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BIOMARCADORES LIQUÓRICOS COMO PREDITORES DE CONVERSÃO DO COMPROMETIMENTO COGNITIVO LEVE AMNÉSTICO À DEMÊNCIA DA DOENÇA DE ALZHEIMER

PORTO ALEGRE

2019

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BIOMARCADORES LIQUÓRICOS COMO PREDITORES DE CONVERSÃO DO COMPROMETIMENTO COGNITIVO LEVE AMNÉSTICO À DEMÊNCIA DA DOENÇA DE ALZHEIMER

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"O navio está seguro quando está no porto, mas não é para isso que se fazem navios"

William Shedd

RESUMO

Introdução: A Doença de Alzheimer é uma desordem neurodegenerativa caracterizada pela formação de placas amilóides e emaranhados neurofibrilares. A correta identificação de indivíduos com risco de progressão para a demência da Doença de Alzheimer (DDA) é crucial em termos de diagnóstico precoce, prognóstico e tratamento. As concentrações liquóricas das proteínas $A\beta_{1-42}$ e p-Tau_{181} são os biomarcadores mais promissores para o diagnóstico da patologia e podem ser identificados precocemente no *continuum* da doença. Indivíduos com Comprometimento cognitivo leve amnéstico (CCL-a) possuem um alto risco de progressão para a DDA. Dessa forma, pesquisas longitudinais com indivíduos com CCL-a representam uma oportunidade para investigar os processos biológicos subjacentes (amiloidose e neurodegeneração) e predizer o potencial risco de progressão para a DDA.

Objetivo: Mensurar os biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} em indivíduos com Comprometimento Cognitivo Leve amnéstico e verificar suas capacidades preditivas para a fase demencial da Doença de Alzheimer em um estudo longitudinal.

Métodos: Quarenta e dois indivíduos diagnosticados com comprometimento cognitivo leve amnéstico ou comprometimento cognitivo subjetivo foram avaliados em 2013 e reavaliados em 2018. Os níveis de $A\beta_{1-42}$ e p-Tau_{181} no líquido cefalorraquidiano foram mensurados por ensaio imunoenzimático e os pontos de corte foram calculados pelo teste de Youden. Além disso, foram realizados testes neuropsicológicos, como o CERADs e o MoCA.

Resultados: 45,2% dos indivíduos com CCL-a progrediram para a DDA em cinco anos. O risco relativo de progressão para indivíduos com CCL-a e $A\beta_{1-42}$ <618,5 pg/mL foi 5,8 vezes maior do que naqueles cujos níveis foram superiores a esse ponto de corte (P = 0,0011). E o risco naqueles cuja razão p-$Tau_{181}/A\beta_{1-42}$ foi maior de 0,135 foi 3,83 vezes maior (P = 0,0001). Os

níveis liquóricos de $A\beta_{1-42}$ e p-Tau_{181} explicaram 47,5% da variância do ΔCERADs (P <0,001), enquanto os níveis da proteína $A\beta_{1-42}$ isoladamente explicaram 38,6% (P < 0,001).

Conclusões: Nesse estudo, a proteína $A\beta_{1-42}$ mensurada no líquor proporcionou o melhor risco cumulativo de progressão do CCL-a para a DDA em cinco anos. A razão p-Tau_{181}/$A\beta_{1-42}$ não foi melhor que a proteína $A\beta_{1-42}$ isolada na predição da progressão do CCL-a para a DDA. No entanto, os níveis de p-Tau_{181} ajudaram a explicar um extra de 9% da variância do ΔCERADs em cinco anos.

Palavras-chave: Doença de Alzheimer; Comprometimento Cognitivo Leve amnéstico, biomarcadores liquóricos; proteína beta-amilóide; proteína Tau.

ABSTRACT

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the formation of amyloid plaques and neurofibrillary tangles. The correct identification of individuals at risk for developing Alzheimer's disease dementia (ADD) is of utmost importance for its early diagnosis and treatment, and for improving prognosis. Cerebrospinal fluid concentrations of $A\beta_{1-42}$ and p-Tau_{181} proteins are the most promising biomarkers for the detection of AD, and may be identified early in its clinical *continuum*. Individuals with amnestic mild cognitive impairment (aMCI) are at high risk for developing ADD. Therefore, longitudinal studies with individuals diagnosed with aMCI provide an opportunity to investigate the underlying biological processes (amyloidosis and neurodegeneration) and predict the potential risk of progression to ADD.

Objective: To measure the cerebrospinal fluid biomarkers $A\beta_{1-42}$ and p-Tau_{181} in individuals with aMCI and to verify whether these biomarkers are able to predict progression to the stage of dementia in Alzheimer's disease via a longitudinal study.

Methods: Forty-two patients diagnosed with aMCI or subjective cognitive decline were evaluated in 2013 and re-evaluated in 2018. $A\beta_{1-42}$ and p-Tau_{181} levels in their cerebrospinal fluid were measured via an immunoenzymatic assay and cutoff points were calculated with the Youden test. Moreover, neuropsychological testing, such as the CERADs and MoCA, was performed.

Results: 45,2% of individuals diagnosed with aMCI progressed to ADD in five years. The relative risk of progression in patients diagnosed with aMCI and with $A\beta_{1-42}$ concentrations of <618,5 pg/mL was 5.8 times greater than that of patients with higher $A\beta_{1-42}$ concentrations (*P* = 0,0011). Those with a p-Tau_{181}/$A\beta_{1-42}$ ratio above 0.135 were 3.83 times at risk of disease progression (*P* = 0,0001). Cerebrospinal fluid concentrations of $A\beta_{1-42}$ and p-Tau_{181} explained

47.5% of the variance observed in the ΔCERADs (P <0,001), whereas the $A\beta_{1-42}$ protein on its own was able to explain 38.6% (P < 0,001).

Conclusions: We hereby demonstrate that $A\beta_{1-42}$ cerebrospinal fluid protein concentration was the best measure for establishing the relative risk of evolving from aMCI to ADD in five years. The p-Tau_{181}/$A\beta_{1-42}$ ratio was not superior to isolated $A\beta_{1-42}$ protein concentration in predicting progression from aMCI to ADD. Nonetheless, p-Tau_{181} protein concentrations aided in accounting for an extra 9% of the variance in ΔCERADs in five years.

Keywords: Alzheimer's disease; amnestic mild cognitive impairment; cerebrospinal fluid biomarkers; beta-amyloid protein; Tau protein.

LISTA DE ABREVIATURAS E SIGLAS

AD: Alzheimer’s disease

ADD: Alzheimer’s disease dementia

aMCI: amnestic Mild Cognitive Impaiment

APOE: Apolipoproteína E

APP: Amyloid Precursor Protein - Proteína Precursora Amilóide

AUC: Area Under the Curve

BACE1: enzima β-secretase

BNT: Boston Naming Test

CCL: Comprometimento Cognitivo Leve

CCL-a: Comprometimento Cognitivo Leve amnéstico

CCl-na: Comprometimento Cognitivo Leve não-amnéstico

CDR: Clinical Dementia Rating

CDT: Clock Drawing Test

CERADs: Consortium to Establish a Registry for Alzheimer’s Disease subscore

CP: Constructional Praxis

CSF: Cerebrospinal Fluid

DA: Doença de Alzheimer

DSM-5: Manual Diagnóstico e Estatístico de Transtornos Mentais - 5ª edição

ELISA: Enzyme-Linked Immunosorbent Assay

GDS: Geriatric Depression Scale

MCI: Mild Cognitive Impairment

MMSE: Mini-Mental State Examination

MoCA: Montreal Cognitive Assessment

NIA-AA: National Institute on Aging and Alzheimer's Association

P1: Presenilina 1

P2: Presenilina 2

PET: Positron Emission Tomography

ROC: Receiver Operating Characteristic Curve

SCI: Subjective Cognitive Impairment

SPSS: Statistical Package for the Social Sciences

VFT: Verbal Fluency Test

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CAPÍTULO I – INTRODUÇÃO

A Doença de Alzheimer (DA) é uma patologia neurodegenerativa progressiva que causa grave comprometimento cognitivo(1, 2). Dentre as doenças mais prevalentes, é uma das que

não possuem prevenção efetiva ou tratamento eficaz(3). Possui uma complexa e heterogênea fisiopatologia e é caracterizada pelo declínio insidioso e irreversível de funções cognitivas, tais como: memória, atenção, linguagem, percepção e funções executivas. O processo patológico costuma evoluir de forma lenta e a sobrevida média após o diagnóstico é curta(4).

Caracteriza-se pela presença de placas de proteína β-amilóide e emaranhados neurofibrilares de proteína *Tau* hiperfosforilada, além de processos oxidativos e inflamatórios no cérebro(5). As alterações patológicas se iniciam muitos anos antes dos primeiros sintomas clínicos(6). A ausência de terapias efetivas instiga a compreensão da fisiopatologia da doença e a busca de novos meios diagnósticos. Um dos aspectos mais importantes da pesquisa na DA é a descoberta de biomarcadores confiáveis que possam auxiliar a estabelecer o curso clínico e o prognóstico da doença(7).

Estudos têm se concentrado principalmente na correta identificação de indivíduos com comprometimento cognitivo leve (CCL), pelo fato desse estágio estar na transição entre a cognição normal e a demência(8, 9). Além disso, pesquisas demonstraram que indivíduos com CCL, especialmente do subtipo amnéstico (CCL-a), têm um risco aumentado de desenvolver a DA(10-12). Esta entidade nem sempre é facilmente distinguível de casos verdadeiramente pré-demenciais. Neste sentido, o desenvolvimento de biomarcadores e métodos confiáveis capazes de identificar o declínio ou a estabilidade cognitiva em pessoas com CCL-a são importantes. Além disso, os biomarcadores podem contribuir tanto para a correta identificação de indivíduos prodrômicos à DA, quanto para a diferenciação de outras causas de CCL(13). Com base no conhecimento atual, quanto mais previamente a DA for diagnosticada, maiores são as chances de um melhor prognóstico(14, 15).

A nova estrutura de pesquisa, proposta pelo *National Institute on Aging and Alzheimer's Association* (NIA-AA), definiu que os processos patológicos subjacentes à DA podem ser

reconhecidos por exame *post-mortem* ou *in vivo* por meio dos biomarcadores(16). Há evidências de que a quantificação das proteínas envolvidas com o processo patológico, $A\beta_{1-42}$ e p-$Tau_{181,}$ possa se tornar um importante instrumento de apoio ao diagnóstico da DA prodrômica(16). Essas proteínas podem atuar como verdadeiros biomarcadores, visto que podem ser detectadas no líquido cefalorraquidiano antes da emergência da sintomatologia clínica da DA(17). Então, a partir deste racional, este estudo foi proposto com o objetivo de verificar a predição dos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} na progressão do CCL-a para o estágio demencial da DA.

A estrutura da apresentação desta tese seguiu as normas do Programa de Pós Graduação em Medicina: Ciências Médicas da Faculdade de Medicina da Universidade Federal do Rio Grande do Sul (UFRGS). A formatação dos artigos foi realizada de acordo com as normas dos periódicos aos quais foram ou serão submetidos.

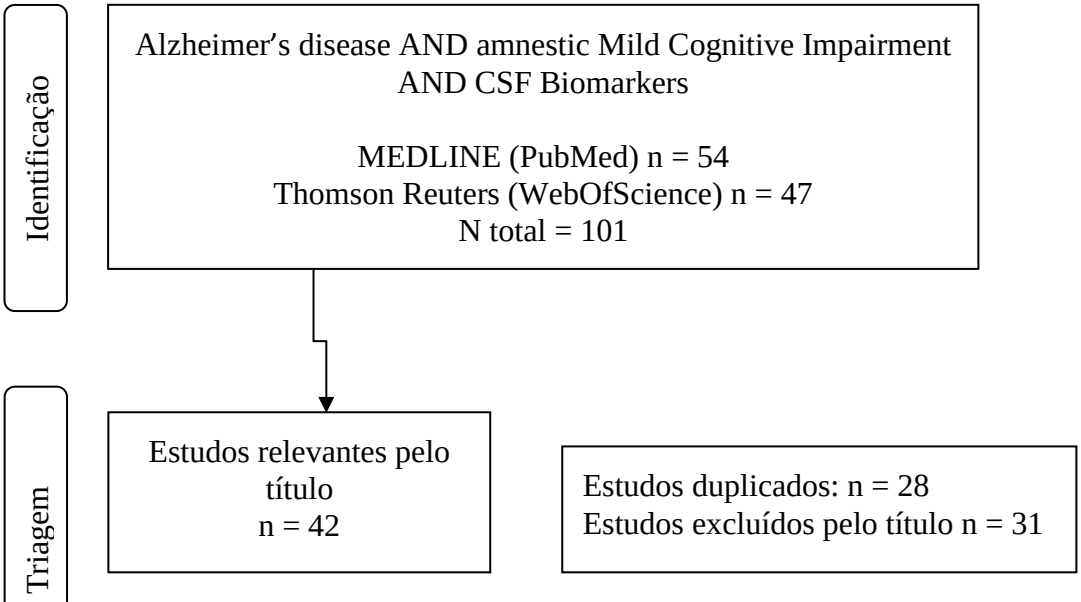
CAPÍTULO II – REVISÃO SISTEMATIZADA DA LITERATURA

1. Estratégias para localizar e selecionar informações

Para a apresentação dos aspectos epidemiológicos, conceituais, fisiológicos e sustentar as conclusões obtidas nesta tese, buscou-se suporte em artigos originais, artigos de revisão e meta-

análises. A revisão sistematizada da literatura foi realizada enfatizando-se os aspectos da DA e dos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181}. As informações foram selecionadas nas bases de dados MEDLINE (PubMed) e Thomson Reuters (WebOfScience) através de combinações dos seguintes descritores: '*Alzheimer's disease*', '*amnestic Mild Cognitive Impairment*' e *'CSF Biomarkers'*. Foram selecionadas publicações dos últimos cinco anos e não foi feita restrição de idioma. As referências dos artigos escolhidos e utilizados foram revisadas de modo a encontrar outras não contempladas na busca. Portanto, alguns estudos comumente referenciados e conceituadas publicações mais antigas foram posteriormente incluídas na revisão.

O critério de inclusão foram estudos que envolveram a relação dos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} na predição da progressão do comprometimento cognitivo leve para a demência da Doença de Alzheimer. O diagrama de fluxo a seguir mostra os resultados obtidos na busca sistematizada. Foram encontrados inicialmente 101 estudos publicados nos últimos cinco anos. Desses, 59 foram excluídos (primeira exclusão) após rastreio por duplicações e leitura de títulos. Todos os restantes 42 artigos tiveram seus resumos cuidadosamente lidos. Dos quais, 13 foram excluídos (segunda exclusão) devido aos critérios de elegibilidade. Todos os 29 artigos restantes foram totalmente lidos e 21 foram finalmente incluídos na revisão sistematizada final e/ou nos artigos científicos.



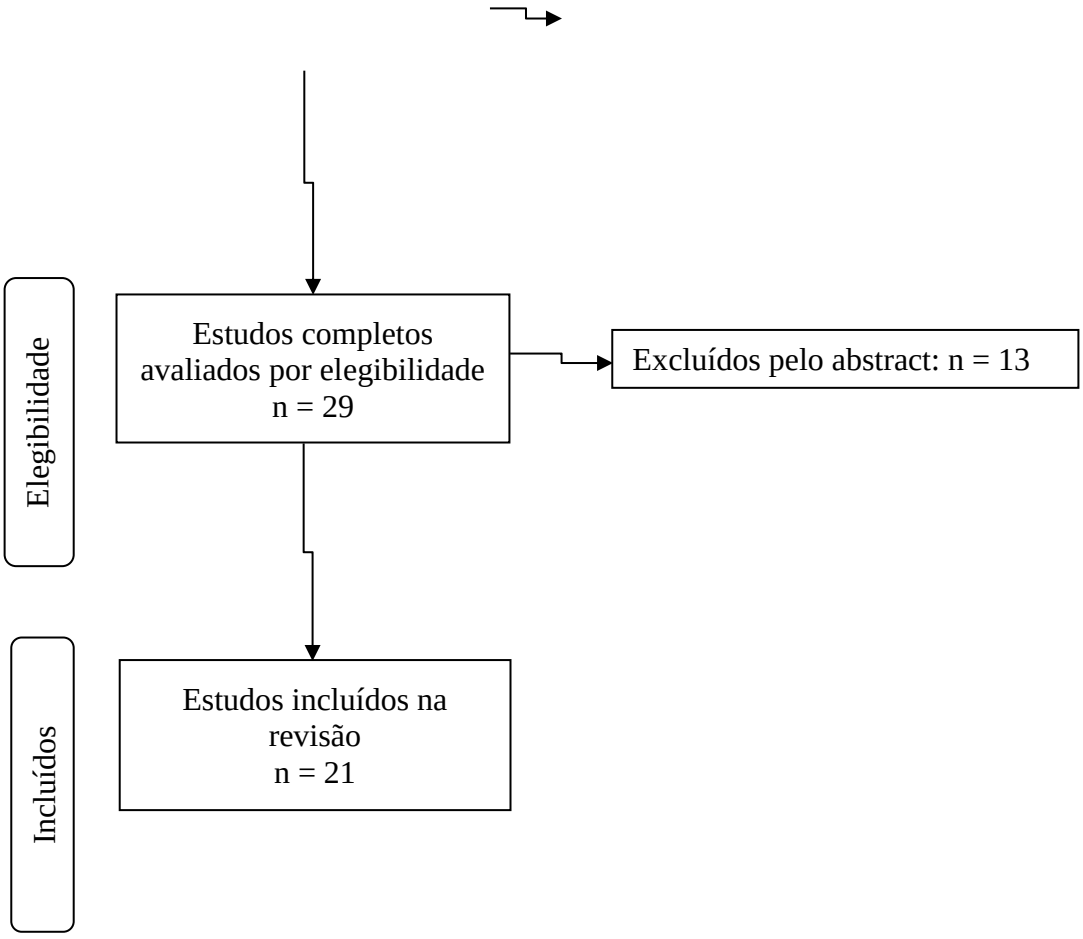


Figura 1: Diagrama de fluxo da revisão sistematizada da literatura.

2. Aspectos Conceituais e Epidemiológicos da Doença de Alzheimer

A DA foi descrita pela primeira vez em 1906 pelo médico psiquiatra Alois Alzheimer(18). Inicialmente acreditava-se tratar de uma doença rara e foram necessários mais de setenta anos desde o seu descobrimento para que ela fosse reconhecida como um tipo demência e uma das principais causas de morte(19). Hoje, a DA é considerada a causa mais prevalente de demência, responsável por cerca de 60% a 80% dos casos(3, 20, 21). A saber, o

termo “Demência” (Transtorno Neurocognitivo Maior), segundo o DSM-5, é uma síndrome clínica, que cursa com deterioração dos domínios cognitivos, alterações de comportamento e prejuízo funcional(22).

As demências em geral, e a DA em particular, são consideradas prioridades de saúde pública em nível global, devido à alta prevalência e ao impacto causado aos indivíduos e suas famílias, à sociedade e à economia(23). O número de pessoas com demência em todo o mundo estimado em 2015 era de 46,8 milhões, sendo que a previsão para 2030 é de 74,7 milhões e de 131,5 milhões para 2050. 68% desse número vivem em países de baixa e média renda(24). No Brasil, as demências afetam aproximadamente 7,5 a 8,0% da população com mais de 65 anos e estima-se a incidência de 55 mil novos casos por ano. A prevalência das demências na comunidade brasileira em geral dobra a cada cinco anos a partir dos 65 anos (2%), para atingir aproximadamente 4% aos 70 anos, 8% aos 75 anos, 15% aos 80 anos e 30% aos 85 anos. A partir dessa faixa etária essa aceleração diminui para dobrar apenas aos 100 anos (60%)(25).

A DA é neurodegenerativa, progressiva e incurável e causa a perda irreversível de funções cognitivas(1). O termo “cognição” refere-se a todo processo cerebral com tomada de consciência e que vise à tentativa de controle dos meios externo ou interno(26). Cinco são os domínios cognitivos primários, conforme descritos por Mesulam: 1) Memória; 2) Linguagem; 3) Atenção; 4) Funções Executivas e 5) Funções Visuoespaciais. Todos esses domínios possuem seus respectivos circuitos cerebrais, os quais se integram em áreas associativas(26). Portanto, o declínio das funções cognitivas pode ser caracterizado pela dificuldade progressiva em fixar fatos recentes, adquirir novos conhecimentos, fazer cálculos numéricos e julgamentos de valor, manter-se alerta, compreender e expressar-se na linguagem adequada e manter a motivação, dentre outras funções corticais superiores(27).

A DA é caracterizada pelo declínio insidioso de funções cognitivas, sendo que o comprometimento do domínio cognitivo memória é o primeiro e principal sintoma. Porém, além da disfunção cognitiva, podem ocorrer mudanças comportamentais e perda da autonomia funcional com o avançar da doença. O processo patológico, em geral, costuma evoluir de forma lenta e a sobrevida média após o diagnóstico clínico é curta(4). Modelos hipotéticos da relação temporal entre os elementos patológicos e os sintomas clínicos têm sido propostos de modo a elucidar etiologia subjacente e propiciar o diagnóstico precoce(23).

3. Fatores de risco

A idade é o principal fator de risco para o desenvolvimento da DA(28). Logo, o aumento da longevidade leva a um aumento no número de casos, os quais têm sobrecarregado os sistemas de saúde pública, principalmente de países em desenvolvimento(3). Ressalta-se, porém, que a DA não faz parte do envelhecimento biológico esperado, assim, somente idade avançada não é suficiente para causar a doença. A maioria dos casos de DA (aproximadamente 95%) se refere ao tipo de início tardio, que ocorre acima dos 65 anos de idade, resultado da interação de fatores ambientais e/ou genéticos.

O principal fator de risco genético para o desenvolvimento da DA de início tardio é ser portador do alelo ε4 do gene da apolipoproteína E (APOE)(29). Localizado no cromossomo 19, o gene da APOE está envolvido com o transporte de colesterol e outros lipídeos na corrente sanguínea(30). Em seres humanos há três alelos diferentes do gene: ε2, ε3 e ε4, os quais são herdados um de cada genitor. O tipo ε3 é o mais comum, seguido do ε4 e do ε2, o qual é relativamente raro. Portar APOE-ε4 constitui o maior fator de risco genético para a DA, sendo que a APOE-ε3 é neutra, não diminui nem aumenta o risco, e a APOE-ε2 é considerada protetora(31). Portar ε4 não garante que um indivíduo irá desenvolver a DA. Todavia, aqueles

que possuem uma cópia de ε4 apresentam três vezes maior risco de desenvolver a DA, enquanto aqueles que possuem duas cópias desse alelo têm risco de oito a doze vezes maior(32, 33). Além disso, indivíduos que possuem o alelo ε4 são mais prováveis de desenvolver a DA em uma idade mais jovem do que aqueles que possuem os outros alelos do gene da APOE(33). Em um estudo recente foi reportado que indivíduos com pelo menos um alelo ε4 têm maior probabilidade de desenvolver CCL do tipo amnéstico(34).

Certas mutações genéticas, como as que ocorrem no gene da proteína precursora amilóide (APP) (cromossomo 21), ou nos genes das proteínas Presenilina 1 (P1) (cromossomo 14) ou Presenilina 2 (P2) (cromossomo 1), ambas componentes da γ-secretase, estão relacionadas ao desenvolvimento da DA com herança autossômica dominante. Uma pequena porcentagem dos casos de DA (estima-se 1% ou menos) a desenvolve como resultado de mutações em qualquer um dos três genes. Mutações na P1 e na APP estão associadas à penetrância completa, significando que todos os indivíduos que têm uma mutação na P1 ou na APP desenvolverão DA ao longo da vida. Em contraste, as mutações na P2 mostram 95% de penetrância, o que significa que nem todos indivíduos com mutação na P2 desenvolverão a doença(35, 36). Mutações na P1 são as mais comuns (30%-70%), seguido das mutações na APP (10%-15%) e na P2 (<5%)(27). Indivíduos com mutações em qualquer um desses três genes tendem a desenvolver DA precocemente, muitas vezes com a idade de 30 anos, por isso é conhecida como DA de início precoce ou pré-senil(36).

Também se identificou que indivíduos com síndrome de Down possuem maior risco de desenvolver DA, o que pode estar relacionado com a cópia adicional do cromossomo 21, cromossomo que codifica a APP. Logo, ter uma cópia extra de cromossomo 21 pode aumentar o número de fragmentos de β-amilóide no cérebro(37). Cerca de 30% das pessoas com síndrome de Down com 50 anos de idade têm DA. E com a expectativa de vida aumentando, aproximadamente 70% delas desenvolverão a DA ao longo da vida(38). Outros 4% dos casos

de DA de inicio precoce desenvolvem a doença devido a uma herança complexa, baseada na combinação de fatores de risco ambientais e genéticos de múltiplas causas.

Embora fatores de risco como idade e genéticos não possam ser alterados, outros podem ser modificados para reduzir a chance de desenvolver a doença: os fatores de risco ambientais(39). Um relatório que avaliou fatores de risco modificáveis concluiu que a atividade física regular e a gestão de fatores de risco cardiovasculares (especialmente diabetes, obesidade, tabagismo e hipertensão) podem reduzir o risco de declínio cognitivo e demência. Além de também contribuírem: o seguimento de uma dieta e de um estilo de vida saudável(40) e a aprendizagem ao longo da vida, o chamado treinamento cognitivo(41). É de amplo conhecimento que quanto maior o nível de escolaridade do indivíduo, maior é sua reserva cognitiva e menor o risco de desenvolver DA(42). Essa variação individual na reserva cognitiva pode, muitas vezes, obscurecer ou mascarar a relação entre a gravidade da patologia e o desempenho cognitivo apresentado pelo indivíduo(43).

Além disso, há evidências emergentes de que os mecanismos epigenéticos (modificações no genoma que são herdadas pelas próximas gerações, mas que não alteram a sequência do DNA) contribuem para a DA. Alterações epigenéticas, sejam elas protetoras ou prejudiciais, podem ajudar a explicar, por exemplo, por que um membro da família desenvolve a doença e o outro não. Cientistas estão estudando cada vez mais sobre epigenética relacionada à DA, com a esperança de desenvolver manejos individualizados(44-47).

4. Características Anatomopatológicas

A patologia da DA é caracterizada por atrofia cerebral difusa não uniforme, além de estresse oxidativo generalizado, disfunção mitocondrial e neuroinflamação(48). Os níveis dos

neurotransmissores acetilcolina, serotonina, noradrenalina e somatostatina encontram-se reduzidos, enquanto que os de glutamato geralmente estão elevados(49). Nos estágios iniciais primariamente o lobo temporal medial é afetado, seguindo progressivamente para as áreas associativas neocorticais(50).

A neuropatologia, segundo estágios de Braak, é caracterizada pela presença de emaranhados neurofibrilares que se iniciam envolvendo o córtex entorrinal (estágios I e II), progredindo para os hipocampos (III) e a ínsula (IV), para finalmente envolverem o neocórtex de forma inicial (V) ou avançada (VI)(51, 52). Essa progressão neuropatológica explica a evolução típica da forma clássica da doença, da anosmia à amnésia anterógrada - especialmente para fatos recentes – e desorientação espacial (fase III), depressão e outras alterações neuropsiquiátricas como delírios (IV), para finalmente levar a alterações nas "funções corticais superiores" (fases V e VI), incluindo afasia anômica, agnosias, apraxias, além de progressiva perda da memória autobiográfica(26). Naturalmente, a ordem da apresentação dos sintomas pode variar substancialmente de um indivíduo para outro.

Ao microscópio observa-se perda de neurônios e degeneração sináptica, os quais são resultado do processamento incorreto de proteínas do sistema nervoso central (53). Duas são as principais proteínas envolvidas com a patologia da DA: a proteína β-amilóide e a proteína *Tau*(54). Essas proteínas formam as placas amilóides e os emaranhados neurofibrilares que foram vistas por Alois Alzheimer ao estudar o cérebro de Auguste Deter, porém foram descritas cientificamente somente mais tarde, em 1984(55) e 1986(56, 57) respectivamente.

5. A Proteína β-amilóide

Desde o primeiro relato de caso da DA, as marcas neuropatológicas eram conhecidas por serem compostas de “amilóide”, isto é, depósitos proteicos que podem ser corados com corantes como o Congo Red. Somente após o desenvolvimento de um método para purificar as placas amilóides, em meados de 1984, pode-se observar a sequência completa da proteína purificada de 40 aminoácidos (4kDa) que formava placas no tecido cerebral. Com base no seu peso molecular, a proteína foi inicialmente denominada proteína A4 amilóide, a qual é hoje conhecida como β-amilóide ou Aβ. A identificação da sequência de aminoácidos da proteína β-amilóide facilitou posteriormente a clonagem do gene da APP(58).

β-amilóide é resultante da clivagem de uma grande proteína transmembrana, a APP, pela enzima β-secretase (BACE1), sequencialmente seguida de clivagens por γ-secretases. O peptídeo formado (sAPPβ) pode, sob condições normais, ser degradado por enzimas, tais como: a neprisilina, a enzima degradadora da insulina ou ainda pela enzima conversora de endotelina; ou ser eliminado pelo líquido instersticial cerebral através da barreira hematoencefálica. Alternativamente, pode ser transportado ao líquido cefalorraquidiano para ser eliminado por essa via. No entanto, se houver alguma deficiência na degradação e/ou eliminação, ou ainda, aumento da produção de peptídeos β-amilóide, ele irá se agregar ao longo do tempo, e produzirá as conhecidas e neurotóxicas placas amilóides(59). O aumento da produção de β-amilóide por mutações ou duplicações no gene da APP e nas presenilinas está relacionado com à DA familial, enquanto que a diminuição da degradação da β-amilóide está mais relacionada à DA esporádica(60). A função fisiológica da proteína β-amilóide ainda não está bem elucidada, mas sabe-se que em animais jovens e saudáveis a presença desses peptídeos é importante para o aprendizado e a memória(61). A APP, alternativamente, poderá seguir outra via de clivagem, na qual a enzima α-secretase atuará, seguida de clivagens por γ-secretases, o que resultará em peptídeos sAPPα, com função não-amiloidogênica e neuroprotetora(20, 62) (Figura 2).

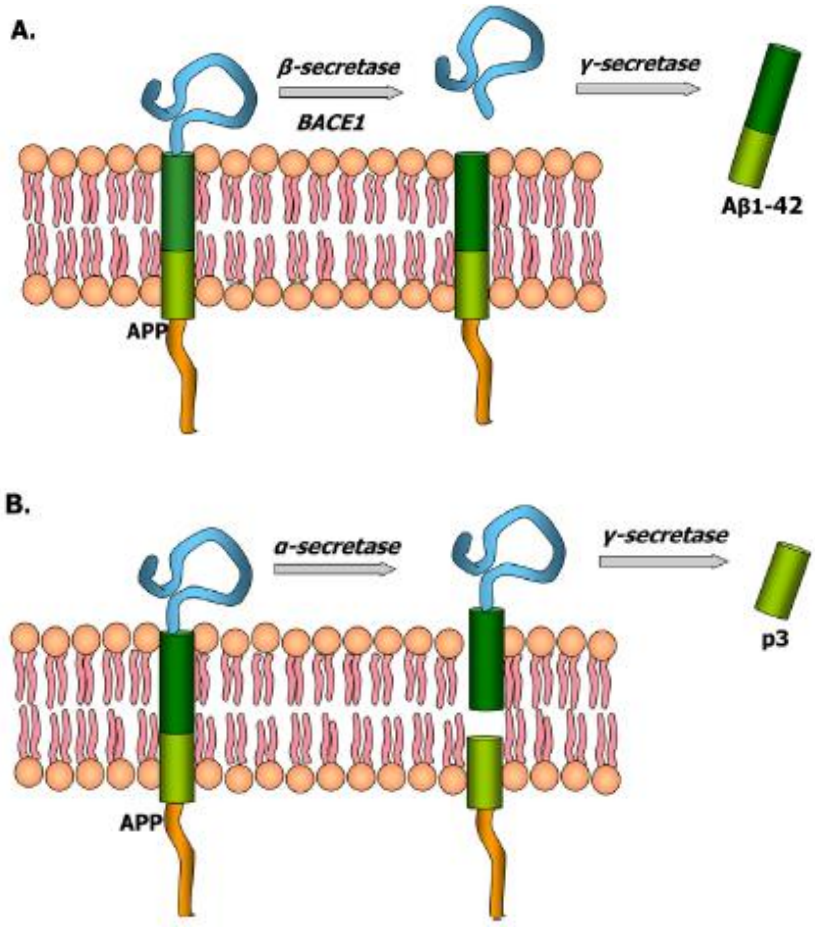


Figura 2. A) Proteólise de APP por ações enzimáticas seqüenciais de β e γ-secretases, gerando peptídeos sAPPβ e liberando $A\beta_{1-42}$. B) Proteólise da APP pela α-secretase, gerando peptídeos sAPPα e liberando o peptídeo p3 extracelular(63).

Os peptídeos β-amilóide são produzidos no cérebro e estão presentes também no liquido cefalorraquidiano e no plasma. Há vários tipos, o tipo Aβ40 é o mais abundante, no entanto o tipo Aβ42 tende a se agregar mais facilmente, por ser mais hidrofóbico e mais dinâmico(64). Aβ42 é o principal peptídeo envolvido na patologia da DA e parece ser o principal tipo envolvido 'hipótese da cascata amilóide', a qual é a teoria mais aceita para a explicação da etiopatogenia da doença(65).

A 'hipótese da cascata de amilóide' na gênese da DA, foi proposta em 1991 por John Hardy e David Allsop(66) e revisada por Tanzi e Bertram em 2005(67). Tal hipótese postula que a iniciação da DA acontece pelo excesso de peptídeos β-amilóide. Mutações no gene da

APP estariam associadas às formas familiares da DA com herança autossômica dominante, enquanto interações genéticas complexas e riscos ambientais contribuiriam para a acumulação de espécies tóxicas de β-amilóide na DA de início tardio. Esse excesso e agregação de peptídeos β-amilóide no tecido cerebral desencadearia a patologia. Após esse processo ocorreria a ativação neuroglial, a formação de emaranhados neurofibrilares com a proteína *Tau*, a neuroinflamação, a disfunção sináptica e neurítica e finalmente a morte celular neuronal, resultando em declínio cognitivo e demência(68).

Essa teoria de patogênese linear da DA já foi bastante questionada pelo fato de que outros mecanismos complexos estão envolvidos na etiopatogenia da DA. Entre esses fatores destacam-se: a neuroinflamação(69), o estresse oxidativo, a redução do metabolismo energético e a redução da função sináptica, os quais interagem uns com os outros(70). No entanto, a proteína β-amilóide é considerada o principal biomarcador para a detecção precoce da DA(71). Além disso, resultados de meta-análises sugerem que a proteína β-amilóide é útil tanto para a discriminação da DA de outras demências quanto do CCL(72-74).

6. A Proteína *Tau*

Tau é uma proteína associada aos microtúbulos do citoesqueleto dos neurônios e está diretamente envolvida na estabilização celular, portanto, desempenha importante papel na manutenção da morfologia neuronal e no transporte intracelular(64). Em condições normais, o equilíbrio entre a fosforilação e a desfosforilação da *Tau* coordena a sua ligação ou não aos microtúbulos, o que mantém a estabilidade neuronal e auxilia no transporte de cargas ao longo do axônio. Assim, a perda de sua funcionalidade, geralmente por hiperfosforilação, leva a deficiências funcionais nos neurônios e, consequentemente, das sinapses(21).

A hiperfosforilação diminui a capacidade da *Tau* se ligar aos microtúbulos, o que resulta em um anormal aumento dos níveis de *Tau* não ligada. Logo, com as suas concentrações citosólicas aumentadas, a probabilidade de formar oligômeros e agregados é maior. Na DA ela se hiperfosforila e se torce formando filamentos helicoidais emparelhados, os chamados emaranhados neurofibrilares. Em 1986, foi demonstrado que os emaranhados neurofibrilares são compostos da forma anormal da proteína *Tau*, com cerca de três vezes mais sítios fosforilados do que *Tau* normal(56, 58). Esses emaranhados de *Tau* hiperfosforilada desestabilizam os neurônios e consequentemente levam à neurodegeneração(75).

A chamada *Tau* total não se mostrou específica para a DA, pois é considerada indicadora de danos que podem derivar de uma variedade de etiologias(76), como: lesão cerebrovascular, demência fronto-temporal(77), traumatismo craniano, acidente cardiovascular agudo, doença de Creutzfeldt-Jakob(78), doença de Pick(21), entre outras. A *Tau* total é considerada um "marcador de estado", refletindo a intensidade da neurodegeneração ou a gravidade de um dano neuronal agudo. De fato, após danos cerebrais agudos, os níveis de *Tau* total aumentam em poucos dias após a lesão, permanecendo elevados por semanas até a normalização(58).

A única patologia que mostra consistentemente um aumento de *Tau* hiperfosforilada no líquor é a DA, este biomarcador encontra-se normal em outros distúrbios neurodegenerativos ou em danos cerebrais como acidente vascular cerebral isquêmico agudo(58, 79). A proteína *Tau* hiperfosforilada se mostrou eficaz no diagnóstico diferencial entre a DA, CCL e outros tipos demenciais(80, 81). Além disso, constatou-se que concentrações liquóricas de *Tau* hiperfosforilada podem estar aumentadas em aproximadamente 200% em indivíduos com DA quando comparadas a indivíduos controles(82).Em conjunto, esses dados indicam que a *Tau* total reflete a intensidade da lesão neuronal em um ponto específico, enquanto a *Tau* hiperfosforilada elevada no líquor reflete um estado patológico anormal associado à formação de filamentos helicoidais emparelhados. A proteína *Tau* hiperfosforilada é uma importante

proteína envolvida na patologia da DA, visto que emaranhados neurofibrilares estão mais intimamente associados com a gravidade da demência do que as próprias placas amilóides(83). Esse biomarcador é indicativo de um processo patológico mais tardio em relação ao causado pela proteína β-amilóide, logo, se torna dinâmico somente pouco tempo antes dos primeiros sinais clínicos da doença(6, 84).

Outra teoria proposta na etiopatogenia da DA é a 'teoria *Tau*', que tem como argumento que o grau de severidade das alterações cognitivas da DA correlaciona-se melhor com a severidade da patologia *Tau* do que com a da proteína β-amilóide. Além de que o depósito de placas amilóides não se correlaciona bem com a perda de neurônios. Um possível ponto de conciliação entre a 'teoria Tau' e a 'teoria da cascata amilóide', em termos de plausibilidade e constatação neuropatológica, seria o fato de que a proteína β-amilóide, especialmente em sua forma oligomérica, é necessária, mas não suficiente para causar apoptose e os efeitos adversos da DA(85).

Com efeito, neurônios de modelos transgênicos de camundongos hiperexpressando a proteína β-amilóide, mas geneticamente modificados para não expressar a proteína *Tau* hiperfosforilada, não desenvolveram neurodegeneração(54). Além disso, os primeiros biomarcadores que se tornam alterados em portadores de mutações determinísticas da DA são os do tipo β-amilóide(27, 86, 87). Observa-se então que a proteína β-amilóide pode ser superior à proteína *Tau* na patogênese da DA e desencadeia a conversão de *Tau* de um estado normal para um estado tóxico, isto é, promovendo tauopatia e neurodegeneração, que conduzem finalmente à deterioração cognitiva(85). Porém, deve-se considerar que a *Tau* hiperfosforilada também pode aumentar a toxicidade da proteína β-amilóide através mecanismos de feedback(54).

Alguns estudos hipotetizam que as duas proteínas envolvidas na patologia da DA podem iniciar independentemente o processo patológico, e há proposições de que uma incidente ‘betaopatia’ pode acelerar uma antecedente ‘tauopatia’(6, 88). Em suma, as proteínas que definem a DA como uma doença única entre as muitas que podem levar à demência podem não ser a via causal primária da patologia. Porém, fornecem uma explicação mecanicista para o desenvolvimento de ambas as proteinopatias diagnósticas, bem como a neurodegeneração e os sintomas clínicos da DA(16).

7. Terapêutica da DA

O diagnóstico precoce da DA é decisivo para o tratamento adequado e eficaz. A base da terapêutica tem se concentrado em retardar a evolução, tratar os sintomas e controlar as mudanças comportamentais. No entanto, não tem tido sucesso no desenvolvimento de terapias modificadoras da história natural da doença(89). Por esse motivo, dezenas de medicamentos e novas terapias estão em estudo(90, 91). As principais drogas que conseguem melhorar temporariamente os sintomas da DA agem alterando a disponibilidade de neurotransmissores, sendo que a eficácia desses fármacos varia de pessoa para pessoa. Atualmente existem apenas três modalidades terapêuticas para a DA, a saber: 1) anticolinesterásicos como o donepezil, a rivastigmina e a galantamina, 2) a memantina, única molécula antagonista NMDA aprovada para uso em humanos(49, 92) e 3) alguma evidência para o uso da vitamina E como neuroprotetora na DA(93).

A terapia colinérgica é a mais utilizada, e pode efetivamente melhorar a cognição e a funcionalidade cerebral em cerca de 20% a 30%. Na DA há uma marcada alteração do sistema colinérgico, principalmente com a diminuição (40%-90%) na atividade da colina acetiltransferase (enzima sintetizadora), sobretudo no córtex cerebral e hipocampo. Também

estão diminuídas as atividades da precursora acetilcoenzimaA, resultando em importante diminuição dos níveis de acetilcolina. Já a butilcolinesterase, que tal como a acetilcolinesterase (enzima metabolizadora) degrada a acetilcolina, está significativamente aumentada na doença. Os medicamentos inibidores de acetilcolinesterase agem diminuindo a queda dos níveis de acetilcolina, logo, aumentando a atividade colinérgica do neurotransmissor nos indivíduos com doença(94). A DA também libera moléculas de glutamato em excesso, o que permite a entrada de grande quantidade de cálcio no neurônio pós-sináptico, podendo levá-lo à morte. A Memantina (fármaco antagonista dos receptores de glutamato) coloca-se no canal de entrada para o cálcio no neurônio pós-sináptico, bloqueando sua entrada e a consequente morte neuronal(92).

Todas os fármacos aprovados para uso são indicadas para pacientes que estão nas fases iniciais e intermediárias da doença. São elegíveis os pacientes que ainda mantêm algum grau de cognição e de independência funcional, por este motivo a importância do diagnóstico precoce. Estes medicamentos são essencialmente sintomáticos e não há nenhuma evidência de que eles retardam a progressão da patologia. As perspectivas para o futuro almejam que novas drogas sejam mais seguras e capazes de interferir efetivamente no retardo da evolução natural da doença(95). As terapias mais promissoras consistem: na terapia anti-amilóide, no metabolismo da proteína *Tau* e nas neurotrofinas. Além dessas estratégias terapêuticas há estudos com relação à função mitocondrial, ao uso de antioxidantes, anti-inflamatórios e fitomedicamentos(91).

As terapias anti-amilóide consistem: na inibição tanto da β-secretase quanto γ-secretase, as quais são as duas enzimas que favorecem a via amiloidogênica; terapias antiagregação do β-amilóide em placas senis ou ainda imunoterapias, ativa ou passiva, de modo a aumentar a eliminação do β-amilóide do tecido cerebral com o uso de anticorpos para impedir a formação de placas(91, 96). Já as terapias que envolvem a proteína *Tau* se baseiam na inibição ou da

hiperfosforilação ou da agregação em emaranhados neurofibrilares. Sabe-se também que neurônios colinérgicos são sensíveis à ação do fator de crescimento neuronal. Dessa maneira, alguns estudos testam essa nova possibilidade terapêutica no tratamento da DA, reduzindo a perda de neurônios colinérgicos no córtex e hipocampo(93).

As falhas que se observam nos estudos de fármacos no sentido de alterar o curso natural da doença se devem ao fato da DA ser uma doença extremamente complexa, além da fisiopatologia não estar completamente elucidada(93, 97). Sendo assim, as pesquisas focadas em modelos lineares de mecanismos de ação, que seguem uma linha reducionista - em que o fármaco candidato agirá em um único alvo ligado à patogênese da doença - dificilmente obterão êxito na possível cura da DA. Assim, o melhor entendimento da patogenia da doença e o desenvolvimento de fármacos que possam se ligar a diferentes alvos, seguindo um modelo de rede e/ou multialvo, e com menores efeitos colaterais, poderiam prover melhores resultados na possível cura ou interrupção da progressão da patologia(91). O uso dos biomarcadores, na identificação precoce de indivíduos com alto risco de desenvolverem a DA, é um promissor caminho para que haja um completo entendimento da patologia e o desenvolvimento de drogas específicas, que consigam reverter ou interromper o processo patológico da doença.

8. A Pesquisa na Doença de Alzheimer

A partir do momento em que se evidenciou a ampla abrangência da DA na população mundial, a mesma tornou-se um foco significativo de pesquisa. Em 1984, DA foi inicialmente definida como uma entidade clínico-patológica e era diagnosticada somente *post-mortem* na autópsia ou *in vivo* como possível ou provável(98). O diagnóstico era basicamente clínico, obtido com a identificação de uma síndrome demencial, seguido da exclusão de outras etiologias por meio de uma variedade de abordagens. Algumas ferramentas utilizadas na

exclusão incluíam: histórico médico e familiar (incluindo história psiquiátrica de mudanças cognitivas e comportamentais), testes cognitivos, avaliação física e exclusão de doenças cerebrovasculares ou características de outros tipos demenciais(99).

A pesquisa que se seguiu revelou que a neuropatologia da DA se inicia anos antes do aparecimento dos primeiros sintomas(100). As alterações cerebrais associadas à DA podem iniciar cerca de vinte anos ou mais antes dos sinais clínicos(6, 27) (Figura 3). Portanto, diagnosticar a DA de forma precoce se tornou decisivo para um melhor manejo da doença.

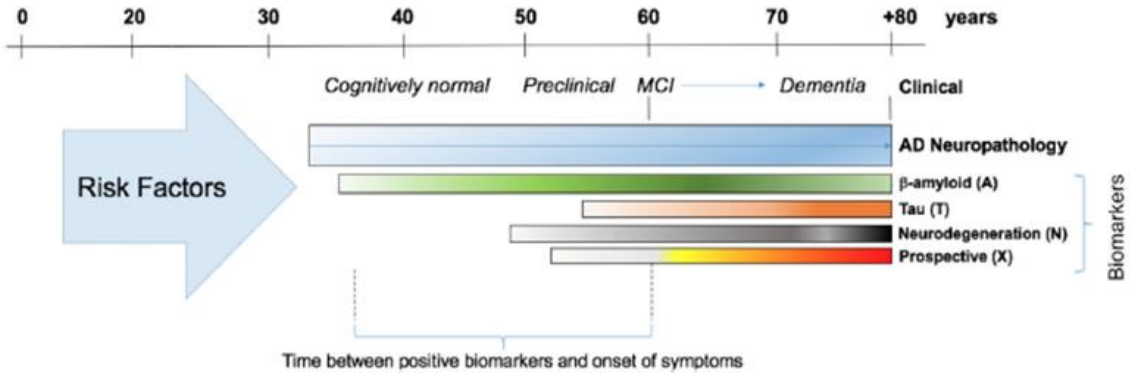


Figura 3. Neuropatologia da Doença de Alzheimer pode ser detectada cerca de 20 a 30 anos antes dos primeiros sintomas clínicos por meio de biomarcadores(101).

Em 2011, o NIA-AA atualizou as recomendações diagnósticas para a DA de 1984(98) e considerou a doença em três principais estágios: 1) pré-clínico, 2) comprometimento cognitivo leve e 3) sintomático ou demencial(102). Além do mais, com a disponibilidade de biomarcadores específicos para a patologia *in vivo* houve suporte para a mudança de definição da DA: de uma entidade clínico-patológica para uma entidade clínico-biológica.

Os estágios da DA passaram a ser caracterizados na pesquisa como segue: 1) pré-clínico: no qual os indivíduos são objetivamente caracterizados como cognitivamente normais, é pré-sintomático(20); 2) fase de CCL: fenótipo clínico específico da síndrome amnéstica do tipo hipocampal(103). Em indivíduos com CCL devido à DA há um declínio cognitivo progressivo maior na memória do que o esperado para a idade e nível de escolaridade, mas sem sinais

clínicos de habilidades funcionais prejudicadas(20); e 3) demencial: onde há prejuízo funcional nas atividades da vida diária (20). A partir dessas recomendações os biomarcadores passaram a ser fortemente sugeridos nas pesquisas com a DA. Verificou-se que a aplicação conjunta de critérios clínicos com os biomarcadores resulta num maior nível de evidência no suporte à etiologia da doença. Outrossim, os biomarcadores auxiliariam na determinação da probabilidade de progressão cognitiva e funcional de um indivíduo com declínio cognitivo a um estágio demencial num determinado período de tempo(104).

O progresso científico nesse ínterim levou a uma iniciativa de atualização e unificação das diretrizes de 2011. Então, em 2018, o NIA-AA propôs uma nova estrutura de pesquisa para a DA. A partir da qual a DA fica definida pelos seus processos patológicos subjacentes, que podem ser documentados por exame *post mortem* ou *in vivo* por biomarcadores. O diagnóstico não é mais baseado nas consequências clínicas da doença - ou seja, nos sintomas - mas sim em um *continuum* da doença baseado em biomarcadores. Além disso, essa nova estrutura muda a definição da DA para pessoas vivas: de processo sindrômico para um construto biológico(16).

O novo quadro de investigação centra-se em biomarcadores agrupados nos diferentes processos patológicos da DA e que podem ser mensurados em pessoas vivas com tecnologia de imagem e/ou análise de líquido cefalorraquidiano. A proposta recomenda que o termo DA seja alocado a pessoas que tenham a comprovada presença biomarcadores positivos(23). Os biomarcadores são agrupados nos de deposição β-amilóide (A), *Tau* hiperfosforilada (T) e neurodegeneração (N) no sistema [AT(N)]. Sendo que β-amilóide e *Tau* hiperfosforilada são os depósitos proteicos anormais que definem a DA como uma doença neurodegenerativa única entre diferentes distúrbios que podem levar à demência(105). Enquanto que os biomarcadores de neurodegeneração, - a saber: dosagem de *Tau* total no líquido cefalorraquidiano, hipometabolismo cerebral com fluordesoxiglicose no PET e atrofia cerebral verificada na ressonância magnética - não são equivalentes àqueles que refletem a acumulação de β-amilóide

e *Tau* hiperfosforilada(76). Os indicadores de neurodegeneração ou lesão neuronal podem ser resultantes de várias causas, não são específicos para neurodegeneração devido à DA. No entanto, a combinação de todos esses biomarcadores pode fornecer uma predição de declínio cognitivo futuro muito mais poderosa do que o estudo das proteínas alteradas isoladamente. Logicamente, uma vez que a neurodegeneração é a alteração neuropatológica da DA que mais se correlaciona com os sintomas da patologia(106). Essa nova estrutura estabelece que quanto mais avançada a doença definida pelos biomarcadores, maior é a probabilidade e mais rápida é a predição de declínio cognitivo. Assim, há sólidas evidências de que as combinações dos biomarcadores alterados são úteis para o estagiamento do *continuum* da DA(16).

Além disso, como a DA é considerada como um *continuum*, o estagiamento cognitivo também passa a ser feito com medidas contínuas. Para esse estagiamento são disponibilizados dois esquemas: um, usando as três categorias sindrômicas tradicionais: sem comprometimento cognitivo, com CCL e demência - a qual pode ser subdividida em leve, moderada ou severa - (aplicável a todos os membros de uma coorte, ou seja, inclui todos os perfis de biomarcadores), ou outro, de estagiamento clínico numérico com seis etapas (aplicável apenas àqueles no *continuum* da DA, útil para ensaios clínicos)(101). Dessa forma, ao considerar a DA como um construto biológico, se observa uma melhor caracterização e compreensão da sequência de eventos que levam ao comprometimento cognitivo, bem como da etiologia multifatorial da demência(16).

9. O advento dos biomarcadores

Biomarcadores são cruciais para a implementação da medicina personalizada, podendo ser mensurados objetivamente em fluidos biológicos como sangue e líquido cefalorraquidiano ou em tecidos periféricos. São indicadores biológicos que aprimoram a compreensão da doença

e podem fornecer informações sobre: presença/ausência de patologia, susceptibilidade, prognóstico ou ainda resposta a um tratamento. As características de um biomarcador ideal podem resumidas em: sensibilidade e especificidade de pelo menos 80%, valor preditivo positivo próximo a 90%, fácil dosagem, confiável, não invasivo, barato, estar envolvido na patologia, permitir a detecção precoce, representar a fisiopatologia e auxiliar a diferenciar a patologia estudada de outras semelhantes(107).

Diferentes biomarcadores, em particular proteínas mensuradas no líquido cefalorraquidiano, demonstraram ser ferramentas úteis para a detecção da DA *in vivo* e estão ganhando atenção na prática clínica(16). Existem múltiplas vantagens associadas à identificação de indicadores prognósticos preditivos de progressão à DA. Por exemplo, o conhecimento da patologia subjacente permite que pacientes e familiares se prepararem para o futuro, enquanto que a correta alocação de indivíduos em ensaios clínicos impede que indivíduos de improvável progressão à DA se exponham a um risco indevido.

A busca por biomarcadores que possibilitem o diagnóstico preciso da DA é uma das áreas de maior concentração de pesquisas atualmente. Principalmente busca-se biomarcadores acurados para os estágios iniciais da doença, onde medicamentos terão maior eficácia. Muitas das falhas nos ensaios clínicos se devem à imprecisão da correta identificação de indivíduos em vias de desenvolvimento da DA. É possível que mais de 30% dos indivíduos recrutados para ensaios clínicos, somente pela história natural e clínica, possuem patologias diferentes da DA(101). Além do diagnóstico precoce, o emprego dos biomarcadores pode trazer novas informações a respeito dos mecanismos fisiopatológicos envolvidos na doença, sendo vantajosos no prognóstico, no desenvolvimento de um tratamento e no monitoramento da sua eficácia(13).

Na medicina clínica os biomarcadores de fluidos corporais influenciam em até 70% das decisões médicas. Para distúrbios cerebrais, incluindo a DA, o desenvolvimento de biomarcadores iniciou-se adotando-se o líquido cefalorraquidiano como matriz, o qual, comparado ao sangue, tem a vantagem da sua proximidade com o parênquima cerebral e de receber as proteínas secretadas diretamente do espaço extracelular cerebral(58).

Há muitos anos os testes diagnósticos por meio de reações imunoenzimáticas são utilizados para a quantificação das proteínas β-amilóide e *Tau* no líquor(58). Tais estudos estabeleceram um padrão biomarcador para a DA, muitas vezes denominado "perfil para a DA", sendo que este é caracterizado pela diminuição da concentração líquórica da proteína β-amilóide e pelo aumento da proteína *Tau*. Esses principais biomarcadores evoluíram desde os primeiros estudos baseados em ensaios simples ao seu status atual, com instrumentos totalmente automatizados e extensiva validação de desempenho(58). Os biomarcadores movem a pesquisa sobre a DA na direção de uma medicina personalizada através da codificação de alterações patológicas. As informações fornecidas pelos biomarcadores, combinada com os elementos genéticos e clínicos, promovem uma medicina personalizada(17).

Há algumas diferenças entre os biomarcadores liquóricos e os de imagem. Os biomarcadores de líquido cefalorraquidiano refletem as taxas de concentrações protéicas de produção, degradação ou remoção em um determinado ponto no tempo. As medidas de imagem, por outro lado, representam a magnitude da carga ou dano neuropatológico acumulado ao longo do tempo(108). Discordâncias entre os tipos de biomarcadores poderão ocorrer. Porém, um estado patológico ativo contínuo, denotado pelas concentrações liquóricas, e o acúmulo de carga neuropatológica, denotado pelos exames de imagem, serão concordantes a longo prazo(16). As diretrizes atuais sugerem que ambos podem ser usados na clínica, sendo que a escolha pode ser baseada na disponibilidade, nos custos, ou nas estimativas de risco (exposição

à radiação *versus* cefaleia pós-punção lombar), juntamente com as preferências do médico e do paciente(58).

Embora numerosas pesquisas tenham relatado excelente desempenho diagnóstico por meio dos biomarcadores liquóricos, ainda se observam diferenças marcantes nos níveis absolutos relatados entre os estudos, mesmo quando se utiliza a mesma variante da técnica. A ampla variação nas medidas desses biomarcadores entre estudos e laboratórios é uma das dificuldades na determinação de pontos de corte homogêneos(72). Essas diferenças podem ser causadas tanto pelos os critérios de seleção de casos e resultante diferença nas características da amostra, quanto por diferentes procedimentos pré-analíticos utilizados como, por exemplo: tipo de tubos utilizados e cronograma de congelamento e descongelamento. Além disso, a variabilidade laboratorial também pode ser devida as discrepâncias nos procedimentos analíticos entre os laboratórios ou nos procedimentos de fabricação dos imunoensaios, resultando em variações de lote para lote. Devido a essas diferenças ainda não há o estabelecimento de pontos de corte, o que dificulta a implementação generalizada de biomarcadores na rotina clínica(58). Atualmente sugere-se que grupos de pesquisa utilizem baterias de testes cognitivos e pontos de corte para os biomarcadores encontrados em suas próprias amostras de pesquisa, de modo a melhor atender os objetivos de cada estudo(16). A padronização e o controle de qualidade desses procedimentos podem reduzir a disparidade e aumentar a utilidade dos biomarcadores(109).

A pesquisa clínica sem biomarcadores continua válida e fornece informações fundamentais sobre a sobrecarga social da incapacidade cognitiva e fatores que contribuem para o comprometimento cognitivo. No entanto, as variantes sindrômicas clássicas não são sinônimos da presença de deposição de β-amilóide e da neurodegeneração. Além do mais, os critérios diagnósticos puramente clínicos para DA apresentam baixa acurácia, com valores de sensibilidade e especificidade em torno de 70-80% quando comparados à neuropatologia(58).

Portanto, estudos sem biomarcadores, que inferem associações biológicas com a DA, podem causar confusão de diagnóstico(16).

10. O Comprometimento Cognitivo Leve e os biomarcadores liquóricos

O CCL é caracterizado pela presença de déficits neuropsicológicos objetivos, em um ou mais domínios cognitivos, sem comprometimento funcional nas atividades da vida diária(9). A pesquisa na DA tem se concentrado principalmente na correta identificação de indivíduos com CCL, por ser um estágio de transição entre a cognição normal e a demência(8, 9, 103). Porém, nem todas as pessoas com CCL desenvolverão a DA(34). Estima-se que indivíduos com CCL têm um risco aumentado, com taxas de progressão anuais que variam de 5-20% e quinquenais de 40-60%(10-12). Em autópsias cerebrais de casos com CCL foi constatado que mais de 70% das amostras analisadas estavam em vias de desenvolvimento da DA(110).

Essa entidade nem sempre é facilmente distinguível de quadros verdadeiramente pré-demenciais em idosos, podendo ser confundida, por exemplo, com uma depressão com sintomas cognitivos, o que é muito mais comum de ocorrer em idosos do que em adultos de meia idade(9). Portanto, a correta identificação de indivíduos com CCL que sejam considerados prodrômicos à DA é de extrema importância(103). Com base no conhecimento atual, quanto mais previamente a DA for diagnosticada, as chances de um melhor prognóstico são maiores(14, 15). Afere-se que quando terapias modificadoras da história natural da doença estiverem disponíveis, essas pessoas com CCL serão as principais beneficiadas(13).

Na fase prodrômica, a avaliação clínico-neuropsicológica tem acurácia limitada para a predição de conversão de CCL para a demência da DA(111). Até o momento, não há métodos claramente estabelecidos para prever a progressão para DA em pessoas com CCL(72). Para

superar esse limite, os biomarcadores diagnósticos, como a análise do líquido cefalorraquidiano e a neuroimagem, foram incluídos nos critérios atuais de pesquisa(111). A utilização desses biomarcadores auxilia na resposta a questões fundamentais, no suporte à etiologia da síndrome clínica em indivíduos com CCL e na determinação da probabilidade da progressão cognitiva e funcional de um paciente com CCL a um estágio demencial, juntamente com a chance de que essa progressão irá ocorrer em um definido período de tempo(104).

Os critérios clínicos para a caracterização de sujeitos com CCL foram propostos inicialmente por Petersen em 1999(112) e atualizados em 2004(113, 114). Igualmente se enquadram nas recomendações do NIA-AA (16, 104) e nos critérios para o diagnóstico de DA no Brasil(99). Tais critérios se resumem em: a) queixa cognitiva, de preferência corroborada por informante; b) prova objetiva de perda cognitiva; c) funcionamento cognitivo geral essencialmente preservado; d) não estar demente. Os indivíduos que se enquadram nos quesitos acima ainda podem ser classificados em uma das seguintes categorias: CCL-a (CCL - amnéstico) quando principalmente o desempenho nos testes neuropsicológicos de memória episódica for insatisfatório ou CCL-na (CCL - não amnéstico) no caso de mau desempenho em testes neuropsicológicos relativos a outros domínios cognitivos, tais como as funções executivas, linguagem ou habilidades visuo-espaciais(9).

O padrão específico de comprometimento da memória episódica, ou seja, “amnésia do tipo hipocampal”, é o critério clínico central para o diagnóstico precoce da DA(104). Esse perfil amnéstico, caracterizado por baixa capacidade de aprendizado e declínio rápido da memória em um período de tempo relativamente curto, é tipicamente associado ao envolvimento precoce do lobo temporal medial, que é o principal local anatômico para consolidação e armazenamento de memória(115). Pesquisas recentes revelam que esse padrão característico de amnésia é reconhecível em pacientes com CCL-a.

Indivíduos com CCL-a, cujo desempenho na memória episódica é pior do que em outros domínios cognitivos, têm maior probabilidade de progredir para DA(116, 117). A saber, a memória episódica pode ser subdividida de acordo com as diferentes etapas do processo de reprodução (por exemplo: recordação livre, recordação dirigida ou reconhecimento) e essa análise múltipla pode ser usada para descrever o risco real de progressão de um individuo com CCL à DA(116). A recordação tardia é o mais importante preditor neuropsicológico da conversão de CCL para a DA(116, 118).

Embora o CCL seja uma entidade bem conhecida, pode ser difícil para não especialistas diagnosticar adequadamente essa condição em sua prática somente pela clínica(119). Estudos longitudinais com pacientes com CCL representam uma oportunidade para investigar a intensidade dos processos biológicos subjacentes (amiloidose e neurodegeneração) e a sua relevância para a progressão para DA. Alguns estudos avaliaram o valor dos biomarcadores liquóricos na predição da progressão do CCL para a fase demencial da DA(7, 10, 73, 79, 120-122). Porém, as dificuldades em prever a progressão do CCL para a DA podem ser influenciadas pela heterogeneidade intrínseca dos indivíduos com CCL.

CCL é uma condição amplamente heterogênea, com uma gama de diferentes mecanismos neurodegenerativos subjacentes, o que dificulta a predição precoce do risco para o desenvolvimento da DA(123). Dessa forma, a generalização da pesquisa científica para a prática clínica é limitada. Os estudos envolvendo a dosagem dos biomarcadores $A\beta_{1-42}$ e p-Tau_{181} disponíveis compreendem, em sua maioria, indivíduos com diagnóstico de CCL geral, não fazendo diferenciação entre os subtipos amnéstico e não-amnéstico, o que gera uma ampla variabilidade de resultados e nenhuma concordância no reconhecimento do risco factual para o desenvolvimento da DA(73, 74, 79, 111, 124). Nesse sentido, buscou-se nesse estudo original reportar o risco relativo da progressão de um grupo composto exclusivamente de indivíduos

com CCL amnéstico para a demência da DA, baseado nos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181}.

CAPÍTULO III

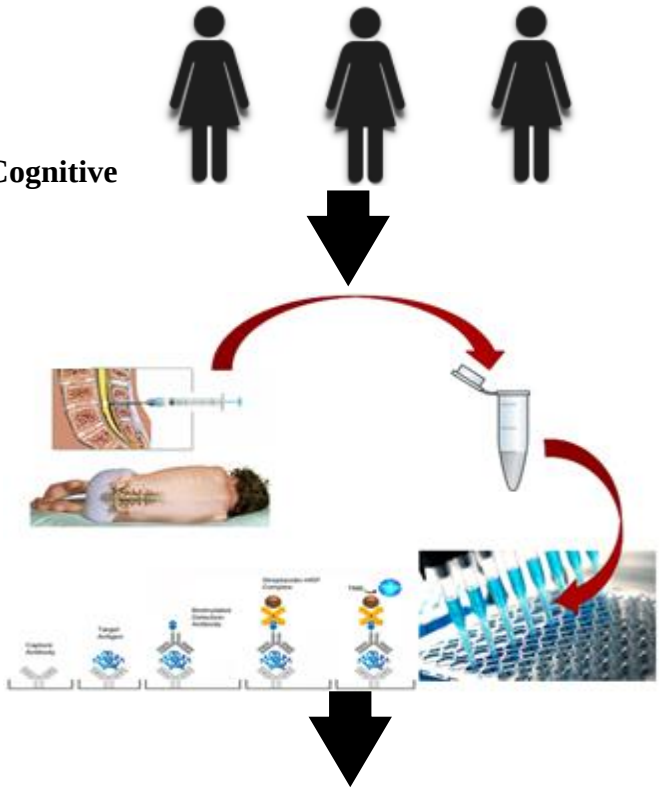
1. JUSTIFICATIVA

A DA é causa direta de grandes prejuízos pessoais, sociais e financeiros. Portanto, estudos que auxiliem na correta identificação de indivíduos com risco de progressão à fase demencial da doença são de máxima importância. O uso dos biomarcadores na identificação precoce de indivíduos com alto risco de desenvolverem a DA, é um promissor caminho para que haja um completo entendimento da patologia e o desenvolvimento de tratamentos específicos que consigam reverter ou interromper o processo patológico da doença. Com base no conhecimento atual, quanto mais previamente a DA for diagnosticada, maior é a chance de um melhor prognóstico. Além disso, muitas das falhas nos ensaios clínicos se devem à imprecisão na identificação de indivíduos em vias de desenvolvimento da DA.

Embora o CCL seja uma entidade bem conhecida, pode ser difícil diagnosticar adequadamente essa condição somente pelos aspectos clínicos. Estudos longitudinais com pacientes com CCL representam uma oportunidade para investigar a intensidade dos processos biológicos subjacentes e a sua relevância na progressão para DA. A capacidade dos biomarcadores em definir adequadamente o estado de risco pode, em última análise, permitir que agentes terapêuticos finalmente atinjam o status modificador da história natural da doença. Portanto, esse estudo justifica-se pela avaliação da contribuição dos biomarcadores liquóricos $A\beta_{1\text{-}42}$ e p-Tau_{181} na predição do risco de progressão à fase demencial da DA em um grupo de alto risco, composto exclusivamente por indivíduos com CCL do tipo amnéstico, o qual que terá maior vantagem prognóstica ao ser corretamente identificado.

2. MAPA CONCEITUAL

amnestic Mild Cognitive



Impairment

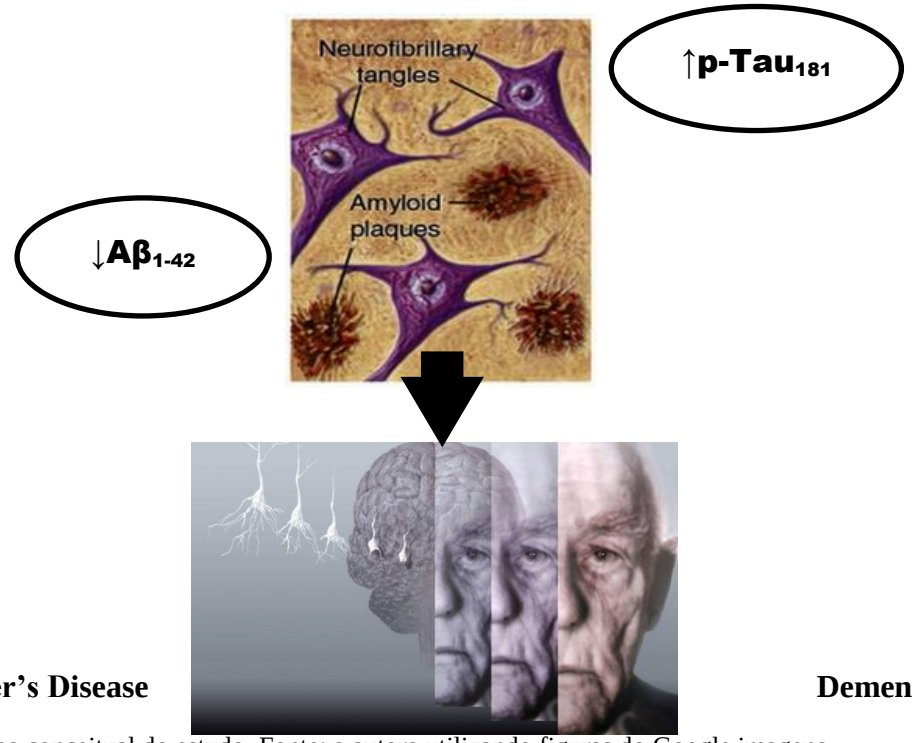


Figura 4. Mapa conceitual do estudo. Fonte: a autora utilizando figuras do Google imagens.

3. OBJETIVOS

3.1 Objetivo Geral

- Mensurar os biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} em indivíduos com Comprometimento Cognitivo Leve amnéstico e verificar suas capacidades preditivas para a fase demencial da Doença de Alzheimer em um estudo longitudinal.

3.2 Objetivos Específicos

- Identificar e comparar os perfis dos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} em indivíduos com Comprometimento Cognitivo Leve amnéstico e com Comprometimento Cognitivo Subjetivo;

- Verificar a relação e a concordância desses biomarcadores com testes neuropsicológicos selecionados;

- Predizer o risco de progressão do Comprometimento Cognitivo Leve amnéstico para a fase demencial da Doença de Alzheimer em cinco anos de seguimento.

REFERÊNCIAS DA REVISÃO DE LITERATURA

1. Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol. 2014;88(4):640-51.

2. Molinuevo JL, Ayton S, Batrla R, Bednar MM, Bittner T, Cummings J, et al. Current state of Alzheimer's fluid biomarkers. Acta Neuropathol. 2018.

3. Rizzi L, Rosset I, Roriz-Cruz M. Global epidemiology of dementia: Alzheimer's and vascular types. Biomed Res Int. 2014;2014:908915.

4. Association As. 2014 Alzheimer's disease facts and figures. Alzheimers Dement. 2014;10(2):e47-92.

5. Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ, Hampel H. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement. 2015;11(1):58-69.

6. Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013;12(2):207-16.

7. Lange C, Suppa P, Pietrzyk U, Makowski MR, Spies L, Peters O, et al. Prediction of Alzheimer's Dementia in Patients with Amnestic Mild Cognitive Impairment in Clinical Routine: Incremental Value of Biomarkers of Neurodegeneration and Brain Amyloidosis Added Stepwise to Cognitive Status. J Alzheimers Dis. 2018;61(1):373-88.

8. Hugo J, Ganguli M. Dementia and cognitive impairment: epidemiology, diagnosis, and treatment. Clin Geriatr Med. 2014;30(3):421-42.

9. Petersen RC, Caracciolo B, Brayne C, Gauthier S, Jelic V, Fratiglioni L. Mild cognitive impairment: a concept in evolution. J Intern Med. 2014;275(3):214-28.

10. Tondelli M, Bedin R, Chiari A, Molinari MA, Bonifacio G, Lelli N, et al. Role of cerebrospinal fluid biomarkers to predict conversion to dementia in patients with mild cognitive impairment: a clinical cohort study. Clin Chem Lab Med. 2015;53(3):453-60.

11. Schjønning Nielsen M, Simonsen AH, Siersma V, Hasselbalch SG, Høgh P. Are CSF Biomarkers Useful as Prognostic Indicators in Diagnostically Unresolved Cognitively Impaired Patients in a Normal Clinical Setting. Dement Geriatr Cogn Dis Extra. 2016;6(3):465-76.

12. Schott JM, Petersen RC. New criteria for Alzheimer's disease: which, when and why? Brain. 2015;138(Pt 5):1134-7.

13. Reiman EM. Alzheimer disease in 2016: Putting AD treatments and biomarkers to the test. Nat Rev Neurol. 2017;13(2):74-6.

14. Karakaya T, Fußer F, Schröder J, Pantel J. Pharmacological Treatment of Mild Cognitive Impairment as a Prodromal Syndrome of Alzheimer´s Disease. Curr Neuropharmacol. 2013;11(1):102-8.

15. Gauthier S, Albert M, Fox N, Goedert M, Kivipelto M, Mestre-Ferrandiz J, et al. Why has therapy development for dementia failed in the last two decades? Alzheimers Dement. 2016;12(1):60-4.

16. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535-62.

17. Knopman DS, Haeberlein SB, Carrillo MC, Hendrix JA, Kerchner G, Margolin R, et al. The National Institute on Aging and the Alzheimer's Association Research Framework for Alzheimer's disease: Perspectives from the Research Roundtable. Alzheimers Dement. 2018;14(4):563-75.

18. Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde Band, Verlag von Georg Reimer: Allgemeine Zeitschrift für Psychiatrie und psychisch-gerichtliche Medizin; 1907. p. 146-8.

19. Katzman R. Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. Arch Neurol. 1976;33(4):217-8.

20. Raskin J, Cummings J, Hardy J, Schuh K, Dean RA. Neurobiology of Alzheimer's Disease: Integrated Molecular, Physiological, Anatomical, Biomarker, and Cognitive Dimensions. Curr Alzheimer Res. 2015;12(8):712-22.

21. Yoshiyama Y, Lee VM, Trojanowski JQ. Therapeutic strategies for tau mediated neurodegeneration. J Neurol Neurosurg Psychiatry. 2013;84(7):784-95.

22. American Psychiatric Association. DSM-V: Manual Diagnóstico e Estatístico de Transtornos Mentais. 5 ed. Porto Alegre: Artmed; 2014. 992 p.

23. Petersen RC. How early can we diagnose Alzheimer disease (and is it sufficient)? The 2017 Wartenberg lecture. Neurology. 2018;91(9):395-402.

24. Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M. World Alzheimer Report 2015: an analysis of prevalence, incidence, cost and trends. London: Alzheimer's Disease International; 2015.

25. Burlá C, Camarano AA, Kanso S, Fernandes D, Nunes R. [A perspective overview of dementia in Brazil: a demographic approach]. Cien Saude Colet. 2013;18(10):2949-56.

26. Mesulam M-M. Principles of Cognitive and Behavioral Neurology. New York: Oxford University Press; 2002.

27. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795-804.

28. Chen ST, Siddarth P, Ercoli LM, Merrill DA, Torres-Gil F, Small GW. Modifiable risk factors for Alzheimer disease and subjective memory impairment across age groups. PLoS One. 2014;9(6):e98630.

29. Lim YY, Kalinowski P, Pietrzak RH, Laws SM, Burnham SC, Ames D, et al. Association of β-Amyloid and Apolipoprotein E ε4 With Memory Decline in Preclinical Alzheimer Disease. JAMA Neurol. 2018.

30. Mahley RW. Central Nervous System Lipoproteins: ApoE and Regulation of Cholesterol Metabolism. Arterioscler Thromb Vasc Biol. 2016;36(7):1305-15.

31. Huang YA, Zhou B, Wernig M, Südhof TC. ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and Aβ Secretion. Cell. 2017;168(3):427-41.e21.

32. Loy CT, Schofield PR, Turner AM, Kwok JB. Genetics of dementia. Lancet. 2014;383(9919):828-40.

33. Spinney L. Alzheimer's disease: The forgetting gene. Nature. 2014;510(7503):26-8.

34. Michaud TL, Su D, Siahpush M, Murman DL. The Risk of Incident Mild Cognitive Impairment and Progression to Dementia Considering Mild Cognitive Impairment Subtypes. Dement Geriatr Cogn Dis Extra. 2017;7(1):15-29.

35. Goldman JS, Hahn SE, Catania JW, LaRusse-Eckert S, Butson MB, Rumbaugh M, et al. Genetic counseling and testing for Alzheimer disease: joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet Med. 2011;13(6):597-605.

36. Van Cauwenberghe C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. Genet Med. 2016;18(5):421-30.

37. Wiseman FK, Al-Janabi T, Hardy J, Karmiloff-Smith A, Nizetic D, Tybulewicz VL, et al. A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome. Nat Rev Neurosci. 2015;16(9):564-74.

38. Hartley D, Blumenthal T, Carrillo M, DiPaolo G, Esralew L, Gardiner K, et al. Down syndrome and Alzheimer's disease: Common pathways, common goals. Alzheimers Dement. 2015;11(6):700-9.

39. Imtiaz B, Tolppanen AM, Kivipelto M, Soininen H. Future directions in Alzheimer's disease from risk factors to prevention. Biochem Pharmacol. 2014;88(4):661-70.

40. Vemuri P, Lesnick TG, Przybelski SA, Knopman DS, Roberts RO, Lowe VJ, et al. Effect of lifestyle activities on Alzheimer disease biomarkers and cognition. Ann Neurol. 2012;72(5):730-8.

41. Baumgart M, Snyder HM, Carrillo MC, Fazio S, Kim H, Johns H. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. Alzheimers Dement. 2015;11(6):718-26.

42. Stern Y. Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol. 2012;11(11):1006-12.

43. Rentz DM, Locascio JJ, Becker JA, Moran EK, Eng E, Buckner RL, et al. Cognition, reserve, and amyloid deposition in normal aging. Ann Neurol. 2010;67(3):353-64.

44. Carafa V, Rotili D, Forgione M, Cuomo F, Serretiello E, Hailu GS, et al. Sirtuin functions and modulation: from chemistry to the clinic. Clin Epigenetics. 2016;8:61.

45. Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: the royal road to cutting the therapeutic Gordian Knot. Alzheimers Dement. 2012;8(4):312-36.

46. Marques SC, Lemos R, Ferreiro E, Martins M, de Mendonça A, Santana I, et al. Epigenetic regulation of BACE1 in Alzheimer's disease patients and in transgenic mice. Neuroscience. 2012;220:256-66.

47. Li Y, Daniel M, Tollefsbol TO. Epigenetic regulation of caloric restriction in aging. BMC Med. 2011;9:98.

48. Godoy JA, Rios JA, Zolezzi JM, Braidy N, Inestrosa NC. Signaling pathway cross talk in Alzheimer's disease. Cell Commun Signal. 2014;12:23.

49. Tan CC, Yu JT, Wang HF, Tan MS, Meng XF, Wang C, et al. Efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis. 2014;41(2):615-31.

50. Gispert JD, Rami L, Sánchez-Benavides G, Falcon C, Tucholka A, Rojas S, et al. Nonlinear cerebral atrophy patterns across the Alzheimer's disease continuum: impact of APOE4 genotype. Neurobiol Aging. 2015;36(10):2687-701.

51. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-59.

52. Kretzschmar H. Brain banking: opportunities, challenges and meaning for the future. Nat Rev Neurosci. 2009;10(1):70-8.

53. Humpel C, Hochstrasser T. Cerebrospinal fluid and blood biomarkers in Alzheimer's disease. World J Psychiatry. 2011;1(1):8-18.

54. Bloom GS. Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. 2014;71(4):505-8.

55. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984;120(3):885-90.

56. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem. 1986;261(13):6084-9.

57. Brion JP, Flament-Durand J, Dustin P. Alzheimer's disease and tau proteins. Lancet. 1986;2(8515):1098.

58. Blennow K, Zetterberg H. Biomarkers for Alzheimer disease - current status and prospects for the future. J Intern Med. 2018.

59. Morley JE, Farr SA. The role of amyloid-beta in the regulation of memory. Biochem Pharmacol. 2014;88(4):479-85.

60. Blennow K, Mattsson N, Schöll M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. Trends Pharmacol Sci. 2015;36(5):297-309.

61. Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L. A physiological role for amyloid-beta protein:enhancement of learning and memory. J Alzheimers Dis. 2010;19(2):441-9.

62. Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 2010;362(4):329-44.

63. Rizzi L, Roriz-Cruz M. Sirtuin 1 and Alzheimer's disease: An up-to-date review. Neuropeptides. 2018;71:54-60.

64. Šimić G, Babić Leko M, Wray S, Harrington C, Delalle I, Jovanov-Milošević N, et al. Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. Biomolecules. 2016;6(1):6.

65. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297(5580):353-6.

66. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991;12(10):383-8.

67. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell. 2005;120(4):545-55.

68. De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. Subcell Biochem. 2012;65:329-52.

69. Rizzi L, Roriz-Cruz M. Cerebrospinal fluid inflammatory markers in amnestic mild cognitive impairment. Geriatr Gerontol Int. 2017;17(2):239-45.

70. Pimplikar SW. Neuroinflammation in Alzheimer's disease: from pathogenesis to a therapeutic target. J Clin Immunol. 2014;34 Suppl 1:S64-9.

71. Bjerke M, Engelborghs S. Cerebrospinal Fluid Biomarkers for Early and Differential Alzheimer's Disease Diagnosis. J Alzheimers Dis. 2018;62(3):1199-209.

72. Tang W, Huang Q, Wang Y, Wang ZY, Yao YY. Assessment of CSF Aβ42 as an aid to discriminating Alzheimer's disease from other dementias and mild cognitive impairment: A meta-analysis of 50 studies. J Neurol Sci. 2014;345(1-2):26-36.

73. Ferreira D, Rivero-Santana A, Perestelo-Pérez L, Westman E, Wahlund LO, Sarría A, et al. Improving CSF Biomarkers' Performance for Predicting Progression from Mild Cognitive Impairment to Alzheimer's Disease by Considering Different Confounding Factors: A Meta-Analysis. Front Aging Neurosci. 2014;6:287.

74. Ferreira D, Perestelo-Pérez L, Westman E, Wahlund LO, Sarría A, Serrano-Aguilar P. Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. Front Aging Neurosci. 2014;6:47.

75. Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. J Alzheimers Dis. 2013;33 Suppl 1:S123-39.

76. Wirth M, Madison CM, Rabinovici GD, Oh H, Landau SM, Jagust WJ. Alzheimer's disease neurodegenerative biomarkers are associated with decreased cognitive function but not β-amyloid in cognitively normal older individuals. J Neurosci. 2013;33(13):5553-63.

77. Irwin DJ, Trojanowski JQ, Grossman M. Cerebrospinal fluid biomarkers for differentiation of frontotemporal lobar degeneration from Alzheimer's disease. Front Aging Neurosci. 2013;5:6.

78. Skillbäck T, Rosén C, Asztely F, Mattsson N, Blennow K, Zetterberg H. Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease: results from the Swedish Mortality Registry. JAMA Neurol. 2014;71(4):476-83.

79. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673-84.

80. Schoonenboom NS, Reesink FE, Verwey NA, Kester MI, Teunissen CE, van de Ven PM, et al. Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. Neurology. 2012;78(1):47-54.

81. Tang W, Huang Q, Yao YY, Wang Y, Wu YL, Wang ZY. Does CSF p-tau181 help to discriminate Alzheimer's disease from other dementias and mild cognitive impairment? A meta-analysis of the literature. J Neural Transm (Vienna). 2014;121(12):1541-53.

82. Hampel H, Lista S, Teipel SJ, Garaci F, Nisticò R, Blennow K, et al. Perspective on future role of biological markers in clinical therapy trials of Alzheimer's disease: a long-range point of view beyond 2020. Biochem Pharmacol. 2014;88(4):426-49.

83. Giacobini E, Gold G. Alzheimer disease therapy--moving from amyloid-β to tau. Nat Rev Neurol. 2013;9(12):677-86.

84. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280-92.

85. Chabrier MA, Blurton-Jones M, Agazaryan AA, Nerhus JL, Martinez-Coria H, LaFerla FM. Soluble aβ promotes wild-type tau pathology in vivo. J Neurosci. 2012;32(48):17345-50.

86. Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol. 2013;12(4):357-67.

87. Fagan AM, Xiong C, Jasielec MS, Bateman RJ, Goate AM, Benzinger TL, et al. Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. Sci Transl Med. 2014;6(226):226ra30.

88. Wischik CM, Harrington CR, Storey JM. Tau-aggregation inhibitor therapy for Alzheimer's disease. Biochem Pharmacol. 2014;88(4):529-39.

89. Cummings J, Aisen PS, DuBois B, Frölich L, Jack CR, Jones RW, et al. Drug development in Alzheimer's disease: the path to 2025. Alzheimers Res Ther. 2016;8:39.

90. Cummings J, Lee G, Mortsdorf T, Ritter A, Zhong K. Alzheimer’s disease drug development pipeline: 2017. Alzheimer’s & Dementia: Translational Research & Clinical Interventions. 2017;3(3):367-84.

91. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. Lancet Neurol. 2010;9(7):702-16.

92. Matsunaga S, Kishi T, Iwata N. Memantine monotherapy for Alzheimer's disease: a systematic review and meta-analysis. PLoS One. 2015;10(4):e0123289.

93. Franco R, Cedazo-Minguez A. Successful therapies for Alzheimer's disease: why so many in animal models and none in humans? Front Pharmacol. 2014;5:146.

94. Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev. 2006(1):CD005593.

95. Schneider LS, Mangialasche F, Andreasen N, Feldman H, Giacobini E, Jones R, et al. Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. J Intern Med. 2014;275(3):251-83.

96. Lobello K, Ryan JM, Liu E, Rippon G, Black R. Targeting Beta amyloid: a clinical review of immunotherapeutic approaches in Alzheimer's disease. Int J Alzheimers Dis. 2012;2012:628070.

97. Blennow K. Biomarkers in Alzheimer's disease drug development. Nat Med. 2010;16(11):1218-22.

98. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34(7):939-44.

99. Frota NAF, Nitrini R, Damasceno BP, Forlenza O, Dias-Tosta E, Silva AB, et al. Critérios para o diagnóstico de doença de Alzheimer. Dement Neuropsychol. 2011;5(1):5-10.

100. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry. 2012;69(1):98-106.

101. Silverberg N, Elliott C, Ryan L, Masliah E, Hodes R. NIA commentary on the NIA-AA Research Framework: Towards a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):576-8.

102. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-9.

103. Dubois B. The Emergence of a New Conceptual Framework for Alzheimer's Disease. J Alzheimers Dis. 2018;62(3):1059-66.

104. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-9.

105. Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87(5):539-47.

106. Wirth M, Villeneuve S, Haase CM, Madison CM, Oh H, Landau SM, et al. Associations between Alzheimer disease biomarkers, neurodegeneration, and cognition in cognitively normal older people. JAMA Neurol. 2013;70(12):1512-9.

107. El Kadmiri N, Said N, Slassi I, El Moutawakil B, Nadifi S. Biomarkers for Alzheimer Disease: Classical and Novel Candidates' Review. Neuroscience. 2018;370:181-90.

108. Gordon BA, Friedrichsen K, Brier M, Blazey T, Su Y, Christensen J, et al. The relationship between cerebrospinal fluid markers of Alzheimer pathology and positron emission tomography tau imaging. Brain. 2016;139(Pt 8):2249-60.

109. Samtani MN, Raghavan N, Shi Y, Novak G, Farnum M, Lobanov V, et al. Disease progression model in subjects with mild cognitive impairment from the Alzheimer's disease neuroimaging initiative: CSF biomarkers predict population subtypes. Br J Clin Pharmacol. 2013;75(1):146-61.

110. Markesbery WR. Neuropathologic alterations in mild cognitive impairment: a review. J Alzheimers Dis. 2010;19(1):221-8.

111. Caminiti SP, Ballarini T, Sala A, Cerami C, Presotto L, Santangelo R, et al. FDG-PET and CSF biomarker accuracy in prediction of conversion to different dementias in a large multicentre MCI cohort. Neuroimage Clin. 2018;18:167-77.

112. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 1999;56(3):303-8.

113. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.

114. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256(3):240-6.

115. De Simone MS, Perri R, Fadda L, Caltagirone C, Carlesimo GA. Predicting progression to Alzheimer's disease in subjects with amnestic mild cognitive impairment using performance on recall and recognition tests. J Neurol. 2018.

116. Russo MJ, Campos J, Vázquez S, Sevlever G, Allegri RF, Initiative AsDN. Adding Recognition Discriminability Index to the Delayed Recall Is Useful to Predict Conversion from Mild Cognitive Impairment to Alzheimer's Disease in the Alzheimer's Disease Neuroimaging Initiative. Front Aging Neurosci. 2017;9:46.

117. Jungwirth S, Zehetmayer S, Hinterberger M, Tragl KH, Fischer P. The validity of amnestic MCI and non-amnestic MCI at age 75 in the prediction of Alzheimer's dementia and vascular dementia. Int Psychogeriatr. 2012;24(6):959-66.

118. Gainotti G, Quaranta D, Vita MG, Marra C. Neuropsychological predictors of conversion from mild cognitive impairment to Alzheimer's disease. J Alzheimers Dis. 2014;38(3):481-95.

119. Brandt J. Mild cognitive impairment in the elderly. Am Fam Physician. 2001;63(4):620, 2, 5-6.

120. Parnetti L, Chiasserini D, Eusebi P, Giannandrea D, Bellomo G, De Carlo C, et al. Performance of aβ1-40, aβ1-42, total tau, and phosphorylated tau as predictors of dementia in a cohort of patients with mild cognitive impairment. J Alzheimers Dis. 2012;29(1):229-38.

121. Frölich L, Peters O, Lewczuk P, Gruber O, Teipel SJ, Gertz HJ, et al. Incremental value of biomarker combinations to predict progression of mild cognitive impairment to Alzheimer's dementia. Alzheimers Res Ther. 2017;9(1):84.

122. Prestia A, Caroli A, Wade SK, van der Flier WM, Ossenkoppele R, Van Berckel B, et al. Prediction of AD dementia by biomarkers following the NIA-AA and IWG diagnostic criteria in MCI patients from three European memory clinics. Alzheimers Dement. 2015;11(10):1191-201.

123. Shin S, Kim JH, Cho JH, Kim GS, Choi SA, Lee JH, et al. Mild cognitive impairment due to alzheimer disease is less likely under the age of 65. Alzheimer Dis Assoc Disord. 2015;29(1):26-31.

124. Mounsey AL, Zeitler MR. Cerebrospinal Fluid Biomarkers for Detection of Alzheimer Disease in Patients with Mild Cognitive Impairment. Am Fam Physician. 2018;97(11):714-5.

CAPÍTULO IV

1. ARTIGO CIENTÍFICO ORIGINAL I

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Title of the paper: CSF $A\beta_{1-42}$, but not p-Tau_{181}, differentiates aMCI from SCI.

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Abstract and Keywords

Aim: Individuals with amnestic mild cognitive impairment (aMCI) are at a high risk to develop Alzheimer's disease (AD). We compared CSF levels of biomarkers of amyloidosis ($A\beta_{1-42}$) and neurodegeneration ($p\text{-}Tau_{181}$) in individuals with aMCI and with subjective cognitive impairment (SCI) in order to ascertain diagnostic accuracy and predict the odds ratio associated with aMCI.

Methods: We collected CSF of individuals clinically diagnosed with aMCI (33) and SCI (12) of a memory clinic of Southern Brazil. Levels of $A\beta_{1-42}$ and $p\text{-}Tau_{181}$ were measured by immunoenzymatic assay. Participants also underwent neuropsychological testing including the verbal memory test subscore of the Consortium to Establish a Registry for Alzheimer's Disease (VM-CERAD).

Results: CSF concentration of $A\beta_{1-42}$ was significantly lower (p: 0.007) and $p\text{-}Tau_{181}/A\beta_{1-42}$ ratio higher (p: 0.014) in aMCI individuals than in SCI. However, isolate $p\text{-}Tau_{181}$ levels were not associated with aMCI (p: 0.166). There was a statistically significant association between $A\beta_{1-42}$ and $p\text{-}Tau_{181}$ (R^2: 0.177; β: -4.43; p: 0.017). ROC AUC of CSF $A\beta_{1-42}$ was 0.768 and of the $p\text{-}Tau_{181}/A\beta_{1-42}$ ratio equals 0.742. Individuals with $A\beta_{1-42}$ <823 pg/mL levels were 6.0 times more likely to be diagnosed with aMCI (p: 0.019), with a 68.9% accuracy. Those with $p\text{-}Tau_{181}/A\beta_{1-42}$ ratio >0.071 were at 4.6 increased odds to have aMCI (p: 0.043), with a 64.5% accuracy. VM-CERAD was significantly lower in aMCI than among SCI (p: 0.041).

Conclusion: CSF $A\beta_{1-42,}$ but not $p\text{-}Tau_{181,}$ level was significantly associated with aMCI.

Keywords: Alzheimer; Amyloid; CSF Biomarkers; MCI; Tau.

1. Introduction

Mild cognitive impairment (MCI) is a disorder situated in the continuum between normal cognition and dementia. According to National Institute on Aging and the Alzheimer's Association (NIA-AA), the clinical characterization of MCI requires the presence all items below: (1) self- or informant -reported cognitive complain, (2) objective cognitive impairment, (3) preserved independence in functional abilities, and (4) no dementia. Individuals with MCI ascertained according to these core clinical criteria could be further sub classified into two categories: amnestic MCI (aMCI) if performance on neuropsychological tests of episodic memory is poor, and non-amnestic MCI (naMCI) in the case of poor performance on tests covering cognitive domains other than memory(Petersen et al., 2014).

Only a proportion of individuals with MCI progress to AD (Alzheimer's disease). According to criteria established by NIA-AA, the use of biomarkers may aid in identifying etiological MCI subtypes by differentiating between MCI due to AD (i.e.: prodromal AD) and MCI that is unlikely to be due to AD(Albert et al., 2011; Dubois et al., 2014).

Many biomarkers have been studied to support the clinical diagnosis of AD. Nonetheless, few have successfully defined AD signature. The most consistent ones have been related to compounds of neuritic plaques such as the β-amyloid protein ($A\beta_{1-42}$) and the main constituent of the neurofibrillary tangles, i.e., the hyperphosphorylated Tau protein (p-Tau_{181})(Blennow et al., 2014). They are reported to be altered at least 5-10 years before dementia diagnosis(Jack et al., 2013). However, diagnostic accuracies of CSF biomarkers in MCI are still to be defined.

Although aMCI is a well-defined entity, it may be more difficult for non-specialists to proper diagnose this condition in their practice. In this sense, an exam that helps to discriminate normal aging from aMCI would be welcomed(Brandt, 2001). Moreover, accurate identification

of prodromal AD would become crucial when a disease-modyfing drug becomes a reality(Cavedo et al., 2014).

Based on core criteria above we measured and compared the concentration of ($A\beta_{1-42}$ and p-Tau_{181}), in individuals clinically diagnosed with aMCI and with subjective cognitive impairment (SCI) in a manner to ascertain the diagnostic accuracy and verify the odds ratio to have aMCI.

2. Results

Table 1 compares the demographics characteristics and neuropsychological scores between aMCI and SCI groups. Mean age was 67.9±5.4 years and the majority was women (71.1%). There was a significant difference in median verbal memory test subscore of the Consortium to Establish a Registry for Alzheimer's Disease (VM-CERAD)(Bertolucci et al., 1998)between aMCI and SCI groups (p: 0.041). No differences were found when we analyzed scores of Geriatric Depression Scale (GDS) (Almeida and Almeida, 1999) (p: 0.724) and Clock-Drawing Test (CDT)(Shulman et al., 1993) (p: 0.825) between aMCI and SCI. Our sample was composed mostly of a Caucasian population, as which reflects the demographics of the Southern Brazilian population. Only one individual in each group was African-Brazilian.

Table 2 describes CSF biomarkers concentration between aMCI and SCI. CSF concentration of $A\beta_{1-42}$ protein (p: 0.007), but not p-Tau protein (p: 0.166), was significantly different between the two groups. The p-Tau_{181}/$A\beta_{1-42}$ ratio displayed significant difference between groups (p: 0.014). A linear regression analysis demonstrated significant association between $A\beta_{1-42}$ and p-Tau_{181} protein (R^2: 0.100; β: 95.19; p: 0.034), even when adjusted for age (R^2: 0.177; β: -4.43; p: 0.017). When the two outliers showed in the Figure 1 were removed, the analysis remains significant (R^2: 0.171; β: 1.77; p: 0.023, adjusted for age).

$A\beta_{1-42}$ ROC AUC was 0.768 (CI 95%: 0.618 – 0.918; p: 0.007), as shown at Table 3. Cutoff value determined by the Youden index, that stretches the maximum potential effectiveness of a biomarker, was 823 pg/mL, with sensibility of 66.7% (CI 95%: 49.6- 80.2), specificity of 75% (CI95%: 46.7 - 91.1) and predictive positive value of 88% (CI95%: 73.67 - 95.05%). The logistic linear regression analysis revealed that individuals with $A\beta_{1-42}$ <823 pg/mL were 6.0 increased odds (p: 0.019) to have aMCI. When adjusted for age, the odds ratio almost did not change (6.2; p: 0.022). At this cutoff level, 31 of 45 individuals (68.9%) were correctly classified. ROC AUC of the ratio p-Tau_{181}/$A\beta_{1-42}$ was 0.742 (CI 95%: 0.589 – 0.896; p: 0.014). The optimal cutoff value was 0.071, with sensibility of 60.6% (CI 95%: 43.6 - 75.3), specificity of 75% (CI95%: 46.7 - 91.1) and predictive positive value of 87% (CI95%: 71.60 - 94.63%). Individuals with the ratio p-Tau_{181}/$A\beta_{1-42}$ >0.071 were at 4.6 increased odds (p: 0.043) to have aMCI. At this cutoff 29 of 45 individuals (64.5%) were correctly classified, but all of them were also adequately classified according to $A\beta_{1-42}$ levels alone.

3. Discussion

As expected, the concentration of $A\beta_{1-42}$ protein was significantly diminished in aMCI subjects than among SCI. However, p-Tau_{181} protein was not different between groups. These results are in accord with the recently proposed biomarker behavior hypothetical model of AD(Jack et al., 2010). According to this model, CSF $A\beta_{1-42}$ levels declines before the p-Tau_{181} increases. It is possibly that CSF p-Tau_{181} levels only start to increases expressively as neurodegeneration advances. Considering this model, we can infer that at the moment the markers were measured at our sample, CSF p-Tau_{181} concentration in aMCI may not have raised expressively yet. In fact, p-Tau_{181} only increase in the CSF later than $A\beta_{1-42}$ starts to decrease(Buchhave et al., 2012).

Although we found no significant difference in levels of p-Tau$_{181}$, we verified a linear association of this protein with A$\beta_{1-42,}$ suggesting that they could be influenced by common mechanisms and/or influence each other. p-Tau$_{181}$/Aβ_{1-42} ratio displayed significant difference between groups at our study. This coefficient is frequently utilized because it presumes a link between β-amyloid and p-Tau pathways(Shaw et al., 2009). Indeed, p-Tau$_{181}$/Aβ_{1-42} ratio was already reported (1) to predict cognitive decline in cognitively normal elderly(Fagan et al., 2007), (2) to differentiate patients with AD from individuals without objective cognitive impairment(Ferreira et al., 2014), and (3) to predict MCI progression to AD(Ferreira et al., 2014).

Patients with AD dementia tends to have lower levels of CSF Aβ_{1-42} and higher levels of CSF p-Tau$_{181}$ than individuals without cognitive impairment(Blennow et al., 2010). It has been reported Aβ_{1-42} levels are reduced in about 50% among individuals with AD relatively to age-matched people without cognitive impairment (Holtzman, 2011). Although there are no established cutoff values for these CSF biomarkers general acceptable, some studies suggests cutoffs for CSF Aβ_{1-42} positivity in AD, which below 650 pg/mL when utilizing immunoenzymatic assays(Blennow et al., 2015). p-Tau$_{181}$ thresholds usually vary above 60 and 80 pg/mL at the demential phase of the disease(Tang et al., 2014b).

Cutoff points for Aβ_{1-42} and p-Tau$_{181}$ are still scarce and even unclear for aMCI individuals. A recent meta-analysis described that the levels of Aβ_{1-42} in aMCI individuals ranged from 172.6±53.5 to 622.9±275.6 pg/mL, whereas among healthy cognitively people they vary between 383.5±101.8 and 1020±230 pg/mL. However, none of these studies have direct compared aMCI to SCI. This meta-analysis suggests that there is no established threshold which can distinguish AD or aMCI from healthy cognitively individuals(Mo et al., 2015). Although this study does not differentiate aMCI from SCI, some older studies compared CSF Aβ_{1-42} levels in general MCI individuals and healthy cognitively people(Herukka et al., 2005;

Maruyama et al., 2001). One reported that CSF $A\beta_{1-42}$ levels did not differ significantly between cognitively normal subjects and MCI groups(Maruyama et al., 2001), and other showed that values of CSF $A\beta_{1-42}$ were significantly lower in progressive MCI group than in progressive and stable MCI groups(Herukka et al., 2005).

The broad variation at the measurements of these biomarkers between studies and laboratories is only one of the difficulties in determining homogenous cutoff points(Tang et al., 2014a). It might results from differences in CSF sample handling techniques, analytical procedures and kits. Cases selection criteria and resultant difference in sample characteristics are other problems. Standardization and quality control of these procedures may reduce the disparity and increase the utility of these CSF biomarkers(Samtani et al., 2013). To find the best cutoff values we used the Youden index. This is a standardized and objective method to determine diagnostic cutoff points. Youden index evaluates the maximum potential effectiveness of a biomarker by choosing the best values in terms of test accuracy, taking in consideration both sensibility and specificity (Ruopp et al., 2008; YOUDEN, 1950). Such cutoff points, however, are dependent of several variables, such as study population and phase of disease. In this sense, it would be important that other cross-sectional studies on aMCI be conducted in different regions of the globe in order to find their best own distinctive cutoff point to differentiate aMCI from SCI.

At our study, individuals with $A\beta_{1-42}$ <823 pg/mL were 6.0 times more likely to be diagnosed with aMCI, with reasonable accuracy. At the ratio p-$Tau_{181}/A\beta_{1-42,}$ individuals with >0.071 were at 4.6 increased odds to have aMCI. Thus, among the CSF biomarkers studied here, CSF $A\beta_{1-42}$ concentration was the best analyte for detection of aMCI subjects (ROC AUC: 0.768). Thereby, indicating that CSF $A\beta_{1-42}$ might be the most single informative biomarker for prodromal AD. To our knowledge, no previous study has measured CSF biomarkers that discriminates exclusively aMCI individuals from SCI and identified cut off values and odds

ratio to have aMCI, independently from previous neuropsychological and clinical evaluation. Our finding is important in order to correctly identify people who are at an imminent risk to progress to Alzheimer's disease dementia. We also aim to help non-specialists to properly diagnose aMCI and differentiate it from normal aging in clinical practice.

As expected, performance on VM-CERAD was significantly lower at aMCI group than at SCI. This finding probably means that aMCI cases and SCI individuals were well allocated. Conversely, GDS and CDT were not different between aMCI and SCI. Depression may be a cause of cognitive deficits or simulate aMCI. Our result probably means that depressed individuals were adequately excluded from our sample. In turn, CDT evaluates more executive function and visuospacial organization than memory itself. Vascular mild cognitive impairment is characterized by more impairment in executive functions and gait apraxia than is AD(Román and Royall, 1999). Therefore, it was also expected that there will be no differences in CDT scores between our pure aMCI individuals and SCI. In fact, this finding also suggests that we have appropriately excluded MCI cases other than the purely amnestic ones.

Limitations of our study include the relatively small sample size and the fact that it is a case-control study, therefore general causality cannot be inferred. Strengths include simultaneous analysis of CSF biomarkers and neuropsychological evaluation in a group at high risk for developing AD. Moreover, biomarkers measures and cognitive and affective tests were conducted by blind persons, using validated methods.

3.1 Conclusion

To our knowledge, this is the first study that evaluated CSF $A\beta_{1\text{-}42}$ to discriminate exclusively aMCI individuals from SCI and identified cut off values and odds ratio of to have aMCI. Moreover, we found no value for CSF p-Tau_{181} *vis-à-vis* CSF $A\beta_{1\text{-}42}$ alone in predicting aMCI diagnosis.

4. Methods and Materials

4.1 Study participants and clinical classification

We included thirty three subjects with aMCI, and twelve individuals with SCI, without evidence of objective impairment, in the study. All were sixty years or older. They were referred to a memory clinic of Southern Brazil called “Hospital de Clínicas de Porto Alegre”. The sample size was based in a conservative estimation of AD’s pathology among aMCI (70%) and SCI (10%). Considering a power of 90%, significance of 5% and 1/3 of losses(Markesbery, 2010).

Participants were submitted to an evaluation that was performed blindly by a behavioral neurologist and a neuropsychologist, and included a structured clinical interview, a full neurological examination, and neuropsychological assessment. NIA-AA criteria for aMCI was used(Albert et al., 2011; Petersen et al., 2014) and corroborated by Clinical Dementia Rating (CDR)(Morris, 1993). Cases fulfilled the criteria for aMCI and scored 0.5 on CDR, and SCI were individuals who had no evidence of objective cognitive decline by NIA-AA criteria and scored CDR: 0.

We excluded subjects who had evidence of dementia, stroke, cerebrovascular disease, Parkinson’s disease, depression or other neurological disease that potentially cause cognitive impairment. A questionnaire was applied in order to assess the following socio demographic data: age, sex, occupation and years of schooling.

4.2 Neuropsychological assessments

We utilized the VM-CERAD, Brazilian validated adaptation(Bertolucci et al., 1998). In order to assess verbal episodic memory function this evaluation was performed by a certified and blind neuropsychologist. The final score was composed by a possible sum of 30 points for the word list learning, 10 points for word list recall and 10 points for word list recognition (total of 50 possible points)(Chandler et al., 2005). The 0-5 points version of CDT was also utilized(Shulman et al., 1993). The GDS 15-item version, translated and validated to Brazilian portuguese was applied (Almeida and Almeida, 1999). Presence of significant depressive symptomatology was considered for all subjects who scored ≥6 points in the scale.

4.3 CSF analysis

All participants underwent a fasted lumbar puncture to obtain CSF samples. Briefly, CSF (5ml) was collected in polypropylene tubes and immediately centrifuged (4,000 g X 10 minutes) at 4°C and then stored at -80°C until assay. We used commercially available ELISA kits to determine levels of $A\beta_{1-42}$ (Innotest β-amyloid1-42, Fujirebio-Europe, Gent, Belgium) and p-Tau_{181} (Innotest Phospho-Tau181P, Fujirebio-Europe) following the manufacturers' recommendations. CSF tests were performed blindly after clinical evaluation, in duplicate, and the laboratory technician was also blinded to any clinical and demographic information about the study participants.

4.4 Statistical analysis

Statistical analyses were performed using SPSS (the Statistical Package for Social Sciences) version 20. To compare gender distribution between the groups, a Chi-square test was performed. Subsequently, Mann–Whitney U tests were performed to compare clinical, neuropsychological and biomarker data between the groups. A linear regression analysis was used to verify the relationship between proteins. Receiver operating characteristic (ROC) curve analyses were used to obtain area under the curve (AUC), that express the probability of a

positive case test randomly chosen will exceed the outcome of a randomly chosen negative case. The optimal cutoff values were determined by calculating the maximal sum of sensitivity and specificity (i.e., maximizing the Youden index (YOUDEN, 1950). The accuracy of the tests was determined by the proportion of true results (both true positives and true negatives) among the total number of cases examined. Logistic linear regression was performed to verify the odds ratio. All results were showed as CI95% and p value <0.05 was considered significant. Values were adjusted for age.

4.5 Ethical considerations

All participants gave their written consent, and the study was approved by the local ethics committee. This study was conducted in accord with the Declaration of Helsinki.

5. Acknowledgements

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6. Disclosure statement

All authors declare no actual or potential conflict of interests including any financial, personal or other relationships with other people or organizations.

7. References

Albert, M.S., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., 2011. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 7**,** 270-9.

Almeida, O., Almeida, S., 1999. Confiabilidade da versão brasileira da Escala de Depressão em Geriatria (GDS) versão reduzida. Arq Neuro-Psiquiatr. 57**,** 421-6.

Bertolucci, P.H.F., Okamoto, I.H., Toniolo Neto, J., Ramos, L.R., Brucki, S.M.D., 1998. Desempenho da populacao brasileira na bateria neuropsicologica do Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Vol. 25, ed.^eds. *Revista de Psiquiatria Clínica*, São Paulo, pp. 80-83.

Blennow, K., Hampel, H., Weiner, M., Zetterberg, H., 2010. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol. 6**,** 131-44.

Blennow, K., Dubois, B., Fagan, A.M., Lewczuk, P., de Leon, M.J., Hampel, H., 2014. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement.

Blennow, K., Mattsson, N., Schöll, M., Hansson, O., Zetterberg, H., 2015. Amyloid biomarkers in Alzheimer's disease. Trends Pharmacol Sci. 36**,** 297-309.

Brandt, J., 2001. Mild cognitive impairment in the elderly. Am Fam Physician. 63**,** 620, 622, 625-6.

Buchhave, P., Minthon, L., Zetterberg, H., Wallin, A.K., Blennow, K., Hansson, O., 2012. Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry. 69**,** 98-106.

Cavedo, E., Lista, S., Khachaturian, Z., Aisen, P., Amouyel, P., Herholz, K., Jack, C.R., Sperling, R., Cummings, J., Blennow, K., O'Bryant, S., Frisoni, G.B., Khachaturian, A., Kivipelto, M., Klunk, W., Broich, K., Andrieu, S., de Schotten, M.T., Mangin, J.F., Lammertsma, A.A., Johnson, K., Teipel, S., Drzezga, A., Bokde, A., Colliot, O., Bakardjian, H., Zetterberg, H., Dubois, B., Vellas, B., Schneider, L.S., Hampel, H., 2014. The Road Ahead to Cure Alzheimer's Disease: Development of Biological Markers and Neuroimaging Methods for Prevention Trials Across all Stages and Target Populations. J Prev Alzheimers Dis. 1**,** 181-202.

Chandler, M.J., Lacritz, L.H., Hynan, L.S., Barnard, H.D., Allen, G., Deschner, M., Weiner, M.F., Cullum, C.M., 2005. A total score for the CERAD neuropsychological battery. Neurology. 65**,** 102-6.

Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., DeKosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y.,

Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 13**,** 614-29.

Fagan, A.M., Roe, C.M., Xiong, C., Mintun, M.A., Morris, J.C., Holtzman, D.M., 2007. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol. 64**,** 343-9.

Ferreira, D., Perestelo-Pérez, L., Westman, E., Wahlund, L.O., Sarría, A., Serrano-Aguilar, P., 2014. Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. Front Aging Neurosci. 6**,** 47.

Herukka, S.K., Hallikainen, M., Soininen, H., Pirttilä, T., 2005. CSF Abeta42 and tau or phosphorylated tau and prediction of progressive mild cognitive impairment. Neurology. 64**,** 1294-7.

Holtzman, D.M., 2011. CSF biomarkers for Alzheimer's disease: current utility and potential future use. Neurobiol Aging. 32 Suppl 1**,** S4-9.

Jack, C.R., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C., Trojanowski, J.Q., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 9**,** 119-28.

Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C., Trojanowski, J.Q., 2013. Tracking pathophysiological processes in

Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 12**,** 207-16.

Markesbery, W.R., 2010. Neuropathologic alterations in mild cognitive impairment: a review. J Alzheimers Dis. 19**,** 221-8.

Maruyama, M., Arai, H., Sugita, M., Tanji, H., Higuchi, M., Okamura, N., Matsui, T., Higuchi, S., Matsushita, S., Yoshida, H., Sasaki, H., 2001. Cerebrospinal fluid amyloid beta(1-42) levels in the mild cognitive impairment stage of Alzheimer's disease. Exp Neurol. 172**,** 433-6.

Mo, J.A., Lim, J.H., Sul, A.R., Lee, M., Youn, Y.C., Kim, H.J., 2015. Cerebrospinal fluid β-amyloid1-42 levels in the differential diagnosis of Alzheimer's disease--systematic review and meta-analysis. PLoS One. 10**,** e0116802.

Morris, J.C., 1993. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology. 43**,** 2412-4.

Petersen, R.C., Caracciolo, B., Brayne, C., Gauthier, S., Jelic, V., Fratiglioni, L., 2014. Mild cognitive impairment: a concept in evolution. J Intern Med. 275**,** 214-28.

Román, G.C., Royall, D.R., 1999. Executive control function: a rational basis for the diagnosis of vascular dementia. Alzheimer Dis Assoc Disord. 13 Suppl 3**,** S69-80.

Ruopp, M.D., Perkins, N.J., Whitcomb, B.W., Schisterman, E.F., 2008. Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. Biom J. 50**,** 419-30.

Samtani, M.N., Raghavan, N., Shi, Y., Novak, G., Farnum, M., Lobanov, V., Schultz, T., Yang, E., DiBernardo, A., Narayan, V.A., Initiative, A.s.D.N., 2013. Disease progression model in subjects with mild cognitive impairment from the Alzheimer's disease neuroimaging initiative: CSF biomarkers predict population subtypes. Br J Clin Pharmacol. 75**,** 146-61.

Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Siemers, E., Potter, W., Lee, V.M., Trojanowski, J.Q., Initiative, A.s.D.N., 2009. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 65**,** 403-13.

Shulman, K.I., Gold, D.P., Cohen, C.A., Zucchero, C.A., 1993. Clock-drawing and dementia in the community

A longitudinal study, International Journal of Geriatric Psychiatry Volume 8, Issue 6. In International Journal of Geriatric Psychiatry. Vol. 8, ed.^eds., pp. 487-496.

Tang, W., Huang, Q., Wang, Y., Wang, Z.Y., Yao, Y.Y., 2014a. Assessment of CSF Aβ42 as an aid to discriminating Alzheimer's disease from other dementias and mild cognitive impairment: A meta-analysis of 50 studies. J Neurol Sci. 345**,** 26-36.

Tang, W., Huang, Q., Yao, Y.Y., Wang, Y., Wu, Y.L., Wang, Z.Y., 2014b. Does CSF p-tau181 help to discriminate Alzheimer's disease from other dementias and mild cognitive impairment? A meta-analysis of the literature. J Neural Transm (Vienna). 121**,** 1541-53.

YOUDEN, W.J., 1950. Index for rating diagnostic tests. Cancer. 3**,** 32-5.

Table 1

Demographics characteristics and neuropsychological scores.

	aMCI	**SCI**	**ρ value**
n	33	12	
Age (years): median (min-max)	68 (61-78)	63.5 (60-76)	.053
Education (years): median (min-max)	11 (1-18)	11 (5-18)	.211
Gender, (male/female): n	10/23	3/9	.120
GDS: median (min-max)	2 (0-5)	1 (0-5)	.724
VM-CERAD: median (min-max)	27 (18-37)	31 (23-40)	.041
CDT: median (min-max)	4 (1-5)	4 (1-5)	.825

†aMCI: amnestic Mild Cognitive Impairment; CDT: Clock Drawing Test; GDS: Geriatric Depression Scale; SCI: Subjective Cognitive Impairment; VM-CERAD: Verbal Memory test subscore of the Consortium to Establish a Registry for Alzheimer’s Disease. ‡ Mann-Whitney test was used, except for sex (Chi-Square Test).

Table 2

CSF biomarkers features.

	aMCI	**SCI**	**ρ value**
n	33	12	
Aβ $_{1-42}$ (pg/mL): median (P25 – P75)	677.82 (493.72 - 913.35)	985.99 (757.67 - 1096.57)	0.007
p-Tau$_{181}$ (pg/mL): median (P25 – P75)	64.94 (50.32 - 85.08)	55.26 (48.54 - 69.87)	0.166
p-Tau$_{181}$ / Aβ $_{1-42}$ ratio: median(P25 – P75)	0.075 (0.059 - 0.167)	0.059 (0.051 - 0.074)	0.014

†aMCI: amnestic Mild Cognitive Impairment; SCI: Subjective Cognitive Impairment. P25: percentile 25; P75: percentile 75. ‡ Mann-Whitney test was used.

Table 3

ROC Curve Parameters.

Parameters	**Aβ$_{1-42}$**	**p-Tau$_{181}$/Aβ$_{1-42}$**
ROC AUC (CI95%)	0.768 (0.618 – 0.918)	0.742 (0.589 – 0.896)
Threshold value (pg/mL)	823	0.071
Sensitivity (%) (CI95%)	66.7 (49.6 - 80.2)	60.6 (43.6 - 75.3)
Specificity (%) (CI95%)	75.0 (46.7 - 91.1)	75.0 (46.7 - 91.1)
Test accuracy (%)	68.9	64.5

†ROC: receiver operating characteristic; AUC: area under the curve ‡Youden index was used. §Both specificities were exactly the same.

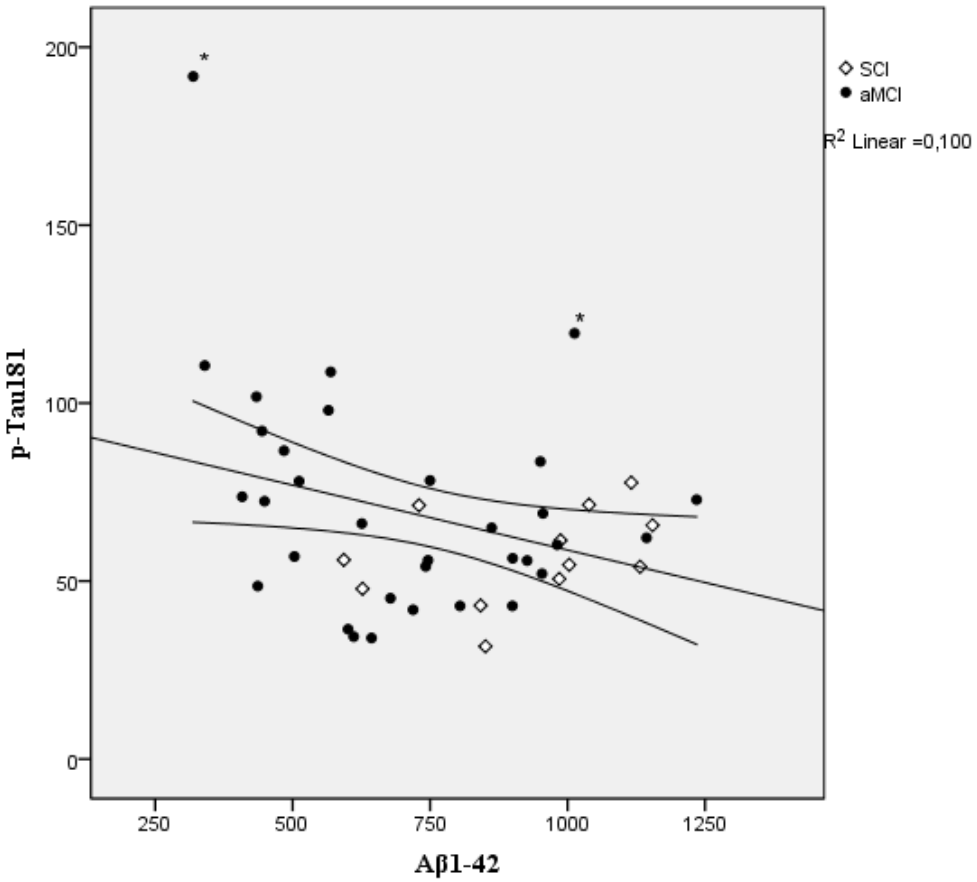

Figure 1. Linear regression analysis between $A\beta_{1\text{-}42}$ and p-Tau_{181} (pg/mL) (β: 95.19; p: 0.034), showing a linear dependence. Distribution at SCI and MCI cases. When adjusted for age, R^2: 0.177; β: -4.43; p: 0.017. Even when the two outliers (*) showed at the figure were removed, the linear regression remains significant (R^2: 0.171; β: 1.77; p: 0.023, adjusted for age). †aMCI: amnestic Mild Cognitive Impairment; SCI: Subjective Cognitive Impairment.

2. ARTIGO CIENTÍFICO ORIGINAL II

Estudo longitudinal de três anos de seguimento da coorte com CCL-a disponível online na revista NeuroMolecular Medicine em 10.10.2018 (https://doi.org/10.1007/s12017-018-8516-8).

Title: CSF $A\beta_{1-42}$, but not p-Tau_{181}, predicted progression from amnestic MCI to Alzheimer's disease dementia

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ABSTRACT

Objective: To determine whether $A\beta_{1-42}$ and p-Tau_{181} cerebral spinal fluid (CSF) levels can predict progression from amnestic Mild Cognitive Impairment (aMCI) to Alzheimer's disease dementia (ADD) in a three-year follow-up study.

Methods: All participants were evaluated blindly by a behavioral neurologist and a neuropsychologist, and classified according to the Petersen criteria for aMCI and according to the Clinical Dementia Rating (CDR) scale. Individuals were also submitted to lumbar puncture at baseline. Levels of $A\beta_{1-42}$ and p-Tau_{181} were measured by immunoenzymatic assay. Values were adjusted for age and sex.

Results: Thirty-one of 33 (93.9%) participants completed follow-up. Approximately 39% of aMCI individuals progressed to ADD. The relative risk of developing ADD in those with $A\beta_{1-42}$ CSF levels lower than 618.5 pg/mL was 17.4 times higher than in those whose levels were higher than 618.5 pg/mL ($P = 0.003$). p-Tau_{181} alone did not predict progression to ADD ($P = 0.101$). The relative risk in those with a p-Tau_{181}/$A\beta_{1-42}$ ratio higher than 0.135 was 5.7 times greater ($P < 0.001$). $A\beta_{1-42}$ and p-Tau_{181} explained 40.1% of the verbal memory test subscore of the Consortium to Establish a Registry for Alzheimer's Disease (ΔCERADs) variance ($P = 0.008$).

Conclusion: $A\beta_{1-42}$ strongly predicted progression from aMCI to ADD. p-Tau_{181} alone, or its relation to $A\beta_{1-42}$, was inferior than $A\beta_{1-42}$ alone as a predictor of progression to ADD.

Key words: Alzheimer's disease; amyloid protein; CSF biomarkers; MCI; Tau.

INTRODUCTION

The correct identification of people at risk to develop Alzheimer's disease dementia (ADD) is crucial in terms of early diagnosis (prodromal Alzheimer), prognosis and treatment. Research efforts to improve the positive predictive value of progression from amnestic mild cognitive impairment (aMCI) to ADD are needed, since many trials on disease modifying drugs fail because of low progression rates. In this sense, efforts to correctly identify individuals with prodromal AD are of utmost importance(McGeer and McGeer, 2013; Molinuevo et al., 2014). Even so, it is often difficult to identify who among those with aMCI will progress to ADD based solely on neuropsychological evaluations(Petersen et al., 2014).

Different biomarkers demonstrated to be useful diagnostic tools *in vivo*, and are now gaining attention in the clinical practice after being incorporated into research criteria for AD(Albert et al., 2011; Dubois et al., 2014; Morris et al., 2014). The combination of decreased concentrations of beta amyloid protein ($A\beta_{1-42}$) and increased concentrations of hyperphosphorylated Tau protein ($p\text{-}Tau_{181}$) in the CSF is related to AD pathology and may predict the progression to ADD(Jack et al., 2013; Jack et al., 2010).

Many studies have evaluated the value of CSF biomarkers in predict progression from MCI to ADD(Ferreira et al., 2014b; Prestia et al., 2015; Ritchie et al., 2014; Tondelli et al., 2015; Vos et al., 2013). However, no study has evaluated the relative risk to develop ADD in an exclusively amnestic MCI patient sample in a three-year follow-up. This study consisted of measuring the concentrations of both CSF $A\beta_{1-42}$ and $p\text{-}Tau_{181}$ proteins in a sample of exclusive amnestic MCI subjects and analyzing to which extent these biomarkers could predict progression to ADD in a three-year follow-up. The relationship and agreement of these biomarkers with neuropsychological tests was then verified.

MATERIAL AND METHODS

Participants

Thirty-three subjects diagnosed with aMCI from a memory clinic in Southern Brazil were included in the study. All individuals were evaluated by a behavioral/geriatric neurologist and the National Institute on Aging – Alzheimer's Association (NIA-AA) criteria for MCI(Albert et al., 2011), corroborated by the Clinical Dementia Rating scale (CDR)(Morris, 1993). aMCI diagnoses were based on criteria by Petersen and colleagues(Petersen, 2004; Petersen et al., 2014). We excluded those who had diagnosis of dementia, stroke, Parkinson's disease, depression or other neurological conditions that could possibly cause cognitive impairment. Furthermore, we also excluded individuals with normal cognition. A questionnaire was applied in order to obtain sociodemographic data.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the local research ethics committee and is in accordance with the Declaration of Helsinki. All participants gave their written consent in order to participate in this study.

CSF analyses

All participants underwent a fasted lumbar puncture to measure $A\beta_{1-42}$ and p-Tau_{181} proteins in the CSF. CSF (5ml) was collected in polypropylene tubes and immediately centrifuged (4,000 *g* x 10 minutes) at 4°C and then stored at -80°C until the assay. Commercially-available ELISA kits were used to determine the levels of $A\beta_{1-42}$ (Innotest β-amyloid1-42, Fujirebio-Europe,

Gent, Belgium) and p-Tau_{181} (Innotest Phospho-Tau181P, Fujirebio-Europe). Tests were performed in duplicate and the laboratory technician was blinded to any clinical and demographic information about the participants.

Neuropsychological tests

At baseline, participants were submitted to a battery of neuropsychological evaluations performed by a certified neuropsychologist. We utilized the validated adaptation for Brazilian patients version of the verbal memory test subscore of the Consortium to Establish a Registry for Alzheimer's Disease (CERADs)(Morris et al., 1989)(Bertolucci et al., 1998). The final score was composed by a possible sum of 30 points for word list learning, 10 points for word list recall and 10 points for word list recognition (total of 50 possible points)(Chandler et al., 2005). The Geriatric Depression Scale (GDS) 15-item version(Yesavage et al., 1982), which was translated and validated to Brazilian portuguese(Almeida and Almeida, 1999), was utilized to screen for depressive symptoms.

After three years of follow-up, subjects were reassessed in order to verify whether or not they progressed to ADD. Evaluation was performed blindly by the same behavioral neurologist and neuropsychologist, and included a structured clinical interview, a full neurological examination, and neuropsychological evaluation. At this time, they were analyzed by NIA-AA criteria for ADD(McKhann et al., 2011), corroborated by the CDR, and were classified in two groups: progressors (P), and non-progressors (NP) to ADD. The neuropsychological tests, besides those performed at baseline, included the Montreal Cognitive Assessment (MoCA)(Memória et al., 2013; Nasreddine et al., 2005), Boston Naming Test (BNT)(Kaplan et al., 1983; Miotto et al., 2010), Verbal Fluency Test, animals category (VFT)(Brucki et al., 1997; Isaacs and Kennie, 1973) and Constructional Praxis (CP)(Bertolucci et al., 2001; Rosen et al., 1984), all translated

and validated to Brazilian Portuguese. Instead of MMSE, we preferred to utilize MoCA because it has been shown to be more accurate in detecting MCI than MMSE(Trzepacz et al., 2015).

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20. To compare sex distribution, a Chi-square test was performed. Mann–Whitney tests were performed to compare clinical, neuropsychological and biomarker data. Receiver operating characteristic (ROC) curve analyses were used to obtain the area under the curve (AUC) and other parameters. The optimal cutoff values were determined by calculating the maximal sensitivity and specificity (i.e., maximizing the Youden index(Youden, 1950)). The Generalized Estimating Equation Model with the Bonferrroni *post-hoc* test was applied to verify the mean of CERADs score between groups, discriminated by time. Linear regressions were performed to evaluate the relationship among ΔCERADs and CSF biomarkers. Values were adjusted for age and sex. All results were showed as confidence intervals CI 95% and *P* values <0.05 were considered significant.

RESULTS

Thirty-one of 33 (93.9%) participants completed the follow-up. Of these, twelve (38.4%) progressed to ADD after three years. Mean age was 68.1 ± 5.1 years and 67.75% were female. No participants deceased during the follow-up. Table 1 lists the demographic characteristics and baseline neuropsychological scores between P and NP. There was no significant difference between groups when baseline characteristics were analyzed.

Table 2 lists the neuropsychological scores after three years of follow-up and CSF biomarker features. $A\beta_{1-42}$ concentrations in CSF were significantly different between groups ($P < 0.001$), as opposed to p-Tau_{181} concentrations ($P = 0.101$). The p-Tau_{181} /$A\beta_{1-42}$ ratio also displayed a significant difference between groups ($P < 0.001$). The CERADs and MoCA tests differed significantly at endpoint as well ($P = 0.006$ and $P = 0.025$, respectively).

In Table 3, cutoff values were determined by Youden's index, which stretches the maximum potential effectiveness of a biomarker. For $A\beta_{1-42,}$ the threshold value was 618.5 pg/mL, with 91.7% sensitivity (CI95%: 64.6 – 98.5) and 94.7% specificity (CI95%: 75.4 – 99.0). At this cutoff level, 29 of 31 individuals (93.5%) were correctly classified in P or NP. The relative risk of developing ADD in those directly exposed ($A\beta_{1-42} < 618.5$ pg/mL) was 17.4 times higher than among those who were not exposed (CI95%: 2.5 – 118.2; $P = 0.003$). In other words, those who had values above this concentration had their risk of developing ADD reduced by 94.3%. The optimal cutoff value for the p-Tau_{181}/$A\beta_{1-42}$ ratio was 0.135, with 66.7% sensitivity (CI95%: 39.0 – 86.2) and 100% specificity (CI95%: 83.2 – 100.0). At this cutoff value, 27 of 31 individuals (87%) were correctly classified as P or NP. The relative risk of developing ADD in those whose p-Tau_{181}/$A\beta_{1-42}$ ratio was higher than 0.135 was 5.7 times greater when compared to those with a lower p-Tau_{181}/$A\beta_{1-42}$ ratio (CI95%: 2.3 – 14.0; $P < 0.001$).

As measure of experimental validity, Fig. 1 displays the mean and standard errors of the CERADs score for each group discriminated by the baseline and follow-up times in years (baseline in 2013; follow-up in 2016). When the time was set and the groups were compared, there was no difference in the CERADs score of the NP group ($P = 0.437$), but there was a significant difference in the P group ($P < 0.001$).When the groups were fixed and times were compared, there was no difference between groups in 2013 ($P = 0.575$), but, in 2016, a significant difference between P and NP was observed ($P = 0.004$).

ΔCERADs was defined as the difference between CERADs score from 2016 and 2013. ΔCERADs was statistically different between P and NP groups (P = 0.002) and correlated with $A\beta_{1-42}$ levels (P = 0.011), p-Tau_{181} levels (P = 0.045) and p-Tau_{181}/$A\beta_{1-42}$ ratio (P = 0.010). $A\beta_{1-42}$ and p-Tau_{181} levels explained 40.1% of ΔCERADs variance (B:-6.2; SE: 13.7; P = 0.008). As demonstrated in Fig. 2, $A\beta_{1-42}$ alone explained 26.8% of ΔCERADs variance (P = 0.036), whereas p-Tau_{181} alone accounted for 27.5% of ΔCERADs variance (P = 0.032).

DISCUSSION

In this study, 38.7% of aMCI individuals progressed to ADD, a rate which is consistent with previous studies(Schjønning Nielsen et al., 2016). CSF concentration of $A\beta_{1-42}$ was significantly diminished in the P group (P <0.001), whereas there was no difference in p-Tau_{181} levels between groups (P = 0.101). These results are in line with the model of dynamic pathophysiological biomarkers in AD(Jack et al., 2013), in which $A\beta_{1-42}$ levels in the CSF becomes abnormal 5–10 years or before the diagnosis of dementia. A meta-analysis of fifty studies confirmed that $A\beta_{1-42}$ is a good biomarker for discriminating ADD from MCI and other dementias(Ferreira et al., 2014a). Moreover, our group recently demonstrated that $A\beta_{1-42}$, but not p-Tau_{181}, is significantly associated with aMCI(Rizzi et al., 2018). Previous studies agree that p-Tau_{181} levels in the CSF become progressively abnormal, and have better predictive power only 0–5 years before progression to ADD(Buchhave et al., 2012). The p-Tau_{181}/$A\beta_{1-42}$ ratio also showed significant difference (P < 0.001) between patients that progressed to ADD versus those who did not. The literature supports the evidence that the combination of $A\beta_{1-42}$ and p-Tau_{181} protein levels is a suitable measure for MCI prognosis(Ferreira et al., 2014a).

In the CSF, the $A\beta_{1-42}$ concentration displayed a cutoff value of 618.5 pg/mL for aMCI cases, with greater sensitivity and specificity when compared to similar reports in the

literature(Ferreira et al., 2014a; Ritchie et al., 2014). From this value, we can infer that the relative risk to develop ADD in those whose CSF Aβ $_{1-42}$ levels was lower than 618.5 pg/mL was about 17.4 times higher than in those whose level was above this value. Other studies described different values associated with the relative risk of developing ADD from MCI. Michaud and colleagues(Michaud et al., 2015) found a hazard ratio of developing ADD among MCI patients with high-risk biomarker levels about 4 times greater than in MCI patients with low-risk biomarker values. Tondelli and collaborators(Tondelli et al., 2015) established that $A\beta_{1-42}$ was helpful to discriminate between MCI and dementia, with a relative risk of 1.01 in MCI patients. Here we emphasizes that our cutoff value was specific for amnestic MCI patients, even though our sample size was small. The interpretation of any comparative results should be made with caution due to the heterogeneity of the population that was evaluated in this study.

For the p-Tau_{181}/$A\beta_{1-42}$ ratio in CSF, the optimal cutoff value was 0.135, with less sensitivity than $A\beta_{1-42}$ alone, but with 100% of specificity. The relative risk of developing ADD in those whose ratio was higher than 0.135 was 5.7 times greater than in those whose ration was lower than 0.135. Michaud and colleagues found that $A\beta_{1-42}$ and p-Tau_{181} were the best combination among CSF biomarkers to predict the overall risk of developing ADD among MCI patients with an area under the curve of 0.77(Michaud et al., 2015). In our study, $A\beta_{1-42}$ alone (AUC: 0.961) performed better than the p-Tau_{181}/$A\beta_{1-42}$ ratio (AUC: 0.864) in predicting overall risk, but the combination of the two proteins showed a better positive predictive value (100%). Prestia and colleagues found that the best predictive accuracy was achieved by combinations of amyloidosis and neurodegeneration biomarkers(Prestia et al., 2015). Moreover, in a recent meta-review(Ferreira et al., 2014a) it was found that the p-Tau_{181}/Aβ $_{1-42}$ ratio was the most accurate CSF measure in predicting ADD progression from MCI. Our finding that $A\beta_{1-42}$ levels alone is a superior, more accurate, measure when compared to the p-Tau_{181}/Aβ $_{1-42}$ ratio needs

to be confirmed in further studies with larger samples sizes and a more heterogeneous population.

Although several different possible cut-off values were proposed, there is a lack of agreement on which would be the best discriminant value. This controversy might be due to the variability in CSF measurements between laboratories that utilize different techniques(Samtani et al., 2013; Tang et al., 2014). We decided to find the best cut-off values for our sample by utilizing the Youden test(Youden, 1950), differently from other reports in which the threshold values were based on laboratory cut-off points that were based on different populations and methodologies(Ritchie et al., 2014). For instance, difficulties in predicting MCI progression to ADD could be influenced by the intrinsic heterogeneity of MCI individuals.

After three years of follow-up, only CERADs and MoCA tests reflected differences between the P and NP groups. These results agree with our baseline selection of exclusively aMCI cases, since episodic memory is the major cognitive domain impaired in this subtype of MCI patients(Gifford et al., 2015). There was no significant difference in the GDS test, although some patients who did not progress to ADD presented depressive symptoms after three years.

The CERADs test was performed at baseline and again after three years. As Fig. 1 shows, there was no difference between groups at baseline, but over time it was observed a significant decline in the episodic memory of those who progressed to ADD. This pattern was not verified in the NP group, which performed similarly at baseline and follow-up. Additionally, ΔCERADs correlated with the two proteins alone, besides correlating with their combination ratio. This finding reinforces the early and specific involvement of episodic memory in the prodromal phase of AD. Haldenwanger and colleagues already emphasized the existence of a significant correlation between $A\beta_{1-42}$ in the CSF and memory performance for aMCI patients, but not for non-amnesic MCI(Haldenwanger et al., 2010). Rami and collaborators also suggested that

memory performance is first related with $A\beta_{1-42}$ levels and then with t-Tau or p-Tau_{181}(Rami et al., 2011). Moreover, a recent study suggested that, in particular, $A\beta_{1-42}$ protein is associated with a delayed memory performance CERAD(Haapalinna et al., 2016), agreeing with our results. $A\beta_{1-42}$ and p-Tau_{181} levels explained 40.1% of ΔCERADs variance, better than each one alone.

Often, MCI represents an intermediate stage between normal cognition and dementia(Petersen et al., 2014). In one year, about 10-15% of MCI patients develop ADD. In 5 years, this number can raise up to 40-60%(Schjønning Nielsen et al., 2016; Tondelli et al., 2015). To our knowledge, this is the first study that evaluated the relative risk of both $A\beta_{1-42}$ and p-Tau_{181}/$A\beta_{1-42}$ ratio in predicting progression from aMCI to ADD in an exclusively amnestic MCI sample, in a three-year follow-up.

An important limitation of our study is the relatively small sample size. Another possible methodologic drawback is the absence of controls. Nonetheless, in this study we did not aim to evaluate differences between individuals with aMCI and controls, but, instead, to analyze the role of CSF $A\beta_{1-42}$ and p-Tau_{181} in predicting progression from aMCI to ADD. In another words, we tried to answer a common question that physicians find in everyday practice: what is the predictive value of analyzing $A\beta_{1-42}$ and p-Tau_{181} in the CSF of individuals with aMCI?

CONCLUSION

Taken together, our results suggest that the CSF biomarkers analyzed herein provide predictive information about progression from aMCI to ADD. $A\beta_{1-42}$ strongly predicted progression from aMCI to ADD, whereas p-Tau_{181} alone or its relation to $A\beta_{1-42}$ were no better than $A\beta_{1-42}$ alone

as a progression to ADD predictor. However, the CSF p-Tau_{181}/$A\beta_{1-42}$ ratio did improve the specificity and negative predictive value as compared to CSF $A\beta_{1-42}$ levels alone.

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DECLARATION OF INTEREST

The authors hereby declare that there are no actual or potential conflicts of interest that may have affected the discussion presented.

REFERENCES

Albert, M.S., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., 2011. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3), 270-279.

Almeida, O., Almeida, S., 1999. Confiabilidade da versão brasileira da Escala de Depressão em Geriatria (GDS) versão reduzida. Arq Neuro-Psiquiatr 57, 421-426.

Bertolucci, P.H., Okamoto, I.H., Brucki, S.M., Siviero, M.O., Toniolo Neto, J., Ramos, L.R., 2001. Applicability of the CERAD neuropsychological battery to Brazilian elderly. Arq Neuropsiquiatr 59(3-A), 532-536.

Bertolucci, P.H.F., Okamoto, I.H., Toniolo Neto, J., Ramos, L.R., Brucki, S.M.D., 1998. Desempenho da populacao brasileira na bateria neuropsicologica do Consortium to Establish a Registry for Alzheimer's Disease (CERAD) *Revista de Psiquiatria Clínica*, São Paulo, pp. 80-83.

Brucki, S.M., Malheiros, S.M., Okamoto, I.H., Bertolucci, P.H., 1997. [Normative data on the verbal fluency test in the animal category in our milieu]. Arq Neuropsiquiatr 55(1), 56-61.

Buchhave, P., Minthon, L., Zetterberg, H., Wallin, A.K., Blennow, K., Hansson, O., 2012. Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry 69(1), 98-106.

Chandler, M.J., Lacritz, L.H., Hynan, L.S., Barnard, H.D., Allen, G., Deschner, M., Weiner, M.F., Cullum, C.M., 2005. A total score for the CERAD neuropsychological battery. Neurology 65(1), 102-106.

Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., DeKosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014.

Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 13(6), 614-629.

Ferreira, D., Perestelo-Pérez, L., Westman, E., Wahlund, L.O., Sarría, A., Serrano-Aguilar, P., 2014a. Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. Front Aging Neurosci 6, 47.

Ferreira, D., Rivero-Santana, A., Perestelo-Pérez, L., Westman, E., Wahlund, L.O., Sarría, A., Serrano-Aguilar, P., 2014b. Improving CSF Biomarkers' Performance for Predicting Progression from Mild Cognitive Impairment to Alzheimer's Disease by Considering Different Confounding Factors: A Meta-Analysis. Front Aging Neurosci 6, 287.

Gifford, K.A., Liu, D., Damon, S.M., Chapman, W.G., Romano Iii, R.R., Samuels, L.R., Lu, Z., Jefferson, A.L., Initiative, A.s.D.N., 2015. Subjective memory complaint only relates to verbal episodic memory performance in mild cognitive impairment. J Alzheimers Dis 44(1), 309-318.

Haapalinna, F., Paajanen, T., Penttinen, J., Kokki, H., Kokki, M., Koivisto, A.M., Hartikainen, P., Solje, E., Hänninen, T., Remes, A.M., Herukka, S.K., 2016. Low Cerebrospinal Fluid Amyloid-Beta Concentration Is Associated with Poorer Delayed Memory Recall in Women. Dement Geriatr Cogn Dis Extra 6(2), 303-312.

Haldenwanger, A., Eling, P., Kastrup, A., Hildebrandt, H., 2010. Correlation between cognitive impairment and CSF biomarkers in amnesic MCI, non-amnesic MCI, and Alzheimer's disease. J Alzheimers Dis 22(3), 971-980.

Isaacs, B., Kennie, A.T., 1973. The Set test as an aid to the detection of dementia in old people. Br J Psychiatry 123(575), 467-470.

Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C., Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 12(2), 207-216.

Jack, C.R., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C., Trojanowski, J.Q., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9(1), 119-128.

Kaplan, E., Goodglass, H., Weintraub, S., 1983. Boston naming test. Lea & Febiger, Philadelphia.

McGeer, P.L., McGeer, E.G., 2013. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. Acta Neuropathol 126(4), 479-497.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack, C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor, M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3), 263-269.

Memória, C.M., Yassuda, M.S., Nakano, E.Y., Forlenza, O.V., 2013. Brief screening for mild cognitive impairment: validation of the Brazilian version of the Montreal cognitive assessment. Int J Geriatr Psychiatry 28(1), 34-40.

Michaud, T.L., Kane, R.L., McCarten, J.R., Gaugler, J.E., Nyman, J.A., Kuntz, K.M., Initiative, A.s.D.N., 2015. Risk Stratification Using Cerebrospinal Fluid Biomarkers in Patients with Mild Cognitive Impairment: An Exploratory Analysis. J Alzheimers Dis 47(3), 729-740.

Miotto, E.C., Sato, J., Lucia, M.C., Camargo, C.H., Scaff, M., 2010. Development of an adapted version of the Boston Naming Test for Portuguese speakers. Rev Bras Psiquiatr 32(3), 279-282.

Molinuevo, J.L., Blennow, K., Dubois, B., Engelborghs, S., Lewczuk, P., Perret-Liaudet, A., Teunissen, C.E., Parnetti, L., 2014. The clinical use of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers Dement.

Morris, J.C., 1993. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43(11), 2412-2414.

Morris, J.C., Blennow, K., Froelich, L., Nordberg, A., Soininen, H., Waldemar, G., Wahlund, L.O., Dubois, B., 2014. Harmonized diagnostic criteria for Alzheimer's disease: recommendations. J Intern Med 275(3), 204-213.

Morris, J.C., Heyman, A., Mohs, R.C., Hughes, J.P., van Belle, G., Fillenbaum, G., Mellits, E.D., Clark, C., 1989. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39(9), 1159-1165.

Nasreddine, Z.S., Phillips, N.A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L., Chertkow, H., 2005. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53(4), 695-699.

Petersen, R.C., 2004. Mild cognitive impairment as a diagnostic entity. J Intern Med 256(3), 183-194.

Petersen, R.C., Caracciolo, B., Brayne, C., Gauthier, S., Jelic, V., Fratiglioni, L., 2014. Mild cognitive impairment: a concept in evolution. J Intern Med 275(3), 214-228.

Prestia, A., Caroli, A., Wade, S.K., van der Flier, W.M., Ossenkoppele, R., Van Berckel, B., Barkhof, F., Teunissen, C.E., Wall, A., Carter, S.F., Schöll, M., Choo, I.H., Nordberg, A., Scheltens, P., Frisoni, G.B., 2015. Prediction of AD dementia by biomarkers following the NIA-AA and IWG diagnostic criteria in MCI patients from three European memory clinics. Alzheimers Dement 11(10), 1191-1201.

Rami, L., Fortea, J., Bosch, B., Solé-Padullés, C., Lladó, A., Iranzo, A., Sánchez-Valle, R., Molinuevo, J.L., 2011. Cerebrospinal fluid biomarkers and memory present distinct associations along the continuum from healthy subjects to AD patients. J Alzheimers Dis 23(2), 319-326.

Ritchie, C., Smailagic, N., Noel-Storr, A.H., Takwoingi, Y., Flicker, L., Mason, S.E., McShane, R., 2014. Plasma and cerebrospinal fluid amyloid beta for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). Cochrane Database Syst Rev(6), CD008782.

Rizzi, L., Maria Portal, M., Batista, C.E.A., Missiaggia, L., Roriz-Cruz, M., 2018. CSF Aβ1–42, but not p-Tau181, differentiates aMCI from SCI. Brain Research 1678, 27-31.

Rosen, W.G., Mohs, R.C., Davis, K.L., 1984. A new rating scale for Alzheimer's disease. Am J Psychiatry 141(11), 1356-1364.

Samtani, M.N., Raghavan, N., Shi, Y., Novak, G., Farnum, M., Lobanov, V., Schultz, T., Yang, E., DiBernardo, A., Narayan, V.A., Initiative, A.s.D.N., 2013. Disease progression model in subjects with mild cognitive impairment from the Alzheimer's disease neuroimaging initiative: CSF biomarkers predict population subtypes. Br J Clin Pharmacol 75(1), 146-161.

Schjønning Nielsen, M., Simonsen, A.H., Siersma, V., Hasselbalch, S.G., Høgh, P., 2016. Are CSF Biomarkers Useful as Prognostic Indicators in Diagnostically Unresolved Cognitively Impaired Patients in a Normal Clinical Setting. Dement Geriatr Cogn Dis Extra 6(3), 465-476.

Tang, W., Huang, Q., Wang, Y., Wang, Z.Y., Yao, Y.Y., 2014. Assessment of CSF Aβ42 as an aid to discriminating Alzheimer's disease from other dementias and mild cognitive impairment: A meta-analysis of 50 studies. J Neurol Sci 345(1-2), 26-36.

Tondelli, M., Bedin, R., Chiari, A., Molinari, M.A., Bonifacio, G., Lelli, N., Trenti, T., Nichelli, P., 2015. Role of cerebrospinal fluid biomarkers to predict conversion to dementia in

patients with mild cognitive impairment: a clinical cohort study. Clin Chem Lab Med 53(3), 453-460.

Trzepacz, P.T., Hochstetler, H., Wang, S., Walker, B., Saykin, A.J., Initiative, A.s.D.N., 2015. Relationship between the Montreal Cognitive Assessment and Mini-mental State Examination for assessment of mild cognitive impairment in older adults. BMC Geriatr 15, 107.

Vos, S.J., van Rossum, I.A., Verhey, F., Knol, D.L., Soininen, H., Wahlund, L.O., Hampel, H., Tsolaki, M., Minthon, L., Frisoni, G.B., Froelich, L., Nobili, F., van der Flier, W., Blennow, K., Wolz, R., Scheltens, P., Visser, P.J., 2013. Prediction of Alzheimer disease in subjects with amnestic and nonamnestic MCI. Neurology 80(12), 1124-1132.

Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., Leirer, V.O., 1982. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17(1), 37-49.

Youden, W.J., 1950. Index for rating diagnostic tests. Cancer 3(1), 32-35.

Table 1. Patients characteristics and baseline neuropsychological scores.

	Progressors	Non-Progressors	*P* value
n (%)	12 (38.7%)	19 (61.3%)	
Age (years): median (min –max)	69 (63-76)	67 (61-78)	0.765
Education (years): median (min –max)	9.5 (3-18)	11.0 (1-16)	0.191
Gender (male/female): n	3/9	7/12	0.492
GDS: median (min –max)	2.5 (0-5)	2.0 (0-5)	0.562
CERADs: median (min –max)	26.5 (18-37)	27.0 (22-34)	0.535

GDS: Geriatric Depression Scale; CERADs: Consortium to Establish a Registry for Alzheimer's Disease. All results were obtained performing the Mann-Whitney test, with the exception of gender, in which the Chi-Square Test was used.

Table 2. Relationship between baseline CSF $A\beta_{1-42}$, $p\text{-}Tau_{181}$, and neuropsychological scores at follow-up.

	Progressors	Non progressors	*P* value
n (%)	12 (38.7%)	19 (61.3%)	
$A\beta_{1-42}$ (pg/mL): median (min –max)	466.5 (319.1 – 742.0)	900.1 (503.3 – 1234.6)	<0.001
$p\text{-}Tau_{181}$ (pg/mL): median (min –max)	82.3 (34.3 – 191.7)	56.4 (33.9 – 119.6)	0.101
$p\text{-}Tau_{181}$ / $A\beta_{1-42}$ ratio: median (min –max)	0.17 (0.05 – 0.60)	0.06 (0.04 – 0.11)	<0.001
GDS: median (min –max)	2 (1 – 5)	2 (0 – 11)	0.857
CERADs: median (min –max)	19 (5 – 36)	27 (18 – 35)	0.006
MoCA: median (min –max)	18.5 (9 – 27)	23.0 (14 – 28)	0.025
BNT: median (min –max)	13 (9 – 15)	14 (8 – 15)	0.509
VFT: median (min –max)	15 (5 – 27)	15 (8 – 20)	0.646
CP: median (min –max)	6 (2 – 10)	7 (2 – 11)	0.326

GDS: Geriatric Depression Scale; CERADs: Consortium to Establish a Registry for Alzheimer's Disease; MoCA: Montreal Cognitive Assessment; BNT: Boston Naming Test; VFT: Verbal Fluency Test, animals category; CP: Constructional Praxis. All results were obtained via the Mann-Whitney test.

Table 3. ROC curve parameters of CSF biomarkers in predicting progression from aMCI to ADD.

Threshold values	Progressors	Non-Progressors	ROC AUC (CI95%)	Sensitivity (%) (CI95%)	Specificity (%) (CI95%)	Test accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
$A\beta_{1-42}$ < 618.5 pg/mL	11	1	0.961 (0.898 - 1.00)	91.7 (64.6 – 98.5)	94.7 (75.4 – 99.0)	93.5	91.7	94.7
$A\beta_{1-42}$ > 618.5 pg/mL	1	18						
$p\text{-}Tau_{181}$ / $A\beta_{1-42}$ > 0.135	8	0	0.864 (0.721 - 1.00)	66.7 (39.0 – 86.2)	100 (83.2 – 100.0)	87.0	100.0	82.6
$p\text{-}Tau_{181}$ / $A\beta_{1-42}$ < 0.135	4	19						

aMCI: amnestic mild cognitive impairment; ADD: Alzheimer's disease dementia; ROC: receiver operating characteristic; AUC: area under the curve. Youden index was used.

Figures

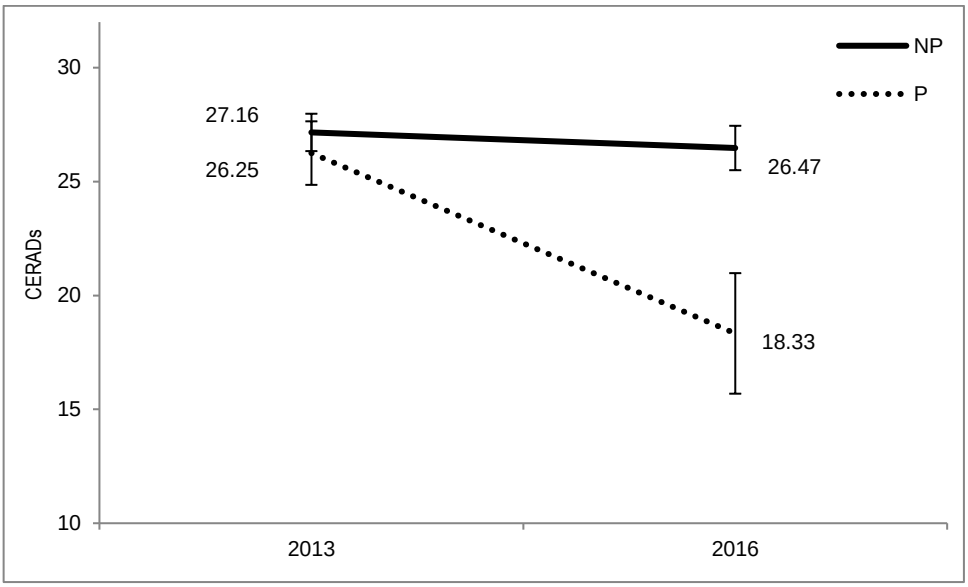

Fig. 1 Line chart of the means and standard errors of the CERADs score of each group. There was an association between variables(P<0.001).When the time was set and the groups were compared, there was no difference in the NP group (P=0.437) but there was a significant difference in the P group (P< 0.001). When the groups were fixed and times were compared in 2013 (baseline), there was no difference between groups (P = 0.575). When times were compared in 2016 (follow-up), there was a statistical difference between groups (P = 0.004). P: Progressors; NP: Non-progressors; CERADs: Consortium to Establish a Registry for Alzheimer's Disease. Generalized Estimating Equation Model with the Bonferroni *post-hoc* test was used

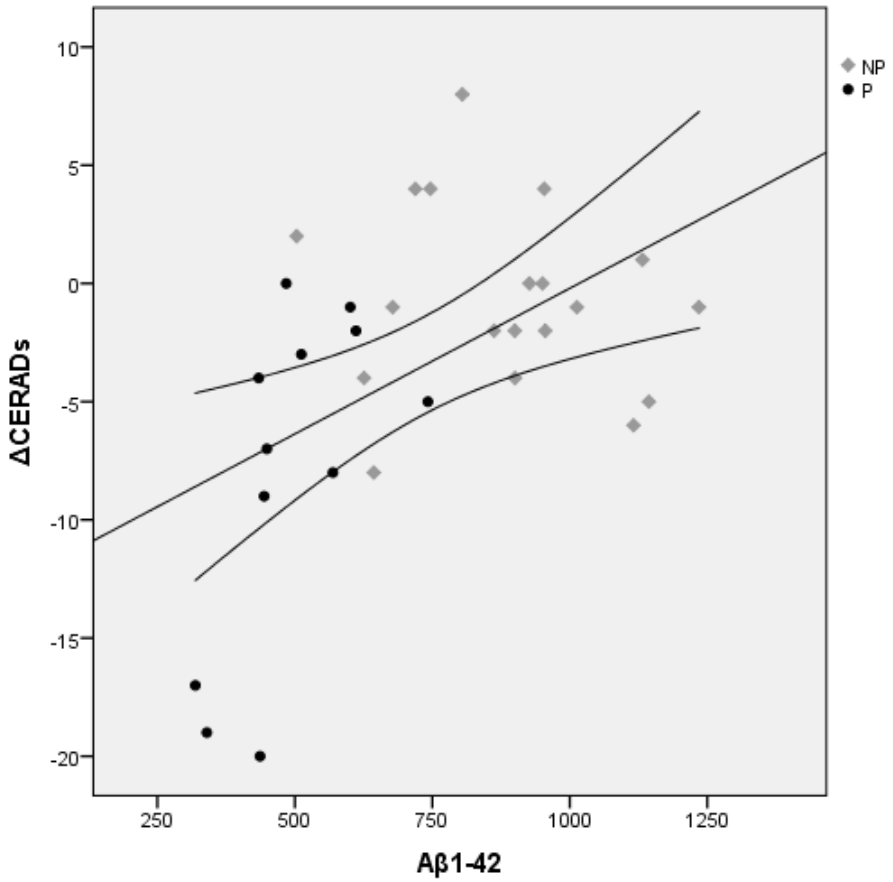

Fig. 2 Linear relationship between $A\beta_{1\text{-}42}$ (pg/mL)and ΔCERADs (R^2: 0.268; *P* = 0.036). Values were adjusted for age and sex. Distribution at P and NP. P: Progressors; NP: Non progressors; CERADs: Consortium to Establish a Registry for Alzheimer's Disease

3. ARTIGO CIENTÍFICO ORIGINAL III

Estudo longitudinal final de cinco anos de seguimento da coorte inicial a ser submetido.

Title: Value of CSF biomarkers in predicting risk of progression from aMCI to ADD: a 5-year follow-up study

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ABSTRACT

Objective: To approach cerebrospinal fluid (CSF) $A\beta_{1-42}$ and p-Tau_{181} as risks factors in predicting progression from amnestic mild cognitive impairment (aMCI) to Alzheimer's disease dementia (ADD) in a 5-year follow-up.

Methods: Forty-two individuals diagnosed as aMCI or SCI were evaluated in 2013 and reevaluated in 2018. CSF $A\beta_{1-42}$ and p-Tau_{181} were measured by immunoenzymatic assay, and cutoffs were calculated with the Youden test. Differences in performance between endpoint and baseline were verified by neuropsychological tests, such as the Consortium to Establish a Registry for Alzheimer's Disease (CERADs) and Montreal Cognitive Assessment (MoCA). Values were adjusted for age.

Results: 45.2% of the aMCI individuals progressed to ADD. The cumulative risk to develop ADD in individuals with aMCI and $A\beta_{1-42} < 618.5$ pg/mL was 5.8 times higher than in those whose levels were above this cutoff ($P = 0.0011$). Moreover, the relative risk among those in which the p-$Tau_{181}/A\beta_{1-42}$ ratio was higher than 0.135 was 3.83 times greater ($P = 0.0001$). Both $A\beta_{1-42}$ and p-Tau_{181} levels explained 47.5% of ΔCERADs variance ($P < 0.001$), whereas $A\beta_{1-42}$ alone explained 38.6% ($P < 0.001$).

Conclusion: The p-$Tau_{181}/A\beta_{1-42}$ ratio was no better than $A\beta_{1-42}$ alone in predicting progression from aMCI to ADD. However, p-Tau_{181} levels helped to explain an extra 9% of ΔCERADs variance in 5 years.

Key words: Alzheimer's disease; amyloid protein; CSF biomarkers; MCI; Tau.

INTRODUCTION

The new research framework, proposed by the National Institute on Aging and Alzheimer's Association (NIA-AA), defined that Alzheimer's disease (AD) underlying pathologic processes can be acknowledged by *postmortem* examination or *in vivo* by biomarkers[20]. Especially, cerebrospinal fluid (CSF) concentrations of $A\beta_{1-42}$ and p-Tau_{181} proteins seem to be the most promising biomarkers related to AD and can be early identified in the disease continuum[37]. Therefore, it is extreme important to estimate the added value of these biomarkers to identify the real risk of progression to the dementia phase of this disease.

Longitudinal research on mild cognitive impairment (MCI) individuals represent an opportunity to investigate the underlying biological processes (amyloidosis and neurodegeneration) and their relevance in the progression to AD. However, MCI remains a largely heterogeneous condition with a range of different underlying neurodegenerative mechanisms, which blurs both the ability to predict an individual's risk and the early detection of AD in its course. For this reason, individuals with amnestic mild cognitive impairment (aMCI) are the focus of ongoing research, since they are more likely to develop Alzheimer's disease dementia (ADD)[47].

Reports from studies regarding dominantly inherited AD genetic mutations support the concept of a protracted preclinical period during which biomarkers become abnormal[25]. It has been observed that the first changes in CSF $A\beta_{1-42}$ begin at least 20 years prior to the first symptoms, and CSF p-Tau_{181} almost 15 years before[21]. However, few studies have evaluated the specific role of these biomarkers in patients diagnosed with aMCI regarding prediction of progression to ADD[13, 14, 24, 35, 36, 40, 50]. We have previously demonstrated the relative risk of developing ADD in 3 years, by following-up an exclusively aMCI cohort[43]. Here, we report the 5-year follow-up of this sample. The overarching aim of the present study was to

analyze whether or not these biomarkers, $A\beta_{1-42}$ and p-$Tau_{181,}$ might predict the cumulative risk of progressing from aMCI to ADD in a 5-year follow-up.

MATERIALS AND METHODS

Subjects

In 2013, we began a longitudinal assessment of 33 patients diagnosed with aMCI and 12 patients diagnosed with subjective cognitive impairment (SCI) at a memory clinic in Southern Brazil. This cohort underwent lumbar puncture followed by CSF biomarkers evaluation and was enrolled in the study until 2018. The baseline cross-sectional and the 3-year follow-up studies have already been previously published [42, 43]. In brief, patients were enrolled in a systematic way with clinical evaluations by a behavioral/geriatric neurologist and a neuropsychological specialist in order to detect progression to ADD in the aMCI group. Those who followed-up until they developed dementia or until they had been deemed cognitively stable comprised the longitudinal study-group. This study was approved by the local research ethics committee (number 13-0009) and is in accordance with the Declaration of Helsinki. All participants gave their written consent in order to participate in this study.

Clinical and Neuropsychological Procedures

At baseline, aMCI diagnoses were based on criteria established by Petersen [38, 39] and on NIA-AA criteria for MCI[1]. SCI individuals included were those without objective evidence of cognitive impairment. All diagnoses were corroborated by the Clinical Dementia Rating scale (CDR)[31], in which aMCI scored 0.5 on CDR, and SCI scored 0 on CDR. As an exclusion criteria for enrolment we considered the diagnosis of dementia, stroke, Parkinson's

disease, depression or other neurological conditions that could possibly cause cognitive impairment.

Diagnostic investigation included a standard clinical evaluation and a battery of neuropsychological tests performed by a certified neuropsychologist. At baseline, the comprehensive diagnostic battery-protocol included: the verbal memory test subscore of the Consortium to Establish a Registry for Alzheimer's Disease (CERADs)[6, 32] to test memory performance, the 0-5 points version of the CDT (Clock Drawing Test)[3, 48] to measure executive functions, and the 15-item version of the Geriatric Depression Scale (GDS)[2, 53] to measure depressive symptomatology, all translated and validated to Brazilian Portuguese. The final CERADs score was composed by a possible sum of 30 points for word list learning, 10 points for word list recall and 10 points for word list recognition (total of 50 possible points)[10]. The presence of significant depressive symptomatology on GDS was considered for all subjects who scored ≥5 points in the scale. All the available information was used to reach a consensus diagnosis.

A similar approach was used in the follow-up evaluations, which were performed blindly by the same behavioral neurologist and neuropsychologist, and included a structured clinical interview, a full neurological examination, and other neuropsychological tests. Besides those performed at baseline, other the tests included were: the Montreal Cognitive Assessment (MoCA)[27, 34], Boston Naming Test (BNT)[22, 28], Verbal Fluency Test, animals category (VFT)[8, 19] and Constructional Praxis (CP)[5, 44], all translated and validated to Brazilian Portuguese. At the endpoint, individuals were reassessed in order to verify whether or not they had progressed to ADD. At this time, aMCI individuals were evaluated to verify whether they fulfilled the NIA-AA criteria for ADD[26], corroborated by changes in global CDR rating from 0.5 to 1 or more, in order to confirm the cognitive profile of dementia. Afterwards, the aMCI

group was subdivided accordingly into two groups: those who progressed to ADD (Progressors) or those who did not (Non-Progressors).

Laboratory Determinations

CSF samples were collected from all participants. They underwent a fasted lumbar puncture from the L3/L4 or L4/L5 intervertebral region. No adverse events were reported. CSF (5ml) was collected in sterile polypropylene tubes and immediately centrifuged (4,000 *g* x 10 minutes) at 4°C, aliquoted in polypropylene Eppendorfs, and then stored at -80°C until analysis. CSF $A\beta_{1\text{-}42}$ and p-Tau_{181} were measured separately by commercially-available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Innotest, Fujirebio-Europe, Ghent, Belgium). Tests were performed in duplicate and the laboratory technician was blinded to any clinical and demographic information about the participants.

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 20.0) (IBM SPSS, Chicago, IL). Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Non-parametric tests were used. The Kruskal-Wallis followed by the Dunn *post-hoc* tests were utilized to compare clinical, neuropsychological and biomarker data among the groups. ANCOVA test followed by the Bonferroni *post-hoc* test was performed to assess the statistical significance adjusted for age. Groups' differences between categorical variables were examined using the Chi-square ($\chi 2$) test. Receiver operating characteristic (ROC) curve analyses were performed to obtain the area under the curve (AUC) and related parameters. The optimal cutoff values were determined by the Youden test[54]. The

Generalized Estimating Equation Model with the Bonferrroni *post-hoc* test was applied to verify the mean of CERADs between those who were above and under the cutoff established for Aβ and in the SCI group, discriminated by time. Linear regressions were performed to evaluate the relationship between ΔCERADs and CSF biomarkers. Values were adjusted for age. All results are showed as confidence intervals CI 95% and *P* values <0.05 were considered significant.

RESULTS

At baseline, 33 aMCI and 12 SCI individuals were included in the study. Initially, these two groups differed only in the CERADs ($P = 0.041$; $P_{adj.} = 0.009$). Thirty-one aMCI and 11 SCI individuals completed the follow-up. After five years, 45.16% of the aMCI individuals progressed to ADD, corresponding to an annual conversion rate of 9.03%. The group of aMCI individuals was then dichotomized between those who were cognitively stable in the last observation, called Non-Progressors (17), and those who evolved to ADD, called Progressors (14). Additionally, we also verified that two (18.18%) SCI individuals progressed to aMCI in five years. None developed another kind of dementia. Moreover, our sample was composed mostly of a Caucasian population, which reflects the demographics of the Southern Brazilian population[33]. Only one individual in each group was African-Brazilian. No participants deceased during the follow-up, and those missed were due to no-shows.

Baseline demographic, neuropsychological and CSF biomarker data of the study population that completed the follow-up are presented in Table 1. The groups did not show significant differences in gender, educational level, and age. However, we corrected all data for age to discard any possible bias. CSF $A\beta_{1-42}$ and p-$Tau_{181}/A\beta_{1-42}$ ratio were significantly different between groups. Table 2 describes the demographic and neuropsychological endpoint

data of the study population. ΔCERADs, defined as the difference between CERADs scores in 2018 compared to 2013, and the MoCA test were significantly different between aMCI Progressors and the SCI group (P =0.001 and $P < 0.001$, respectively).

Table 3 describes ROC curve parameters of CSF biomarkers for predicting progression from aMCI to ADD. The best cutoff values were determined as described previously[43]. For $A\beta_{1\text{-}42,}$ the threshold value was 618.5 pg/mL, with 78.5% sensitivity (CI95%: 52.2 – 92.4) and 94.1% specificity (CI95%: 73.0 – 98.9). The relative risk of developing ADD after five years among those with aMCI and $A\beta_{1\text{-}42} < 618.5$ pg/mL was 5.8 times higher than among those who were above this cutoff value (IC95%: 2.02 - 16.62; P =0.0011). The optimal cutoff value for the p-$Tau_{181}/A\beta_{1\text{-}42}$ ratio was 0.135, with 57.1% sensitivity (CI95%: 32.6 – 78.6) and 100% specificity (CI95%: 81.6 – 100.0). The relative risk of developing ADD after five years of follow-up in those whose p-$Tau_{181}/A\beta_{1\text{-}42}$ ratio was higher than 0.135 was 3.83 times higher when compared to those with a lower p-$Tau_{181}/A\beta_{1\text{-}42}$ ratio (IC95%: 1.92 - 7.62; P =0.0001).

Table 4 summarizes the demographic and neuropsychological endpoint data between those who were above and below the cutoff for Aβ. At this time, ΔCERADs was significantly different in those were below the cutoff for Aβ as compared to those who were above the cutoff, or in the SCI group ($P < 0.001$). MoCA and BNT tests differed from those who were under the cutoff for Aβ and in the SCI group ($P < 0.001$ and $P = 0.035$, respectively). Although the ΔGDS, defined as the difference between GDS scores in 2018 compared to 2013, presented some variation throughout this time, it did not affect the group with Aβ < 618.5 pg/mL.

The CERADs was applied three times during the follow-up. Figure 1 displays the line chart of CERADs in those who were above and below the cutoff for Aβ, and in the SCI group. The figure shows significant differences only in those who presented Aβ levels < 618.5 pg/mL over time ($P < 0.001$). Moreover, in 2013 (baseline), when the groups were fixed and times

were compared, there was no difference between groups ($P = 0.078$), but there was a statistical significant difference at the 3-year (2016) and 5-year (2018) follow-up ($P = 0.004$ and $P = 0.002$, respectively). Furthermore, both $A\beta_{1-42}$ and p-Tau_{181} levels explained 47.5% of ΔCERADs variance (B:-13.13; SE: 10.52; $P < 0.001$), whereas $A\beta_{1-42}$ alone explained 38.6% ($P < 0.001$), and p-Tau_{181} accounted for 23.1% ($P = 0.006$). p-Tau_{181} levels helped explain an extra 9% ($P = 0.015$) of ΔCERADs variance in 5 years.

DISCUSSION

We verified that the 5-year cumulative risk of developing ADD in individuals with aMCI and $A\beta_{1-42} < 618.5$ pg/mL was 5.8 times higher than among those whose levels were above this cutoff. Similarly, in those whose p-Tau_{181}/$A\beta_{1-42}$ ratio was greater than 0.135, the relative risk was 3.83 times higher of developing ADD. Interestingly, these relative risk values are lower than the ones we have reported in our 3-year follow-up study[43]. Moreover, we verified that 45.16% of aMCI individuals who completed the follow-up progressed to ADD after five years, corresponding to an annual conversion rate of approximately 9%, and which is in agreement with previous studies[29, 46, 49, 50]. Prediction of ADD in a short period of time appears much more relevant in a clinical perspective than prediction of dementia in a more distant future. The proportion of aMCI patients progressing to ADD is not constant over time, is highest during the first years of follow-up, and decreases at longer follow-up intervals[14, 24]. Additionally, most people with MCI will not progress to dementia even after 10 years of follow-up[29].

$A\beta_{1-42}$ was the best biomarker to predict ADD, which is also supported by others in clinical and neuropathological studies[18, 20, 45]. Furthermore, human and animal model data suggest a causal upstream role for $A\beta_{1-42}$ in the pathogenesis of AD[21]. This resembles studies

of AD-related neurodegeneration, in which amyloid deposition acts as the trigger for further disease progression[11]. Although $A\beta_{1-42}$ alone is insufficient to cause cognitive deterioration directly, it may be sufficient to cause tauopathy and neurodegeneration, which ultimately leads to cognitive deterioration[4, 7]. Biomarkers of neuronal injury appeared to best predict conversion from shorter time intervals before developing ADD[9].

It is important to note that, in the present study, the p-$Tau_{181}/A\beta_{1-42}$ ratio showed good predictive value in the progression from aMCI to ADD. Moreover, it combines measures of two different pathological processes into a single diagnostic biomarker. This result is also consistent with previous studies, in which the p-$Tau_{181}/A\beta_{1-42}$ ratio was found to be an accurate measure for progression to ADD[12, 23, 40]. It was also demonstrated that, in subjects with MCI and evidence of amyloid pathology, the injury markers, such as CSF p-Tau_{181}, can predict further cognitive decline[52]. In our study, we verified that p-Tau_{181} levels helped to explain an extra 9% of ΔCERADs variance in 5 years.

In line with the approach of a proxy routine clinical practice, we exclusively included patients with aMCI at baseline, since the focus in implementing biomarkers, and in developing new treatments, when available, is specially in this clinical setting[20]. Despite few studies that have been conducted in this field, [13, 14, 24, 35, 36, 40, 50], this is, to our knowledge, the first study to verify the relative risk of CSF $A\beta_{1-42}$ and of the p-$Tau_{181}/A\beta_{1-42}$ ratio in predicting ADD in an exclusively aMCI sample in a 5-year follow-up.

Decline in episodic memory is one of the hallmark features of AD and is also a defining feature of aMCI[15]. To assess episodic memory, we applied the verbal memory test of the CERAD. Figure 1 clearly demonstrates that, in those with $A\beta_{1-42} < 618.5$ pg/mL, CERADs declined dramatically along time, which did not occur in the other groups. Moreover, this decline happened mainly in the first three years, demonstrating that those who have low levels of $A\beta_{1-42}$ have an earlier impairment in episodic memory than those whose levels are above

618.5 pg/mL [16, 41]. Both $A\beta_{1-42}$ and p-Tau_{181} levels explained 47.5% of CERADs variance, whereas $A\beta_{1-42}$ alone explained 38.6%, adjusted for age. This data is in agreement with our rigorous inclusion criteria of aMCI individuals and with the statement that a decline in episodic memory correlates with $A\beta_{1-42}$ in other aMCI studies[17].

At baseline, there was no difference in the GDS. It was important to include this scale, since depressive symptoms are very prevalent in subjects with MCI. Since it depression displays a similar pattern of symptoms as AD, it could be a possible bias[30]. Although some individuals developed depression during the follow-up, there were no significant differences between groups at the endpoint. Moreover, the ΔGDS showed that the variation in the score along time did not affect the aMCI Progressors group, and, therefore, did not influence our results. Additionally, we observed that there were no significant differences in other cognitive domains, such as executive functions measured by CDT, which are more associated with other forms of MCI[38, 39].

The MoCA test is a cognitive screening scale that assesses several cognitive domains[27, 34]. Instead of the Mini-Mental State Examination (MMSE), we preferred to utilize MoCA because it has been shown to be more accurate in detecting MCI than MMSE [51]. In our study, we observed that, at the endpoint, this test showed a significant difference between those who had $A\beta_{1-42}$ levels below the cutoff point as compared with the SCI group. This endorses that the prodromal aMCI individuals had higher global cognition deterioration than those who had normal CSF $A\beta_{1-42}$ levels. The BNT scale was different between those who had CSF $A\beta_{1-42}$ < 618.5 pg/mL and the SCI group. Nonetheless, because of the non-homoscedasticity of the data, we cannot make conclusions regarding this finding.

Strengths of this study are the rigorous methodology adopted in the inclusion criteria of exclusively aMCI or SCI individuals, and in their progression, avoiding misclassifications. Moreover, the use of neuropsychological instruments that were validated and administered by

the same experienced neuropsychologist may improve reliability and diagnostic consistency. Differently from studies that used threshold values based on laboratory standards, and that evaluated different populations with diverse methodologies, we preferred to utilize our own cut-offs to improve the reliability of our results. The main limitations of this study are, in the first place, the small sample size. Secondly, since only the amnestic subtype of MCI was considered, the generalization of the results to more heterogeneous population should be made with caution. And, lastly, we have measured the aforementioned CSF biomarkers only in the baseline.

CONCLUSIONS

In this study, $A\beta_{1-42}$ provided the best cumulative risk to the progression from aMCI to ADD in a 5-year follow-up cohort. p-Tau_{181}/$A\beta_{1-42}$ ratio was no better than $A\beta_{1-42}$ alone in predicting progression from aMCI to ADD. However, p-Tau_{181} levels helped to explain an extra 9% of ΔCERADs variance in 5 years. The value of CSF biomarkers in predicting progression to ADD in aMCI individuals is more relevant during the first three years of follow-up than at longer intervals.

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DECLARATION OF INTEREST

The authors hereby declare that there are no actual or potential conflicts of interest that may have affected the discussion presented.

REFERENCES

1. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:270-279

2. Almeida O, Almeida S (1999) Confiabilidade da versão brasileira da Escala de Depressão em Geriatria (GDS) versão reduzida. Arq Neuro-Psiquiatr 57:421-426

3. Aprahamian I, Martinelli JE, Neri AL, Yassuda MS (2010) The accuracy of the Clock Drawing Test compared to that of standard screening tests for Alzheimer's disease: results from a study of Brazilian elderly with heterogeneous educational backgrounds. Int Psychogeriatr 22:64-71

4. Barage SH, Sonawane KD (2015) Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides 52:1-18

5. Bertolucci PH, Okamoto IH, Brucki SM, Siviero MO, Toniolo Neto J, Ramos LR (2001) Applicability of the CERAD neuropsychological battery to Brazilian elderly. Arq Neuropsiquiatr 59:532-536

6. Bertolucci PHF, Okamoto IH, Toniolo Neto J, Ramos LR, Brucki SMD (1998) Desempenho da populacao brasileira na bateria neuropsicologica do Consortium to Establish a Registry for Alzheimer's Disease (CERAD). In:*Revista de Psiquiatria Clínica*, São Paulo, pp 80-83

7. Bjerke M, Engelborghs S (2018) Cerebrospinal Fluid Biomarkers for Early and Differential Alzheimer's Disease Diagnosis. J Alzheimers Dis 62:1199-1209

8. Brucki SM, Malheiros SM, Okamoto IH, Bertolucci PH (1997) [Normative data on the verbal fluency test in the animal category in our milieu]. Arq Neuropsiquiatr 55:56-61

9. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O (2012) Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry 69:98-106

10. Chandler MJ, Lacritz LH, Hynan LS, Barnard HD, Allen G, Deschner M, Weiner MF, Cullum CM (2005) A total score for the CERAD neuropsychological battery. Neurology 65:102-106

11. Ekman U, Ferreira D, Westman E (2018) The A/T/N biomarker scheme and patterns of brain atrophy assessed in mild cognitive impairment. Sci Rep 8:8431

12. Ferreira D, Perestelo-Pérez L, Westman E, Wahlund LO, Sarría A, Serrano-Aguilar P (2014) Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. Front Aging Neurosci 6:47

13. Ferreira D, Rivero-Santana A, Perestelo-Pérez L, Westman E, Wahlund LO, Sarría A, Serrano-Aguilar P (2014) Improving CSF Biomarkers' Performance for Predicting Progression from Mild Cognitive Impairment to Alzheimer's Disease by Considering Different Confounding Factors: A Meta-Analysis. Front Aging Neurosci 6:287

14. Frölich L, Peters O, Lewczuk P, Gruber O, Teipel SJ, Gertz HJ, Jahn H, Jessen F, Kurz A, Luckhaus C, Hüll M, Pantel J, Reischies FM, Schröder J, Wagner M, Rienhoff O, Wolf S, Bauer C, Schuchhardt J, Heuser I, Rüther E, Henn F, Maier W, Wiltfang J, Kornhuber J (2017) Incremental value of biomarker combinations to predict progression of mild cognitive impairment to Alzheimer's dementia. Alzheimers Res Ther 9:84

15. Gainotti G, Quaranta D, Vita MG, Marra C (2014) Neuropsychological predictors of conversion from mild cognitive impairment to Alzheimer's disease. J Alzheimers Dis 38:481-495

16. Haapalinna F, Paajanen T, Penttinen J, Kokki H, Kokki M, Koivisto AM, Hartikainen P, Solje E, Hänninen T, Remes AM, Herukka SK (2016) Low Cerebrospinal Fluid Amyloid-Beta Concentration Is Associated with Poorer Delayed Memory Recall in Women. Dement Geriatr Cogn Dis Extra 6:303-312

17. Haldenwanger A, Eling P, Kastrup A, Hildebrandt H (2010) Correlation between cognitive impairment and CSF biomarkers in amnesic MCI, non-amnesic MCI, and Alzheimer's disease. J Alzheimers Dis 22:971-980

18. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Thies B, Trojanowski JQ, Vinters HV, Montine TJ (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1-13

19. Isaacs B, Kennie AT (1973) The Set test as an aid to the detection of dementia in old people. Br J Psychiatry 123:467-470

20. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R, Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 14:535-562

21. Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Pankratz VS, Donohue MC, Trojanowski JQ (2013) Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 12:207-216

22. Kaplan E, Goodglass H, Weintraub S (1983) Boston naming test. Lea & Febiger, Philadelphia

23. Landau SM, Harvey D, Madison CM, Reiman EM, Foster NL, Aisen PS, Petersen RC, Shaw LM, Trojanowski JQ, Jack CR, Weiner MW, Jagust WJ, Initiative AsDN (2010) Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 75:230-238

24. Lange C, Suppa P, Pietrzyk U, Makowski MR, Spies L, Peters O, Buchert R, Initiative AsDN (2018) Prediction of Alzheimer's Dementia in Patients with Amnestic Mild Cognitive Impairment in Clinical Routine: Incremental Value of Biomarkers of Neurodegeneration and Brain Amyloidosis Added Stepwise to Cognitive Status. J Alzheimers Dis 61:373-388

25. Mantzavinos V, Alexiou A (2017) Biomarkers for Alzheimer's Disease Diagnosis. Curr Alzheimer Res 14:1149-1154

26. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263-269

27. Memória CM, Yassuda MS, Nakano EY, Forlenza OV (2013) Brief screening for mild cognitive impairment: validation of the Brazilian version of the Montreal cognitive assessment. Int J Geriatr Psychiatry 28:34-40

28. Miotto EC, Sato J, Lucia MC, Camargo CH, Scaff M (2010) Development of an adapted version of the Boston Naming Test for Portuguese speakers. Rev Bras Psiquiatr 32:279-282

29. Mitchell AJ, Shiri-Feshki M (2009) Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand 119:252-265

30. Monastero R, Mangialasche F, Camarda C, Ercolani S, Camarda R (2009) A systematic review of neuropsychiatric symptoms in mild cognitive impairment. J Alzheimers Dis 18:11-30

31. Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43:2412-2414

32. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C (1989) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39:1159-1165

33. Moura RR, Coelho AV, Balbino VeQ, Crovella S, Brandão LA (2015) Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. Am J Hum Biol 27:674-680

34. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H (2005) The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53:695-699

35. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, Hölttä M, Rosén C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 15:673-684

36. Parnetti L, Chiasserini D, Eusebi P, Giannandrea D, Bellomo G, De Carlo C, Padiglioni C, Mastrocola S, Lisetti V, Calabresi P (2012) Performance of aβ1-40, aβ1-42, total tau, and phosphorylated tau as predictors of dementia in a cohort of patients with mild cognitive impairment. J Alzheimers Dis 29:229-238

37. Petersen RC (2018) How early can we diagnose Alzheimer disease (and is it sufficient)? The 2017 Wartenberg lecture. Neurology 91:395-402

38. Petersen RC (2004) Mild cognitive impairment as a diagnostic entity. J Intern Med 256:183-194

39. Petersen RC, Caracciolo B, Brayne C, Gauthier S, Jelic V, Fratiglioni L (2014) Mild cognitive impairment: a concept in evolution. J Intern Med 275:214-228

40. Prestia A, Caroli A, Wade SK, van der Flier WM, Ossenkoppele R, Van Berckel B, Barkhof F, Teunissen CE, Wall A, Carter SF, Schöll M, Choo IH, Nordberg A, Scheltens P, Frisoni GB (2015) Prediction of AD dementia by biomarkers following the

NIA-AA and IWG diagnostic criteria in MCI patients from three European memory clinics. Alzheimers Dement 11:1191-1201

41. Rami L, Fortea J, Bosch B, Solé-Padullés C, Lladó A, Iranzo A, Sánchez-Valle R, Molinuevo JL (2011) Cerebrospinal fluid biomarkers and memory present distinct associations along the continuum from healthy subjects to AD patients. J Alzheimers Dis 23:319-326

42. Rizzi L, Maria Portal M, Batista CEA, Missiaggia L, Roriz-Cruz M (2018) CSF Aβ1–42, but not p-Tau181, differentiates aMCI from SCI. Brain Research 1678:27-31

43. Rizzi L, Missiaggia L, Roriz-Cruz M (2018) CSF Aβ 1-42 , but not p-Tau181, predicted progression from amnestic MCI to Alzheimer's Disease Dementia. Neuromolecular Med

44. Rosen WG, Mohs RC, Davis KL (1984) A new rating scale for Alzheimer's disease. Am J Psychiatry 141:1356-1364

45. Santana I, Baldeiras I, Santiago B, Duro D, Freitas S, Pereira MT, Almeida MR, Oliveira CR (2018) Underlying Biological Processes in Mild Cognitive Impairment: Amyloidosis Versus Neurodegeneration. J Alzheimers Dis 64:S647-S657

46. Schjønning Nielsen M, Simonsen AH, Siersma V, Hasselbalch SG, Høgh P (2016) Are CSF Biomarkers Useful as Prognostic Indicators in Diagnostically Unresolved Cognitively Impaired Patients in a Normal Clinical Setting. Dement Geriatr Cogn Dis Extra 6:465-476

47. Shin S, Kim JH, Cho JH, Kim GS, Choi SA, Lee JH, Initiative AsDN (2015) Mild cognitive impairment due to alzheimer disease is less likely under the age of 65. Alzheimer Dis Assoc Disord 29:26-31

48. Shulman KI, Gold DP, Cohen CA, Zucchero CA (1993) Clock-drawing and dementia in the community: A longitudinal study. International Journal of Geriatric Psychiatry 8:487-496

49. Steenland K, Zhao L, John SE, Goldstein FC, Levey A, Alvaro A, Initiative AsDN (2018) A 'Framingham-like' Algorithm for Predicting 4-Year Risk of Progression to Amnestic Mild Cognitive Impairment or Alzheimer's Disease Using Multidomain Information. J Alzheimers Dis 63:1383-1393

50. Tondelli M, Bedin R, Chiari A, Molinari MA, Bonifacio G, Lelli N, Trenti T, Nichelli P (2015) Role of cerebrospinal fluid biomarkers to predict conversion to dementia in patients with mild cognitive impairment: a clinical cohort study. Clin Chem Lab Med 53:453-460

51. Trzepacz PT, Hochstetler H, Wang S, Walker B, Saykin AJ, Initiative AsDN (2015) Relationship between the Montreal Cognitive Assessment and Mini-mental State Examination for assessment of mild cognitive impairment in older adults. BMC Geriatr 15:107

52. van Rossum IA, Vos SJ, Burns L, Knol DL, Scheltens P, Soininen H, Wahlund LO, Hampel H, Tsolaki M, Minthon L, L'italien G, van der Flier WM, Teunissen CE, Blennow K, Barkhof F, Rueckert D, Wolz R, Verhey F, Visser PJ (2012) Injury markers

predict time to dementia in subjects with MCI and amyloid pathology. Neurology 79:1809-1816

53. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO (1982) Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17:37-49

54. Youden WJ (1950) Index for rating diagnostic tests. Cancer 3:32-35

Table 1. Baseline demographic, neuropsychological and CSF biomarker data of the study population that completed the follow-up.

	SCI	aMCI Non-Progressors	aMCI Progressors	*P* value	Adjusted *P* value
n	11	17	14		
Age (years): median (min – max)	62 (60 – 76)	66 (60 – 78)	69 (63 – 76)	0.069	
Education (years): median (min – max)	11 (5 – 18)	11 (1 – 20)	11 (3 – 18)	0.354	
Gender (female/male): n	8/3	12/5	9/5	0.237	
$A\beta_{1-42}$ (pg/mL): median (P25 – P75)	850.58 (593.06 – 1002.89)[b]	900.17 (732.17 – 1064.29)[b]	497.91 (436.03 – 627.89)[a]	<0.001	<0.001
p-Tau_{181} (pg/mL): median (P25 – P75)	55.94 (47.84 – 71.45)	56.40 (47.54 – 67.58)	80.80 (47.72 – 103.54)	0.202	0.279*
p-Tau_{181}/$A\beta_{1-42}$ ratio: median(P25 – P75)	0.062 (0.051 – 0.094)[b]	0.060 (0.053 – 0.075)[b]	0.156 (0.071 – 0.214)[a]	0.003	0.001*
CERADs: median (min – max)	29 (23 – 40)	27 (22 – 34)	26.5 (18 – 37)	0.137	0.128
GDS: median (min – max)	1 (0 – 5)	2 (0 – 5)	2.5 (0 – 5)	0.728	0.820
CDT: median (min – max)	4 (1 – 5)	4 (1 – 5)	5 (1 – 5)	0.061	0.293

†aMCI: amnestic Mild Cognitive Impairment; CDT: Clock Drawing Test; CERADs: Consortium to Establish a Registry for Alzheimer's Disease subscore; GDS: Geriatric Depression Scale; SCI: Subjective Cognitive Impairment; P25: percentile 25; P75: percentile 75. ‡ Results were obtained performing the Kruskal-Wallis test followed by the Dunn *post-hoc* test. [a,b] Equal letters do not differ according to the Dunn *post-hoc* test at 5% significance. Regarding gender, the Chi-Square Test was used. *P* values adjusted for age were obtained performing the ANCOVA test, *with logarithmic transformation.

Table 2. Demographic and neuropsychological endpoint data of the study population.

	SCI	aMCI Non-Progressors	aMCI Progressors	*P* value	Adjusted *P* value
n	11	17	14		
Age (years): median (min – max)*	66 (63 – 80)	70 (64 – 82)	73.5 (67 – 80)	0.057	
ΔCERADs: median (min – max)	-2 (-11 – 7)[a,b]	3 (-9 – 9)[b]	-6.5 (-22 – 4)[a]	0.002	0.001
ΔGDS: median (min – max)	1 (3 – 11)	1 (-2 – 11)	0 (-5 – 3)	0.120	0.127
MoCA: median (min – max)	27 (18 – 29)[b]	23 (17 – 27)[a,b]	16.5 (5 – 28)[a]	0.001	<0.001
BNT: median (min – max)	15 (13 – 15)	15 (11-15)	13 (5-15)	0.057	0.040[¥]
VFT: median (min – max)	16 (11 – 19)	12 (8 – 21)	13 (0 – 20)	0.306	0.185
CP: median (min – max)	7 (3 – 11)	7 (0 – 11)	4.5 (2 – 8)	0.058	0.102
CDT: median (min – max)	5 (1 – 5)	4 (1 – 5)	4 (0 – 5)	0.150	0.311

†aMCI: amnestic Mild Cognitive Impairment; BNT: Boston Naming Test; CDT: Clock Drawing Test; CERADs: Consortium to Establish a Registry for Alzheimer's Disease subscore; CP: Constructional Praxis; GDS: Geriatric Depression Scale; MoCA: Montreal Cognitive Assessment; SCI: Subjective Cognitive Impairment; VFT: Verbal Fluency Test; Δ: values from 2018 minus 2013; P25: percentile 25; P75: percentile 75. ‡ Results were obtained performing the Kruskal-Wallis test followed by the Dunn *post-hoc* test. [a, b] Equal letters do not differ according to the Dunn *post-hoc* test at 5% significance. *P* values adjusted for age were obtained performing the ANCOVA test. [¥] Levene's Test for Equality of Variances: $P = 0.004$. *Differences in median age from the baseline to endpoint were due to small differences between time of reevaluation and birthdate.

Table 3. ROC curve parameters of CSF biomarkers in predicting progression from aMCI to ADD.

Threshold values‡	aMCI Progressors	aMCI Non-Progressors	ROC AUC (CI95%)	Sensitivity (%) (CI95%)	Specificity (%) (CI95%)	Test accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
$A\beta_{1-42}$ < 618.5 pg/mL	11	1	0.912 (0.676 – 0.964)	78.5 (52.4 – 92.4)	94.1 (73.0 – 98.9)	87.1	91.7	84.2
$A\beta_{1-42}$ > 618.5 pg/mL	3	16						
p-Tau_{181} / $A\beta_{1-42}$ > 0.135	8	0	0.849 (0.71–0.984)	57.1 (32.6 – 78.6)	100.0 (81.6 – 100.0)	80.6	100.0	73.9
p-Tau_{181} / $A\beta_{1-42}$ < 0.135	6	17						

† ADD: Alzheimer's disease dementia; aMCI: amnestic mild cognitive impairment; AUC: area under the curve; ROC: receiver operating characteristic. ‡ Youden index was used.

Table 4. Demographic and neuropsychological endpoint data between those above and under the cutoff for Aβ.

	SCI	Aβ > 618.5 pg/mL	Aβ < 618.5 pg/mL	*P* value	Adjusted *P* value
n	11	19	12		
Age (years): median (min – max)	66 (63 – 80)	70 (64 – 82)	72.5 (67 – 80)	0.067	
Education (years): median (min – max)	11 (5– 18)	11 (1 – 20)	9.5 (3 – 18)	0.243	
Gender (female/male): n	8/3	12/7	9/3	0.749	
ΔCERADs: median (min – max)	-2 (-11 – 7) [b]	2 (-9 – 9) [b]	-7 (-22 – 3) [a]	0.001	<0.001
ΔGDS: median (min – max)	1 (-2 – -7) [a,b]	1 (-1 – 11) [b]	0 (-5 – 3) [a]	0.046	0.066
MoCA: median (min – max)	27 (18– 29) [b]	23 (16 – 28) [a,b]	16.5 (5 – 27) [a]	0.001	<0.001
BNT: median (min – max)	15 (13– 15) [b]	15 (11 – 15) [a,b]	12.5 (5 – 15) [a]	0.035	0.016[¥]
VFT: median (min – max)	16 (11– 19)	13 (8 – 21)	13 (0 – 18)	0.194	0.062
CP: median (min – max)	7 (3– 11)	7 (0 – 11)	4.5 (2 – 8)	0.118	0.166
CDT: median (min – max)	5 (1– 5)	4 (1 – 5)	4 (0 – 5)	0.145	0.312

†aMCI: amnestic Mild Cognitive Impairment; BNT: Boston Naming Test; CDT: Clock Drawing Test; CERADs: Consortium to Establish a Registry for Alzheimer's Disease subscore; CP: Constructional Praxis; GDS: Geriatric Depression Scale; MoCA: Montreal Cognitive Assessment; SCI: Subjective Cognitive Impairment; VFT: Verbal Fluency Test; Δ: values from 2018 minus 2013; P25: percentile 25; P75: percentile 75. ‡ Results were obtained performing the Kruskal-Wallis followed by the Dunn *post-hoc* test. [a, b] Equal letters do not differ according to the Dunn *post-hoc* test at 5% significance. *P* values adjusted for age were obtained performing the ANCOVA test. [¥] Levene's Test of Equality of Variances: $P = 0.005$.

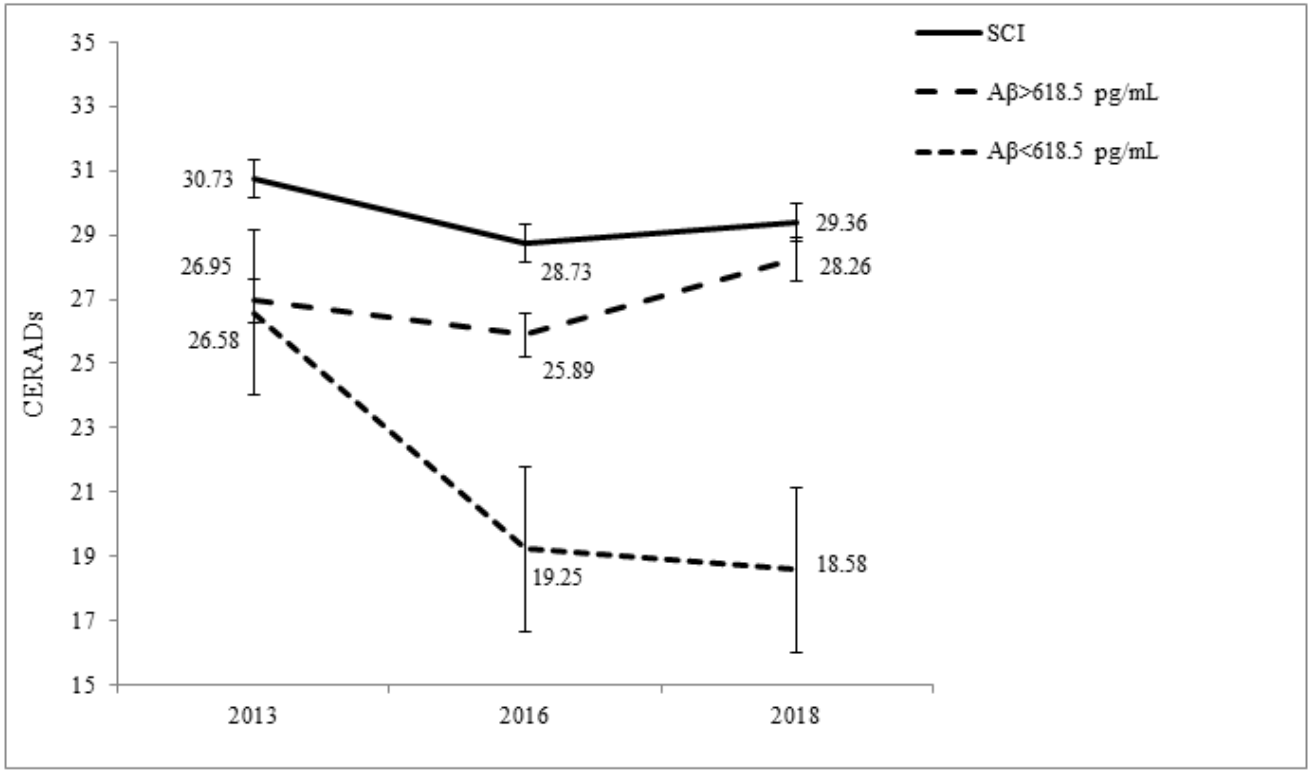


Fig. 1 Line chart of the means and standard errors of CERADs among those above and under the cutoff for Aβ, and the SCI group. There was an interaction between variables ($P = 0.002$). When the time was set and the groups were compared, there was no difference in the SCI ($P = 0.316$) and Aβ > 618.5 pg/mL ($P = 0.063$) groups, but there was a significant difference in the Aβ < 618.5 pg/mL group ($P < 0.001$; 2013 versus 2016 and $P = 0.001$; 2013 versus 2018 $P < 0.001$). When the groups were fixed and times were compared, there was no difference between groups in 2013 ($P = 0.078$), but there was a significant difference at the follow-up in 2016 ($P = 0.004$; Aβ < 618.5 pg/mL versus SCI $P = 0.005$), and in 2018 ($P = 0.002$; Aβ < 618.5 pg/mL versus SCI $P = 0.001$; Aβ < 618.5 pg/mL versus Aβ > 618.5 pg/mL $P = 0.002$). †CERADs: Consortium to Establish a Registry for Alzheimer's Disease subscore; SCI: Subjective Cognitive Impairment. ‡Generalized Estimating Equation Model followed by the Bonferroni *post-hoc* test.

CAPÍTULO V

1. CONSIDERAÇÕES FINAIS

A presente tese agregou conhecimento na avaliação dos biomarcadores liquóricos $A\beta_{1-42}$ e p-Tau_{181} na predição do risco de progressão à demência da DA em um grupo de indivíduos com CCL-a. Em nosso estudo, 45,2% dos indivíduos com CCL-a progrediram para a DDA em cinco anos. A proteína $A\beta_{1-42}$ mensurada no líquor proporcionou o melhor risco cumulativo de progressão do CCL-a para a fase demencial da DA. Enquanto que a razão p-Tau_{181}/$A\beta_{1-42}$ não foi melhor que a proteína $A\beta_{1-42}$ isolada na predição da progressão do CCL-a para a DDA. No entanto, os níveis de p-Tau_{181} ajudaram a explicar um extra de 9% da variância ΔCERADs em cinco anos. Além disso, verificou-se que, numa perspectiva clínica, o valor dos biomarcadores liquóricos na predição da progressão de indivíduos com CCL-a para demência da DA é mais relevante durante os primeiros anos de acompanhamento do que em intervalos mais longos.

Um progresso substancial foi feito ao longo das últimas décadas na compreensão DA e no uso dos biomarcadores. No entanto, ainda não há padronização na utilização dos mesmos de modo que possam ser implementados efetivamente na prática clínica, o que atrasa o diagnóstico e, consequentemente, as intervenções farmacológicas e terapêuticas que poderiam retardar ou impedir a progressão da patologia da DA. Este estudo parte de uma linha de pesquisa cuja abordagem visa integrar a pesquisa à assistência e colabora para que o conhecimento gerado seja transferido ao paciente e à sociedade, auxiliando no diagnóstico clínico. Desta forma, buscou-se contribuir com o desenvolvimento científico e tecnológico e, com isto, auxiliar no fortalecimento da pesquisa brasileira no cenário nacional e internacional.

2. PERSPECTIVAS FUTURAS

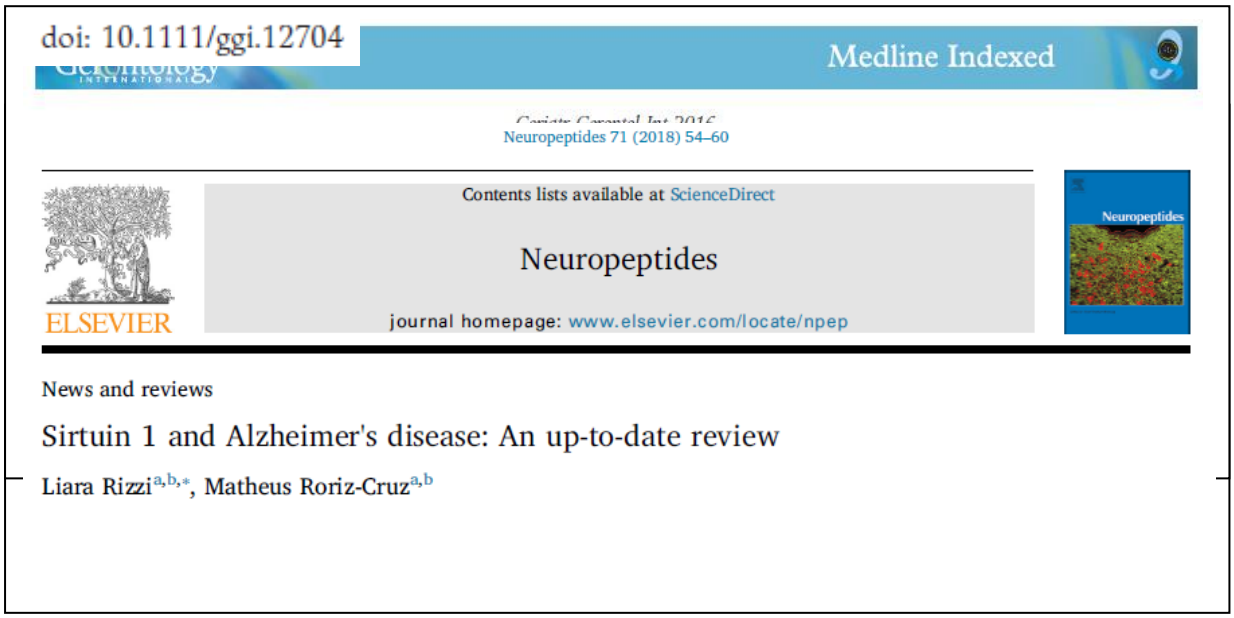
Embora numerosas pesquisas tenham relatado bom desempenho diagnóstico por meio dos biomarcadores, ainda observam-se diferenças marcantes entre os estudos. Dessa forma, mais pesquisas nessa área se tornam necessárias, com amostras populacionais maiores e com rigor na seleção de indivíduos, de modo a melhor esclarecer a performance dos biomarcadores e promover a sua implementação generalizada na prática clínica.

Este estudo encoraja novas pesquisas com os biomarcadores na DA, envolvendo amostras maiores, bem como estudos de coorte com dosagens iniciais e seriadas das proteínas β-amilóide e Tau, juntamente com avaliações clínicas e neuropsicológicas intermitentes de modo a melhor elucidar os mecanismos etiopatogênicos e fisiopatológicos da doença. As perspectivas futuras consistem no seguimento desses indivíduos visando reavaliação quanto à progressão à DA em um período maior de tempo, além de novas dosagens e possíveis correlações com outros biomarcadores.

CAPÍTULO VI

1. ANEXOS

1.1 Artigos científicos publicados durante o período de doutoramento



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News and reviews

Sirtuin 1 and Alzheimer's disease: An up-to-date review

Liara Rizzi[a,b,*], Matheus Roriz-Cruz[a,b]

https://doi.org/10.1016/j.npep.2018.07.001

Alzheimer's, Dementia & Cognitive Neurology

Research Article

Haemodialysis improves uraemic patients' cognition: a pilot study

Rodrigo T Starosta[1*], Marcos Vinícius Vidor[1], Liara Rizzi[2], Fabrício Marques[2] and Matheus Roriz-Cruz[3]

doi: 10.15761/ADCN.1000101 *Alzheimers Dement Cogn Neurol*, 2016

1.2 Guideline STARD

STARD checklist for the reporting of studies of diagnostic accuracy.

Section and Topic	Item #		On page #

TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	99, 100
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	102
METHODS			
Participants	3	Describe the study population: The inclusion and exclusion criteria, setting and locations where the data were collected.	102
	4	Describe participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	102, 103
	5	Describe participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected.	102, 103
	6	Describe data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	102, 103, 104
Test methods	7	Describe the reference standard and its rationale.	104
	8	Describe technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	104
	9	Describe definition of and rationale for the units, cutoffs and/or categories of the results of the index tests and the reference standard.	104, 106
	10	Describe the number, training and expertise of the persons executing and reading the index tests and the reference standard.	104
	11	Describe whether or not the readers of the index tests and reference standard were	

		blind (masked) to the results of the other test and describe any other clinical information available to the readers.	104
Statistical methods	12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	104, 105
	13	Describe methods for calculating test reproducibility, if done.	NA
RESULTS			
Participants	14	Report when study was done, including beginning and ending dates of recruitment.	102, 105
	15	Report clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, comorbidity, current treatments, recruitment centers).	105
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	105
Test results	17	Report time interval from the index tests to the reference standard, and any treatment administered between.	NA
	18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	106
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	123
	20	Report any adverse events from performing the index tests or the reference standard.	NA
Estimates	21	Report estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	106, 123
	22	Report how indeterminate results, missing responses and outliers of the index tests were	NA

		handled.	
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	NA
	24	Report estimates of test reproducibility, if done.	NA
DISCUSSION	25	Discuss the clinical applicability of the study findings.	108

*NA: Not applicable.