SDS-PAGE and western blotting	ADAM10 antibody (ref NR, Santa Cruz Biotechnology)	-Higher ADAM10 levels in platelets of both MCI and AD groups, compared to the Control. -Higher ratio of ADAM10/BACE1 in MCI, compared to AD.
Manzine et al. (2013a); Brazil	*AD* (n = 30; CDR1, n = 10 age = 75.0 (66-84), 70% female; CDR2, n = 11; age = 76.0 (69-89), 64% female; CDR3, n=9, age = 74.5 (60-90), 67% female)	*Control* (n = 25; age = 75.7 (64-84); 64% female)	NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	ADAM10 antibody (ref NR, Santa Cruz Biotechnology)	-Lower ADAM10f levels in platelets of AD, compared to Control -Lower ADAM10f level, according to the severity of the dementia -Positive correlation between ADAM10 levels and Clock Drawing Test
Manzine et al. (2013b); Brazil	*AD* (n = 30; CDR1, n = 10; age = 75.0 (66-84); 70% female; CDR2, n=11; age = 76.0 (69-89); 64% female; CDR3, n=9; age = 74.5 (60-90); 67% female)	*Control* (n = 25; age = 75.7 (64-84); 64% female)	NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	ADAM10 antibody (ref NR, Santa Cruz Biotechnology)	-Lower ADAM10f levels in platelets of AD, compared to Control -Lower ADAM10f level, according to the severity of the dementia -Positive correlation between ADAM10 levels and MMSE
Manzine et al. (2013c); Brazil	*AD* (n = 30; CDR1, n = 10; age = 75.0 (66-84); 70% female; CDR2, n=11; age = 76.0 (69-89); 64% female; CDR3, n=9; age = 74.5 (60-90); 67% female)	*Control* (n = 25; age = 75.7 (64-84); 64% female)	NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	ADAM10 antibody (ref NR, Santa Cruz Biotechnology)	-Lower ADAM10f levels in platelets of AD, compared to Control -Lower level, according to the severity of the dementia.
Tang et al. (2006); USA	*AD* (n = 31; 76.0 ± 8.7 years old; % female NR; mild and severe dementia)	*Control* (n = 10; 76.3 ± 4.4 years old; % female NR)	NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	ADAM10 antibody (ref NR, Chemicon)	-Lower ADAM10f levels in platelets of mild and severe AD, compared to Control -No differences between mild and severe AD.

Colciaghi et al. (2004); Italy	*VmAD - Very mild AD* (n = 11; 67.8 ± 6.3 years old; 63% female; very mild stage of dementia); *mAD - mild AD* (n = 20; 68.0 ± 6.3 years old; 60% female; mild dementia)	*Control* (n = 15; 67.7 ± 4.2 years old; 60% female)	NINCDS-ACRDA	Platelets	SDS-PAGE and western blotting	ADAM 10 (ref NR, Proscience Inc.)	-Lower ADAM10f levels in platelets of vmAD and mAD, compared to Control -Lower levels are more evident in mAD than vmAD
Colciaghi et al. (2002); Italy	*AD* (n = 33; 68.1 ± 6.1 years old; 60 % female; stage dementia= NR)	*Control* (n = 26; 63.1 ± 6.1 years old; 65% female)	NINCDS-ACRDA	Platelets and CSF	SDS-PAGE and western blotting	C-terminal ADAM10 antibody (ref NR)	-Lower ADAM10f levels in platelets and CSF of AD, compared to Control

Note: AD: Alzheimer's disease; *CSF*: cerebrospinal fluid; *MMSE*: Mini Mental State Examination; *NIA-AA*: National Institute of Aging and the Guidelines of Alzheimer's Association; *NINCDS-ACRDA*: National Institute of Neurological and Communicative Disordes and Stroke-Alzheimer's Disease and Related Disorders Association; *NR*: not reported; *T-tau*: total tau; *TMSE*: Thai Mental State Examination; *P-tau*: phosphorylated tau; qPCR: quantitative polymerase chain reaction

For the AD diagnosis, eleven studies used the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ACRDA) (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Bermejo-Bescós, Martín-Aragón et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Manzine, Barham et al. 2013b, Manzine, Marcello et al. 2015, Sarno, Talib et al. 2017, de França Bram, Talib et al. 2019, de Oliveira, Erbereli et al. 2020), three used the Aβ42 and tau levels, that are established AD biomarkers (Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020, Vatanabe, Peron et al. 2021), one used the National Institute of Aging and the Guidelines of Alzheimer's Association criteria (NIA-AA) (Yu, Liu et al. 2021), other used the Thai clinical practice guidelines for dementia (Wongchitrat, Pakpian et al. 2019) and one did not report (Vinothkumar, Krishnakumar et al. 2018).

The included studies investigated ADAM10 protein levels (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Bermejo-Bescós, Martín-Aragón et al. 2013, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Sarno, Talib et al. 2017, Sogorb-Esteve, García-Ayllón et al. 2018, Vinothkumar, Krishnakumar et al. 2018, de França Bram, Talib et al. 2019, Agüero, Sainz et al. 2020, de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021, Yu, Liu et al. 2021) or ADAM10 mRNA levels (Manzine, Marcello et al. 2015, Wongchitrat, Pakpian et al. 2019) in different biological samples.

ADAM10 protein levels were measured using the technique SDS-PAGE followed by western blotting in all studies, except Oliveira et al. (2020), which used ELISA and DµP Assay, and Yu et al. (2021) that, in addition to the SDS-PAGE and western blotting, included the proteomic analysis TMT-labeled peptides using LC-MS/MS (Yu, Liu et al. 2021). ADAM10 mRNA expression was measured using the RT-qPCR (Manzine, Marcello et al. 2015, Wongchitrat, Pakpian et al. 2019)

Methodological quality

Table 2 present the methodological quality evaluation of the 17 studies included in this review. All studies were considered “good”, of which fifteen presented score eight and two showed score 7. The difference between these only two studies was in the “do not justify the sample size” (Tang, Hynan et al. 2006) and the “do not define the exposure measures in detail” (Vinothkumar, Krishnakumar et al. 2018). It is important to emphasize that the main questions that presented negative answers were related to que questions 6 and 7. Question 6 is related to the “exposure assessed before outcome measurement”, and question 7 refers to the “sufficient timeframe to see an effect”. In the case of cross-sectional analyses, the exposures and outcomes are assessed simultaneously, so the response is "no" for all studies.

Table 2. Quality of the studies selected for the review using the NIH’s Quality Assessment of Cohort and Cross-sectional Studies.

S	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TS	OQ
CROSS-SECTIONAL STUDIES																
Vatanabe et al. (2021)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Yu et al. (2021)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Oliveira et al. (2020)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Agüero et al. (2020)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Bram et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Wongchitrat et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Vinothkumar et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	No	N/A	Yes	N/R	N/A	Yes	7	Good
Sogorb-Esteve et al. (2018)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Sarno et al. (2017)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Manzine et al. (2015)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Bermejo et al. (2013)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Manzine et al. (2013a)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Manzine et al. (2013b)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Manzine et al. (2013c)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Tang et al. (2006)	Yes	Yes	Yes	Yes	No	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	7	Good
Colciaghi et al. (2004)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good
Colciaghi et al. (2002)	Yes	Yes	Yes	Yes	Yes	No	No	N/A	Yes	N/A	Yes	N/R	N/A	Yes	8	Good

CD*: cannot determine;* N/R: not reported; N/A: not applicable. Inherent to the design, cross-sectional studies automatically score “not applicable” on criteria 8, 10 and 13; TS: Total Score; OQ: Overall Quality

ADAM10 protein levels

ADAM10 protein levels were analyzed from platelets (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Bermejo-Bescós, Martín-Aragón et al. 2013, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Sarno, Talib et al. 2017, Vinothkumar, Krishnakumar et al. 2018, de França Bram, Talib et al. 2019, Yu, Liu et al. 2021), leukocytes (de França Bram, Talib et al. 2019), plasma (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021), serum (de Oliveira, Erbereli et al. 2020) and CSF (Colciaghi, Borroni et al. 2002, Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020, de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021).

Platelets and leukocytes

Platelets were the most biological sample source adopted by the studies. All studies have found lower levels of ADAM10 in platelets of AD (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Sarno, Talib et al. 2017, Vinothkumar, Krishnakumar et al. 2018, de França Bram, Talib et al. 2019, Yu, Liu et al. 2021) and MCI (Yu, Liu et al. 2021), compared to control. Except the study conducted by Bermejo-Bescós et al. (2013), which reported higher levels of ADAM10 in platelets of AD and MCI, compared to control.

The lower levels of ADAM10 were found at severe AD (Tang, Hynan et al. 2006, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013), but also at moderate (Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013) and early stage of AD (Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013).

Leukocytes have not been explored across studies. The only study investigating ADAM10 levels in leukocytes did not find differences between AD and control, although reported differences in the platelet ADAM10 (de França Bram, Talib et al. 2019).

Plasma and serum

Both studies with plasma have found higher ADAM10 levels in AD compared to the control (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021). When the comparison was made between MCI and control, only Oliveira et al. (de Oliveira, Erbereli et al. 2020) reported higher levels in MCI. In addition, higher ADAM10 levels were found at different stages of the dementia (Vatanabe, Peron et al. 2021). The only study conducted with serum also reported a higher level of ADAM10 in AD and MCI than in the control (de Oliveira, Erbereli et al. 2020).

Cerebrospinal fluid (CSF)

Five studies investigated ADAM10 levels in CSF. Of them, two studies reported higher levels (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021) and three have found lower levels (Colciaghi, Borroni et al. 2002, Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020) of ADAM10 in AD, compared to control. It is essential to highlight that Oliveira et al. (2020) and Vatanabe et al. (2021) investigated the soluble ADAM10 levels, Colciaghi et al. (2002) the full ADAM10, and Sogorb-Esteve et al. (2018) and Aguero et al. (2020) both species of ADAM10.

ADAM10 mRNA levels

Two studies investigated ADAM10 mRNA expression, and the results are inconsistent. On the one hand, Wongchitrat et al. (2019) have found lower mRNA ADAM10 levels in the plasma of

participants with AD compared to healthy persons. On the other hand, no differences were found between AD, MCI and control in the study conducted by Manzine et al. (2015).

Discussion

The main goal of this systematic review was to analyse studies that investigated the protein or mRNA levels of ADAM10 in AD and to verify its potential as a biomarker for the disease. From the 148 articles found in the databases, 17 met the inclusion criteria. To the best of our knowledge, this is the first systematic review summarizing the functions of ADAM10 as a biomarker for AD. It is important to highlight that all studies included in this systematic review were considered "good" quality. The studies investigated the levels of ADAM10 in different types of biological samples, including plasma, serum, and CSF. The findings suggested that ADAM10 levels are altered in platelets, plasma, serum, and CSF of patients with AD, and controversial results were found regarding altered ADAM10 mRNA levels in plasma.

Considering the mRNA levels, no significant differences in ADAM10 gene expression in total blood or platelet were observed by Manzine et al. (2015). A further study performed by this research group explored and validated 21 miRNAs directly or indirectly related to the AD pathophysiology and to the ADAM10 gene, which was differentially expressed in the total blood of AD participants, compared with cognitively healthy matched controls. In addition, using SH-SY5Y cells, the study described for the first time the role of miR-221 as a regulator of ADAM10 at a translational level, providing new insights to understand the reduced protein levels of ADAM10 in the AD (Manzine, Pelucchi et al. 2017). In other words, despite no alteration found in mRNA ADAM10 levels between AD and healthy participants, a different expression of miR-221 was reported in AD participants, which could partially explain the effects on ADAM10 protein levels.

Human platelets are the largest source of circulating APP in the peripheral tissues (Padovani, Borroni et al. 2001, Veitinger, Varga et al. 2014) and the second most important source of APP, besides the brain (Veitinger, Varga et al. 2014). Studies have shown that blood APP is processed by

the same amyloidogenic and non-amyloidogenic pathways in the brain (Colciaghi, Marcello et al. 2004, Veitinger, Varga et al. 2014). Accordingly, the evidence for lower levels of ADAM10 in platelets of AD patients was more substantial, since platelets were the most common biological sample used by the studies and all presented results in the same way (Colciaghi, Borroni et al. 2002, Colciaghi, Marcello et al. 2004, Tang, Hynan et al. 2006, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013, Sarno, Talib et al. 2017, Vinothkumar, Krishnakumar et al. 2018, de França Bram, Talib et al. 2019, Yu, Liu et al. 2021), except for the work of Bermejo-Bescós et al. (2013), who found increased levels of ADAM10 in both MCI and AD groups compared with age-matched controls. Authors explain this result as a possible compensation mechanism and cite other studies in which ADAM10 mRNA levels are up-regulated in the hippocampus of severe AD cases (Gatta, Albertini et al. 2002).

In this regard, it is important to point out that a crucial factor in analysing ADAM10 levels in different samples is the type and nature of the antibody used in the experiments. There are a variety of commercial antibodies available to detect the protein, ranging from polyclonal to monoclonal and produced against several regions of the molecule. ADAM10 is a large multidomain protein with multiple cleavage sites and, therefore, can be present in biological samples of different sizes and isoforms. C-terminal end recognizing antibodies usually reveal bands with a molecular mass of ~60kDa in platelets, corresponding to the mature full-length form of the enzyme. On the other hand, N-terminal end recognizing antibodies commonly reveal an isoform with the apparent molecular weight of ~50kDa (Pereira Vatanabe, Peron et al. 2021).

Most studies reported ADAM10 isoform in platelets with a molecular mass of ~60kDa (Tang, Hynan et al. 2006, Manzine, Barham et al. 2013, Manzine, Barham et al. 2013, Manzine, de Franca Bram et al. 2013), while another study showed a ~68kDa molecular mass for the protein (Colciaghi, Borroni et al. 2002), which was detected by specific C-terminal and N-terminal antibodies and may represent the mature full-length form of the protein.

Previous studies have reported higher plasma ADAM10 levels in AD compared to

cognitively healthy participants (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021). Both studies corroborate with a recent work that has found evidence that higher ADAM10 plasma levels are predictors of cognitive worsening in older adults (Monteiro, Oliveira et al. 2021), suggesting the potential role of this protein as an AD biomarker. In their study, Vatanabe et al. (2021) showed that ~50kDa plasma and CSF levels of soluble ADAM10 were significantly increased in mild AD and that in these samples, this protease is inactive. In addition, ADAM10 reached its principal activity in the plasma membrane fraction, where the mature form (~65kDa) of the enzyme was detected. The mature full-length protein isoform can also be seen in leukocytes; however, different from platelets, there is no evidence of altered ADAM10 levels in AD compared to healthy older adults (de França Bram, Talib et al. 2019). The authors suggested that leukocytes could not reflect the pathophysiological processes of AD, although it contains the necessary machinery for Aβ formation (de França Bram, Talib et al. 2019).

Mature full-length active and inactive soluble ADAM10 isoforms were also investigated in CSF, and all studies agree that the mature ADAM10 isoform levels are reduced in AD (Colciaghi, Borroni et al. 2002, Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020). On the other hand, findings regarding soluble form are contradictory, as studies have reported lower (Sogorb-Esteve, García-Ayllón et al. 2018, Agüero, Sainz et al. 2020) or higher levels of ADAM10 (de Oliveira, Erbereli et al. 2020, Vatanabe, Peron et al. 2021) in CSF of people with AD, compared to cognitively healthy participants. The differences found can be explained by a myriad of reasons, from different sample processing – including, but not limited to, the conditions of sample collections, such as the type of tubes used for collection, the temperatures of sample storing, centrifugation steps, freeze and thaw cycles and so on – to the use of different antibodies, and further studies with this type of biological sample are needed.

This review has some limitations that should be acknowledged. Firstly, many studies did not present means and standard deviations related to ADAM10 levels. In combination with the high heterogeneity of samples, antibodies and lack of data normalization, we prevented advancing to a

detailed meta-analysis report. In addition, there are limitations related to the language restrictions since we did not review the grey literature. Yet, the information provided in this systematic review can help understand the biological roles of the main a-secretase involved in non-amyloidogenic cleavage of APP and offer further insight on this potential biomarker candidate for earlier AD diagnosis.

Conclusion

In conclusion, ADAM10 levels are altered in platelets, plasma, serum, and CSF of persons with AD. The alteration was evident in all stages of the disease, and therefore, the protein may represent a promising biomarker for the disease. Studies are still scarce, and there are no established cut-off values to discriminate participants with AD from controls. The review also highlights the importance of standardizing the experiments and choosing the appropriate antibodies for ADAM10 detection to use this protein as a complementary tool for AD diagnosis.

References

Agüero, P., M. J. Sainz, M.-S. García-Ayllón, J. Sáez-Valero, R. Téllez, R. Guerrero-López, J. Pérez-Pérez, A. Jiménez-Escrig and E. Gómez-Tortosa (2020). "α-Secretase nonsense mutation (ADAM10 Tyr167*) in familial Alzheimer's disease." Alzheimer's Research & Therapy **12**(1): 1-9.

Bermejo-Bescós, P., S. Martín-Aragón, K. Jiménez-Aliaga, J. Benedí, E. Felici, P. Gil, J. M. Ribera and Á. M. Villar (2013). "Processing of the platelet amyloid precursor protein in the mild cognitive impairment (MCI)." Neurochemical research **38**(7): 1415-1423.

Blennow, K. and H. Zetterberg (2018). "The Past and the Future of Alzheimer's Disease Fluid Biomarkers." J Alzheimers Dis **62**(3): 1125-1140.

Colciaghi, F., B. Borroni, L. Pastorino, E. Marcello, M. Zimmermann, F. Cattabeni, A. Padovani and M. Di Luca (2002). "α-Secretase ADAM10 as Well as α APPs Is Reduced in Platelets and CSF of Alzheimer Disease Patients." Molecular medicine **8**(2): 67-74.

Colciaghi, F., E. Marcello, B. Borroni, M. Zimmermann, C. Caltagirone, F. Cattabeni, A. Padovani and M. Di Luca (2004). "Platelet APP, ADAM 10 and BACE alterations in the early stages of Alzheimer disease." Neurology **62**(3): 498-501.

Colciaghi, F., L. Pastorino, B. Borroni, A. Padovani, F. Cattabeni and M. Di Luca (2001). "Alpha and beta secretase activity in platelets of Alzheimer's disease patients." Journal of Neurochemistry **77**: 21-21.

de França Bram, J. M., L. L. Talib, H. P. G. Joaquim, T. A. Sarno, W. F. Gattaz and O. V. Forlenza (2019). "Protein levels of ADAM10, BACE1, and PSEN1 in platelets and leukocytes of Alzheimer's disease patients." European archives of psychiatry and clinical neuroscience **269**(8): 963-972.

de Oliveira, T. R., C. R. Erbereli, P. R. Manzine, T. N. Magalhães, M. L. Balthazar, M. R. Cominetti and R. C. Faria (2020). "Early diagnosis of Alzheimer's disease in blood using a disposable electrochemical microfluidic platform." ACS sensors **5**(4): 1010-1019.
Gatta, L. B., A. Albertini, R. Ravid and D. Finazzi (2002). "Levels of beta-secretase BACE and alpha-secretase ADAM10 mRNAs in Alzheimer hippocampus." Neuroreport **13**(16): 2031-2033.
Koychev, I., K. Jansen, A. Dette, L. Shi and H. Holling (2020). "Blood-Based ATN Biomarkers of Alzheimer's Disease: A Meta-Analysis." J Alzheimers Dis.
Lammich, S., E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass and F. Fahrenholz (1999). "Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease." Proc Natl Acad Sci U S A **96**(7): 3922-3927.
Manzine, P. R., E. J. Barham, F. A. Vale, H. S. Selistre-de-Araujo, S. C. Pavarini and M. R. Cominetti (2013). "Platelet a disintegrin and metallopeptidase 10 expression correlates with clock drawing test scores in Alzheimer's disease." Int J Geriatr Psychiatry.
Manzine, P. R., E. J. Barham, F. d. A. C. d. Vale, H. S. Selistre-de-Araujo, S. C. Iost Pavarini and M. R. Cominetti (2013b). "Correlation between mini-mental state examination and platelet ADAM10 expression in Alzheimer's disease." Journal of Alzheimer's Disease **36**(2): 253-260.
Manzine, P. R., E. J. Barham, A. Vale Fde, H. S. Selistre-de-Araujo, S. C. Iost Pavarini and M. R. Cominetti (2013). "Correlation between mini-mental state examination and platelet ADAM10 expression in Alzheimer's disease." J Alzheimers Dis **36**(2): 253-260.
Manzine, P. R., J. M. de Franca Bram, E. J. Barham, A. do Vale Fde, H. S. Selistre-de-Araujo, M. R. Cominetti and S. C. Iost Pavarini (2013). "ADAM10 as a biomarker for Alzheimer's disease: a study with Brazilian elderly." Dement Geriatr Cogn Disord **35**(1-2): 58-66.
Manzine, P. R., E. Marcello, B. Borroni, W. Kamphuis, E. Hol, A. Padovani, C. C. Nascimento, P. de Godoy Bueno, F. Assis Carvalho Vale and S. C. Iost Pavarini (2015). "ADAM10 gene expression in the blood cells of Alzheimer's disease patients and mild cognitive impairment subjects." Biomarkers **20**(3): 196-201.
Manzine, P. R., S. Pelucchi, M. A. Horst, F. A. C. Vale, S. C. I. Pavarini, M. Audano, N. Mitro, M. Di Luca, E. Marcello and M. R. Cominetti (2017). "microRNA 221 Targets ADAM10 mRNA and is Downregulated in Alzheimer's Disease." J Alzheimers Dis.
Manzine, P. R., I. P. Vatanabe, R. Peron, M. M. Grigoli, R. V. Pedroso, C. M. C. Nascimento and M. R. Cominetti (2020). "Blood-based Biomarkers of Alzheimer's Disease: The Long and Winding Road." Curr Pharm Des **26**(12): 1300-1315.
Marcinkiewicz, M. and N. G. Seidah (2000). "Coordinated expression of beta-amyloid precursor protein and the putative beta-secretase BACE and alpha-secretase ADAM10 in mouse and human brain." J Neurochem **75**(5): 2133-2143.
Monteiro, M. P. A. O., D. S. S. Oliveira, P. R. Manzine, C. M. C. Nascimento, A. A. dos Santos Orlandi, G. A. de Oliveira Gomes, F. dos Santos Orlandi, M. S. Zazzetta, H. Pott-Junior and M. R. Cominetti (2021). "ADAM10 plasma levels predict worsening in cognition of older adults: a 3-year follow-up study." Alzheimer's Research & Therapy **13**(1): 1-8.
NHLBI, N. (2014). "Quality assessment tool for observational cohort and cross-sectional studies." Nhlbi. nih. gov.
Padovani, A., B. Borroni, F. Colciaghi, L. Pastorino, S. Archetti, E. Cottini, L. Caimi, F. Cattabeni and M. Di Luca (2001). "Platelet amyloid precursor protein forms in AD: a peripheral diagnostic tool and a pharmacological target." Mechanisms of ageing and development **122**(16): 1997-2004.
Page, M. J., J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L. Shamseer, J. M. Tetzlaff, E. A. Akl and S. E. Brennan (2021). "The PRISMA 2020 statement: an updated guideline for reporting systematic reviews." Bmj **372**.
Pereira Vatanabe, I., R. Peron, M. Mantellatto Grigoli, S. Pelucchi, G. De Cesare, T. Magalhaes, P. R. Manzine, M. L. Figueredo Balthazar, M. Di Luca, E. Marcello and M. R. Cominetti (2021). "ADAM10 Plasma and CSF Levels Are Increased in Mild Alzheimer's Disease." Int J Mol Sci **22**(5).

Sarno, T. A., L. L. Talib, H. P. G. Joaquim, J. M. d. F. Bram, W. F. Gattaz and O. V. Forlenza (2017). "Protein expression of BACE1 is downregulated by donepezil in Alzheimer's disease platelets." Journal of Alzheimer's Disease **55**(4): 1445-1451.
Schuck, F., D. Wolf, A. Fellgiebel and K. Endres (2016). "Increase of alpha-Secretase ADAM10 in Platelets Along Cognitively Healthy Aging." J Alzheimers Dis **50**(3): 817-826.
Skovronsky, D. M., D. B. Moore, M. E. Milla, R. W. Doms and V. M. Lee (2000). "Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network." J Biol Chem **275**(4): 2568-2575.
Sogorb-Esteve, A., M.-S. García-Ayllón, J. Gobom, J. Alom, H. Zetterberg, K. Blennow and J. Sáez-Valero (2018). "Levels of ADAM10 are reduced in Alzheimer's disease CSF." Journal of neuroinflammation **15**(1): 1-9.
Tang, K., L. S. Hynan, F. Baskin and R. N. Rosenberg (2006). "Platelet amyloid precursor protein processing: a bio-marker for Alzheimer's disease." Journal of the neurological sciences **240**(1-2): 53-58.
Vatanabe, I. P., R. Peron, M. M. Grigoli, S. Pelucchi, G. Cesare, T. Magalhães, P. R. Manzine, M. L. F. Balzthazar, M. Di Luca, E. Marcello and M. R. Cominetti (2021). "ADAM10 plasma and CSF levels are increased in mild Alzheimer's disease " International Journal of Molecular Sciences **in press**.
Veitinger, M., B. Varga, S. B. Guterres and M. Zellner (2014). "Platelets, a reliable source for peripheral Alzheimer's disease biomarkers?" Acta neuropathologica communications **2**(1): 1-15.
Vinothkumar, G., S. Krishnakumar, G. Shivashekar, S. Sreedhar, S. Dinesh, A. Sundaram, D. Balakrishnan and P. Venkataraman (2018). "Therapeutic impact of rHuEPO on abnormal platelet APP, BACE 1, presenilin 1, ADAM 10 and Aβ expressions in chronic kidney disease patients with cognitive dysfunction like Alzheimer's disease: A pilot study." Biomedicine & Pharmacotherapy **104**: 211-222.
Wongchitrat, P., N. Pakpian, K. Kitidee, K. Phopin, P. A. Dharmasaroja and P. Govitrapong (2019). "Alterations in the expression of amyloid precursor protein cleaving enzymes mRNA in Alzheimer peripheral blood." Current Alzheimer Research **16**(1): 29-38.
Yu, H., Y. Liu, B. He, T. He, C. Chen, J. He, X. Yang and J. Z. Wang (2021). "Platelet biomarkers for a descending cognitive function: A proteomic approach." Aging cell **20**(5): e13358. Zetterberg, H. and S. C. Burnham (2019). "Blood-based molecular biomarkers for Alzheimer's disease." Mol Brain **12**(1): 26.

REFERÊNCIAS

Blennow, K. and H. Zetterberg (2018). "The Past and the Future of Alzheimer's Disease Fluid Biomarkers." J Alzheimers Dis 62(3): 1125-1140.

Camarano, A.A.; Kanso, S. Envelhecimento da população brasileira: uma contribuição demográfica. In: FREITAS, E.V.; PY, L. (Eds.) Tratado de geriatria e gerontologia 4. ed. Rio de Janeiro: Guanabara Koogan, 2016. p. 52-65.

Diniz, B.S.O.; Nunes, P.V.; Yassuda, M.S.; Forlenza, O.V. Diagnosis of mild cognitive impairment revised after one year: preliminary results of a prospective study. Dement. Geriatr. Cogn. Disord., 27(3):224-231, 2009.

Lammich, S., E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass and F. Fahrenholz (1999). "Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease." Proc Natl Acad Sci U S A 96(7): 3922-3927.

Lanctôt, K. L. et al. Neuropsychiatric signs and symptoms of Alzheimer’s disease: Newtreatment paradigms. Alzheimer’s and Dementia: Translational Research and Clinical Interventions, v. 3, n. 3, p. 440–449, 2017.

Neto, João Bastos Freire et al., Cuidados em Domicílio/ Conceitos e Práticas – In: FREITAS, E.V.; PY, L. (Eds.) Tratado de geriatria e gerontologia. 4. ed. Rio de Janeiro: Guanabara Koogan, 2016, p. 2004-2010.

Netto, Mateus Papaléo, Estudo da velhice / Histórico, definição de campo e termos básicos - In: FREITAS, E.V.; PY, L. (Eds.) Tratado de geriatria e gerontologia. 4. ed. Rio de Janeiro: Guanabara Koogan, 2016, p. 74-87.

Teixeira, Jane Blanco et. al Doença de Alzheimer: estudo da mortalidade no Brasil, 2000-2009- Cad. Saúde Pública, Rio de Janeiro, 31(4):1-12, abr, 2015.

Zetterberg, H. and S. C. Burnham (2019). "Blood-based molecular biomarkers for Alzheimer's disease." Mol Brain 12(1): 2.